The Force for Poleward Chromosome Motion in *Haemanthus* Cells Acts along the Length of the Chromosome during Metaphase but Only at the Kinetochore during Anaphase

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Abstract. The force for poleward chromosome motion during mitosis is thought to act, in all higher organisms, exclusively through the kinetochore. We have used time-lapse, video-enhanced, differential interference contrast light microscopy to determine the behavior of kinetochore-free "acentric" chromosome fragments and "monocentric" chromosomes containing one kinetochore, created at various stages of mitosis in living higher plant (*Haemanthus*) cells by laser microsurgery. Acentric fragments and monocentric chromosomes generated during spindle formation and metaphase both moved towards the closest spindle pole at a rate (~1.0 μm/min) similar to the poleward motion of anaphase chromosomes. This poleward transport of chromosome fragments ceased near the onset of anaphase and was replaced, near midanaphase, by another force that now transported the fragments to the spindle equator at 1.5–2.0 μm/min. These fragments then remained near the spindle midzone until phragmoplast development, at which time they were again transported randomly poleward but now at ~3 μm/min. This behavior of acentric chromosome fragments on anastral plant spindles differs from that reported for the astral spindles of vertebrate cells, and demonstrates that in forming plant spindles, a force for poleward chromosome motion is generated independent of the kinetochore. The data further suggest that the three stages of non-kinetochore chromosome transport we observed are all mediated by the spindle microtubules. Finally, our findings reveal that there are fundamental differences between the transport properties of forming mitotic spindles in plants and vertebrates.

During mitosis the two sister kinetochores on each replicated chromosome acquire a bundle of microtubules (Mt),[1] known as a kinetochore fiber, that securely tethers them to opposing spindle poles (for review see Salmon, 1989; Rieder, 1990). These kinetochore fibers are required for producing the forces that move chromosomes poleward throughout mitosis and, as a result, all current models envision that the poleward force on a chromosome acts exclusively on the kinetochore (for review see Mitchison, 1989; McIntosh and Hering, 1991; Ault and Rieder, 1994; Inoue and Salmon, 1995). Currently, the most popular mechanism envisions that this force is produced by kinetochore-associated molecular motors (for review see McIntosh and Pfarr, 1991; Sawin and Endow, 1993; Rieder and Salmon, 1994) acting on kinetochore Mts, which themselves are coordinately disassembling within the kinetochore (for review see Mitchison and Salmon, 1992; Desai and Mitchison, 1995).

In animal somatic cells the equatorial alignment of chromosomes on the forming spindle also appears to involve another force that acts on the chromosome arms to counterbalance the sister kinetochore-based poleward-directed forces (for review see Rieder and Salmon, 1994; Cassimeris et al., 1994; Fuller, 1995). This force, referred to as the polar ejection force or "polar wind," transports chromosome arms away from the closest pole during spindle formation (i.e., prometaphase). The presence of this ejection force can be easily demonstrated by severing chromosome arms from the kinetochore region with a laser microbeam during prometaphase. Under this condition the kinetochore-free (acentric) chromosome fragments (ACF) are transported away from the closest pole at ~2 μm/min (Rieder et al., 1986; Rieder and Salmon, 1994).

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1. *Abbreviations used in this paper:* ACF, acentric chromosome fragment; Mt, microtubule.

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The molecular mechanism behind the polar ejection force is unknown. The most popular hypothesis is that chromosome arms contain Mt plus-end motors that interact with astral and half-spindle Mts growing from the pole (Salmon, 1989; Ault et al., 1991; Carpenter, 1991). This hypothesis is supported circumstantially by Wang and Adler's (1995) recent finding that chromosome arms in chick cells become decorated with a kinesin-like molecule (chromokinesin) as the cell enters mitosis. It is also supported by functional studies in *Drosophila* (Afshar et al., 1995; Murphy and Karpen, 1995) and *Xenopus* (Vernos et al., 1995) that implicate chromosome-associated kinesin-like proteins (NOD and Xklp 1) in chromosome positioning on the meiotic (oocyte) spindle. Alternatively, the fact that the strength of the ejection force is related to Mt density, and depends on the normal behavior of Mt plus ends, suggests that it arises from the constant growth of dynamically unstable polar-nucleated Mt ends as they impact and push on the chromosome (Ault et al., 1991; Cassimeris et al., 1994; Rieder and Salmon, 1994).

An important consideration in mitosis research is the extent to which mechanistic findings in one system are applicable to other systems (Rieder et al., 1993). In this regard it is currently unknown whether the polar ejection force is a feature only of the astral mitosis of vertebrate cells, or whether it is also present during spindle formation in other cell types or organisms. Included here are the astral meiotic spindles of animal spermatocytes and the anastral spindles of many animal oocytes (e.g., mouse, *Drosophila*, *Xenopus*) and most plants. Unlike astral spindles, which are formed from two radial (astral) arrays of centrosomal Mts, the anastral spindles of some oocytes and higher plants are organized primarily by the chromosomes from random Mt arrays (for review see Smirnova and Bajer, 1992; Rieder et al., 1993; Vernos and Karsenti, 1995). Since forming anastral spindles lack well-developed arrays of polar Mts, and since the polar ejection force has only been demonstrated for spindles formed from astral Mt arrays, it is possible that these two distinct pathways of spindle morphogenesis produce spindles that utilize fundamentally different mechanisms to position chromosomes, i.e., that astral and anastral spindles exert different non–kinetochore-based forces on the chromosomes.

To evaluate this possibility, we have examined the behavior of ACFs and monocentric chromosomes containing a single kinetochore, created at various stages of mitosis by laser microsurgery in *Haemanthus* endosperm. We chose this higher plant system because, unlike the anastral spindles found in some animal oocytes, chromosome behavior on the forming anastral spindle can be clearly followed by video–light microscopy in living *Haemanthus* cells. Our observations reveal that *Haemanthus* spindles possess stage-specific non–kinetochore-based chromatin transport systems that are neither present during astral mitosis nor previously demonstrated for any other animal spindle. Unlike during astral mitosis, we show clearly for the first time that a poleward force is exerted along the entire length of *Haemanthus* chromosomes during spindle formation and chromosome congression. Then, at the onset of anaphase, this force disappears and is replaced by an opposite force that is similar to the polar ejection force seen in animal somatic cells. From our results we conclude that there are fundamental differences in how chromosomes become positioned on the forming mitotic spindle in plant and animal cells.

Materials and Methods

Endosperm Preparation

Endosperm from the African blood lily, *Haemanthus katherinae Bak*, were extruded onto agar-coated coverslips and mounted for live cell time-lapse video microscopy as described by Bajer and Molè-Bajer (1986). Some preparations were fixed and stained for the immunogold localization of spindle microtubules by light microscopy as described by De Mey et al. (1982).

Laser Microsurgery and Video-enhanced Microscopy

The differential interference contrast video-enhanced light microscopic/laser microsurgery system, described in detail by Cole et al. (1995), was used to sever chromosome arms and to follow their behavior in living *Haemanthus* endosperm cells. This system is centered around an inverted differential interference contrast light microscope (Diaphot 200; Nikon Inc., Garden City, NY) equipped with a motorized stage (Ludl MAC 2000; Ludl Electronics, Ltd., Hawthorne, NY). In brief, *Haemanthus* cells were followed with heat-filtered and shuttered 546-nm light using a ×60 1.4 NA differential interference contrast objective and a 0.85 NA condenser. Sequential images were obtained every 4 s by integrating two video frames directly onto a chip (P100 CCD; Paultek, Princeton, NJ). This integrated frame was then routed through Image I (Universal Imaging Corp., West Chester, PA) for image processing before storage on a laser videodisk recorder (LVR-3300M; Sony Corp. of America, Montvale, NJ). Electronic and optical noise within the system was reduced by background subtraction and by recording an eight-frame jumping average.

The chromosome microsurgery system used in our study was based on a pulsed (5 ns) Neodymium-YAG (yttrium-aluminum-garnet) laser (Surelite II; Continuum, Santa Clara, CA). The 1064-nm output of this laser was frequency doubled to 532 nm and filtered to remove stray 1064-nm light. This beam was then steered into the epiport of the Diaphot 200 where it was reflected, via a custom-made dichroic mirror (Omega Optical, Brattleboro, VT), through the Wollaston prism and onto the back aperture of the 1.4 NA objective. The objective then focused the beam to a diffraction-limited spot of ~0.5-μm diam (Cole et al., 1995). As demonstrated by Berns and others (for review see Berns et al., 1991; Cole et al., 1995; Rieder et al., 1995), pulsed 532-nm laser light can be used to selectively destroy chromatin and chromatin-associated organelles (e.g., kinetochores, nucleolar organizers) in living mitotic cells without deleteriously affecting spindle Mts or the process of mitosis.

The focused laser spot at the specimen plane was set near the center of a video screen, and its exact position was determined by irradiating a dried film of RBC5. Once located, the position of the laser was marked with cross-hairs on the video monitor. Cutting was then achieved by using the Ludl motorized stage to slowly pass the specimen through the fixed laser beam path. In our system optimal chromosome cutting was achieved by operating the laser at 10 Hz with 5-ns pulses, and each pulse contained ~500 nJ of power as measured at the focal point of the objective lens. In practice it took ~4–8 s, or 40–80 laser pulses, to completely sever a *Haemanthus* chromosome across its short axis.

Tracking Chromosomes and Chromosome Fragments

Distance vs. time plots were generated using software contained in the Image I system that calculates the distance between two movable cursors superimposed on sequential video frames. For this process time-lapse videodisk images were rerouted into Image I through a time-base corrector (For.A Corp., Natick, MA). Since dividing *Haemanthus* cells do not normally have focused spindle poles but are nonmotile and stuck to the agar, a stationary point on the video screen was selected as a reference point for each sequence analyzed. The distance between this point, and either the edge of a primary constriction or a distinguishable feature on a chromosome fragment edge (e.g., a barb of chromatin or a sharp edge), was then calculated for each frame.

Results

Both Kinetochores Are Required to Keep a Metaphase Chromosome Positioned on the Haemanthus Spindle Equator

When fully formed in highly flattened *Haemanthus* cells, the spindle contains two diffuse and broad spindle poles (Fig. 1 *A*). At this time it consists of conspicuous kinetochore fiber Mt bundles interspersed within an irregular meshwork of other Mts. By metaphase the sister kinetochore regions on all chromosomes are aligned near the spindle equator, but because of the irregularly shaped polar regions, the kinetochores do not necessarily define a linear metaphase plate across the broad axis of the spindle (Fig. 1 *A*). It is noteworthy that the arms of long chromosomes in these cells are bent near the kinetochore region and project towards one or both spindle poles (Fig. 1 *A*).

When one kinetochore on a bioriented chromosome is destroyed with a laser in animal somatic cells, the now monocentric chromosome moves to the pole to which the nonirradiated kinetochore is attached with a velocity similar to anaphase chromosomes (for review see Rieder et al., 1995). To determine whether this is also true for chromosomes on plant spindles, we selectively ablated one of the kinetochores on 14 bioriented metaphase chromosomes in seven *Haemanthus* cells. This operation produced a monocentric chromosome containing a single kinetochore that, in every case, moved from the metaphase plate into the polar region to which the nonirradiated kinetochore was attached (Fig. 2). At our temporal resolution of 4 s, which can resolve a lateral displacement of ~0.1 μm per frame, the kinetics of this motion was smooth and linear for 10–15 μm (Fig. 3) until the monocentric chromosome reached the end of the clear zone delineating the spindle pole. The velocity of this motion varied between monocentric chromosomes created in the same cell and ranged from 0.5 to 1.1 μm/min with an average of 0.8 μm/min. This is approximately the same poleward velocity exhibited by *Haemanthus* chromosomes during anaphase (our data; see also Bajer, 1966). After a monocentric chromosome reached the edge of the clear zone, which roughly defines the boundary of the spindle pole, its directed motion was replaced by a Brownian "drift" (Fig. 3). By behavioral criteria the irradiated kinetochore on laser-generated monocentric chromosomes was no longer functional since the chromatid

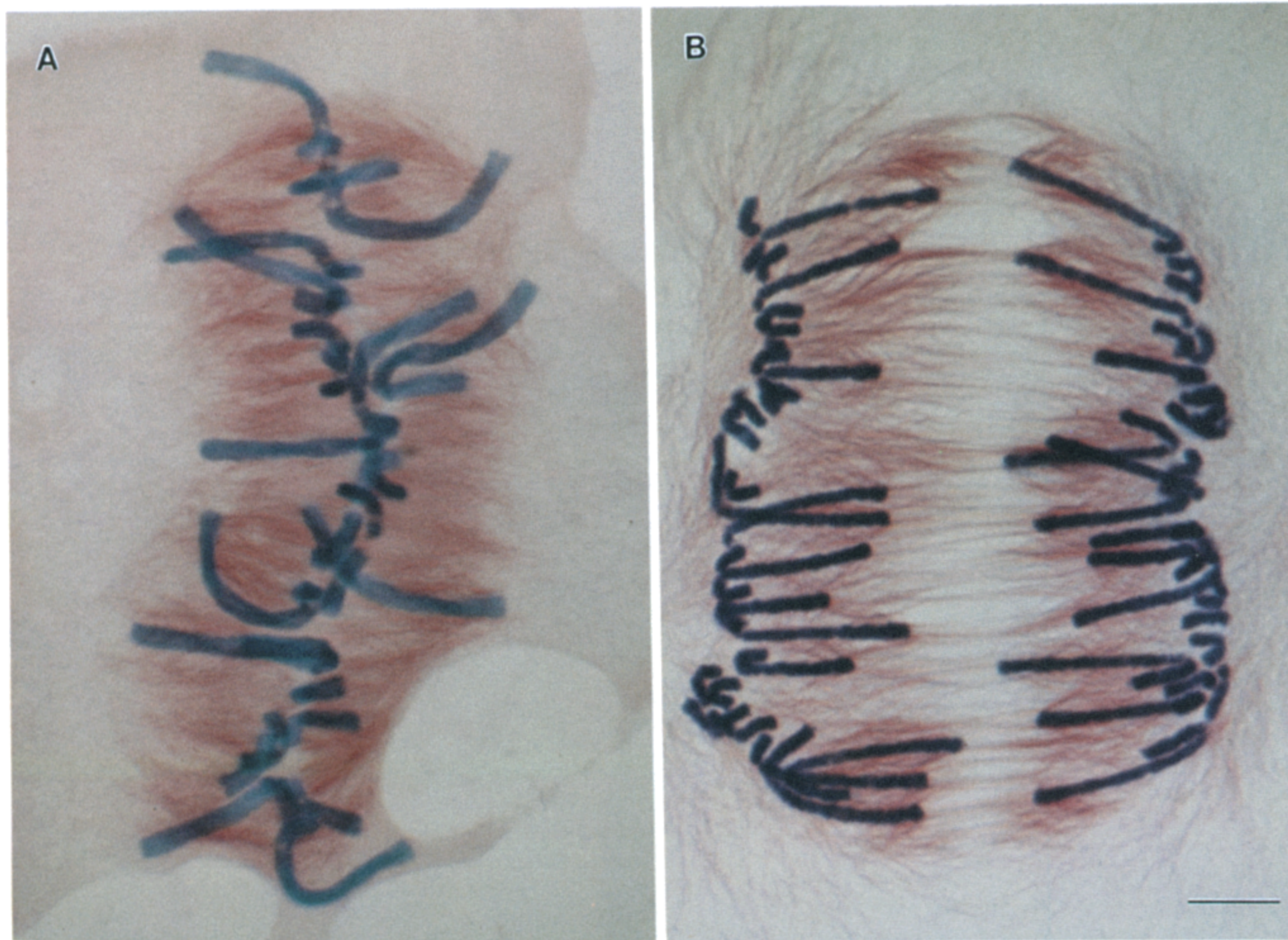

Figure 1. (*A* and *B*) Light micrographs of metaphase (*A*) and late anaphase (*B*) *Haemanthus* spindles stained for the immunogold localization of microtubules (*red*) and counterstained with toluidine blue for the localization of chromosomes. Note that during metaphase (*A*) the chromosome arms are bent in the direction of one or both of the spindle poles, while the kinetochore regions are positioned on the spindle equator. In anaphase (*B*) the arms now trail the kinetochore regions as they move poleward. Bar in *B*, 10 μm.

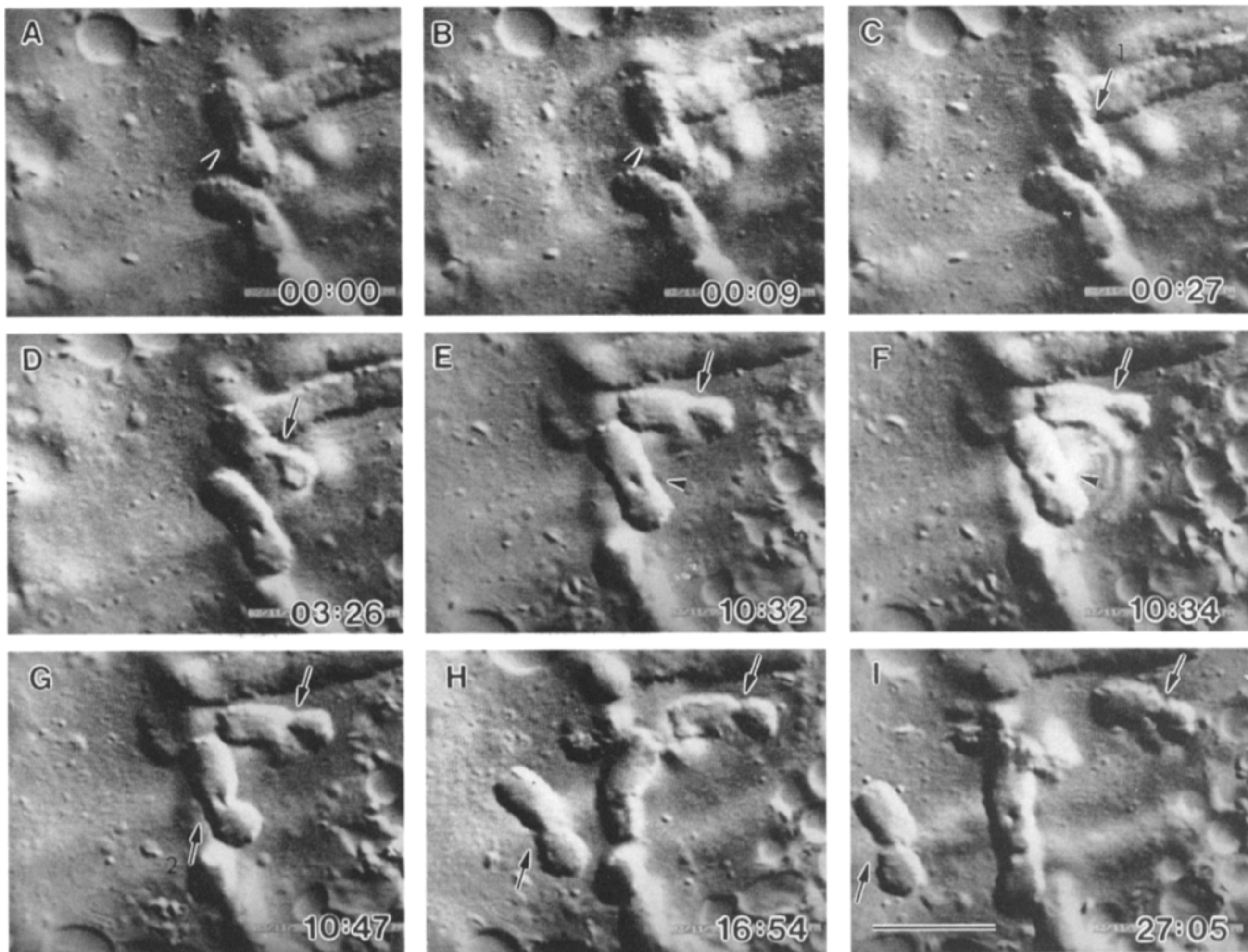

Figure 2. (*A–I*) Selected frames from a time-lapse video sequence of a *Haemanthus* cell in which one of two sister kinetochores was destroyed by the laser on two metaphase chromosomes. The arrowhead in *A* notes a kinetochore region on a bioriented chromosome that is subsequently destroyed by the laser in *B*. As a result of this operation, the now monocentric chromosome initiates motion towards the (right-hand) pole to which the nonirradiated kinetochore (*arrow* in *C–I*) is attached. Note that this chromosome rotates as it moves poleward (*C–E*) so that its long axis is parallel to the direction of poleward motion. The arrowhead in *E* notes one of the kinetochore regions on a second metaphase chromosome, which was subsequently destroyed by the laser in *F*. Again, after laser microsurgery, this now monocentric chromosome began moving towards the (left-hand) pole to which its nonirradiated kinetochore (*lower arrow* in *G–I*) was attached, but it did not rotate significantly during this motion. A kinetic plot for these two chromosomes is presented in Fig. 3. Time in min:s is shown at the lower right corner of each frame. Bar in *I*, 10 μm.

containing the irradiated kinetochore showed no kinetochore-based motility after disjoining from its sister during the ensuing anaphase (see below).

In the majority of cases, monocentric chromosomes rotated around their long axis as they moved poleward so that one arm led the motion; i.e., the kinetochore lost its poleward orientation during poleward motion (chromosome 1 in Fig. 2). The rate of poleward movement did not change during or after this rotational movement. This observation suggests that the force responsible for the poleward motion of monocentric chromosomes in metaphase cells is not generated solely at the kinetochore but also along the chromosome arms.

A Poleward Force Constantly Acts Along the Arms of Metaphase Chromosomes in Haemanthus

To determine if a pole-directed force acts along the arms of chromosomes in forming *Haemanthus* spindles, independent of that acting on the kinetochores, we cut the arms away from the centromeric region of 30 bioriented chromosomes in 20 late prometaphase/metaphase cells. Such an operation in animal somatic cells produces kinetochore-free ACFs that are expelled towards the spindle equator where they remain throughout the duration of mitosis (Rieder et al., 1986, 1995). However, in contrast to animal cells, we found that all ACFs created in prometaphase or metaphase *Haemanthus* cells were transported away from the metaphase plate and toward the closest pole (Fig. 4). The parameters of this poleward motion, including the average velocity (0.8 μm/min; range 0.5–1.8 μm/min), were similar to that of monocentric chromosomes formed in metaphase cells (see above and below).

To compare the poleward movement of both monocentric chromosomes and ACFs more accurately, we used the

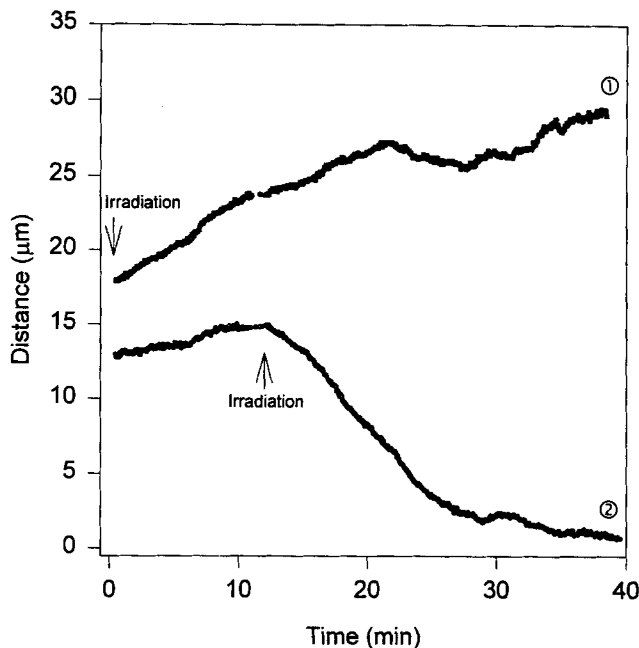

Figure 3. Kinetic plot for the upper (*1*) and lower (*2*) chromosomes described in Fig. 2. The times shown in Fig. 2 are coincident with points along the time axis of this plot. Note that once created by laser irradiation (*arrows*), both of these monocentric chromosomes initiated poleward motion with constant but different velocities (1 = 0.5 µm/min; 2 = 0.9 µm/min). After reaching the polar areas the chromosomes stopped moving and exhibited Brownian drift.

laser to generate, from the same chromosome, a monocentric fragment and multiple ACFs (Fig. 4). Under this condition both the monocentric and ACFs exhibited the same kinetic parameters during poleward motion (Fig. 5). From these observations we conclude that pole-directed forces are generated throughout the metaphase *Haemanthus* spindle, independent of the kinetochores that act along the length of the chromosomes.

The Poleward Force Acting on Haemanthus Chromosome Arms during Metaphase Is Abolished at Anaphase Onset

We next asked if the force that transports ACFs poleward during spindle formation in *Haemanthus* persists throughout mitosis. To address this question, we used the laser to sever one or both arms from the centromere region of 17 chromosomes, all positioned near the spindle equator, as anaphase was initiated in eight cells. By the time that the operation was complete, both the ACFs and the centromere-containing chromosome fragment had disjoined into independent sister component parts (Fig. 6, *A–D*). During the ensuing anaphase the kinetochore-containing (monocentric) chromosome fragments segregated with a normal velocity (~0.9 µm/min) to opposite polar regions, while all of the ACFs remained positioned near the spindle equator and exhibited Brownian motion (Figs. 6, *E–H*, and 7). From these observations we conclude that the pole-directed force acting on *Haemanthus* chromosome arms during metaphase is abolished at the onset of anaphase.

A New Force Arises during Midanaphase in Haemanthus that Transports ACFs away from the Pole and to the Spindle Equator

We then used the laser beam to sever arms from the poleward-moving chromosomes in mid to late anaphase cells. Under this condition we observed, in every instance ($n = 8$ from three cells), that the ACFs were immediately transported back to the spindle equator (data not shown; see below). From this set of observations, we conclude that a new force appears during anaphase that transports ACFs away from the spindle poles (and to the equator).

To determine when this away-from-the-pole force arises relative to anaphase onset, we followed the fate of monocentric and ACFs, generated during late metaphase, as the cell proceeded into and through anaphase. In these cells the monocentric and ACFs were in the process of being transported towards one of the poles and had attained variable positions between the metaphase plate and a polar region at the time anaphase was initiated. Under these circumstances the poleward motion of the ACFs abruptly ceased at chromatid disjunction (anaphase onset) and, after a delay of ~10 min, the ACFs initiated motion towards the spindle equator at 1.5–2.0 µm/min (Figs. 8 and 9). We observed this reversal of motion for all five ACFs followed in three cells, and in each case this away-from-the-pole motion ceased once the fragment reached the spindle equator (Fig. 9). From these experiments we conclude that a force appears during midanaphase that transports ACFs away from the pole and to the spindle equator. This force affects only ACFs positioned inside the spindle since ACFs expelled from the spindle region by the time of anaphase onset were never transported back to the spindle equator (Fig. 8).

Chromosome Fragments Positioned on the Spindle Equator during Anaphase Are Transported Poleward during Phragmoplast Development

Next, we followed the behavior of 20 ACFs, positioned near the spindle equator during anaphase, in seven cells forming phragmoplasts (cell plates). Although these fragments were created at various stages of mitosis, all of them had acquired a stationary position near the equator by late anaphase. When the phragmoplast began to form, these fragments were suddenly and rapidly transported towards the poles with an average velocity 3.0 µm/min (2.5–3.4 µm/min; Figs. 6, *H* and *I*, and 8 *I*). This poleward motion was linear and smooth until the fragments reached the corresponding mass of telophasing chromatin in the polar regions (Fig. 7). During this process the fragments were transported with their long axes parallel to the direction of motion (Figs. 6, *G–L*, and 8, *H* and *I*). In some cases a fragment was caught within the forming phragmoplast before initiating poleward motion. Under this condition the fragment became highly stretched towards the pole once it began to move (Figs. 6, *J–L*, and 8, *H* and *I*). Finally, ACFs that were derived from chromosomes initially positioned in one half-spindle during prometaphase or anaphase were randomly distributed as they moved poleward during telophase. These observations reveal that forces are generated at the time of phragmoplast development that move ACFs to the poles.

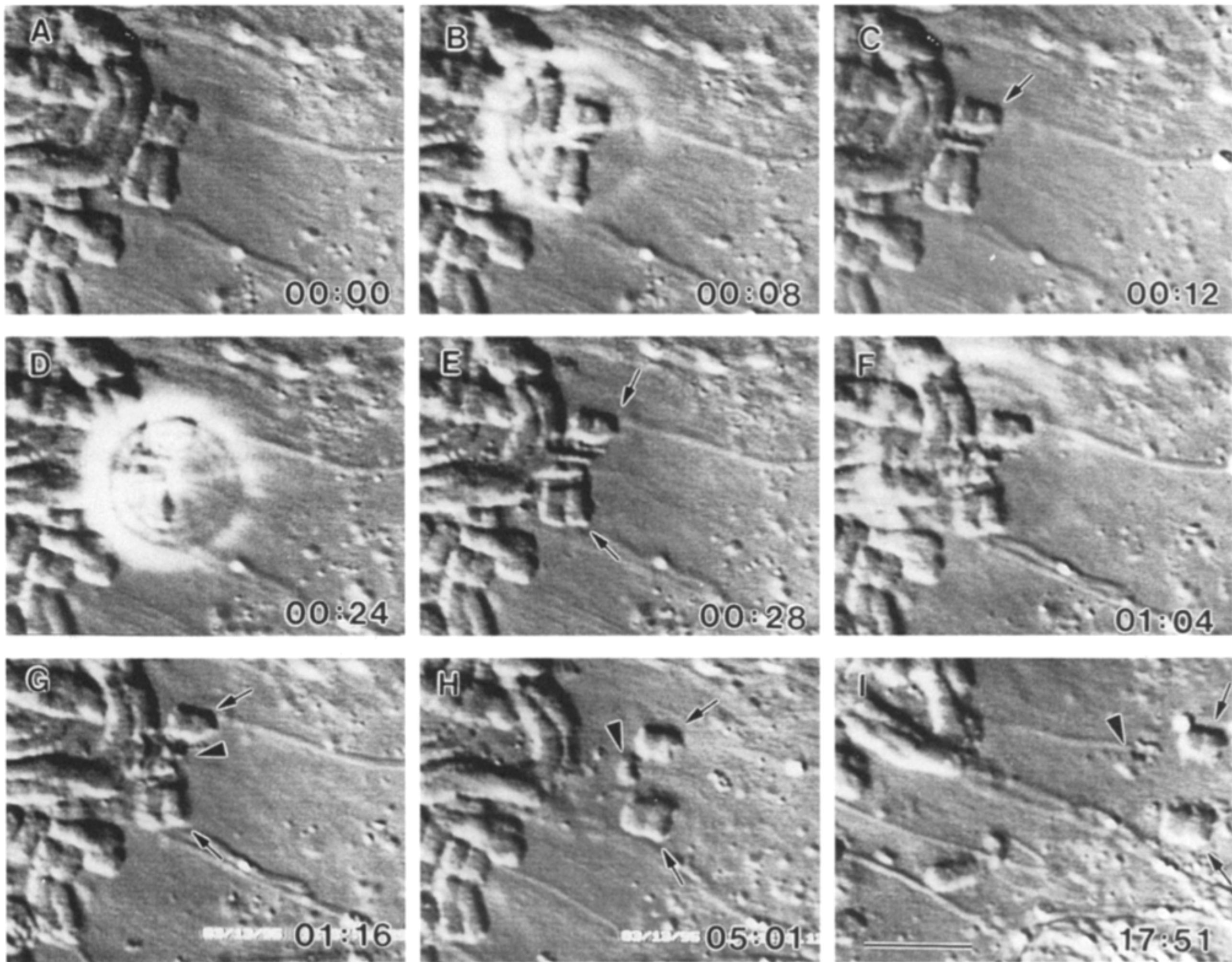

Figure 4. Selected frames from a time-lapse video sequence of a prometaphase *Haemanthus* cell in which the laser was used to sever both arms (*arrows* in *C* and *E*) from the centromere region of a congressing chromosome. After these two operations the distal kinetochore region of the centromere was then destroyed (*F*) to produce a monocentric chromosome fragment containing one kinetochore (*arrowhead* in *G*). This monocentric fragment, as well as both kinetochore-free acentric fragments, were then transported poleward (*H* and *I*). A kinetic plot for these three chromosome fragments is presented in Fig. 5. Time in min:s is shown at lower right corner of each frame. Bar in *I*, 10 μm.

Discussion

Recent models of mitosis envision that the force for poleward chromosome motion is generated by, or acts exclusively on, the kinetochore (McIntosh and Pfarr, 1991; Sawin and Endow, 1993; Inoue and Salmon, 1995). However, the arrangement of chromosome arms in plant spindles, which are usually bent towards the poles (Fig. 1 *A*), suggests that the arms are under the influence of a non–kinetochore-based poleward force (Bajer and Molè-Bajer, 1956). This notion is also consistent with earlier observations on the elimination of persistent nucleoli from forming plant spindles (for review see Bajer and Molè-Bajer, 1956; Fuge, 1990). Subsequently, Bajer (1958; see also Östergren et al., 1960; Bajer, 1966) followed β-irradiated *Haemanthus* endosperm as they entered and progressed through mitosis and found that the chromosome fragments produced were, for the most part, expelled from the spindle during the early stages of mitosis. He also observed "fragments moving out of the spindle in the direction of the poles" during spindle formation—an observation that supported the notion that in plants poleward forces act not just at the kinetochore but also along the length of chromosomes. However, this conclusion from living cells has always been suspect because at the time it was "very difficult to find out whether . . . the fragments [had] kinetochores or not . . ." (Bajer, 1958; p. 323).

Using laser microsurgery we were able to compare unambiguously the behavior of monocentric chromosomes, ACFs, and normal chromosomes within the same region of the spindle and to define changes in their behavior as a function of mitotic stage. Our data reveal that a poleward force acts along the length of the chromosome in forming *Haemanthus* spindles, independent of the kinetochore, and that this force can move large (≥10 μm) ACFs poleward with a velocity of anaphase chromosomes. The direction of this non–kinetochore-based chromosome transport is opposite that of forming spindles in animal somatic cells where ACFs are ejected away from the poles (for review see Rieder and Salmon, 1994). From these observations

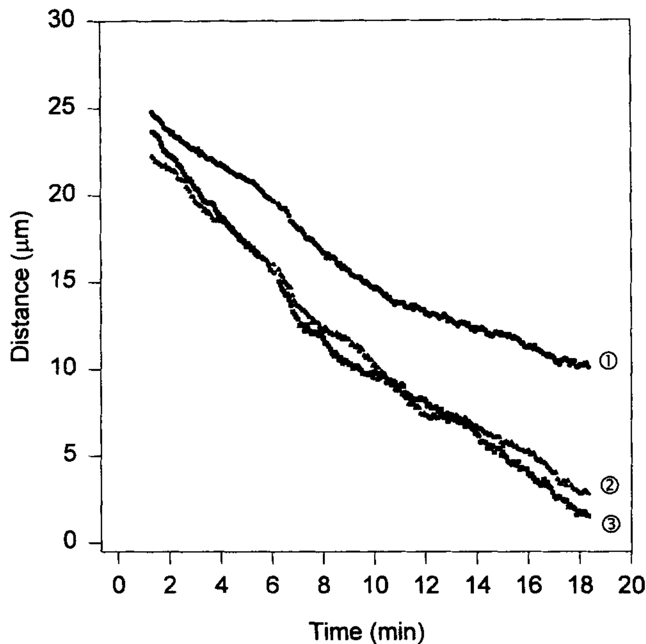

Figure 5. A kinetic plot of the monocentric (*1*) and acentric (*2,3*) chromosome fragments depicted in Fig. 4. The times shown in Fig. 4 are coincident with points along the time axis of this plot. Note that the velocity of poleward motion is similar for all of the fragments (monocentric = 0.8 μm/min; acentric = 1.0 μm/min).

we conclude that there are fundamental differences in the chromosome transport properties between the anastral mitotic spindles of higher plants and the astral mitotic spindles of animals. This raises the possibility that the non–kinetochore-based chromosome transport properties of those anastral spindles formed in animal oocytes (e.g., *Xenopus* and *Drosophila*) are more similar to those we describe for plants than for animal somatic cells.

The reason why plant anastral spindles and animal astral spindles differ in their non-kinetochore chromosome transport properties is unknown, but it may be related to the different pathways by which the polar regions in these two types of spindles are formed. Unlike for astral spindles, in which the poles are derived from preexisting radial arrays of centrosomal Mts, the polar regions in anastral spindles appear to be organized by the action of the chromosomes and Mt minus-end motors (Verde et al., 1991; Sawin and Endow, 1993; Vernos and Karsenti, 1995; McKim and Hawley, 1995). Thus, it is possible that the poleward transport of chromosome arms in *Haemanthus* spindles, which is mediated by and occurs towards the minus ends of spindle Mts (see below), is simply a manifestation of a continuous minus-Mt end transport process required to establish and to maintain spindle bipolarity in the absence of well-defined poles.

In *Haemanthus*, the poleward force acting along the chromosome terminates at the onset of anaphase and is then replaced by an opposite force that now transports ACFs positioned in the spindle to the spindle equator. This away-from-the-pole force is, in turn, replaced during phragmoplast development with another force that transports ACFs poleward. From these observations we conclude that the chromosome arms in *Haemanthus* endosperm experience stage-specific forces throughout mitosis independent of the poleward forces acting on the kinetochores.

Does the poleward force acting on chromosome arms in forming *Haemanthus* spindles also act on other components within the spindle? According to Bajer and Molè-Bajer (1956), some small "particles" in *Haemanthus* metaphase spindles do move poleward with the speed of anaphase chromosomes, but others oscillate irregularly or move to the spindle equator at the same time. Nicklas and Koch (1972) similarly found that 0.7–3.0-μm diam lipid droplets and mitochondria commonly exhibited poleward motions with the speed of anaphase chromosomes when micromanipulated into metaphase spindles of grasshopper spermatocytes. However, they also emphasized that such particles underwent radial motion out of the spindle as often as poleward motion, and the behavior of ACFs was never addressed. Thus, although limited, the current data suggest that when positioned in the spindle, membrane-bound particles undergo stochastic, bidirectional, "saltatory" motions along Mts that differ from the exclusively smooth and poleward motion of ACFs.

What roles do the stage-specific non–kinetochore-based chromosome transport forces play during mitosis in *Haemanthus*? Although we have no direct evidence, we favor the idea that the primary role of the poleward force is to position the chromosomes between the two polar regions during spindle formation, i.e., that it counters the activity of kinetochores (see below). This poleward force then might be terminated at the onset of anaphase so that it does not compete or interfere with the kinetochore-based poleward motion of the chromosomes, which is mandatory for proper chromosome segregation. The away-from-the-pole force that appears in midanaphase may lead to the accumulation of those components needed to construct the cell plate in the region where this structure will ultimately form. Finally, the production of a poleward force during phragmoplast development would transport long chromosome arms towards the nucleus so that they do not become trapped in the forming cell plate. Such a force might also produce periodic genomic changes by ensuring that naturally occurring ACFs ultimately become incorporated into a nucleus.

The Stage-specific Forces that Act along Haemanthus Chromosomes Are Mediated by Spindle Mts

The motion of ACFs is smooth and linear throughout mitosis in *Haemanthus* and, depending on the stage, the velocity ranges from 0.5 to 3.4 μm/min. We also found, as others have (e.g., Bajer, 1958), that ACFs move only when positioned within the spindle (Fig. 8), and that this motion is directed roughly parallel to the long axis of spindle Mts. Structural analyses of fixed cells reveal that ACFs within but not outside the spindle are in contact with numerous Mts (Bajer et al., 1987; Bajer and Vantard, 1988; Fuge, 1990). Finally, all chromosome motion, including the non–kinetochore-based transport that we have described, is inhibited by drugs that disrupt Mt assembly including colchicine (Molè-Bajer, 1958; Bajer, 1966) and chloral hydrate (Molè-Bajer, 1967). Together these findings strongly sup-

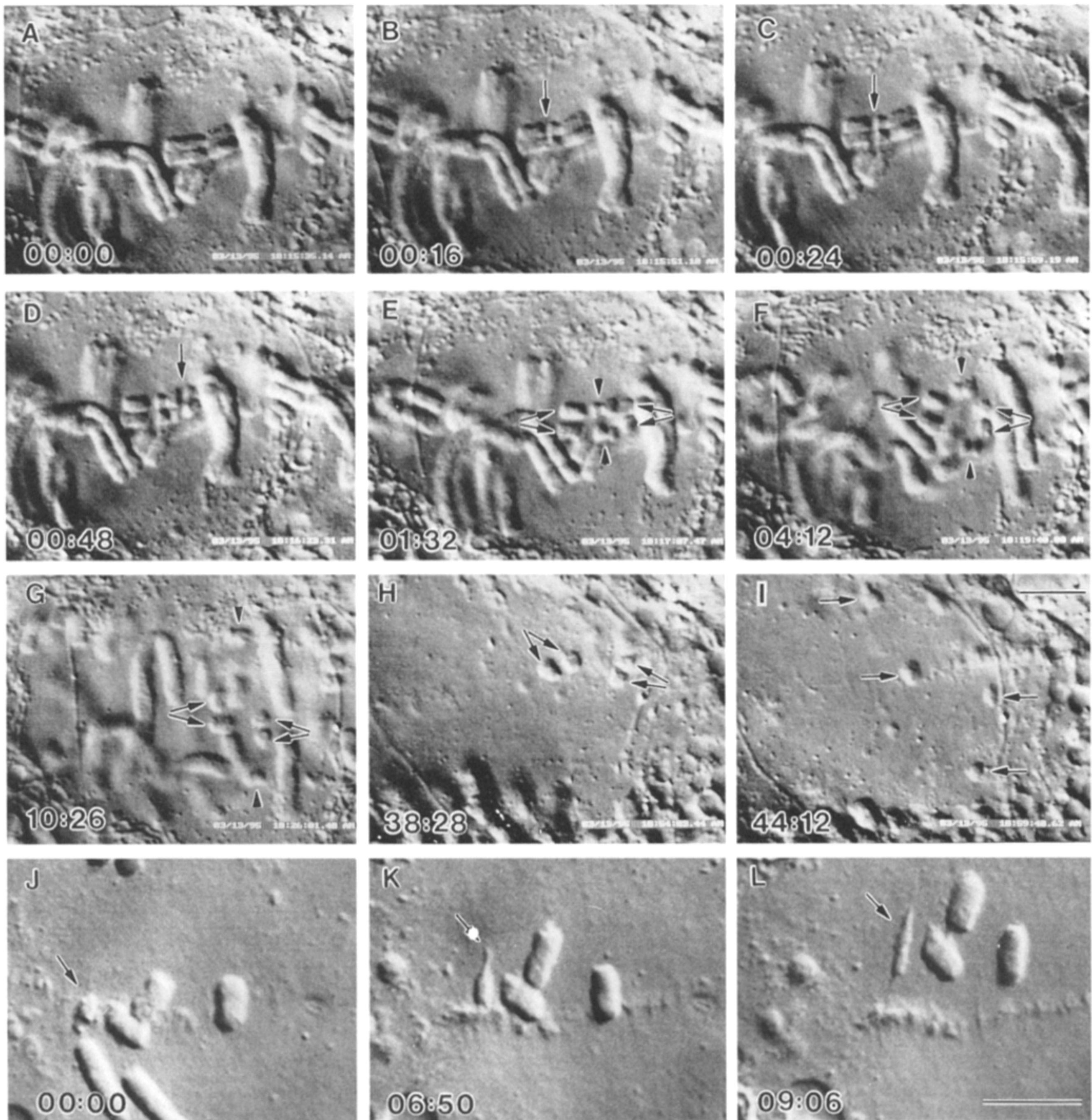

Figure 6. (*A–I*) Selected frames from a time-lapse video sequence of a *Haemanthus* cell in which the laser was used to sever both arms (*arrow* in *B–D*) from the centromeric region of a bioriented chromosome just as the cell was entering anaphase. After chromatid disjunction the two sister chromosome fragments containing the kinetochores (*arrowheads* in *E–G*) segregated to opposite polar regions, while the four acentric fragments (*arrows* in *E–H*) remained near the spindle equator. These fragments were subsequently transported to the poles during phragmoplast formation (*I*). A kinetic plot of these chromosome fragments is presented in Fig. 7. (*J–L*) A series of video frames from another cell illustrating that an acentric chromosome fragment (*arrow* in *J*) may become highly stretched towards a pole (*arrows* in *K* and *L*) if it is transiently stuck in the phragmoplast at the time it initiates poleward motion. Time in min:s is at lower right-hand corner of each picture. Bars in *I* and *L*, 10 μm.

port the contention that the non-kinetochore chromosome transport we observed is mediated by Mts.

As in animals, metaphase *Haemanthus* spindles are organized with the minus ends of their Mts positioned near the two polar regions, while the plus ends overlap across the spindle equator (Euteneuer et al., 1982). The force-producing mechanism for the poleward motion of ACFs in *Haemanthus* is unknown, but there are several possibilities. It could be mediated by minus-end–directed Mt motor molecules associated with the chromosome surface. Alternatively, nonresolvable globule "particles and states" are seen to flow constantly poleward, with about the same

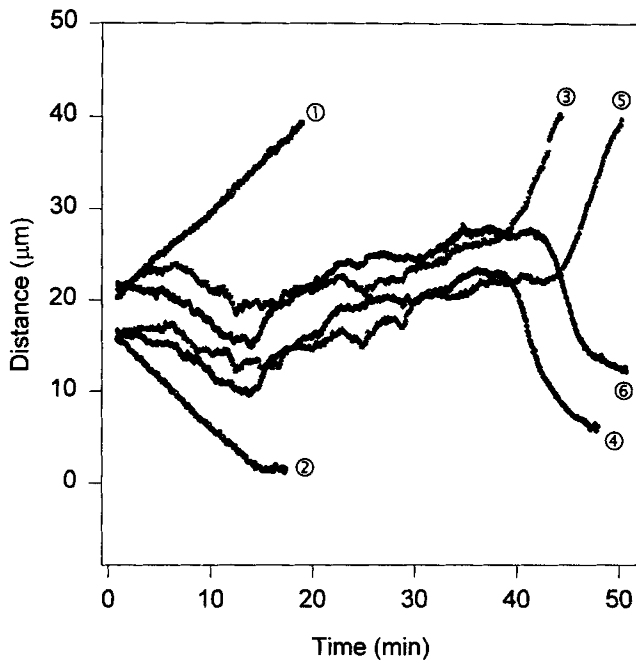

Figure 7. A kinetic plot of the monocentric and acentric chromosome fragments generated in the anaphase cell shown in Fig. 6. The times shown in Fig. 6 are coincident with points along the time axis of this plot. After disjunction the sister monocentric fragments (curves *1* and *2*), each of which contain a single kinetochore, immediately move towards opposite poles with a velocity of 0.9 μm/min. By contrast, the two acentric fragments, which disjoin at anaphase onset into four fragments lacking kinetochores (curves *3–6*), remain at the spindle equator until the phragmoplast begins to form (40-min time point). At this time they are then transported towards the polar regions with a velocity of 3.1–3.3 μm/min. Note that in this example fragments derived from the chromatid segregate to opposite poles (see also Fig. 8).

velocity of anaphase chromosomes, in most if not all plant and animal spindles (Hard and Allen, 1977; Hiramoto and Izutsu, 1977; Salmon, E.D., and C.L. Rieder, unpublished observations). A similar "flowing" motion of submicroscopic material is also seen to occur in association with the Mt arrays of other motility systems including neurons (Allen et al., 1982). Thus, it is possible that the same force-producing mechanism that powers the motion of particles and states also moves ACFs poleward in *Haemanthus.* A logical candidate for this force producer is Mt flux, which is exhibited even by simple self-organized Mt arrays (Sawin and Mitchison, 1994). Although the flux rate of forming *Haemanthus* spindles is unknown, it can approach 2.5 μm/min in *Xenopus* spindles assembled in vitro, well within the range of velocities observed for the poleward transport of ACFs in *Haemanthus.* If flux is involved in the motion of ACFs poleward, then some unidentified chromosome-associated protein(s) must tether the chromatin to the fluxing Mt lattice.

The poleward forces acting on ACFs are terminated at anaphase onset. Such an abrupt cessation of poleward transport may be the result of sudden biochemical changes (e.g., destruction of cyclin B and active maturation promoting factor) that are known to occur at the metaphase–anaphase transition in animal cells (Minshull et al., 1989; Murray, 1994). The fact that kinetochores continue to move poleward at ~1.0 μm/min during anaphase, after the bulk poleward transport of the chromosome ceases, reveals that the mechanisms responsible for kinetochore and non–kinetochore-based chromosome motility differ.

Bajer and his co-workers reported that ACFs located between the separating centromeres in *Haemanthus* spindles moved "only irregularly and but little" (Östergren et al., 1960; see also Bajer and Molè-Bajer, 1956; Bajer, 1958). However, we found that ACFs positioned behind or in front (Fig. 8) of the separating groups of anaphase chromosomes were transported near midanaphase to the spindle equator at 1.5–2 μm/min. Because the polarity of spindle Mts does not change between metaphase and midanaphase (Euteneuer et al., 1982), this away-from-the-pole motion cannot be produced by Mt flux. It may be due to the activation of chromosome-associated kinesin-like Mt plus-end motors and/or to the growth of spindle Mt plus ends toward the cell plate (which appears to occur during midanaphase; Fig. 1 *B*) (Jensen and Bajer, 1973; Bajer and Molè-Bajer, 1982).

Finally, ACFs positioned on the spindle equator during anaphase suddenly move poleward at 2.5–3.4 μm/min as the cell plate begins to form. This poleward motion, which is about three times faster than that observed during metaphase, has been previously described in detail by Bajer (Bajer, 1958; Bajer and Östergren, 1963) who thought it was mediated by the same "traction fiber" forces that move kinetochores during anaphase. Although the data on the polarity of Mts within the phragmoplast are incomplete, they suggest that the plus ends of these Mts are embedded in the cell plate and that the minus ends are directed poleward (Euteneuer and McIntosh, 1980; Vantard et al., 1990; Liu et al., 1993, 1994). The fact that the ACFs within the phragmoplast often become stretched as they move poleward (Fig. 6) reveals that the force for this motion acts either on the front end of the fragment or along its surface. This makes a "pushing" mechanism based on growing Mt ends unlikely. Alternatively, the poleward motion of ACFs during telophase could be due to Mt minus-end motors associated with the phragmoplast (Asada and Shibaoka, 1994) and/or to Mt flux, the latter of which has been shown to occur in the phragmoplast of glycerinated tobacco cells (although at a rate of only 0.1 μm/min) (Asada et al., 1991).

In summary, the stage-specific motions of ACFs during mitosis in *Haemanthus* cannot all be mediated by the same force-producing mechanism. Although an Mt pushing force, based on growing Mt ends (Inoue and Salmon, 1995), may be involved in the motion of ACFs to the spindle equator during anaphase, it cannot provide the basis for poleward motion during metaphase. Mt flux could be involved in the poleward motion of ACFs during metaphase and telophase, but not in the away-from-the-pole motion in anaphase. Finally, if these motions are all mediated solely by chromatin-associated Mt motors, then at least two different (plus- and minus-directed) motors must be involved.

Does Kinetochore Function Differ during Spindle Formation in Plant and Animal Cells?

During mitosis in vertebrates, individual kinetochores

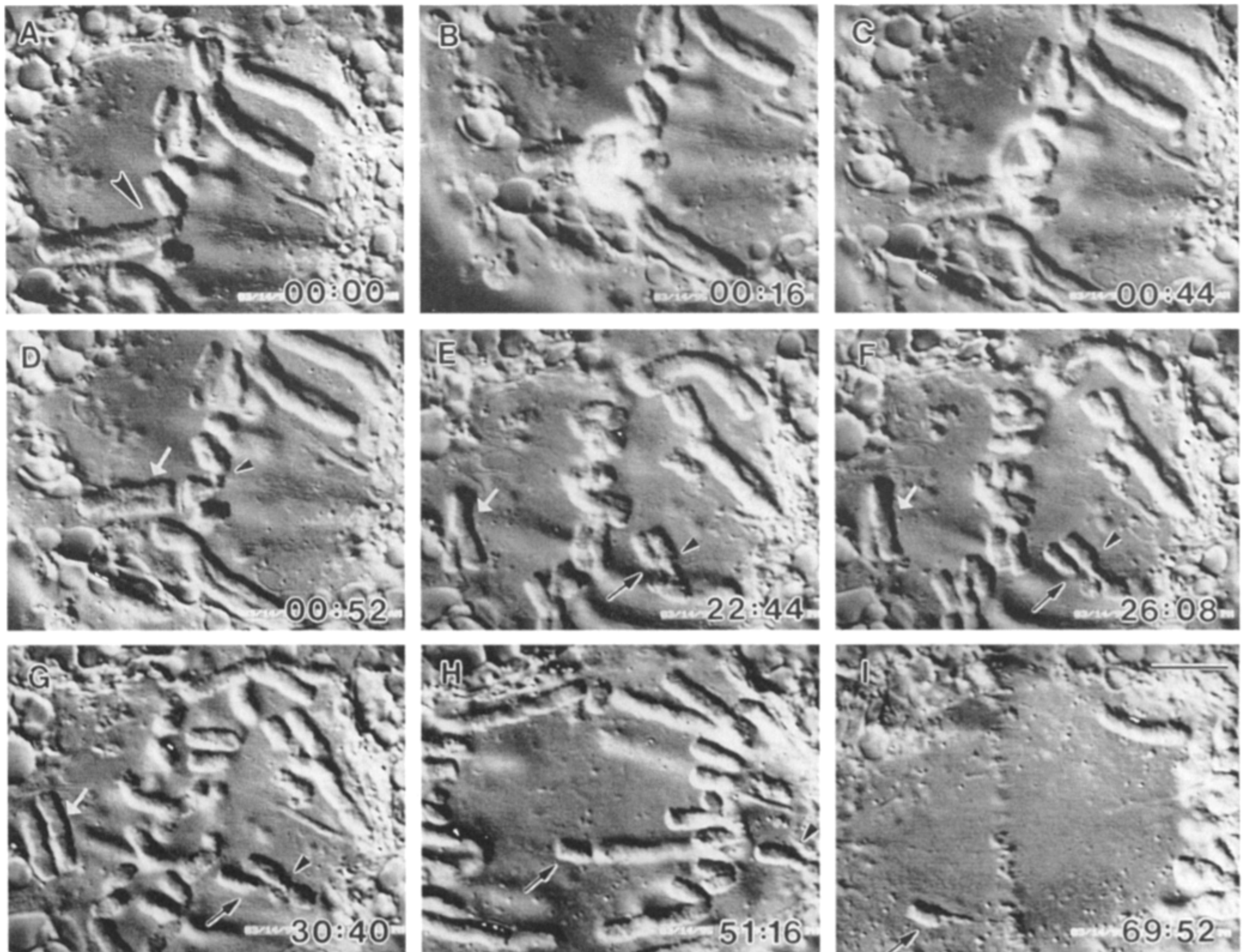

Figure 8. (*A–I*) Selected frames from a time-lapse sequence of a *Haemanthus* cell progressing from metaphase to telophase. During metaphase the long arm on one chromosome (*arrowhead* in *A*) is severed near its centromere with the laser. This arm is then transported into the near pole (*white arrow* in *D–G*). After it reaches the polar region, it stops and does not exhibit directional movement (*E–G*) anymore. In *C* the left-hand kinetochore on the centromere-containing fragment of this chromosome is destroyed by the laser. This produces a monocentric chromosome fragment, which begins to move towards the right-hand pole (*dark arrow/arrowhead* in *D–F*). When anaphase begins (*F*), this chromosome fragment disjoins into a monocentric fragment (*arrowhead* in *F*) and an acentric fragment (*arrow* in *F*). The monocentric fragment continues to move poleward as anaphase proceeds (*arrowhead* in *F–H*), while the acentric fragment (*arrow* in *F–H*) is transported to the spindle equator. Later this fragment is transported to the opposite pole during phragmoplast formation (*arrow* in *I*). A kinetic plot of these chromosome fragments is presented in Fig. 9. Time in min:s is at the lower right of each picture. Bar in *I*, 10 μm.

abruptly switch between states of poleward and away-from-the-pole motion termed "kinetochore directional instability" (Skibbens et al., 1993). This switching is thought to be the result of changing tension levels on the kinetochore, mediated by the motile behavior of the sister kinetochore and by the magnitude of the polar ejection force in the half-spindle containing the chromosome (Skibbens et al., 1993, 1995; Cassimeris et al., 1994). These findings form the basis of a kinetochore directional instability/polar ejection mechanism of chromosome congression that envisions that kinetochores switch into an away-from-the-pole state of motion when they are under sufficiently high tension, and then switch to a state of poleward motion under conditions of lower tension. As summarized by Rieder and Salmon (1994), this model explains the constant and conspicuous oscillatory motions of monooriented and bioriented chromosomes in animal cells, and it is also consistent with a wide variety of other chromosome behavior.

Our results indicate that in its explicit form, the kinetochore directional instability/polar ejection mechansim for chromosome positioning in animal somatic cells is not applicable to higher plants. First, the chromosomes in *Haemanthus* do not exhibit the oscillatory motions characteristically produced by directionally unstable kinetochores (our data; see also Bajer and Molè-Bajer, 1971). Second, unlike in vertebrate spindles where the leading kinetochore on a congressing chromosome is throught to be in a poleward state of motion because it is under low tension, the leading kinetochore on a congressing *Haemanthus* chromosome is moving towards its pole when it is appar-

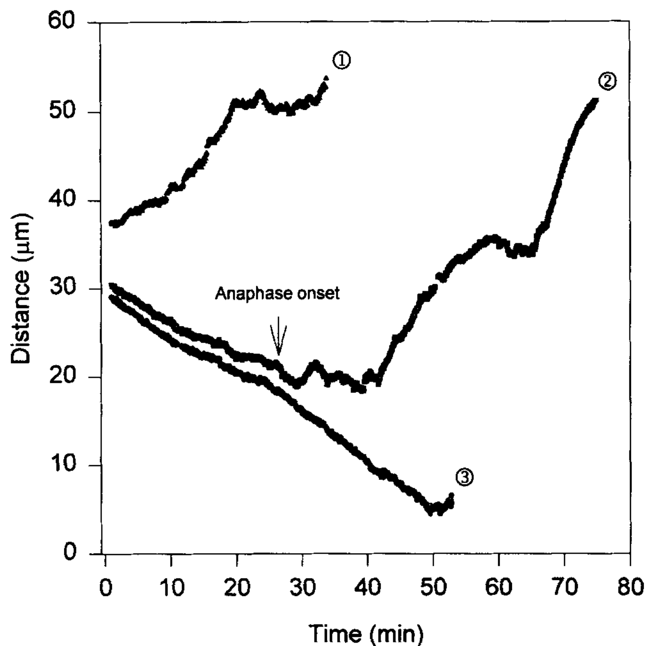

Figure 9. A kinetic plot of the chromosome fragments shown in Fig. 8. The times shown in Fig. 8 are coincident with points along the time axis of this plot. Curve *1* depicts the 0.7–0.8-μm/min poleward motion of the acentric chromosome arm generated during metaphase (see *white arrow* in Fig. 8, *D–G*). Curve *2* illustrates the behavior of the acentric fragment (see *black arrow* in Fig. 8, *D–G*) before and after its disjunction from the monocentric fragment. Note that this fragment stops moving poleward at anaphase onset and then drifts for ~10 min. After this time it is then transported at 1.5 μm/min back to the spindle equator where it stops for a short period of time (between the 58- and 65-min time points) before moving toward the opposite spindle pole at 3.0 μm/min. Curve *3* represents the monocentric chromosome fragment (see *black arrowhead* in Fig. 8, *D–G*), which shows a steady poleward velocity of 0.75 μm/min from the time it was created in metaphase (at the 1-min time point) until the end of anaphase.

ently under conditions of maximum high tension—produced by pole-directed forces within the half-spindle that act on the chromosome arms. Although we do not know the magnitude of these poleward forces relative to the poleward force acting on the leading kinetochore, it is likely that they provide significant drag on a chromosome that is moving away from the closest pole. If this is true, then unlike in vertebrate cells, the kinetochores in plant cells may produce or experience a poleward force only when they are under sufficiently high tension. Although speculative, this hypothesis is consistent with the current data on the behavior of congressing *Haemanthus* chromosomes and should be testable by additional laser microsurgery studies.

We thank Drs. M. Koonce, E.D. Salmon, E.A. Smirnova, R. Vale, and W.Z. Cande for critical comments and helpful discussions. We also thank Mrs. C. Hughes for photographic assistance.

The major part of this work was conducted within the Wadsworth Center's Video-enhanced Light Microscopy Core Facility and was supported by National Institutes of Health grants GM 40198 (to C.L. Rieder), and RR 01219 awarded by the Biomedical Research Technology Program, National Center for Research Resources (Department of Health and Human Resources/Public Health Service), to support the Wadsworth Center's Biological Microscopy and Image Reconstruction facility as a National Biotechnological Resource.

Received for publication 28 November 1995 and in revised form 3 January 1996.

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.

Allen, R.D., J.L. Travis, J.H. Hayden, N.S. Allen, A.C. Breuer, and L.J. Lewis. 1982. Cytoplasmic transport: moving ultrastructural elements common to many cell types revealed by video-enhanced microscopy. *Cold Spring Harb. Symp. Quant. Biol.* 46:85–87.

Asada, T., and H. Shibaoka. 1994. Isolation of polypeptides with microtubule-translocating activity from phragmoplasts of tobacco BY-2 cells. *J. Cell Sci.* 107:2249–2257.

Asada, T., S. Sonobe, and H. Shibaoka. 1991. Microtubule translocation in the cytokinetic apparatus of cultured tobacco cells. *Nature (Lond.).* 350:238–241.

Ault, J.G., and C.L. Rieder. 1994. Centrosome and kinetochore movement during mitosis. *Curr. Opin. Cell Biol.* 6:41–49.

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.

Bajer, A. 1958. Cine-micrographic studies on chromosome movement in β-irradiated cells. *Chromosoma (Berl.).* 9:319–331.

Bajer, A. 1966. Movement within the mitotic spindle. *In* Dynamics of Fluids and Plasmas. Academic Press. Inc., New York. 59–80.

Bajer, A., and J. Molè-Bajer. 1956. Cine-micrographic studies on mitosis in endosperm II. Chromosome, cytoplasmic and brownian movements. *Chromosoma (Berl.).* 7:558–607.

Bajer, A., and J. Molè-Bajer. 1971. Architecture and function of the mitotic spindle. *Adv. Cell Mol. Biol.* 1:213–266.

Bajer, A.S., and J. Molè-Bajer. 1982. Asters, poles, and transport properties within spindlelike microtubule arrays. *Cold Spring Harb. Symp. Quant. Biol.* 46:263–283.

Bajer, A.S., and J. Molè-Bajer. 1986. Reorganization of microtubules in endosperm cells and cell fragments of the higher plant *Haemanthus* in vivo. *J. Cell Biol.* 102:263–281.

Bajer, A., and G. Östergren. 1963. Observation on transverse movements within the phragmoplast. *Hereditas (Lund).* 50:179–195.

Bajer, A.S., and M. Vantard. 1988. Microtubule dynamics determine chromosome lagging and transport of acentric fragments. *Mutat. Res.* 201:271–281.

Bajer, A.S., M. Vantard, and J. Molè-Bajer. 1987. Multiple mitotic transports expressed by chromosome and particle movement. *In* Nature and Function of Cytoskeletal Proteins in Motility and Transport. Gustav Fisher Verlag, New York. 171–186.

Berns, M.W., W.H. Wright, and R. Wiegand Steubing. 1991. Laser microbeam as a tool in cell biology. *Int. Rev. Cytol.* 129:1–44.

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.

Cole, R.W., A. Khodjakov, W.H. Wright, and C.L. Rieder. 1995. A differential interference contrast-based light microscopic system for laser microsurgery and optical trapping of selected chromosomes during mitosis *in vivo. J. Microsc. Soc. Am.* 1:203–215.

De Mey, J., A.M. Lambert, A.S. Bajer, M. Moeremans, and M. De Brabander. 1982. Visualization of microtubules in interphase and mitotic plant cells of *Haemanthus* endosperm with the immuno-gold staining method. *Proc. Natl. Acad. Sci. USA.* 79:1898–1902.

Desai, A., and T. J. Mitchison. 1995. A new role for motor proteins as couplers to depolymerizing microtubules. *J. Cell Biol.* 128:1–4.

Euteneuer, U., and J.R. McIntosh. 1980. Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.* 87:509–515.

Euteneuer, U., W.T. Jackson, and J.R. McIntosh. 1982. Polarity of spindle microtubules in *Haemanthus* endosperm. *J. Cell Biol.* 94:644–653.

Fuge, H. 1990. Non-kinetochore transport phenomena, microtubule-chromosome associations, and force transmission in nuclear division. *Protoplasma.* 158:1–9.

Fuller, M.T. 1995. Riding the polar winds: chromosomes motor down east. *Cell.* 81:5–8.

Hard, R., and R.D. Allen. 1977. Behavior of kinetochore fibers in Haemanthus katherinae during anaphase movements of chromosomes. *J. Cell Sci.* 27:47–56.

Hiramoto, Y., and K. Izutsu. 1977. Poleward movement of "markers" existing in mitotic spindles of grasshopper spermatocytes. *Cell Struct. Funct.* 2:257–259.

Inoue, S., and E. Salmon. 1995. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell.* 6:1619–1640.

Jensen, C., and A. Bajer. 1973. Spindle dynamics and arrangement of microtubules. *Chromosoma (Berl.).* 44:73–89.

Liu, B., J. Marc, H.C. Joshi, and B.A. Palevitz. 1993. A gamma-tubulin-related protein associated with the microtubule arrays of higher plants in a cell cycle-dependent manner. *J. Cell Sci.* 104:1217–1228.

Liu, B., H.C. Joshi, T.J. Wilson, C.D. Silflow, B.A. Palevitz, and D.P. Snustad. 1994. γ-Tubulin in *Arabidopsis*: gene sequence, immunoblot, and immunofluorescence studies. *Plant Cell.* 6:303–314.

McIntosh, J.R., and G.E. Hering. 1991. Spindle fiber action and chromosome movement. *Annu. Rev. Cell Biol.* 7:403–426.

McIntosh, J.R., and C.M. Pfarr. 1991. Mitotic motors. *J. Cell Biol.* 115:577–585.

McKim, K.S., and R.S. Hawley. 1995. Chromosomal control of meiotic cell division. *Science (Wash. DC).* 270:1595–1601.

Minshull, J., J. Pines, R. Golsteyn, N. Standart, S. Mackie, A. Colman, J. Blow, J.V. Ruderman, M. Wu, and T. Hunt. 1989. The role of cyclin synthesis, modification and destruction in the control of cell division. *J. Cell Sci. Suppl.* 12:77–97.

Mitchison, T.J. 1989. Mitosis: basic concepts. *Curr. Opin. Cell Biol.* 1:67–74.

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.

Molè-Bajer, J. 1958. Cine-micrographic analysis of C-mitosis in endosperm. *Chromosoma (Berl.).* 9:332–358.

Molè-Bajer, J. 1967. Chromosome movement in chloral hydrate treated endosperm cells in vitro. *Chromosoma (Berl.).* 22:465–480.

Murphy, T.D., and G.H. Karpen. 1995. Interaction between the nod^{+} kinesin-like gene and extracentromeric sequences are required for transmission of a *Drosophila* minichromosome. *Cell.* 81:139–148.

Murray, A. 1994. Cell cycle checkpoints. *Curr. Opin. Cell Biol.* 6:872–876.

Nicklas, R.B., and C.A. Koch. 1972. Chromosome micromanipulation. IV. Polarized motions within the spindle and models for mitosis. *Chromosoma (Berl.).* 39:1–26.

Östergren, G., J. Molè-Bajer, and A. Bajer. 1960. An interpretation of transport phenomena at mitosis. *Ann. N. Y. Acad. Sci.* 90:381–408.

Rieder, C.L. 1990. Formation of the astral mitotic spindle: ultrastructural basis for the centrosome-kinetochore interaction. *Electron Microsc. Rev.* 3:269–300.

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., E.A. Davison, L.C. Jensen, L. Cassimeris, and E.D. Salmon. 1986. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. *J. Cell Biol.* 103:581–591.

Rieder, C.L., J.G. Ault, U. Eichenlaub-Ritter, and G. Sluder. 1993. Morphogenesis of the mitotic and meiotic spindle: conclusions obtained from one system are not necessarily applicable to the other. *In* Chromosome Segregation and Aneuploidy. NATO-ASI Series. B.K. Vig, editor. Springer Verlag, Berlin. 72:183–197.

Rieder, C.L., R.W. Cole, A.L. Khodjakov, and G. Sluder. 1995. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.* 130:941–948.

Salmon, E.D. 1989. Microtubule dynamics and chromosome movement. *In* Mitosis: Molecules and Mechanisms. J.C. Hyams and B.R. Brinkley, editors. Academic Press, London. 119–181.

Sawin, K.E., and S.A. Endow. 1993. Meiosis, mitosis and microtubule motors. *Bioessays.* 15:399–407.

Sawin, K.E., and T.J. Mitchison. 1994. Microtubule flux in mitosis is independent of chromosomes, centrosomes and antiparallel microtubules. *Mol. Biol. Cell.* 5:217–226.

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.

Skibbens, R.V., C.L. Rieder, and E.D. Salmon. 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.

Smirnova, E.A., and A.S. Bajer. 1992. Spindle poles in higher plant mitosis. *Cell Motil. Cytoskeleton.* 23:1–7.

Vantard, M., N. Levilliers, A.M. Hill, A. Adoutte, and A.M. Lambert. 1990. Incorporation of paramecium axonemal tubulin into higher plant cells reveals functional sites of microtubule assembly. *Proc. Natl. Acad. Sci. USA.* 87: 8825–8829.

Verde, F., J.M. Berrez, C. Antony, and E. Karsenti. 1991. Taxol-induced microtubule asters in mitotic extracts of *Xenopus* eggs: requirement for phosphorylated factors and cytoplasmic dynein. *J. Cell Biol.* 112:1177–1187.

Vernos, I., and E. Karsenti. 1995. Chromosomes take the lead in spindle assembly. *Trends Cell Biol.* 5:297–301.

Vernos, I., J. Raats, T. Hirano, J. Heasman, E. Karsenti, and C. Wylie. 1995. Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. *Cell.* 81:117–127.

Wang, S.-Z., and R. Adler. 1995. Chromokinesin: a DNA-binding, kinesin-like nuclear protein. *J. Cell Biol.* 128:761–768.