Poleward Kinetochore Fiber Movement Occurs during Both Metaphase and Anaphase-A in Newt Lung Cell Mitosis

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Abstract. Microtubules in the mitotic spindles of newt lung cells were marked using local photoactivation of fluorescence. The movement of marked segments on kinetochore fibers was tracked by digital fluorescence microscopy in metaphase and anaphase and compared to the rate of chromosome movement. In metaphase, kinetochore oscillations toward and away from the poles were coupled to kinetochore fiber shortening and growth. Marked zones on the kinetochore microtubules, meanwhile, moved slowly polewards at a rate of ~0.5 μm/min, which identifies a slow polewards movement, or "flux," of kinetochore microtubules accompanied by depolymerization at the pole, as previously found in PtK2 cells (Mitchison, 1989*b*). Marks were never seen moving away from the pole, indicating that growth of the kinetochore microtubules occurs only at their kinetochore ends. In anaphase, marked zones on kinetochore microtubules also moved polewards, though at a rate slower than overall kinetochore-to-pole movement. Early in anaphase-A, microtubule depolymerization at kinetochores accounted on average for 75% of the rate of chromosome-to-pole movement, and depolymerization at the pole accounted for 25%. When chromosome-to-pole movement slowed in late anaphase, the contribution of depolymerization at the kinetochores lessened, and flux became the dominant component in some cells. Over the whole course of anaphase-A, depolymerization at kinetochores accounted on average for 63% of kinetochore fiber shortening, and flux for 37%. In some anaphase cells up to 45% of shortening resulted from the action of flux. We conclude that kinetochore microtubules change length predominantly through polymerization and depolymerization at the kinetochores during both metaphase and anaphase as the kinetochores move away from and towards the poles. Depolymerization, though not polymerization, also occurs at the pole during metaphase and anaphase, so that flux contributes to polewards chromosome movements throughout mitosis. Poleward force production for chromosome movements is thus likely to be generated by at least two distinct molecular mechanisms.

THE physical segregation of sister chromatids at mitosis is mediated by the interaction of their kinetochores with microtubules of the mitotic spindle. An important goal of mitosis research is to determine how the forces that drive segregation are generated and regulated at a molecular level. This requires first understanding the mechanics of the spindle to determine where in the spindle the segregation forces are generated. Over the last few years it has become apparent that the kinetochore itself is a major site of force generation for chromosome movement (reviewed in Mitchison 1988, 1989*a*; McIntosh and Pfarr, 1991; Rieder, 1991; Salmon, 1989). This is particularly clear early in mitosis, when the mechanics of the kinetochore-microtubule interaction can be very simple. Some chromosomes first attach to the spindle when their kinetochores make a side-on interaction with microtubules that emanate from the pole (Rieder and Alexander, 1990; Hayden et al., 1990). This interaction leads to a rapid polewards sliding of the kinetochore over the microtubule lattice (20–50 μm/min in newt cells). Sliding is thought to be driven by dynein molecules attached to the kinetochore (Rieder and Alexander, 1990; Hyman and Mitchison, 1991) and this phase of chromosome movement thus resembles minus end–directed vesicle transport along microtubules.

This rapid poleward sliding, however, is not apparent for the great majority of chromosomes. Instead, chromosome attachment and movement depend primarily on interactions of kinetochores with the ends of polar microtubules. During prometaphase, each kinetochore in a higher eukaryotic spindle becomes attached to the plus end of a bundle of microtubules whose minus ends are mostly located near the spindle pole (Rieder, 1982; McDonald et al., 1992). Since these kinetochore microtubules have been shown in vertebrate spindles to extend continuously between kinetochores and poles, chromosome movement along the spindle axis must be accompanied by a change in their length. In principle,

length changes could be accomplished by polymerization and depolymerization at either the plus ends attached to kinetochores or the minus ends anchored at the poles. Addition and loss of subunits from the interior of the lattice is also a possibility, though it is thought to be unlikely on structural grounds (Mitchison et al., 1986). The site of polymerization dynamics is directly related to the molecular mechanism of chromosome movement. Polewards chromosome movement accompanied by depolymerization at the kinetochore implies force generation at the kinetochore–microtubule interface, which in turn would imply the existence of a motor system at the kinetochore. Polewards chromosome movement accompanied by depolymerization at the pole implies the existence of a fiber translocation mechanism associated with the spindle or the spindle pole.

Localization of the sites of kinetochore microtubule polymerization–depolymerization in mitotic animal cells has been addressed by various microtubule-marking experiments. Injection of biotin- or fluorophore-labeled tubulin into BSC1 or PtK2 cells showed that microtubule ends attached to kinetochores are dynamic, polymerizing in metaphase and depolymerizing in anaphase (Geuens et al., 1989; Mitchison et al., 1986; Mitchison, 1988; Wise et al., 1991; Sheldon and Wadsworth, 1992; Wadsworth et al., 1989). This approach has been extended recently to show that polymerization and depolymerization at kinetochores can account for the majority of prometaphase chromosome movement in PtK2 cells (Kronenbush, P., and G. G. Borisy, unpublished results). Fluorescence photobleaching showed that depolymerization at kinetochores accounts for the majority of anaphase chromosome-to-pole (anaphase-A) movement in cultured pig kidney cells (Gorbsky et al., 1988) and the majority of chromosome-to-pole movement induced in metaphase newt and cultured pig kidney cells when microtubule assembly was blocked with nocodazole (Cassimeris and Salmon, 1991; Centonze and Borisy, 1991). Taken together, these experiments show that kinetochores are the major site of polymerization–depolymerization during mitosis and that they are capable of generating force for chromosome movement. Consistent with this implication, isolated kinetochores have been shown to contain both minus end– and plus end–directed ATPase motor activity (Mitchison and Kirschner, 1985; Pfarr et al., 1990; Steuer et al., 1990; Hyman and Mitchison, 1991). In addition microtubule depolymerization at the attachment site has been shown to move chromosomes in the absence of ATP (Koshland et al., 1988; Coue et al., 1991) indicating that the energetics of depolymerization may play a major role in the production of force for poleward movement.

The kinetochore is not, however, the only site of kinetochore microtubule assembly dynamics. Marking kinetochore microtubules in metaphase PtK2 cells by photoactivation of fluorescence revealed a continuous polewards flux, implying that kinetochore microtubules were depolymerizing at the poles (Mitchison, 1989*b*). The rate of polewards flux was ~0.5 μm/min, slower than anaphase-A movement (1–2 μm/min), but still significant. Polewards flux was also found in reconstituted *Xenopus* spindles, though in this case, it was detected predominantly in non-kinetochore overlap microtubules (Sawin and Mitchison, 1991). The flux rate in *Xenopus* spindles was 3.0 μm/min, enough potentially to account for anaphase-A movement by a non-kinetochore motor system. These flux observations imply that polewards movement of the whole kinetochore fiber could contribute to polewards chromosome movement in both metaphase and anaphase. The actual contribution of kinetochore fiber movement to net chromosome movement was not, however, measured directly in either photoactivation study, primarily for technical reasons.

In the experiments reported in this paper we used photoactivation of fluorescence to visualize the mechanical behavior of kinetochore fiber microtubules in newt lung cells. The large size of the newt spindle (40–50 μm interpolar distance) allows better spatial and temporal analysis of chromosome movement than was possible in the mammalian cell studies, particularly during anaphase-A. Our results show that microtubule depolymerization both at kinetochores and at the poles occurs during net polewards chromosome movement throughout mitosis. We discuss the implications for spindle structure and the mechanisms of force generation.

Materials and Methods

Cell Culture and Microinjection

Newt lung explant cultures were made in Rose chambers as described by Rieder and Hard (1990) and cultured for 1–3 wk before observation. Cells were maintained at 18–23°C at all times. Large, flat cells in late prophase (condensed chromosomes, intact nuclear envelope) or early prometaphase (nuclear envelope broken down, most chromosomes not congressed) were identified by phase contrast microscopy, and the top coverslip of the chamber was removed. The selected cells were microinjected with labeled tubulin, essentially as described (Mitchison, 1989*b*), using a Diavert microscope (Nikon Inc., Garden City, NY) equipped with phase-contrast optics and a hydraulic micromanipulator (Narashige Scientific Laboratory, Tokyo). The injectate contained 45 micromolar tubulin labeled with caged fluorescein and 5 μM tubulin labeled with X-rhodamine, or for some metaphase experiments, 45 μM caged-fluorescein tubulin only. The labeled tubulins were prepared from phosphocellulose purified bovine brain tubulin exactly as described by Hyman et al. (1991). Assuming a 20-fold dilution on microinjection of the large newt cells, this would give ~2 μM concentration of labeled subunits in the cytoplasm, corresponding to between one-fifth and one-tenth of the endogenous pool. After microinjection, the coverslip with the cells attached was inverted onto a drop of medium on a plain glass microscope slide. The coverslip was spaced away from the slide by spacers consisting of two slivers of #1 cover slip mounted on top of each other. The resulting chamber was then sealed with molten beeswax. Control experiments showed that cells would enter mitosis and complete it normally for many hours in such sealed chambers. Injected cells were allowed to proceed into mitosis for at least 20 min before photoactivation, allowing time for incorporation of labeled tubulin subunits into the spindle (Wadsworth and Salmon, 1986). We found that newt cells were quite impervious to these experimental manipulations. Once we gained experience with the injection procedure, almost all injected cells showed rates of congression and anaphase movements that were the same as control cells.

Data Collection

The chamber containing the injected cell was transferred, cover slip up, to an upright (Nikon FX-A Nikon Inc., Garden City, NJ) microscope equipped with the following for the fluorescence studies: (*a*) rotating stage; (*b*) 60× 1.4 NA planapochromat phase-contrast objective and 1.25 NA achromat phase-contrast condenser; (*c*) epi-illumination, with fluorescence filter cubes for DAPI (ex, 350–370 nm; em, 400-nm-long pass), and fluorescein (ex, 450–490 nm; em, 520–560 nm) and X-rhodamine (ex, 560–590 nm; em, 620–680 nm); (*d*) a variable aperture module for the field-diaphragm plane in the epitube (Nikon Inc.) modified to allow mounting of a 50 μm ×13-mm slit (Oriel Corp. of America, Stamford, CT) in the conjugate image plane as well as the various circular apertures. (This modification was simply effected by joining the slit onto the end of the slider which carries the mounted pinholes); (*e*) a variable aperture diaphragm in the epi-tube, along with various neutral density filters to allow modulation of the intensity

of epi-illumination; (*f*) Hg arc epi-illumination and 12 V halogen trans-illumination, both controlled by electronic shutters (Vincent Associates, Rochester, NY) interfaced to a computer-controlled image acquisition system (Data-cube, described in Sawin and Mitchison, 1991); (*g*) a 600-nm-long pass filter in the trans-illumination path to prevent photobleaching and photoactivation by this beam; and (*h*) a SIT video camera (model 68; Dage-MTI, Wabash, WI) interfaced to the image acquisition system.

The injected cell was located using phase contrast optics. Before photoactivation we verified that the spindle was fully labeled by observation in the X-rhodamine channel. We also checked that chromosome movements were normal by time-lapse phase contrast observation. To start a photoactivation run we performed the following sequence of steps: (*a*) Switch to DAPI filter set, open epi-tube aperture diaphragm and remove neutral density to maximize epi-illumination intensity; (*b*) Slide slit into epi-tube field plane; (*c*) Rotate and translate stage to position spindle relative to slit image; (*d*) Open epi-illumination filter for 1 s to admit 360 nm light and photoactivate; (*e*) Switch to fluorescein filter set, lower epi-illumination level, and slide appropriate circular aperture into epi-tube field plane. This aperture allowed illumination of the central 1/3 of the field, sufficient to observe the most of the spindle but minimize stray light; and (*f*) Begin image acquisition sequence within about 3 s of activation.

The image acquisition sequence was as follows: (*a*) Open epi-illumination shutter for sufficient time to sum eight frames on the image processor, then close it. Divide the summed image by four, and store it on an optical memory disc recorder (OMDR) (model TQ-2028F; Panasonic). The SIT camera was manually set on maximum high voltage, and ~50% of maximum analog gain. These settings were held constant throughout a run. The sum/divide parameters and camera gain settings were determined empirically to be those that achieved maximum signal to noise at minimal total observational light dose; (*b*) Open trans-illumination shutter, average eight frames and store the result. The illumination level was set very low so as not to saturate the camera at the high gain setting. Because the optical path was not changed, the fluorescein and phase images were in exact register; (*c*) Switch to the X-rhodamine cube, and collect an image as in *a* above, except that the sum of eight frames was divided by eight; (*d*) Close all shutters, switch back to the fluorescein cube, and wait until the next observation. To limit photobleaching we tried to minimize the number of observations in the fluorescein channel by spacing them every 0.5–2.0 minutes. Control experiments showed that the level of photobleaching in the fluorescein channel was 2–5% per image, depending on the exact level of epi-illumination. We were able to observe the cell continuously by phase contrast, which used dim red light, with no ill effect. We continued collecting images until the cell had entered G1 of the next cell cycle, as visualized by decondensation of the separated chromatids and reformation of the nuclear envelope. Cleavage was frequently abortive in these cells, particularly in the flattest ones selected for observation, as previously reported (Rieder and Hard, 1990). Video-enhanced DIC (VE-DIC) images of uninjected cells were obtained as described by Skibbens et al. (1992).

Image Analysis

The movements of kinetochores and the fluorescent marks on the kinetochore fibers with respect to the spindle poles were measured from the time-lapse video image stored on the OMDR. A mouse-driven video cursor overlaid on the video images was used to visually enter the x and y coordinates into a PC computer. Measurement accuracy was about 0.3 μm for phase images and 0.5 μm for fluorescent images. A custom written computer program, SFM, recorded data, controlled stepping between frames on the OMDR, produced graphs of object motion, and least squares regression analysis of average velocities. The positions of kinetochores were obtained by measuring the leading edge of the centromere regions closest to the poles. The movements of fluorescent marks were tracked by either measuring the position of the poleward edge of marks or in several cases, the position of maximum intensity in the center of mark. In flat cells, the position of the central position of the centrosome was usually clearly visible in either DIC or phase images. Rhodamine spindle images were also used to determine the location of the spindle pole when the centrosome was difficult to see by phase contrast or had been detached from the spindle pole.

In addition, we determined the changes in location and intensity of the fluorescent marks by digital image analysis using a home-built system based on Data-Cube Max-Video boards and custom written software (Cassimeris and Salmon, 1991). Profiles of pixel intensity along kinetochore fibers were obtained from sequential time-lapse images. These profiles were plotted on the same graph to determine the poleward movement and changes in relative fluorescence of the fluorescent marks. We attempted to measure the change in the number of fluorescent microtubules in a marked region of the spindle by integrating the fluorescent values of pixels within a boundary drawn by eye around the marks and subtracting the value obtained from an equivalent area outside the spindle in the cytoplasm. This analysis gave good qualitative measurements of microtubule turnover, but accuracy was limited somewhat by the dynamic range of the video camera and photobleaching. In particular, we adjusted the gain on the video camera and the fluorescence excitation intensity so that the weakly fluorescent marks of the persistent kinetochore fibers were well above the noise floor of the video camera. Intensity values immediately following photoactivation were more than four times the magnitude of the marks on the persistent kinetochore fibers 2–5 min after photoactivation. Intensity values in these early images were "clipped" by the camera electronics so that ratios of fluorescence to initial fluorescence overestimated the persistence of fluorescence after photoactivation.

Photographs of video images recorded on the OMDR were obtained from 35 mm negatives taken from the video screen of a Panasonic WV 5410 monitor using Kodak Plus X film (Eastman Kodak Co., Rochester, NY). The original video images were enlarged twofold using the 2× Zoom function in an Argus 10 (Hamamatsu Photonics, NJ) digital image processor in order to de-emphasize the raster horizontal line scans in the photographic prints. The Argus 10 was also used to superimpose fluorescein and phase-contrast image pairs.

Results

Kinetochore Fibers and Chromosome Movement in Newt Lung Cells

Fig. 1 shows high-resolution VE-DIC micrographs of mitosis in newt lung cells in which the centrosomes, kinetochore fibers, and kinetochore regions of chromosomes are well visualized. In many dividing newt cells, kinetochore fibers can be seen as cables about 250 nm in diameter extending from the kinetochores towards the centrosome as shown in Fig. 1 by VE-DIC microscopy and previously by polarization microscopy (Cassimeris et al., 1988) and EM (Rieder and Hard, 1990). These kinetochore fibers are tight bundles of about 20 kinetochore microtubules which splay apart somewhat toward the pole (Cassimeris et al., 1988; Rieder and Hard, 1990). During metaphase, chromosomes oscillate on the spindle axis, with their kinetochore fibers alternately shortening and elongating. The 0- and 3-min panels of Fig. 1 show two views during metaphase. During the interval, the kinetochore marked with a small arrow moved away from its attached pole as its kinetochore fiber elongated. The kinetochore reversed direction 1.5 min later, moving poleward as its kinetochore fiber shortened (not shown). The 21- and 25-min panels show early and mid-anaphase. During anaphase kinetochore fibers mostly shorten (see for example the one demarcated by the arrows), though occasional elongation occurs in early anaphase (Bajer, 1982; Skibbens, R., and E. D. Salmon, unpublished results). To compare the movement of chromosomes on the spindle axis with the movement of kinetochore fibers we needed data on chromosome movement rates. Measurements on the phase-contrast images collected during the photoactivation runs were used for direct comparisons, but for quantitation of basic movement parameters the data obtained by Skibbens, R., and E. D. Salmon, unpublished results, from VE-DIC images at 2-s intervals is more accurate. These values are shown in Table I. Comparisons between chromosome movement rates in injected and uninjected cells is one way to detect possible perturbation of spindle mechanics by the probe molecule. We did not accurately measure metaphase rates in injected cells due to the 20-s sampling interval, but the overall oscillatory quality of the movement was identical to injected cells. The average

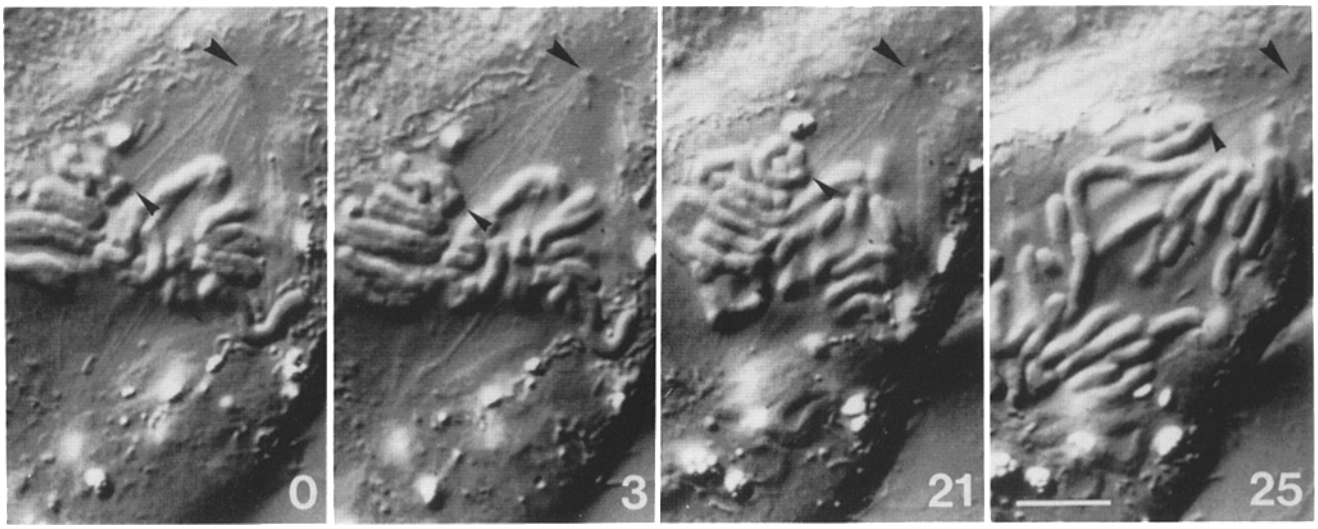


Figure 1. Mitosis in cultured newt lung cells visualized by VE-DIC microscopy. Elapsed time in minutes is shown in each panel, and the arrowheads mark the centrosome and one kinetochore. The 0 and 3 min panels show metaphase, during which time the demarcated kinetochore fiber elongates. The 21 and 25 min panels show early and mid-anaphase, during which time the demarcated kinetochore fiber shortens. Bar, 10 μm.

early anaphase rate of poleward chromosome movement that we measured from our phase contrast images of injected cells, 1.7 μm/min, was slower than the rate found by Skibbens, R., and E. D. Salmon, unpublished results, for uninjected cells, 2.8 μm/min. This difference probably reflects the difficulty in measuring the initial burst of fast poleward movement which occurs shortly after sister centromere separation from the relatively low resolution phase contrast images collected at long sampling intervals during our photoactivation runs. Differences in the age of cultures and temperature of observation may also account for part of the difference in average rates. Early anaphase rates as high as 3.3 μm/min were observed in individual cells (Table I).

Table I. Chromosome and Fluorescent Mark Movement Rates

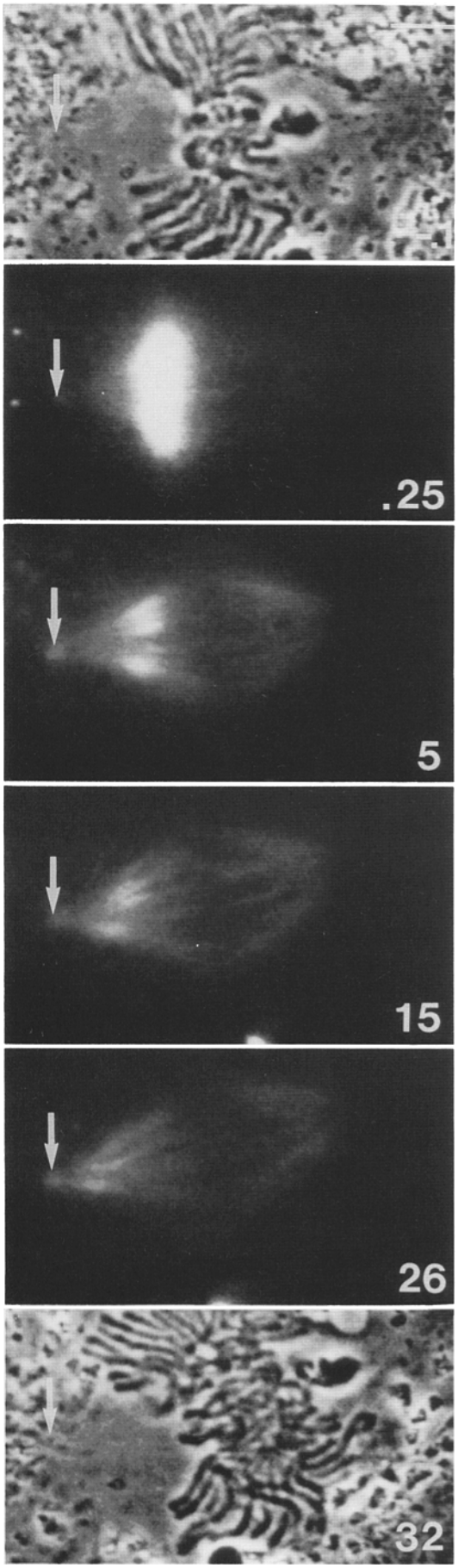
Parameter	Rate (range of observed values shown in parentheses)
	(μm/min)
Metaphase parameters	
Polewards kinetochore movement*	1.8 (0–3.5)
Away from the pole kinetochore movement*	1.7 (0–3.3)
Polewards mark movement, n = 13.7	0.54 (0.29–0.93)
Anaphase parameters	
Polewards kinetochore movement (early anaphase), n = 10.10	1.7 (0.9–3.3)
Polewards kinetochore movement (late anaphase), n = 9.9	0.54 (0.13–1.31)
Polewards mark movement (early anaphase), n = 10.8	0.44 (0.15–0.75)
Polewards mark movement (late anaphase), n = 15.10	0.18 (0.01–0.55)

Asterisks show data from Skibbens, R., and E. D. Salmon, unpublished results. n = number of measurements, number of cells. Early anaphase corresponds to the first half of total chromosome to pole movement.

Overall we saw no indication that mitosis was slow or abnormal in injected cells, and our measured anaphase velocities were within the range of values reported previously by Bajer (1982) and Rieder and Hard (1990) for uninjected cells.

Kinetochore Fiber Movement in Metaphase

We microinjected early mitotic cells with caged fluorescein-labeled tubulin to follow the movement of kinetochore microtubules in metaphase. At least 20 min was allowed for equilibration of the labeled tubulin with spindle microtubules (Wadsworth and Salmon, 1986). Once all the chromosomes had congressed to the metaphase plate, we locally marked spindle microtubules by activation fluorescein in a bar about 4–6-μm wide across the spindle with a 1 s pulse of 366 nm illumination (Fig. 2, 0.25 min). After activation, the fluorescence in the marked region decreased substantially in intensity and resolved into a number of persistent fluorescent cylinders (Fig. 2, 5 min). The rapid decay in intensity that we observed during the first 2 min after activation we interpret as due to turnover of the non-kinetochore microtubules by dynamic instability since the labile non-kinetochore microtubules represent the bulk of microtubules within the spindle (Mitchison et al., 1986; Wadsworth and Salmon, 1986; Rieder and Hard, 1990). From their position, number, morphology, and stability, we interpret the persistent fluorescent cylinders as being marked segments of the kinetochore microtubules, which are known to be much more stable than non-kinetochore microtubules (Mitchison et al., 1986; Cassimeris et al., 1990; Rieder and Hard, 1990). The marked segments of kinetochore fibers moved steadily polewards, indicating polewards flux in these fibers as previously reported in mammalian cells (Mitchison, 1989*b*). Individual marked segments could be followed as they moved all the way from near the kinetochores to the poles, indicating the continuity of many of the microtubules in the kinetochore fibers. This is consistent with EM data showing that the great majority of kinetochore microtubules



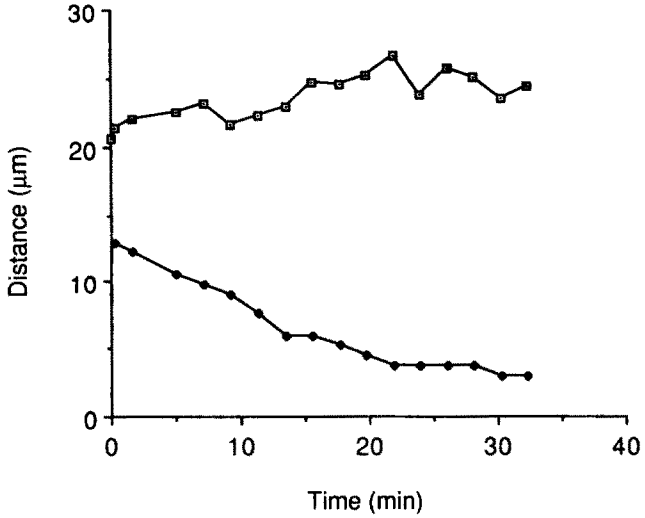


Figure 3. Analysis of the position of kinetochores and fluorescent marks in a metaphase cell. The open squares show the distance between a kinetochore in the lower part of the spindle in Fig. 2 and the leftwards spindle pole as a function of time. The closed diamonds show the distance between the poleward edge of the fluorescent mark on the lower kinetochore fibers and the pole for the same cell at the same plane of focus as the phase contrast images.

extend all the way to the pole (Rieder, 1982; McDonald et al., 1992). Marked segments in individual fibers moved at similar but not identical rates. This can be appreciated from the spread of positions in Fig. 2, 15 min. Marked zones on kinetochore fibers decreased in intensity as they moved poleward, due to a combination of microtubule turnover and some photobleaching. Photobleaching was less than 5% per image with our apparatus (determined by varying the intervals between successive images), and this limited photodamage did not affect chromosome movement rates. However accurate determination of the turnover rate of kinetochore and non-kinetochore microtubules will require further work as discussed in the Materials and Methods section.

The rate of polewards flux in metaphase was determined by tracking the position of the marks as a function of time. A typical plot is shown in Fig. 3. The average rate of polewards flux for all the metaphase cells studied was 0.54 μm/min (Table I). We never observed marked zones moving away from the pole.

Figure 2. Photoactivation marking in metaphase. Time elapsed in minutes from the photoactivation pulse is shown in each panel. The −.1 min panel shows a phase-contrast image of the metaphase spindle with all chromosomes congressed. The arrow marks the centrosome which appears as a black dot in phase contrast images and a bright spot in the fluorescein images several min after photoactivation. The .25–26 panels show fluorescence images in the fluorescein channel at constant illumination intensity and equal printing conditions. Between 0 and 5 min, the initially bright, uniform bar of fluorescence resolves into individual marked kinetochore fibers. The fluorescent marks on the kinetochore fibers then move slowly towards the centrosome. The cell entered anaphase between 26 and 32 min, as can be seen by the early anaphase phase-contrast image at 32 min. Bar, 10 μm.

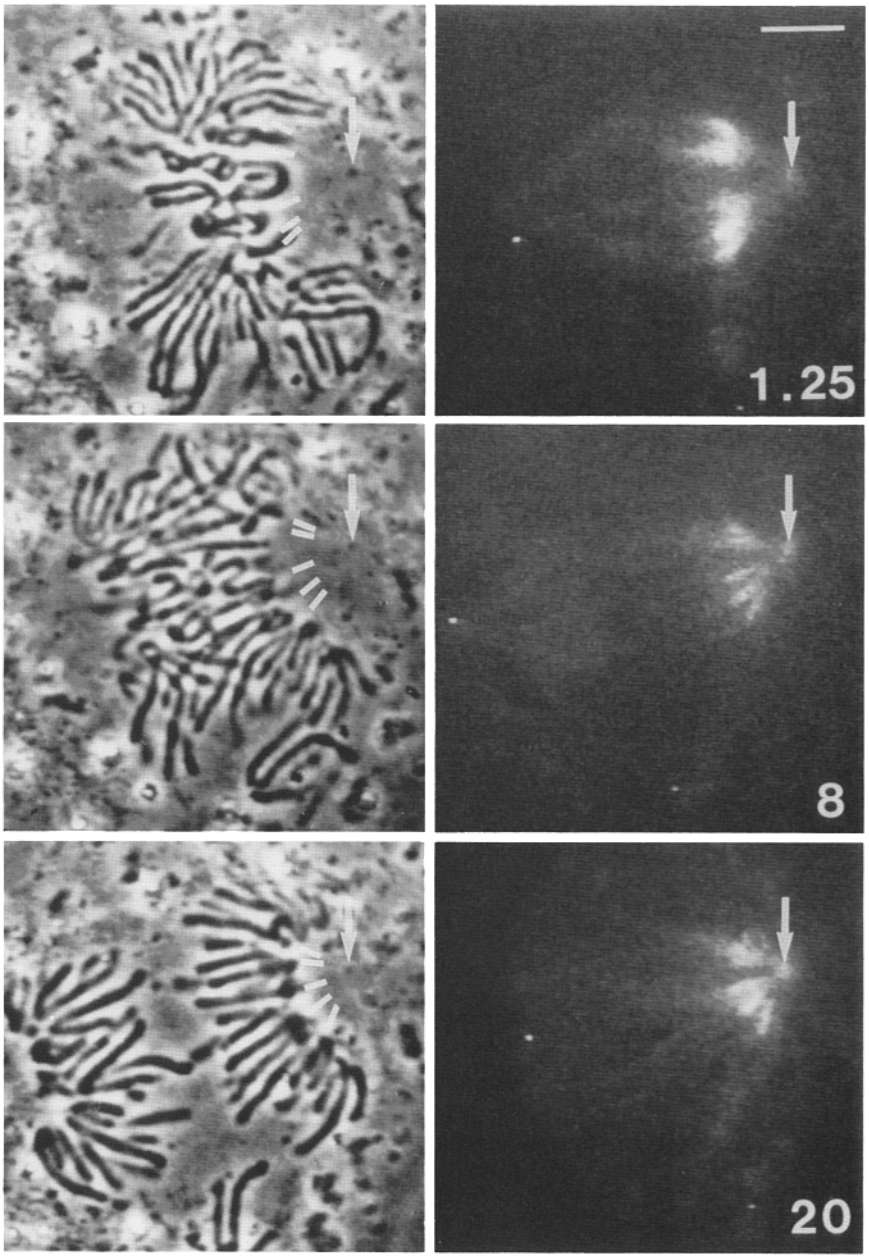


Figure 4. Photoactivation in anaphase. The left hand column shows phase-contrast images and the right hand column corresponding fluorescence images in the fluorescein channel. Time elapsed in minutes from the photoactivation pulse is shown in each fluorescence panel. The corresponding phase image was taken ~1 s later. The fluorescent images at 1.25 and 8 min were taken at constant illumination and printing conditions. The image at 20 min used brighter epi-illumination to bring out the remaining fluorescence in the marked zones. The position of the fluorescent marks is denoted on the phase image by the white lines, and the arrow denotes the position of the centrosome which is visible as a dark dot in the phase-contrast images. Note the movement of both kinetochores and marks towards the spindle pole, and also the movement of kinetochores towards marks. Bar, 10 μm.

Kinetochore Fiber Movement in Anaphase

To follow kinetochore fiber movement during anaphase, we injected cells with a mixture of caged-fluorescein and rhodamine-labeled tubulins early in mitosis. We waited until the separation of sister chromatid arms was clearly visible and then made marks on spindle microtubules by locally activating caged fluorescein as above. Within several minutes after photoactivation, the initially bright homogeneous marks resolved into less intense individual fluorescent cylinders as described for metaphase (Fig. 4). The kinetics of non-kinetochore microtubule turnover appeared somewhat slower than in metaphase, but analysis of this will require more quantitative experiments. We recorded the position of the fluorescent marks as the chromosomes moved polewards by recording pairs of fluorescent and phase contrast images. In flat cells, like the one shown in Fig. 4, the position of the centrosome was visible in both the phase and the fluorescein images (arrows in Fig. 4). To allow accurate comparisons of positions, the positions of the fluorescent marks were overlaid onto the phase contrast images (white bars). Comparing the images at 1.25 and 20 min after activation in Fig. 4, it is clear that the kinetochores moved polewards as expected for anaphase-A, and the kinetochores moved towards the marks as observed previously with photobleached marks (Gorbsky et al., 1988). Unexpectedly, the marks on the kinetochore fibers also moved substantial distances towards the pole, without apparent change in size of the marks. By 20 min, the kinetochores had moved 4–5 μm closer to the marks, while the marks had moved 5–6 μm closer to the pole. As they moved polewards, the marks also changed position in a way expected for kinetochore fibers during the clustering of the chromosomes around the pole, which leads to a spreading out of the kinetochore fibers (Fig. 4, 20 min). As shown in Fig. 4, the fluorescence intensity of the marks decreased substantially during the course of

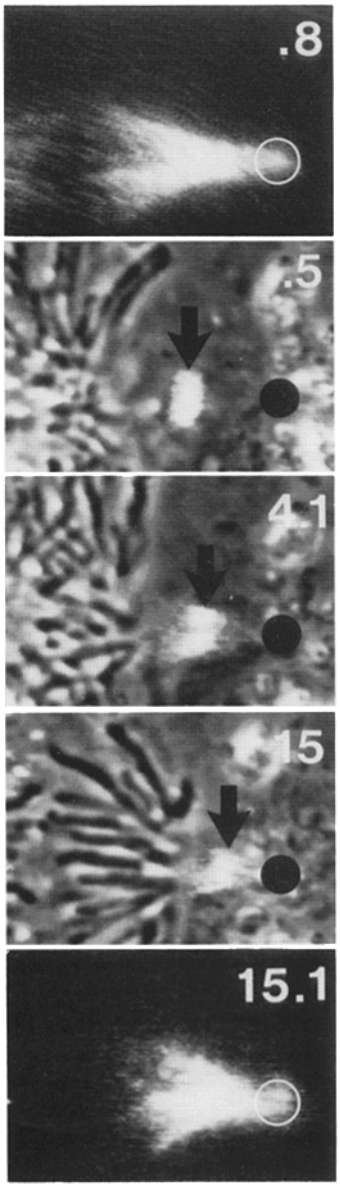


Figure 5. Overlay of fluorescence and phase contrast images showing the right half spindle in a marked anaphase cell. Time elapsed in minutes from the photoactivation pulse is shown in each panel. The panels at .8 and 15.1 min show X-rhodamine fluorescence which reveals the total microtubule distribution. The panels at .5, 4.1, and 15 min show the fluorescein fluorescence overlaid on the paired phase-contrast images. Open circles denote the position of the spindle pole in the rhodamine image, and the dark circles denote this position overlaid onto the phase-contrast images. The arrows denote the fluorescent marks which move towards the pole with time. Magnification as Fig. 4.

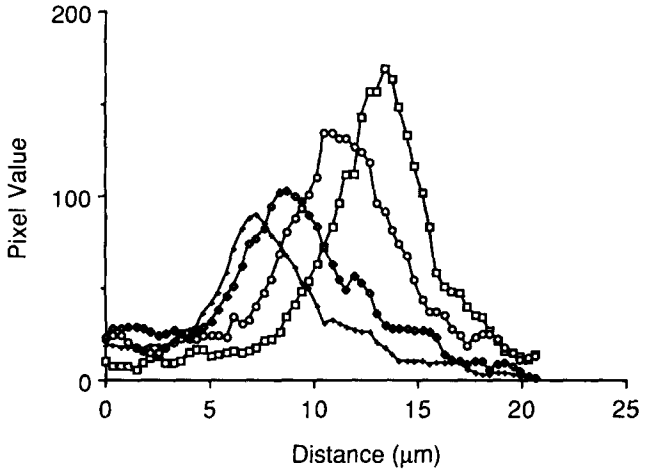


Figure 6. Fluorescence intensity line-scans of a marked anaphase spindle. The fluorescence intensity in the fluorescein channel was measured in arbitrary units for pixels along a line between the equator and the pole through the upper fluorescent marks (*arrow*) in the cell shown in Fig. 5. The length axis shows distance from the spindle pole. The time after activation is shown in minutes for each scan. Epi-illumination level and recording parameters were held constant throughout.

anaphase, and the level of excitation light was increased at the 20 min time point to bring out the morphology of the marks. Photobleaching, microtubule loss and microtubule turnover could contribute to the decrease in fluorescence of the marks, but a decrease in the number of microtubules per kinetochore fiber during anaphase has been documented by EM (Jensen, 1982; McDonald et al., 1992).

We conclude from images such as those in Fig. 4 that poleward transport of kinetochore fibers can make substantial contributions to anaphase-A. We considered two possible artifacts which could erroneously lead to this conclusion: uncertainty in defining the position of the spindle pole and polarized microtubule turnover. Polarized turnover occurs when a microtubule detaches from the kinetochore and shortens only part way back toward the centrosome before regrowth and reattachment (Mitchison, 1989*b*; Wise et al., 1991). This could, in principle, lead to a shift in position of the point of maximum fluorescence intensity without actual microtubule movement. To address the first point, pole location, we used image processing methods to overlay the fluorescein, rhodamine, and phase images in favorable cells. The rhodamine image allowed us to visualize total microtubules and was used to give an unambiguous location of the spindle pole. Fig. 5, -0.8 min shows the rhodamine image of the right half of a spindle in a cell marked in anaphase in which the spindle pole is clearly visible (open circle). The panel at 0.5 min shows an overlay of the fluorescein and phase image, which allows detailed comparison of the position of the mark (arrow) and the chromosomes. Most of the kinetochores at this time are still close to the spindle equator. The position of the pole, determined from the corresponding rhodamine image (not shown), is superimposed as the dark circle. The subsequent overlay images at 4.1 and 15 min clearly show that as the chromosomes approached the pole the marks also moved polewards, though at a slower rate so the chromosomes approached the marks. (See also the cell shown in Fig. 4).

To distinguish microtubule movement from polarized turnover, we measured the fluorescence intensity along kinetochore fibers as a function of time under conditions of constant fluorescence illumination. Kinetochore fiber line scans of the marked anaphase cell in Fig. 5 are shown in Fig. 6. It is clear that the peak of fluorescence intensity moved polewards. However, the peak also moves out from under the original fluorescence profile, despite the progressive decrease in fluorescence intensity. Similar results were obtained from line scans in other cells. We conclude that fluorescently marked tubulin molecules move polewards during anaphase. As in our metaphase experiments, more recordings under conditions of negligible photobleaching will be required to determine how much of the decrease in fluorescence of the marks is due to a combination of microtubule turnover and loss in the kinetochore fibers.

To track the relative positions of kinetochores and marked zones, we measured distances from image sequences like those in Figs. 4 and 5. The results for three separate cells are shown in Fig. 7, and average rates of movement for all the cells we analyzed are given in Table I. The rate kinetochores

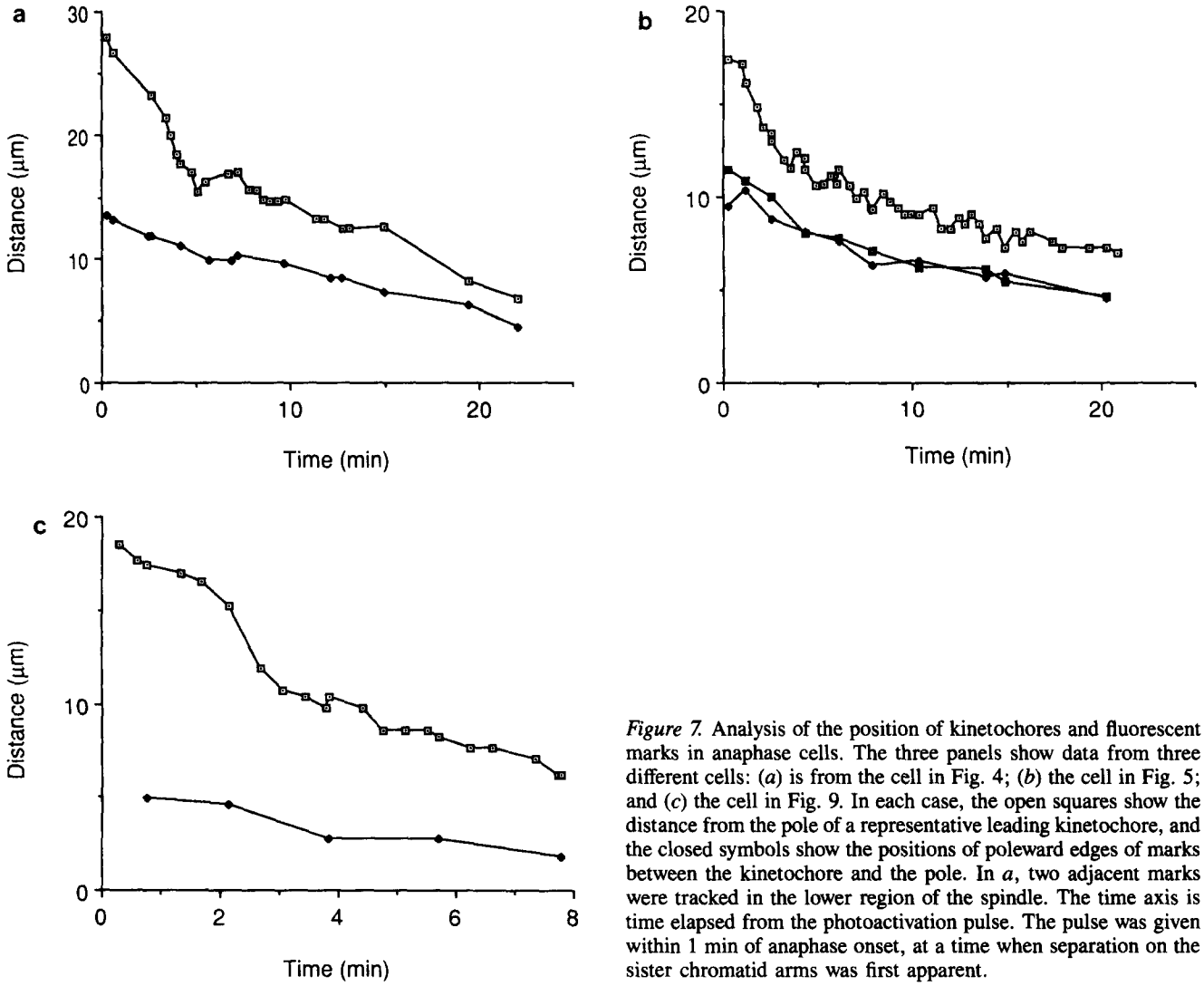


Figure 7. Analysis of the position of kinetochores and fluorescent marks in anaphase cells. The three panels show data from three different cells: (*a*) is from the cell in Fig. 4; (*b*) the cell in Fig. 5; and (*c*) the cell in Fig. 9. In each case, the open squares show the distance from the pole of a representative leading kinetochore, and the closed symbols show the positions of poleward edges of marks between the kinetochore and the pole. In *a*, two adjacent marks were tracked in the lower region of the spindle. The time axis is time elapsed from the photoactivation pulse. The pulse was given within 1 min of anaphase onset, at a time when separation on the sister chromatid arms was first apparent.

move poleward in anaphase-A is bi-phasic in newt cells, with a rapid early phase followed by a slower late phase. This biphasic movement has been noticed previously in newt lung cells (Bajer, 1982) and it is quantified in more detail by Skibbens, R., and E. D. Salmon, unpublished results. The average movement rates measured for our phase contrast images were 1.7 μm/min in the early rapid phase, and 0.54 μm/min in the later slower phase. Currently, we do not know whether this represents two distinct mechanisms, or simply a progressive slowing of poleward movement as the cell cycle progresses from mitosis to interphase. Considering the position of the marked zones, it is evident that polewards movement of the kinetochore fiber occurred throughout anaphase in these cells, though it tended to slow gradually. The average rate of fiber movement was 0.44 μm/min in the early rapid phase of chromosome movement, and 0.18 μm/min in the later slower phase. These average rates are significantly slower than in metaphase, so it appears that although metaphase polewards flux continues into anaphase, it progressively slows down as the cell cycle progresses. These average values for the rate of poleward kinetochore fiber movement conceal considerable variation between individual cells, which was much greater than could be accounted for by uncertainty in measurement (Table I). For example the rate of mark movement in early anaphase varied from 0.15 to 0.75 μm/min.

Comparing kinetochore to marked zone movement, it is evident that kinetochores move substantial distances towards the marks in early anaphase. Thus, microtubule depolymerization at kinetochores is predominant at this stage as previously described (Mitchison et al., 1986; Gorbsky et al., 1988). Late anaphase was more variable, but in some cells the kinetochores and the marks tended to move together, indicating that depolymerization was now mainly at the polewards end of the fiber. We saw examples where the later part of anaphase-A movement was largely attributable to attachment of the kinetochore to a polewards moving kinetochore fiber (see Fig. 7 *a* for example).

Our interpretation of Fig. 7 as showing microtubule depolymerization at kinetochores in early anaphase predicts that if a mark was made sufficiently close to a kinetochore, the mark should disappear as the kinetochore passed over the marked region. This was indeed the case, and an example is shown by the photographs in Fig. 8, using the phase-fluorescein overlay.

Our interpretation of Fig. 7 as also showing microtubule

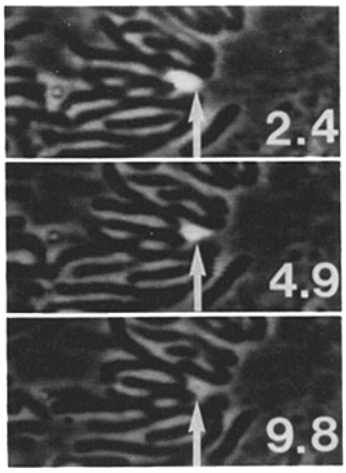


Figure 8. Loss of fluorescence as kinetochores move over a marked region in anaphase. Fluorescein fluorescence and phase-contrast images are overlaid as in Fig. 5. Time elapsed in minutes from the photoactivation pulse is shown in each panel. The arrows denote a fluorescent mark which disappears as kinetochores pass over it. Note that most of the intensity in front of the kinetochores marked by the arrow in the 9.8-min frame is due to the "phase halos" from the chromosomes. Magnification as Fig. 4.

depolymerization at poles implies that the minus-end attachment to a pole must be dynamic, allowing depolymerization while keeping the microtubule attached. Data addressing this point are shown in Fig. 9 for an unusual spindle where the centrosome has detached from the spindle pole. Comparing the position of the marks and the kinetochores it is evident that both are moving towards a focus denoted by the white arrow. The point denoted by the arrow is evidently the site at which kinetochore microtubules terminate and depolymerize, thus it is the spindle pole in a functional sense. It is evident from the rhodamine images, and also the phase images at higher contrast (not shown), however, that the focus of depolymerization and movement is not the centrosome itself. The centrosome, marked by a C, has detached from the functional spindle pole and moved several microns away. This situation was seen several times in older cultures (see also Fig. 23 *a* in Rieder and Hard, 1990). It is clearly anomalous, but since anaphase-A movement continued normally, we consider it informative. Evidently the minus ends of the kinetochore microtubules are anchored to some structure other than the centrosome itself which allows them to depolymerize at their minus ends while remaining fixed in the spindle.

Discussion

Comparison with Previous Work

In this study we have used fluorescence photoactivation to probe the dynamic behavior of kinetochore microtubules in metaphase and anaphase. Before discussing the implications of our results, we need to compare them to previous results, in particular those from photobleaching. First we should emphasize the similarities of our conclusions to previous work.

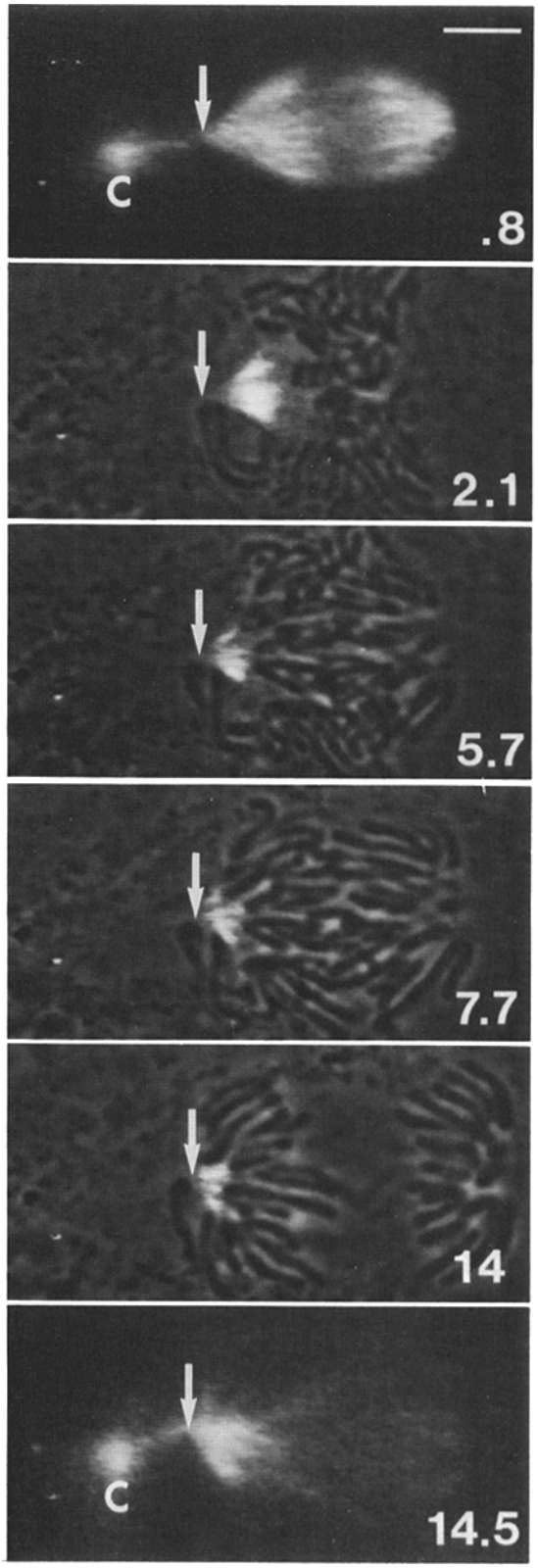


Figure 9. Anaphase marking experiment on a cell in which the centrosome is detached from the spindle pole. Time elapsed in minutes from the photoactivation pulse is shown in each panel. The images at 0.8 and 14.5 min are rhodamine fluorescence (total microtubules) and the remaining images are fluorescein fluorescence overlain on phase contrast. Images were generated as in Fig. 5. The arrow denotes the functional spindle pole, a point at which the kinetochore fibers converge, and towards which the kinetochores move. Note how the length of the fluorescent marks shrink with time once their poleward ends reach this point. The centrosome is marked by a *C*. It is visible in phase-contrast as a dark dot which does not show up at this contrast, and in the rhodamine images as a bright area where astral microtubules converge. Some microtubules extend between the centrosome and the functional spindle pole as evidenced by a bar of fluorescence connecting the two structures. This type of morphology was seen several times in older cultures. Bar, 10 μm.

Results from fluorescence photobleaching, fluorescence photoactivation, biotin/rhodamine tubulin injection, and assembly blocking experiments are in broad agreement when measuring bulk microtubule turnover in the spindle (Salmon et al., 1984*a*,*b*; Saxton et al., 1984; Wadsworth and Salmon, 1986; Hamaguchi et al., 1987; Mitchison et al., 1986; Cassimeris et al., 1988; Gorbsky et al., 1988; Mitchison, 1989*b*; Gorbsky and Borisy, 1989; Cassimeris and Salmon, 1991). The bulk of spindle microtubules are non-kinetochore microtubules which turn over rapidly, exchanging subunits with free monomer with a half-life in the range of 15–90 s (Mitchison et al., 1986; Gelfand and Bershadsky, 1991). Kinetochore microtubules are much more stable and long-lived than the dynamic nonkinetochore microtubules (reviewed in Mitchison, 1988; and Salmon, 1989). In our experiments, as well as in a previous photoactivation study (Mitchison, 1989*b*), the initially bright photoactivated marks resolved within several minutes into weakly fluorescent cylinders corresponding to profiles of the kinetochore fibers seen by high resolution VE-DIC microscopy. The fluorescence of these persisted for many minutes, as expected. Our study, like others (reviewed in Mitchison, 1988; Salmon, 1989), has also identified the kinetochore as the major site of polymerization–depolymerization dynamics during elongation and shortening of the kinetochore fiber.

Our experimental results differ from previous studies in which little or no movement of kinetochore microtubules was detected in metaphase (Wadsworth and Salmon, 1986; Gorbsky and Borisy, 1989; Cassimeris and Salmon, 1991; Centonze and Borisy, 1991) or anaphase (Gorbsky et al., 1988; Nicklas, 1989). We feel that the differences between our results and photobleaching experiments can be accounted for mainly by the increased precision in the work reported here for measuring the dynamics of kinetochore microtubules. Kinetochore microtubules represent a minor population of microtubules in the spindle (McDonald et al., 1992; Wise et al., 1991; Rieder and Hard, 1990). When spindles are marked by photobleaching techniques, fluorescence recovers rapidly in the bleached region to 70–80% of the initial fluorescence as a result of the diffusion of the pool of cytoplasmic tubulin and the rapid turnover of non-kinetochore microtubules. The unrecovered fluorescence corresponding to the differentially stable kinetochore microtubules is low in contrast (Wadsworth and Salmon, 1986). This low contrast could account for previous difficulties detecting slow flux in the face of fast non-kinetochore microtubule turnover. The photoactivation techniques used in our studies avoid this problem and substantially enhance the contrast of the persistent kinetochore microtubules because fluorescence from the cytosol pool of tubulin subunits is nearly absent and marked regions on non-kinetochore microtubules rapidly disappear (see low background levels in the line scans in Fig. 6). As a result, we were able to follow the much weaker signal from the slowly turning-over kinetochore microtubules for a longer period than possible in the photobleaching studies.

Perhaps more important for anaphase analysis, the large size of newt spindles allowed us to measure more accurately the parameters of anaphase-A. In the study by Gorbsky et al. (1988) using cultured pig kidney cells, for example, the total distance moved by chromosomes during anaphase-A movement was typically 6–7 μm, while in this newt study it was 12–14 μm. We found that on average 30%, or 4 μm, of anaphase-A movement was attributable to depolymerization at poles. If the same fraction of depolymerization was occurring in the Gorbsky and colleagues study (1988), total bar movement would have been 2 μm, or one to two times the bar width. This amount of movement should have been detectable, so it is possible that kinetochore fiber movement during anaphase is less prominent, or even nonexistent in cultured pig kidney cells. We note, however, that Gorbsky et al. (1988) did detect limited polewards movement of the bleach zone in some cells and concluded that up to 10% of anaphase-A movement could be due to microtubule depolymerization at poles.

Metaphase Spindle Mechanics

Kinetochores typically oscillate between persistent phases of poleward (P) movement (1.8 microns/min on average) and away from the pole (AP) movement (1.7 μm/min on average) during prometaphase and metaphase in the newt (Bajer, 1982; Skibbens et al., 1992). We use here the P and AP terminology for kinetochore motion defined previously by Pickett-Heaps and colleagues (1982). In addition, photoactivation marking reveals that the kinetochore microtubules are moving continuously polewards at an average rate of $\sim$0.5 μm/min. Thus the motion of the kinetochore relative to the pole must be the sum of two motions: the movement of the kinetochore with respect to the lattice of the kinetochore microtubules and the movement of the kinetochore microtubules with respect to the pole as diagrammed in Fig. 10. For simplicity we have represented the 20 or so kinetochore microtubules typical of newt chromosomes as a single microtubule in the drawing. We have also omitted from the diagram for clarity kinetochore microtubule-by-microtubule turnover through dynamic instability. The kinetochore microtubules are assumed to span the distance between the kinetochore and the pole (Fig. 1; Rieder, 1982; Rieder and Hard, 1990; McDonald et al., 1992).

What should be clear from the diagram in Fig. 10 is that kinetochore oscillations between P and AP movement are due to kinetochore switching between P and AP motions over the microtubule lattice; lattice flux is always poleward. When a kinetochore is in the P phase of movement at 1.8 μm/min, the velocity of the kinetochore over the microtubule lattice is 1.3 μm/min and the poleward movement of the microtubule contributes about 0.5 μm/min. Note in the diagram in Fig. 10, that when a kinetochore is moving poleward, kinetochore microtubule shortening occurs both at the kinetochore and at the pole. When the kinetochore is in the AP phase of movement at 1.7 μm/min away from the pole, the velocity of the kinetochore over the microtubule lattice must be 2.3 μm/min since the poleward movement of the microtubule subtracts 0.5 μm/min. As the kinetochore moves away from the pole, net kinetochore microtubule growth only occurs at the kinetochore since net depolymerization continues at the pole at the flux rate.

This kinetochore switching is the mechanism which uncouples kinetochore motion from the continuous poleward movement of the kinetochore microtubules. Repetitive oscillations between P and AP movements for kinetochores near the spindle equator need not result in any net movement of the kinetochore with respect to the pole even though marks

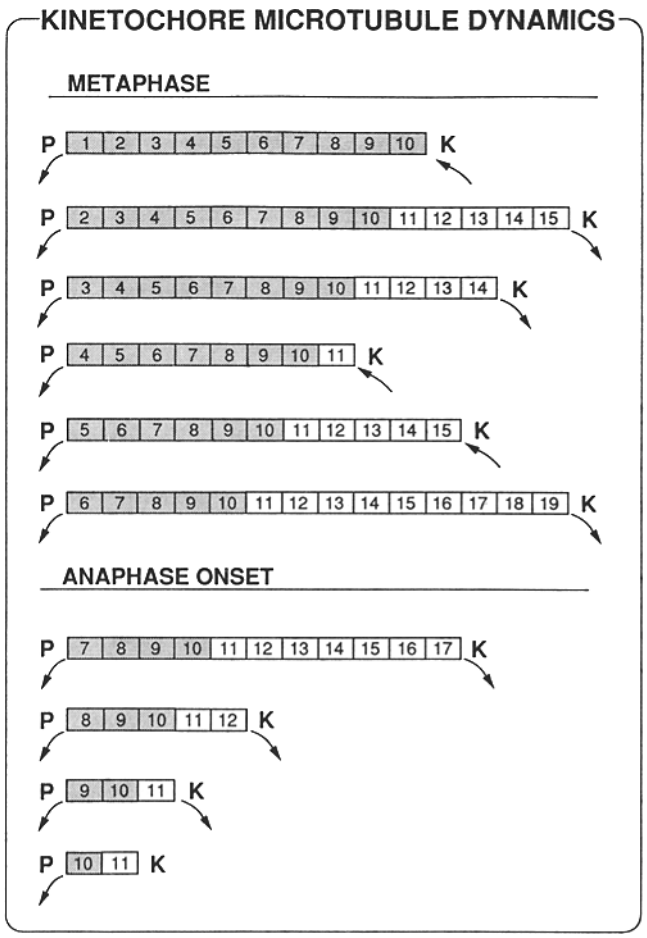


Figure 10. Diagram of kinetochore microtubule dynamics in metaphase and anaphase. The segmented bar represents a typical kinetochore microtubule as a function of time, with individual segments numbered. *P* represents the minus end of end of the microtubule near the pole, and *K* the plus end attached to the kinetochore. Segments represent groups of tubulin subunits in the lattice. The total length of the kinetochore microtubule is about 20 μm, or 32,000 tubulin dimers. Shaded segments are those present at the first timepoint, and open segments are newly polymerized after that time. During metaphase the microtubule elongates and shortens as the kinetochore oscillates between "*P*" and "*AP*" movement as defined in the text. All polymerization is at the kinetochore. Most depolymerization is at the kinetochore, but steady depolymerization also occurs at the pole. During anaphase the microtubule shortens at both ends. Early in anaphase-A depolymerization is predominant at the kinetochore. Later depolymerization at both sites slows down, though depolymerization at the kinetochore may stop first. Not shown in this diagram is the probability that the microtubule will detach from the kinetochore and depolymerize completely by dynamic instability.

on the lattice of the kinetochore microtubules may move all the way to the pole as shown in Figs. 2 and 3. Little is known yet about how the kinetochore switches between P and AP movement along the microtubule lattice, but tension, microtubule motors and phosphorylation mechanisms may be involved (Hyman and Mitchison, 1991; Skibbens, R., and E. D. Salmon, unpublished results). In addition, there are also occasional periods where kinetochores appear stationary with respect to their pole (Bajer, 1982; Skibbens, R., and E. D. Salmon, unpublished results). Whether the kinetochore microtubules on these stationary chromosomes exhibit poleward flux is unknown and more high resolution marking studies are needed to answer this interesting question.

Anaphase Spindle Mechanics

During anaphase, the sister chromatids split, and each moves polewards at a rate of ~1–4 μm/min in early anaphase, slowing to ~0.5 μm/min in later anaphase. Marking experiments reveal that kinetochore microtubules move polewards at an average rate of 0.44 μm/min in early anaphase, slowing to 0.18 μm/min in later anaphase. As with metaphase, net anaphase-A movement of kinetochores on the spindle axis can be partitioned into components due to movement over the lattice of kinetochore microtubules plus movement by virtue of attachment to a poleward moving microtubule lattice. These dynamics are also diagrammed in Fig. 10. We note that turnover numbers are again missing, although in anaphase, kinetochore microtubules which depolymerize by dynamic instability may not in fact be replaced, since their number decreases with time (Jensen, 1982; Gorbsky and Borisy, 1989; McDonald et al., 1992). Although AP movement of kinetochores occurs during anaphase-A in the newt, it is not included in the anaphase-A region of Fig. 10 because P movement is dominant.

The relative importance to anaphase-A of kinetochore-over-microtubule movement versus movement of the whole kinetochore fiber can be expressed in terms of either velocity or distance. At the peak rate we measured for chromosome movement in early anaphase, kinetochore-over-microtubule movement contributed on average 75% of the total rate, and polewards kinetochore fiber movement the remaining 25% (Table I). In late anaphase, the relative contributions were on average 66 and 33%, respectively. A related measure is the contribution of the distance moved by the kinetochore fiber to the total distance moved by the kinetochore. Over the whole course of anaphase-A, polewards movement of the kinetochore fiber accounted for 37% of total polewards kinetochore movement (Table II). The relative contribution of fiber movement is higher in late anaphase by both measures, because microtubule movement slows down more slowly than kinetochore-over-microtubule movement, thus it makes a greater contribution late in anaphase-A.

These numbers are all averages, and we found significant variation between cells which was not attributable to measurement errors. The contribution of fiber movement to the peak rate of movement varied from 14–32% (Table I), and the contribution to total distance covered varied from 20–49% (Table II). There is more variation in the latter num-

Table II. Relationship between Kinetochore Fiber Movement and Total Anaphase-A Movement

Phase of mitosis	Fraction of anaphase-A due to kinetochore fiber movement
Total anaphase, n = 8	37% (20–49%)
Early anaphase, n = 6	25% (14–32%)

The fraction due to kinetochore fiber movement is given by the total distance moved by the mark divided by the total distance moved by the kinetochores. Number in parentheses is the range of observations, and n = number of cells measured. Only flat cells marked early in anaphase were scored. Early anaphase corresponds to the first half of total chromosome to pole movement.

ber because the extent of the second, slow part of anaphase-A was quite variable. The source of this variability is not clear, though different flatness of the cells may have played some role. Flatter cells tended to have larger spindles and a relatively extended late anaphase. Another source of variation in the figure for contribution to total distance moved is the time in anaphase when the mark was made, since later marks tend to emphasize the contribution of fiber movement.

Given these numbers, we conclude that the contribution of polewards kinetochore fiber movement to net chromosome-to-pole movement in anaphase-A was significant in all the cells observed and that this contribution must be taken into account in molecular models of chromosome movement.

Implications for Force Generation

Our data imply that kinetochores move polewards by two distinct mechanisms: one which moves the kinetochore along the microtubule lattice and another that moves the microtubule lattice poleward, pulling the kinetochore with it. The "Pac-man" model proposes a mechanism for chromosome movement with depolymerization at the kinetochore, and the "traction fiber" model proposes a mechanism for movement by attachment to a moving kinetochore fiber, with depolymerization at the pole (reviewed in Salmon, 1989). Previously these models have been considered as alternatives to be distinguished (Gorbsky et al., 1988), but our data show that both may operate simultaneously.

Poleward movement of kinetochores over the microtubule lattice (Pac-man model) is likely to be driven by force generation at the kinetochore for anaphase as well as metaphase. Force could be generated by minus end–directed motor proteins such as dynein (Pfarr et al., 1990; Steuer et al., 1990; Hyman and Mitchison, 1991) or minus end directed kinesins (Walker et al., 1990; McDonald et al., 1990). Alternatively, or in addition, force could be generated directly by the thermodynamic drive towards microtubule depolymerization which results from GTP hydrolysis during polymerization (Inoue and Sato, 1967; Koshland et al., 1988; Coue et al., 1991).

Polewards movement of the kinetochore fiber, carrying the attached kinetochore with it, could be driven by motor proteins acting along the length of the kinetochore microtubules (Nicklas, 1971; Hays et al., 1982; Hays and Salmon, 1990), presumably the same ones that may be responsible for the transient lateral interactions between microtubules in newt spindles (Cassimeris et al., 1988). In principle the motors distributed throughout the spindle could be plus end–directed motor proteins attached to structural elements outside the kinetochore fiber that act on kinetochore microtubules, or minus end–directed motor proteins attached to the fiber itself that act on astral microtubules.

Another possible site for the kinetochore fiber motor is the centrosome, but we consider this unlikely given that removal of the centrosome had little effect on anaphase movement (Fig. 9). This result is perhaps not surprising given that aster removal is known not to affect metaphase or anaphase-A in sand dollar spindles (Hiramoto and Nakano, 1988) and severing the half spindle does not block anaphase-A (Hiramoto and Nakano, 1988; Nicklas, 1989). If they are severed, the new minus ends can apparently act as a new spindle pole. It is currently an open question whether the minus ends of kinetochore fibers are anchored in any unique structure in animal spindles. Holding on to depolymerizing microtubule ends could perhaps best be achieved by motor proteins, and there is evidence that both minus end–directed (Kimble and Church, 1983; Walker et al., 1990; McDonald et al., 1990; Karsenti, 1992) and plus end–directed (Sawin, K. E., and T. J. Mitchison, manuscript submitted for publication) microtubule motor proteins may be involved in holding the polar ends of kinetochore fibers together. Whatever their anchorage, kinetochore microtubules probably need some unique factors interacting with their minus ends to allow depolymerization, since free minus ends are kinetically inert compared to free plus ends in cell extracts (Gard and Kirschner, 1989; Belmont et al., 1990; Spurck et al., 1990; Simon et al., 1992).

One important measure of the relative importance of the two movement mechanisms, Pac-man and traction-fiber, would be the relative magnitude of the force they generate, but unfortunately this is not known. We know from the classic work of Nicklas (1983; see also Alexander and Rieder, 1991) that the total force acting on chromosomes during anaphase-A is an order of magnitude or more higher than that actually required to drag the chromosome arms through the cytoplasm at the observed rate of a few microns per minute. To measure the maximum force generated, it was necessary to pull the chromosome to a complete halt with the tip of a stiff glass needle. Under these conditions all the motors generating polewards forces on the chromosome would simply add together, regardless of the maximum speed at which they can operate. The force-velocity curves measured by Nicklas (1983) were approximately linear, while a system with more than one motor operating at different rates would be predicted to give a more complex curve. The resolution of the Nicklas data might not be sufficient to detect detailed complexity. However his force-velocity curves are probably not consistent with models proposing a weak, fast motor acting together with a much stronger, slower motor.

Why are there two mechanisms pulling kinetochores polewards, and are they redundant? This is a difficult question. Movement of the kinetochore fiber (polewards flux) makes only a minor mechanical contribution to congression movements to the spindle equator in newt cells. Whether kinetochore fiber movement plays a more subtle role in congression, such as generating tension to help signal chromosome position on the spindle, remains to be determined. The relatively small contribution of kinetochore fiber movement to net anaphase-A suggests it is not essential for achieving segregation, and in mammalian cells it was indeed barely detectable (Gorbsky et al., 1988). Fiber movement could simply be a carry-over from polewards flux in metaphase. It may, however, represent a genuine, overlapping anaphase-A mechanism. We do not know whether fiber movement alone could achieve segregation in newt cells, though this might be testable by somehow inactivating depolymerization at the kinetochore.

It is possible that fiber movement could turn out to be the most important anaphase-A mechanism in other species. In reconstituted *Xenopus* spindles, flux was much faster, 3.0 μm/min. If kinetochore fiber movement occurs at that rate in *Xenopus* embryonic spindles, it could account for some aspects of chromosome movement. In the *Xenopus* experiments, though, the marked microtubules were largely of the non-kinetochore microtubule overlap class, which makes

comparison of the systems difficult. Currently, we do not know if the faster flux rate in *Xenopus* spindles is due to a difference in microtubule type, animal species, meiosis vs mitosis, or even artifacts from reconstitution. More marking experiments in large embryonic spindles are needed to address this issue.

Forer's classic UV microbeam marking experiments in crane fly meiotic spindles has indicated that kinetochore fiber movement could largely account for anaphase-A movement (Forer, 1965, 1966), and his experiments were influential in the development of the traction-fiber model of chromosome movement (Nicklas, 1971; Hays et al., 1982; Hays and Salmon, 1990). Other UV marking experiments (Gordon, G., and S. Inoué, unpublished observations) support polewards fiber movement as the predominant action accompanying chromosome movement during insect meiosis. On the other hand, Nicklas (1989) failed to detect any poleward movement of kinetochore fibers during anaphase-A in meiotic spindles of permeabilized grasshopper spermatocytes. Clearly it would be interesting to perform fluorescence photoactivation marking experiments in the insect spermatocyte systems where congression and anaphase chromosome movements have been so well studied.

Perhaps we should not be too surprised by redundancy, since in a sense, animal spindles already possess overlapping, partially redundant mechanisms for segregation in anaphase-A (chromosome-to-pole) and anaphase-B (pole-away-from-pole) movements. Furthermore anaphase-B itself is almost certainly complex given the persuasive arguments that both pulling by asters and pushing by central spindles can give rise to spindle expansion (Aist et al., 1991; discussed in Cande and Hogan, 1989; and Mitchison, 1989*a*). Thus it seems likely that both anaphase-A and -B movements use more than one molecular mechanism so that the number of distinct molecular processes contributing to chromatid segregation in animal cells may be quite large. This complexity could result from an evolutionary drive to maximize the speed and fidelity of segregation. However, we think it likely that complexity results more from the flexibility needed to recover from mistakes. Cells with very different morphologies and lifestyles all use the same basic mitotic mechanism. Furthermore spindles can reassemble and achieve normal segregation even after drastic disruption such as microtubule depolymerization or chromosome detachment (reviewed in Salmon, 1989). Mechanical flexibility operating in conjunction with check-point controls (Hoyt et al., 1991; Li and Murray, 1991) that delay the cell cycle until the spindle is poised for segregation may allow successful mitosis in the face of different starting configurations and environmental insults. We hope that recent progress on the molecular biology of spindle components will allow molecular analysis of the various mechanisms in the near future.

We suspect that another main lesson of polewards microtubule movement concerns the importance of motor proteins in spindle structure and assembly (McIntosh and Pfarr, 1991; Karsenti, 1992; Mitchison, 1992). Circumstantial evidence from inhibitor studies (Sawin and Mitchison, 1991) and genetic investigations (reviewed by Sawin and Scholey, 1991) leads to a model of spindle structure where microtubules are held in place mainly by interactions with motor proteins. These proteins would play a key role in spindle assembly, they would drive flux in metaphase, and they also might contribute to anaphase-A and -B movements. Methods for testing the role of particular motor proteins in these processes are currently being devised. Members of the kinesin (Sawin and Scholey, 1991; Sawin, K. E., and T. J. Mitchison, manuscript submitted for publication) and dynein (Steuer et al., 1990; Pfarr et al., 1990) families have been identified in or near kinetochore fibers. In the future it should be possible to inhibit or modify the action of these proteins in vivo using genetics and in vitro using biochemical approaches, and to assess their roles in the spindle.

We wish to thank Conley Rieder for vigorous discussion and a generous donation of newt cultures, and to acknowledge both practical advice and spiritual guidance from Shinya Inoué. Vicki Skeen also supplied us with excellent newt cultures for our experiments and provided technical assistance to EDS for the VE-DIC microscopy.

This work was generously supported by Nikon Instruments, Inc., through their Marine Biological Laboratory summer fellowship program, and we thank their representatives for help and support. T. J. Mitchison was also supported by National Institutes of Health grant GM29565, and fellowships from the Packard and Searle foundations. E. D. Salmon was supported by National Institutes of Health grant GM24364 and by National Institutes of Health grant R13-GM 31136 to the Physiology Course at M. B.

Received for publication 20 March 1992 and in revised form 28 July 1992.

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