Kinetochore microtubules shorten by loss of subunits at the kinetochores of prometaphase chromosomes

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Summary

The site of tubulin subunit dissociation was determined during poleward chromosome movement in prometaphase newt lung cell mitotic spindles using fluorescence photobleaching techniques and nocodazole-induced spindle shortening. Synchronous shortening of all kinetochore microtubules was produced by incubating cells in 17 μM nocodazole to block microtubule assembly. Under these conditions the spindle poles moved towards the metaphase plate at a rate of 3.6 ± 0.4 μm min^{-1} ($n=3$). On the basis of anti-tubulin immunofluourescent staining of cells fixed after incubation in nocodazole, we found that nonkinetochore microtubules rapidly disappeared and only kinetochore fibers were present after 60–90 s in nocodazole.

To localize the site of tubulin subunit dissociation, a narrow bar pattern was photobleached across one half-spindle in prometaphase–metaphase cells previously microinjected with 5-(4,6-dichlorotriazin-2-yl) amino fluorescein (DTAF)-labeled tubulin. Immediately after photobleaching, cells were perfused with 17 μM nocodazole to produce shortening of kinetochore microtubules. Shortening was accompanied by a decrease in the distance between the bleach bar and the kinetochores. In contrast, there was little or no decrease in the distance between the bleach bar and the pole. Compared to their initial lengths, the average kinetochore to pole distance shortened by 18%, the bleach bar to kinetochore distance shortened by 28% and the average bleached bar to pole distance shortened by 1.6%. The data provide evidence that tubulin subunits dissociate from kinetochore microtubules at a site near the kinetochore during poleward chromosome movement. These results are consistent with models of poleward force generation for chromosome movement in which prometaphase–metaphase poleward force is generated in association with the kinetochore.

Key words: kinetochores, microtubules, mitosis.

Introduction

During prometaphase, one kinetochore of a chromosome becomes attached to the (+) ends of microtubules extending from one spindle pole, while the other kinetochore attaches to the (+) ends of microtubules extending from the opposite pole. Initial attachment at one kinetochore results in kinetochore to pole movement, unless opposed by forces in the opposite direction. When the opposing kinetochore attaches to microtubules from the opposite pole the chromosome begins movement toward the spindle equator (congression). This movement requires lengthening of one kinetochore fiber and shortening of the opposing kinetochore fiber. Chromosome congression to the spindle equator, a position equidistant between the spindle poles, has been best explained by a force-balance mechanism, where chromosomes align at this position because the forces on the chromosome, with respect to opposite poles, are balanced (Nicklas, 1971; Hays *et al.* 1982; Nicklas, 1988). While experimental evidence strongly supports a force–balance mechanism (Nicklas, 1971; Hays *et al.* 1982; Hays and Salmon, 1990), the nature of this mechanism remains controversial.

Two force–balance models have been proposed to explain prometaphase chromosome congression to the metaphase plate: the traction fiber model (Ostergren, 1949, 1950; Ostergren *et al.* 1960) and the kinetochore motor/polar ejection model (Rieder *et al.* 1986; Cassimeris *et al.* 1987; Salmon, 1989*a*). In 1949, Ostergren proposed the traction fiber model in which poleward forces on a chromosome are produced along the length of the kinetochore fibers, with the magnitude of the poleward force increasing with increasing distance from the pole. At the metaphase plate kinetochore fibers are of equal length and, thus, the poleward forces on opposing kinetochores are equal, but of opposite direction, resulting in a net force of zero (Ostergren *et al.* 1960).

An alternative force–balance model has been proposed recently (Rieder *et al.* 1986; Cassimeris *et al.* 1987; Salmon, 1989*a*). In this model poleward force is generated by a motor located at the kinetochore, whose strength depends only on the number of microtubules attached to the kinetochore. In each half-spindle there is also a pushing force directed away from the pole (the ejection force) and it has been suggested that this force is produced by the nonkinetochore microtubules acting on the bulk of the chromosomes. The ejection force decreases in strength with distance away from the poles (Salmon, 1989*a*). The ejection force may be generated by the dynamic instability of polar microtubules or by the action of mechanochemical

translocators acting between the chromatin and the nonkinetochore microtubules. In a bipolar animal spindle each chromosome experiences four forces: two poleward forces at the oppositely oriented kinetochores and two polar ejection forces that push the chromosome arms outward away from the poles. At the metaphase plate, these forces balance for chromosomes with equal numbers of kinetochore microtubules at opposite kinetochores and for polar microtubule arrays of equivalent microtubule distribution and dynamics.

The traction fiber model and the kinetochore motor/polar ejection model predict distinct and different sites of tubulin subunit dissociation from kinetochore microtubules during poleward movement of chromosomes. The simplest interpretation of the traction fiber model predicts that tubulin subunits will dissociate from kinetochore microtubules at the polar area as kinetochores move poleward. The traction forces acting along the kinetochore fiber would pull the fiber poleward and this action, combined with the shortening of the kinetochore fiber, would result in loss of tubulin subunits at the pole. In contrast, the kinetochore motor/polar ejection model predicts that tubulin subunits should dissociate proximal to the kinetochore. As the kinetochore motor moves the chromosome poleward along stationary kinetochore microtubules, tubulin subunits should dissociate at the kinetochore. In support of the kinetochore motor/polar ejection model of chromosome congression, recent observations have shown that tubulin subunits are incorporated into kinetochore microtubules at the kinetochore at metaphase (Mitchison *et al.* 1986; Wise *et al.* 1986; Mitchison, 1988) and that tubulin subunits dissociate from kinetochore microtubules at the kinetochore as the chromosomes move poleward in anaphase (Mitchison *et al.* 1986; Gorbsky *et al.* 1988). Whether prometaphase and anaphase poleward chromosome movement occur by the same mechanism has been a controversial question (Hays *et al.* 1982; Mitchison *et al.* 1986; Mitchison, 1988; Salmon, 1989*a*).

Prometaphase–metaphase kinetochore fibers can be induced to shorten synchronously under conditions where microtubule assembly has been blocked. Microtubule depolymerization induced by cooling, high hydrostatic pressure or colchicine-like drugs results in shortening of metaphase kinetochore microtubules at rates equal to, or greater than, the rate during poleward chromosome movement in anaphase (Inoue, 1952; Inoue and Ritter, 1975; Salmon, 1975; DeBrabander *et al.* 1986).

In this paper we present the results of experiments using drug-induced spindle shortening to determine the site of tubulin subunit dissociation from prometaphase–metaphase kinetochore microtubules during kinetochore to pole shortening. Prometaphase newt lung cells were microinjected with 5-(4,6-dichlorotriazin-2-yl) amino fluorescein (DTAF)-labeled tubulin. Following incorporation of labeled tubulin, a narrow bar pattern was photobleached across one half-spindle to mark a position on the fluorescent microtubules between the kinetochores and the pole (Wadsworth and Salmon, 1986; Gorbsky *et al.* 1988). The position of the bar bleach was recorded using video-enhanced fluorescence microscopy. Immediately after photobleaching and recording the first fluorescent image, cells were perfused with nocodazole to block microtubule assembly (Hoebeke *et al.* 1976; Lee *et al.* 1980), resulting in rapid disassembly of the nonkinetochore microtubules and shortening of the kinetochore fibers. A second fluorescent image was recorded 60–100 s after perfusion with nocodazole. Newt lung cells were used because their large spindles (up to 20 μm kinetochore to pole distance) provided the spatial resolution necessary for these experiments. The data are most consistent with models in which tubulin subunits dissociate from a site(s) proximal to the kinetochore during kinetochore fiber shortening in prometaphase–metaphase.

Materials and methods

Cell culture and microinjection

Primary cultures of newt lung epithelial cells were grown on glass coverslips as described previously (Cassimeris *et al.* 1988*b*). Cells were microinjected with DTAF-tubulin (Wadsworth and Salmon, 1986) and injected cells were allowed to equilibrate for at least 20–30 min before photobleaching.

DIC microscopy and nocodazole perfusion

Uninjected prometaphase cells were observed using video-enhanced differential interference contrast (DIC) microscopy as described previously (Cassimeris *et al.* 1988*b*), without digital enhancement. Cells were perfused with nocodazole (17 μM in culture medium) and the shortening of the spindle was recorded in real time. Images were recorded on ¾ inch video tape with a Sony 5800H recorder. The rate of spindle pole movement was determined using a computer driven video tracking system described previously (Pryer *et al.* 1986; Walker *et al.* 1988). Micrographs were made by photographing images from the video monitor using Pan-X film.

Immunofluorescence

Coverslips with uninjected cells were incubated in nocodazole (17 μM in culture medium) for 60 or 90 s, then lysed and fixed as described previously (Cassimeris *et al.* 1986). Coverslips were incubated with antibodies, examined and photographed as described previously (Cassimeris *et al.* 1986).

Fluorescence microscopy, photobleaching and nocodazole perfusion

Prometaphase–metaphase spindles were photobleached as described previously (Wadsworth and Salmon, 1986; Cassimeris *et al.* 1988*a*), with the following modifications: experiments were performed on a Zeiss inverted microscope stand; images were projected to either a Dage ISIT or a SIT 66 low light level video camera; and images were stored on the hard disk after image processing using a home-built Max-Video digital image processing system (Cassimeris *et al.* 1988*a*; Walker *et al.* 1988). Images were acquired by averaging 32 frames (1 s integration time) to increase the signal to noise ratio.

The experimental protocol for fluorescence redistribution after photobleaching (FRAP) and nocodazole perfusion was as follows. Coverslips with microinjected cells were assembled into Rose chambers filled with 0.6× saline G (Wadsworth and Salmon, 1986). A narrow, 1.8 μm wide, bar pattern was photobleached across one half-spindle (Wadsworth and Salmon, 1986; Cassimeris *et al.* 1988*a*). A fluorescent image marking the original position of the bar pattern was acquired within 5 s after photobleaching, and stored. The saline within the Rose chamber was then quickly exchanged for saline plus nocodazole (17 μM). Transfer of solutions was complete within 20–25 s after photobleaching. Approximately 60–100 s after perfusion with nocodazole, a second fluorescence image was acquired and stored. Micrographs were made by photographing images of the video monitor (Panasonic, model MV 5410) using Pan-X film.

Images were analyzed in two ways; in each case the distances between kinetochores and the center of the pole, between kinetochores and center of the bleached bar, and between the center of the bleached bar and the center of the pole, were measured for images acquired 5 s after photobleaching and images acquired 60–100 s after perfusion with nocodazole. In method 1 images were projected onto a video monitor at a final

magnification of ×2700. Measurements were made by tracing the spindle outline onto transparent acetate sheets and marking the positions of the kinetochores, the center of the bleach bar and the center of the pole on the basis of visual criteria. The position of a kinetochore was assigned to the equatorial end of a fluorescent kinetochore fiber. The center of the bleach bar was assigned a position along the midline of the photobleached bar and the center of the pole was assigned to the center point of the bright fluorescence at the spindle pole. In method 2 images were analyzed by taking fluorescent intensity scan lines along single kinetochore fibers. Four scan lines were taken along a fiber and averaged to reduce the noise in the intensity scan. The resulting curves were smoothed by averaging the value at each pixel position with the values of two pixels on either side. In each case the scan was begun at the equatorial end of a fluorescent fiber and this position was designated as the position of the kinetochore. The center of the pole was assigned to the peak polar brightness and the center of the bleach bar was assigned to the minimium fluorescence along the fiber. The precision of these measurements was estimated to be ~0.5 μm, on the basis of repeated measurements or by separate analysis by two investigators.

Results

Prometaphase–metaphase newt lung spindles shorten when incubated in nocodazole

Prometaphase newt lung cells were perfused with 17 μM nocodazole and the resulting spindle shortening was observed using video-enhanced DIC microscopy. Fig. 1 shows the shortening of one half-spindle after nocodazole was added. The centrosome complex of the spindle pole (arrowhead) moved in towards the metaphase plate at a relatively constant velocity (Fig. 2). For three cells analyzed, the distance between the kinetochores and the poles shortened at 3.6 ($\pm$0.4) μm min^{-1} (S.D.), a velocity that also corresponds to the rate of kinetochore microtubule depolymerization. Our observed spindle shortening rate correlates closely with the rate of kinetochore to pole shortening determined previously for Japanese newt lung epithelial metaphase spindles incubated in 10 μM colcemid (Washio and Sato, 1982).

Rapid disassembly of nonkinetochore microtubules and differential stability of kinetochore microtubules in nocodazole-treated cells

Treatment of cells with 34 μM nocodazole results in rapid (within seconds) disassembly of the nonkinetochore microtubules (Salmon *et al.* 1984). For the experiments presented here, cells were treated with a lower concentration of nocodazole (17 μM) to produce a slower rate of spindle shortening in order to allow enough time for image acquisition during the photobleaching experiments. To determine the microtubule composition of prometaphase–metaphase cells after incubation in the lower concentration of nocodazole (17 μM) used in this study, mitotic cells were fixed 60–90 s after incubation in nocodazole and stained for microtubules by immunofluorescence techniques. As shown in Fig. 3, there was also a large reduction in nonkinetochore microtubule density under these conditions.

Consistent with the immunofluorescent images (Fig. 3B,C), electron microscopic examination of a cell fixed 90 s after incubation in nocodazole (17 μM, data not shown), showed that the remaining microtubules were associated with kinetochore fibers. Therefore, in the photobleaching experiments presented below, the spindle fluorescence that remained after perfusion with nocod-

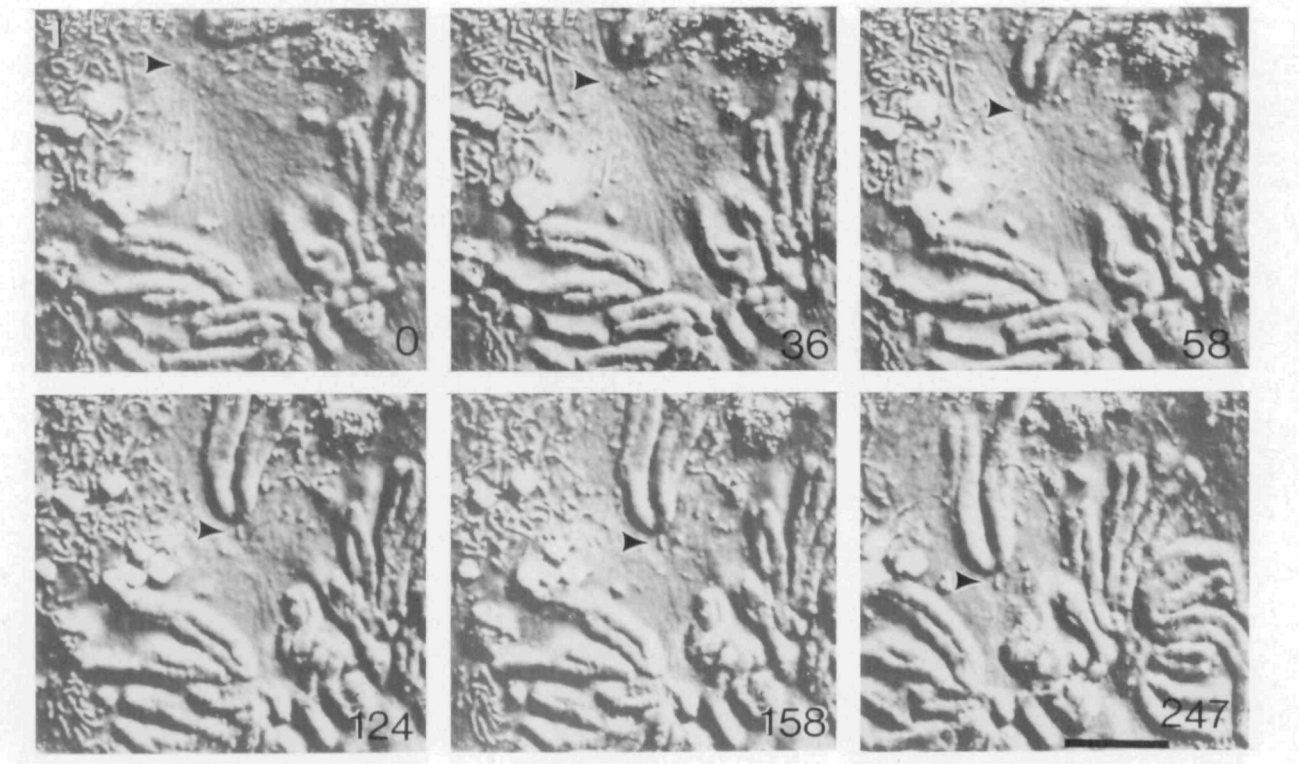


Fig. 1. A series of video-enhanced DIC micrographs taken from a real-time recording of a newt lung prometaphase spindle perfused with medium containing 17 μM nocodazole. Kinetochore fibers shortened as the centrosome complex (arrowheads) moved towards the metaphase plate under these conditions. Nocodazole was added at t=0 and the time in seconds is given in each frame. Bar, 10 μm.

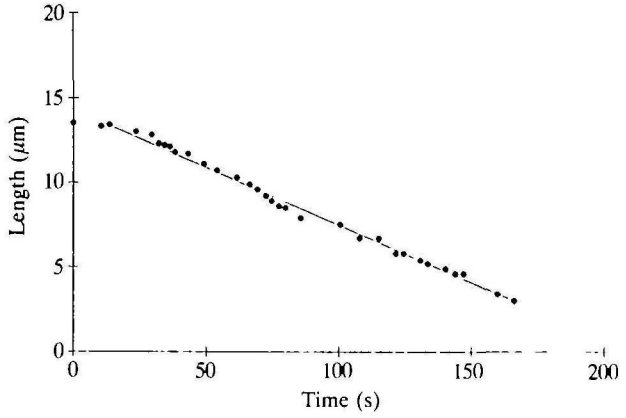


Fig. 2. Kinetics of chromosome fiber shortening for the cell shown in Fig. 1. The centrosome was initially about 13 μm from the metaphase plate and it moved toward the metaphase plate at a rate of approximately 4 μm min^{-1} when nocodazole was added. Time (in seconds) is given, with $t=0$ representing the time when nocodazole was added.

azole represented predominantly kinetochore fiber microtubules.

Tubulin subunits dissociate proximal to the kinetochore as kinetochore microtubules shorten

Fig. 4A shows the fluorescent image of a prometaphase spindle acquired 5 s after photobleaching a bar pattern across the lower half-spindle. As shown in Fig. 4B, 95 s after perfusion with nocodazole, the photobleached bar pattern remained stationary with respect to the spindle pole (the lower pole) as the spindle shortened. In contrast, the distance between the kinetochores and bleached bar shortened. This qualitative impression was confirmed by determining the grey values along the kinetochore fiber (broken lines in Fig. 4A and B). The plots shown in Fig. 4C confirmed that shortening occurred predominately between the kinetochore and the photobleached bar.

Seven spindles were treated by the same protocol and analyzed by tracing spindle outlines onto transparent acetate (method 1, see Materials and methods). Three of these spindles were also analyzed by taking intensity line scans along kinetochore fibers (method 2, see Materials and methods). In all cases, and with either method of analysis, shortening occurred predominantly between the kinetochores and the bar pattern (Tables 1 and 2). On the basis of measurements made by method 1 (see Table 1), the average decreases in length compared to the initial lengths were: 18% between the kinetochores and the center of the pole, 28% between the kinetochores and the center of the bleach bar, and 1.6% between the center of the bleach bar and the center of the pole (Table 1).

On the basis of DIC observations of spindle shortening in uninjected cells treated with nocodazole (Figs 1,2) we predicted that the spindle shown in Fig. 4 should have shortened by 5.7 μm (3.6 μm min^{-1} × 95 s). Direct measurement of spindle length showed that this spindle shortened approximately 5.4 μm (Fig. 4), which agrees well with the predicted value and suggests that the mechanism responsible for spindle shortening is not altered either by microinjection of labelled tubulin or by photobleaching.

Technical problems prevented us from following spindle shortening for more than approximately 100 s. The depolymerization of the nonkinetochore microtubules resulted in a very high background of fluorescent subunits and lower contrast of the persistent kinetochore microtubules.

Discussion

In this study we localized the site of tubulin subunit dissociation from prometaphase–metaphase kinetochore microtubules by taking advantage of the kinetochore fiber shortening that occurs when microtubule assembly is blocked with the tubulin-binding drug nocodazole. Nocodazole rapidly penetrates the plasma membrane and binds to the colchicine-binding site on the tubulin dimer (Hoebeke *et al.* 1976; Lee *et al.* 1980; Salmon *et al.* 1984). This site is not exposed when the dimer is incorporated into the microtubule lattice (Margolis and Wilson, 1977). When microtubule assembly is blocked in prometaphase–

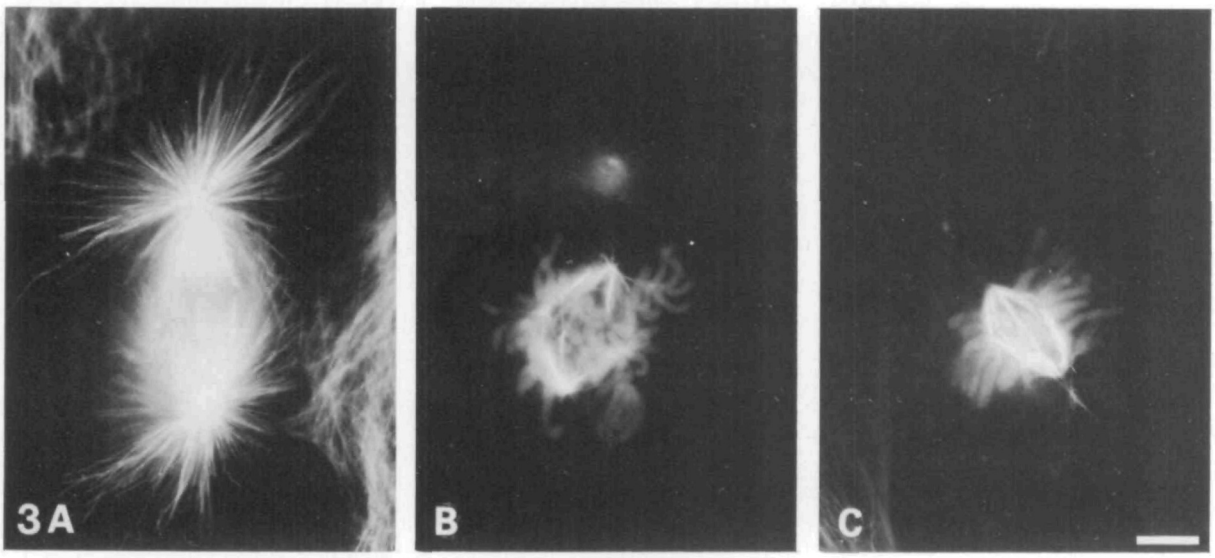
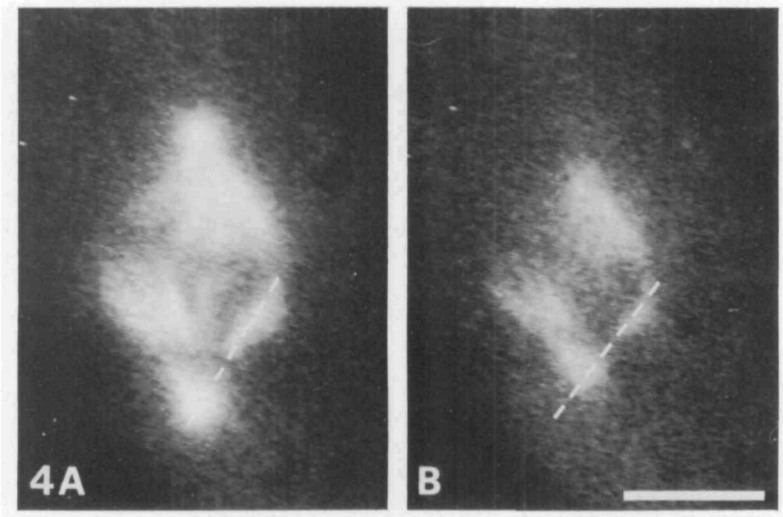


Fig. 3. Immunofluorescent micrographs of newt lung spindles demonstrating the decrease in nonkinetochore microtubule density after incubation in 17 μM nocodazole. (A) Control. (B) Cell fixed after 60 s incubation in nocodazole (17 μM diluted in culture medium). (C) Cell fixed after 90 s incubation in 17 μM nocodazole. Bar, 10 μm.

metaphase cells, the spindle poles move toward the chromosomes (Fig. 1). The mechanism responsible for movement in this direction is probably the same mechanism that generates normal chromosome to pole movements in prometaphase. Each movement requires shortening of the kinetochore fibers, which can only occur by tubulin subunit dissociation from kinetochore microtubules. Molecular attachments (between polar area and microtubules, and between kinetochores and microtubules) and site(s) permitting subunit dissociation should not change in the presence of nocodazole. Therefore, the site of tubulin subunit dissociation determined under spindle shortening conditions should reflect the site of subunit dissociation in untreated cells.

The major source of error in measuring bleach bar movement is assigning the positions of the center of the pole and the center of the bleach bar after nocodazole perfusion. This is an unfortunate outcome of the experimental protocol. Depolymerization of nonkinetochore microtubules releases fluorescent tubulin subunits into the cytoplasm, which increases background fluorescence and decreases the contrast of the persistent kinetochore microtubules. The polar area appears smaller and dimmer after nocodazole perfusion because the astral microtubules have depolymerized. The photobleached bar pattern sometimes shows an apparent widening after nocodazole perfusion (Fig. 4B and C). It is likely that in the image acquired 5 s after photobleaching (Fig. 4A) some nonkinetochore microtubules have elongated into the bleached region, resulting in an apparent narrowing of the photobleached zone. After the nonkinetochore microtubules depolymerize, the photobleached zone appears wider, but this may simply reflect the true width of the original photobleached bar pattern on the kinetochore microtubules. This elongation of nonkinetochore microtubules into the bleach zone would place the apparent center of the bleached zone in the first image (e.g. Fig. 4A) closer to the metaphase plate, and this may account for the small amount of kinetochore fiber shortening that occurred between the pole and the bleached bar. Although there is some difficulty in assigning the positions of the center of the pole and bleached bar pattern, there was



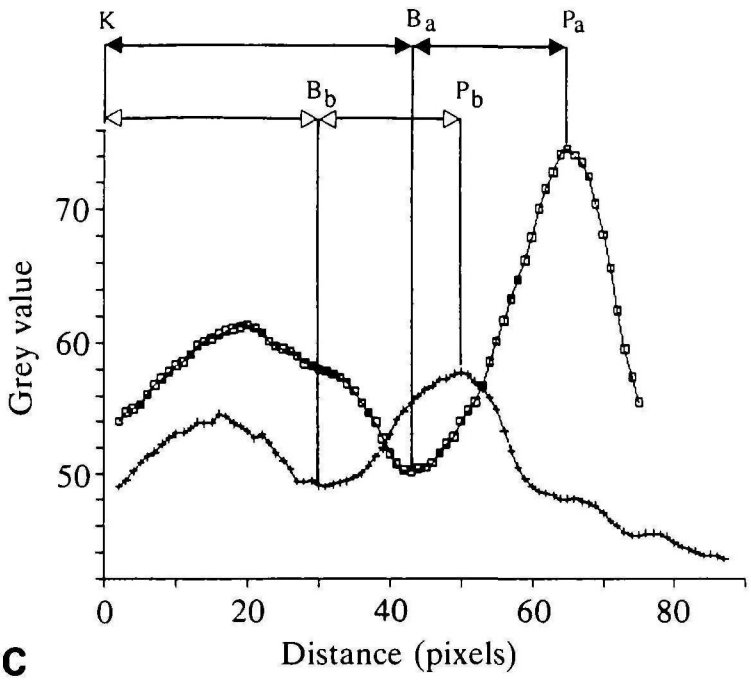


Fig. 4. The direction and magnitude of metaphase kinetochore fiber shortening when microtubule assembly is blocked with 17 μM nocodazole. The cell in A and B was microinjected with DTAF-tubulin and a narrow bar pattern was photobleached across the lower half-spindle as described in Materials and methods. The fluorescence micrograph in A was acquired 5 s after photobleaching. The fluorescence micrograph in B was acquired 95 s after perfusion with 17 μM nocodazole (and 120 s after photobleaching). By this time the spindle shortened to 66 % of its initial length. The plots in C show the grey values along the in-focus kinetochore fiber in the lower right side of the spindle in A (□–□) and B (+–+). The plots in C were generated by obtaining the average values in the direction indicated by the broken lines in A and B. The curves were smoothed by averaging the value at each pixel position with the values for two pixel positions on either side. The scan origin was set at the apparent position of the kinetochore, (K), at the end of the fluorescent fibers. Note that the distance between the equatorial ends of sister kinetochore fibers does not change after incubation in nocodazole. The plots do not extend to zero on the *x*-axis because these first two points are lost during smoothing. The position of the spindle pole, P, was set at the site of peak polar fluorescence and the central position of the bar bleach pattern, B, was set at the position of minimium fluorescence along the kinetochore fiber. The subscripts a and b in C correspond to images A and B, respectively. The reliability of picking the positions of K, B and P is discussed in the text. The Bar in B is 10 μm, which corresponds to 63 pixels on the *x*-axis in C.

Table 1. *Summary of half-spindle shortening and bleach bar movement determined by method 1*

		Initial¶			Final			% Change		
Cell no.	Time*	P–K†	P–B‡	B–K§	P–K	P–B	B–K	P–K	P–B	B–K
1	65	17.4	10.7	6.7	15.9	10.3	5.6	9	4	16
2	95	16.3	10.3	6.0	13.3	10.3	3.0	18	0	50
3	95	8.1	1.5	6.6	6.7	1 5	5.2	17	0	21
4	95	8.1	1.5	6.6	7.0	1.5	5.5	14	0	16
5	95	12.2	2.6	9.6	10.0	2.6	7.4	18	0	23
6	98	10.0	3.0	7.0	6.7	2.8	3.9	33	7	44
7	98	5.6	2.2	3.4	4.8	2.2	2.6	14	0	24

*The time (in seconds) of incubation in nocodazole when the final image was acquired. Photobleaching occurred 20–25 s before nocodazole perfusion was completed.

†P–K denotes the distance (in μm) between the center of the pole and the kinetochores.

‡P–B denotes the distance (in μm) between the center of the pole and the center of the photobleached bar pattern.

§B–K denotes the distance (in μm) between the center of the photobleached bar and the kinetochores.

¶Initial lengths were determined from images acquired 5 s after photobleaching. Final lengths were determined from images acquired after incubation in nocodazole for the times given in the table Lengths were measured by tracing spindle outlines onto transparent acetate (see Materials and methods)

Table 2. *Comparison of half-spindle shortening and bleached bar movement determined by methods 1 and 2*

	% Decrease					
	Method 1			Method 2		
Cell no.	P–K	B–K	P–B	P–K	B–K	P–B
2	18	0	50	13	2	37
3	17	0	21	17	5	18
6	33	7	44	23	6	31

*The percentage decrease (compared to their initial lengths) in half-spindle length (P–K), kinetochore to bleached bar (B–K) and bleached bar to pole (P–B) were determined for three cells using method 1 (tracing spindle outlines onto transparent acetate) and method 2 (measurement of fluorescent intensity along scan lines, see Fig. 4). Cell no. corresponds to the cells listed in Table 1.

consistent evidence from two methods (Table 1 and 2) that the predominent area of spindle shortening occurred between the kinetochore and the photobleached bar.

These experiments demonstrate that as prometaphase–metaphase kinetochore fibers shorten, tubulin subunits dissociate predominately from a site proximal to the kinetochore (Fig. 4, Tables 1 and 2; see also Centonze and Borisy, 1988). In a few experiments there was also evidence that some subunit dissociation may have occurred proximal to the pole. A recent study with a photoactivatable fluorescein-labeled tubulin also suggests that tubulin subunits can dissociate proximal to the pole (Mitchison, 1989). It is possible that the relatively slow poleward flux of tubulin subunits observed by Mitchison (1989) may also account for the small amount of shortening that we observed between the photobleached bar and the pole.

Since it appeared that tubulin subunits dissociated predominately from a site proximal to the kinetochore, our results suggest that poleward force can be generated either by the kinetochore or by components closely associated with the kinetochore during poleward movement of prometaphase–metaphase kinetochores (see also Rieder and Alexander, 1990). Although it is possible that poleward movement in non-drug-treated cells occurs by a separate mechanism, our results support the kinetochore motor/polar ejection model and are not consistent with simple interpretations of the traction fiber model. Since it appears that the motor for anaphase poleward movement is also closely associated with the kinetochore (Mitchison *et al.* 1986; Gorbsky *et al.* 1988; Koshland *et al.* 1988; Nicklas, 1989), it is likely that poleward movement during both prometaphase and anaphase can be driven by the same molecular mechanism.

Although an understanding of the molecular mechanism responsible for force generation at the kinetochore is still lacking, poleward chromosome movement is coupled to the polymerization dynamics of the kinetochore microtubules (Salmon, 1975; Salmon and Begg, 1980; Koshland *et al.* 1988). It is possible that a mechanochemical translocator, such as cytoplasmic dynein, which moves organelles towards microtubule (−) ends (Paschal *et al.* 1987), could be tethered to the kinetochore and generate poleward movement (Salmon, 1989*b*; Rieder and Alexander, 1990). In this model the velocity of poleward chromosome movement would be regulated by the dynamics of depolymerization of the kinetochore microtubules (Salmon, 1975, 1989*b*; Nicklas, 1987).

A very different model was proposed by Hill (1985), in which force production is not directly coupled to nucleotide triphosphate hydrolysis. In this model the kinetochore contains a discrete number of microtubule attachment sites. Each attachment site has the structure of a sleeve and contains a number of sites that interact with the microtubule. The most stable kinetochore position occurs when all these sites are in contact with the microtubule. Poleward movement of the kinetochore occurs by diffusion, but this diffusion is biased because the kinetochore will most probably align at the most stable position. Conditions that promote microtubule disassembly (by tubulin subunit dissociation from the (+) end of the microtubule) would then result in kinetochore translocation towards the microtubule (−) end by this biased diffusional movement.

Koshland *et al.* (1988) recently proposed a modified version of Hill's model in which poleward force production is generated by the energy stored in the microtubule lattice (by the GTP hydrolysis that accompanied the initial microtubule assembly; see Mitchison, 1988; Salmon, 1989*b*). Koshland *et al.* (1988) and Spurck and Pickett-Heaps (1987) have provided suggestive evidence supporting an ATP-independent mechanism of poleward force generation, but this important issue requires further investigation.

It is interesting to consider the rate of kinetochore microtubule shortening that occurred after cells were perfused with nocodazole-containing medium. In three cells we measured an average rate of 3.6 $\mu m\ min^{-1}$, which is approximately three times faster than the rate of

metaphase and anaphase chromosome movement in untreated newt lung cells (Bajer, 1982). The faster rate of chromosome movement in nocodazole-treated cells occurs concurrently with the reduction in the density of the nonkinetochore microtubules. This could represent the effect of reducing a polar ejection force, generated by the nonkinetochore microtubules, which normally opposes chromosome to pole movement (Rieder *et al.* 1986; Salmon, 1989*a*). Alternatively, the faster rate of pole movement could be related to the mechanism of poleward movement generated by the kinetochore. Independent of the mechanism responsible for the faster rate, the data support the hypothesis that the rate-limiting step in chromosome movement is the rate of tubulin subunit dissociation from kinetochore microtubules (Salmon, 1975; Salmon and Begg, 1980; Inoue, 1981; Nicklas, 1988).

Previously, we reported that free microtubule (+) ends depolymerize at a mean rate of $17.3\,\mu m\,min^{-1}$ in interphase newt lung epithelial cells (Cassimeris *et al.* 1988*b*). The rate of microtubule depolymerization has also been estimated at $\sim 20\,\mu m\,min^{-1}$ for nonkinetochre microtubules in the mitotic spindles of sea urchin embryos (Salmon *et al.* 1984). If nonkinetochore microtubules in newt lung cell spindles also depolymerize at ~ 17–$20\,\mu m\,min^{-1}$, then it is likely that capping a microtubule by the kinetochore slows the depolymerization rate by approximately fivefold ($17.3\,\mu m\,min^{-1}$ *versus* $3.6\,\mu m\,min^{-1}$). It is also possible that other factors, including the addition of nocodazole, may alter the rate of microtubule depolymerization at the kinetochore.

In summary, when microtubule assembly is blocked, prometaphase–metaphase kinetochore microtubules are more stable than nonkinetochore microtubules (Salmon and Begg, 1980; Inoue, 1981; Mitchison, 1988), and kinetochore microtubules shorten at a rate approximately five times slower than nonkinetochore microtubules with free (+) ends, presumably because the (+) ends of kinetochore microtubules are capped by the kinetochores. In addition, tubulin subunit dissociation occurs at a site proximal to the kinetochore during kinetochore to pole shortening. These results are consistent with models of poleward force generation in which poleward force for chromosome movement from prometaphase to anaphase is generated in association with the kinetochore (Gorbsky *et al.* 1988; Koshland *et al.* 1988; Mitchison, 1988; Salmon, 1989*a*; Nicklas, 1989)

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