

The Motor for Poleward Chromosome Movement in Anaphase Is in or near the Kinetochore

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Abstract. I have tested two contending views of chromosome-to-pole movement in anaphase. Chromosomes might be pulled poleward by a traction fiber consisting of the kinetochore microtubules and associated motors, or they might propel themselves by a motor in the kinetochore. I cut through the spindle of demembrated grasshopper spermatocytes between the chromosomes and one pole and swept the polar region away, removing a portion of the would-be traction fiber. Chromosome movement continued, and in the best examples, chromosomes moved to within 1 μm of the cut edge. There is nothing beyond the edge to support movement, and a push from the rear is unlikely because cuts in the interzone behind the separating chromosomes did not stop movement. Therefore, I

conclude that the motor must be in the kinetochore or within 1 μm of it. Less conclusive evidence points to the kinetochore itself as the motor. The alternative is an external motor pulling on the kinetochore microtubules or directly on the kinetochore. A pulling motor would move kinetochore microtubules along with the chromosome, so that in a cut half-spindle, the microtubules should protrude from the cut edge as chromosomes move toward it. No protrusion was seen; however, the possibility that microtubules depolymerize as they are extruded, though unlikely, is not ruled out. What is certain is that the motor for poleward chromosome movement in anaphase must be in the kinetochore or very close to it.

THE beautiful, precise movements of chromosomes in mitosis have been known for over a century. Four years after the discovery, Van Beneden (1883) proposed that chromosomes in anaphase are pulled to the poles by traction fibers. In current terms, traction forces pull poleward on the microtubules attached at the kinetochore of each chromosome. This ancient theory naturally suffered some reverses in the course of a century, but by 1960 the traction fiber idea was so well-established that it seemed pretentious to call it a theory. The traction fiber seemed an elementary fact, and the center of speculation shifted to the molecular mechanism of traction (for review, see Inoué, 1981; Nicklas, 1988).

But now the traction fiber idea is once again a theory, a theory that may be wrong. The new alternative is a kinetochore that participates actively in its own movement, rather than being passively dragged toward a pole by traction fibers. *In vitro*, microtubules appear to be pulled toward kinetochores as microtubules disassemble (Koshland et al., 1988), the opposite of a microtubule pulling on a chromosome. However, the relevance of the *in vitro* microtubule movement to chromosome movement in living cells is not certain. Because the movement *in vitro* has not yet been studied in real time, a detailed comparison with movement in living cells is precluded. Also, the structure of the kinetochore-microtubule junction *in vitro* is not yet known, so it is uncertain whether the movement involves the precise structural arrangement seen in cells. Strong evidence from living cells against trac-

tion fibers and in favor of an active kinetochore comes from experiments of Gorbsky et al. (1987, 1988). They reasoned that a traction fiber would move poleward in anaphase along with the chromosome it pulls, so a marker on the kinetochore microtubules should move poleward along with the chromosomes. They made spindle microtubules fluorescent by injecting cells with fluorescent subunits. Then the spindle was irradiated with a band of light to bleach the fluorescence, so that many microtubules were marked by a nonfluorescent band. They found that as chromosomes moved toward the poles, the bleached band did not move much relative to the chromosomes. Particularly striking are instances in which chromosomes caught up with a band and moved through it (Gorbsky et al., 1988). Gorbsky and co-workers concluded that kinetochore microtubules are not pulled poleward and do not pull chromosomes poleward as in a traction fiber model. Instead, kinetochore microtubules are stationary rails on which chromosomes glide, propelled poleward by a motor in the kinetochore (Gorbsky et al., 1987, 1988). While the evidence is impressive, it is not absolutely conclusive. The authors admit that the movement of "one or a very few kinetochore microtubules" cannot be ruled out (Gorbsky et al., 1988). Major concerns are first that some microtubules remain unbleached (cf. Fig. 6 in Gorbsky et al., 1987) and hence are not marked; any movement they undergo is undetectable. Second, the fluorescence increases in the bleached band as chromosomes move toward it (Gorbsky et al., 1988); this

might be due to microtubules that move poleward, so that their unbleached fluorescent parts intrude into the bleached region. Additional concerns have also been raised (Forer, 1988; Vigers et al., 1988; Wolniak, 1988). For me, however, these elegant *in vitro* and *in vivo* experiments are more noteworthy for the novel view they offer of mitosis and the kinetochore than for any shortcomings (Nicklas, 1988). Certainly the recent experiments raise serious doubts about traction fibers, even if they are not a compelling reason to discard a theory that has worked so well for so long (Forer, 1988; Wolniak, 1988).

A novel approach to testing a role of kinetochore microtubules as traction fibers is to cut them off; do chromosomes continue to move when a large part of the putative traction fiber is simply not there? Surprisingly, such direct experiments are possible. Certain insect spermatocytes with large spindles can be demembrated mechanically, allowing free access to the spindle (Nicklas, 1977). The spindle can be cut as desired merely by pressing it against the coverslip with a needle (Nicklas et al., 1989). Remarkably, anaphase chromosome movement continues in cut spindles, even when a large part of the spindle is not present. The characteristics of that movement and the implications for the mechanism of chromosome movement in anaphase are the subjects of this report. The pioneers in cutting the spindle are Hiramoto and Nakano (1988) and Hiramoto and Shoji (1982). They reported continued chromosome movement, but the extent of movement was not clear in their reports, nor was it established that the spindle was severed cleanly and completely.

Materials and Methods

Only certain cells can be demembrated mechanically and yield functional spindles (Nicklas et al., 1989). Chromosome movement has been studied mainly in the exceptionally large spindles of spermatocytes from several grasshoppers of the subfamily Oedipodinae: *Arphia sulphurea* (Fabricius), *A. xanthoptera* (Burmeister), *Chortophaga viridifasciata*, (DeGeer), and *Dissosteira carolina* (L.). Those species were collected as seasonably available from local natural populations. Chromosome-to-pole movement in anaphase is very similar in rate and extent in spermatocytes of all these species, so the results have been pooled. However, for the spindles illustrated, the species are as follows: Figs. 1 and 8, *Dissosteira*; Figs. 2, 3, and 4, *Chortophaga*; Figs. 5 and 6, *Arphia xanthoptera*. Cricket (*Acheta domestica* L.) spermatocytes were used for immunochemical studies (Fig. 7). *Acheta* is available year-round from a commercial supplier (H. O. Brewer, Durham, NC).

Spermatocyte culture, demembration, micromanipulation, and time-lapse movie recording and analysis were carried out as previously described (Nicklas et al., 1989 and references therein). Culture temperature was usually 25–26°C (range: 23–26°C). Spermatocytes are covered with an inert oil and viewed on an inverted microscope; the micromanipulation needle is placed in the oil and lowered down onto the cells.

Spindles were observed by phase contrast, polarized light microscopy, and anti-tubulin immunochemistry. Standard immunochemical procedures were used with mouse monoclonal anti- α -tubulin and anti- β -tubulin (Amersham Corp., Arlington Heights, IL) as described in Nicklas et al. (1989). Zeiss phase contrast optics were used: an inverted "Plankton" microscope was equipped with a 100 \times /1.3 NA (numerical aperture) Neofluar objective and an 0.9 NA long working distance condenser. For polarized light observations, a Nikon "M-stand" inverted microscope was used, equipped with a Nikon rectified 40 \times /0.65 NA achromatic objective and a rectified 0.52 NA long working distance condenser. The polarizers, light source, and heat and green filters were as described earlier (Nicklas, 1979). Unenhanced images were recorded on Kodak Tech Pan 35-mm film exposed at ASA 40 and processed in Kodak HC-110 developer, dilution B, for 12 min at 75°F.

Some spindles were observed by video-enhanced polarized light microscopy, using the procedures for microscope and video adjustment, monitor photography, etc., so well described by Inoué (1986). A series 70 video camera (Dage-MTI, Wabash, MI) with a 1-inch Newvicon tube was used

with an Image 1-AT image processor (Universal Imaging Corp., Media, PA). Video images were processed as follows: 8 or 16 frames were summed to reduce noise, an out-of-focus background image was subtracted to cancel inhomogeneities in illumination, and contrast was optimized. Processed images were recorded on an optical memory disk recorder (model TQ-2021FCB; Panasonic Video Systems, Secaucus, NJ).

The only spindles of interest are those in which both the pole of the uncut half-spindle and the cut edge in the other half were clearly visible. In such spindles, measurements of distance and position were reproducible to within 0.25–0.5 μ m. Cutting spindles removes some of the usual bench marks for measurements. When one pole was cut away, everything was measured and plotted relative to the only available marker, the pole of the uncut half-spindle (defined by centrioles or the convergence of spindle birefringence). Images of focal levels in which the pole was not visible (e.g., Fig. 1, upper row) were aligned to an image showing the pole at nearly the same time (within a minute). The two images (one at the level of the pole and one at another focal level) were aligned using either the cut edge or other markers, if the spindle had not moved.

Measuring and plotting everything relative to the centrioles of the uncut half-spindle has a peculiar effect when spindle elongation is present. Only the chromosomes in the cut half-spindle appear to be moved by spindle elongation (e.g., Fig. 2). Actually, of course, elongation moves the chromosomes in the two spindle halves an equal distance away from the center of the spindle. This plotting artifact is easily recognized and corrected and has no effect on the measurements reported below. As is conventional, distances in the graphs are marked off relative to the equator (e.g., Fig. 2), with the pole at a constant distance from the equator. The pole-to-equator distance was measured soon after the spindle was cut; the equator was defined as a line midway between the separating kinetochores.

Results

Cut Half-Spindles

A well-spread spermatocyte was selected, allowed to enter anaphase, and then demembrated. Anaphase chromosome movement invariably continues after demembration in such a preselected cell, though the rate and the extent are variable (Nicklas, 1977; Nicklas et al., 1989). The spindle was cut simply by laying a glass micromanipulation needle across the spindle where the cut was desired and then lowering the needle to press the spindle tightly against the coverslip. In most experiments, the spindle was cut between the chromosomes and one pole and then the polar piece was swept away. In all illustrations and graphs, the cut half-spindle is uppermost, and to provide a bench mark for movement, the pole of the uncut half-spindle is displayed or plotted at a constant position.

Chromosome movement is most easily analyzed when no spindle elongation occurs, as in Fig. 1. The top 5 μ m of this spindle was cut away at -1 min and swept out of the field of view. In the lower row of prints, the pole of the uncut half-spindle is in focus. The white lines indicate the position of the cut edge above and the pole of the uncut half-spindle below. Between 1 and 20 min, the distance from the lower pole to the cut edge did not change; there was no spindle elongation. The chromosome indicated by the arrow in the top row of prints (0–10 min) quickly moved almost all the way to the cut edge, covering 4 μ m at a velocity of 0.45 μ m/min and stopping within 1 μ m of the edge. This velocity is within the range for uncut spindles in normal cells (Table I).

Spindle elongation is often absent or minimal in spindles from demembrated cells (Nicklas, 1977), but it can be appreciable, especially in *Chortophaga* spindles. In normal cells with intact spindles, elongation produces further separation of the chromosomes, but it does not move them any closer to the poles. Therefore, elongation is distinct from

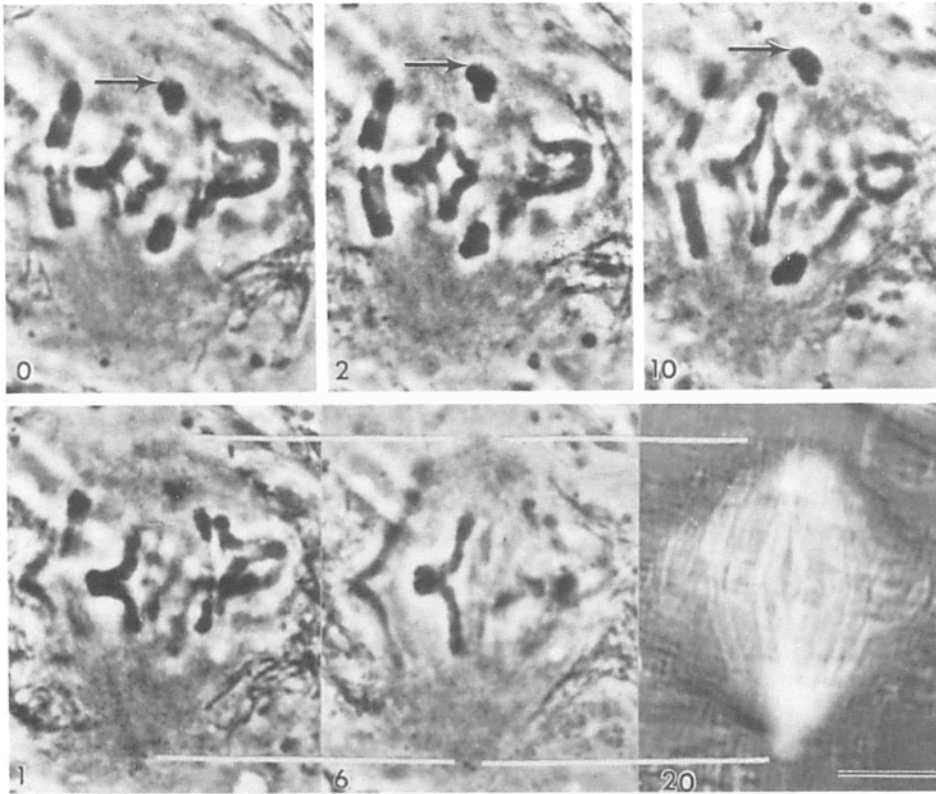


Figure 1. Chromosome movement in a spindle with the upper polar region cut away. The spindle was cut at -1 min. The cut half of the spindle is uppermost in each print and the time in minutes is given on each print. *Top row* (0–10 min), the chromosome indicated by the arrow moves to within $1 \mu\text{m}$ of the cut edge. *Bottom row* (1–20 min; at another focal level), horizontal white lines near the top connect the cut edge in adjacent prints and similar lines near the bottom connect the pole, defined by centriole(s) (1 and 6 min) or by the convergence of spindle birefringence (20 min). There was no change in the distance between the cut edge and the pole. The 20-min print shows the spindle by polarization microscopy; the others are phase contrast views. Bar, $10 \mu\text{m}$.

chromosome-to-pole movement. This might still be true after cutting off a pole, but it is necessary to be positive. Chromosome movement in a cut spindle in which substantial elongation occurred is shown in Fig. 2. The spindle was cut like the one in Fig. 1, but somewhat closer to the chromosomes. After 3 min (Fig. 2), most of the movement was due to spindle elongation. The distance between the pole of the uncut half-spindle and the cut edge increased by $4.5 \mu\text{m}$ between 3.6 and 12.8 min, but the chromosomes in the cut half-spindle moved no closer to the cut edge (Fig. 2, *upper section*). Hence, just as in uncut spindles, movement due to

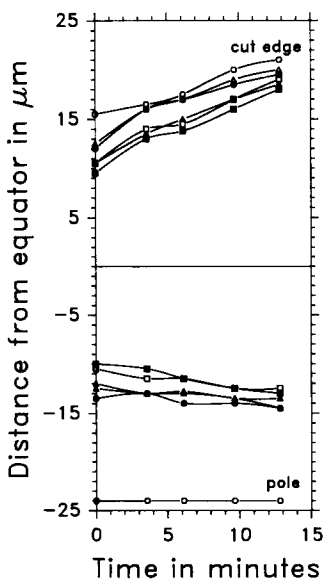


Figure 2. Graph of chromosome movement in a spindle in which great spindle elongation occurred after the upper polar region was cut away. After 3 min, the chromosomes in the cut half-spindle (*top*) moved no closer to the cut edge (\circ), though spindle elongation continued to move the chromosomes and cut edge as a unit away from the pole in the uncut half-spindle (*bottom*). Everything is plotted relative to the pole, which makes spindle elongation seem to affect only the chromosomes in the cut half-spindle (see Materials and Methods).

elongation can be distinguished from movement to the pole or to the cut edge. Chromosome velocity and the extent of movement were measured only in spindles in which the cut edge and the pole in the uncut half were visible, so that any chromosome displacement due to spindle elongation could be detected. The values for the extent and velocity of movement in this report are for chromosome-to-edge or chromosome-to-pole movement only; the contribution, if any, of spindle elongation has been subtracted.

For later discussion, it may be noted that two chromosomes in Fig. 2 were not displaced as much as the others by spindle elongation (the two curves just below the cut edge curve in Fig. 2).

Several chromosomes moved close to the cut edge in the spindle shown in Figs. 3 and 4. The chromosome at the arrow in Fig. 3 moved to $1 \mu\text{m}$ of the cut edge by 17 min (it appears farther from the cut edge as plotted in Fig. 4 because the position of the cut edge was measured along the central spindle axis, not along the path the chromosome at the arrow was moving). It was still closer to the edge at 26 min. However, this might be the result of edge-to-chromosome movement, since the spindle length decreased slightly after 17 min (Fig. 4). The chromosomes in both the cut and the uncut halves of the spindle moved substantial distances and at respectable velocities. The chromosome indicated by the arrow in Fig. 3, for instance, moved $4.3 \mu\text{m}$ at a velocity of $0.4 \mu\text{m}/\text{min}$ (velocity for the first 9 min); its partner in the uncut half-spindle moved $6 \mu\text{m}$ at $0.45 \mu\text{m}/\text{min}$.

Chromosomes in cut spindles, as in normal spindles, move as individuals; they may start together, but their speeds often vary. Such individual variation is important in judging the importance of processes that affect the position of all chromosomes equally. For instance, spindle elongation moves

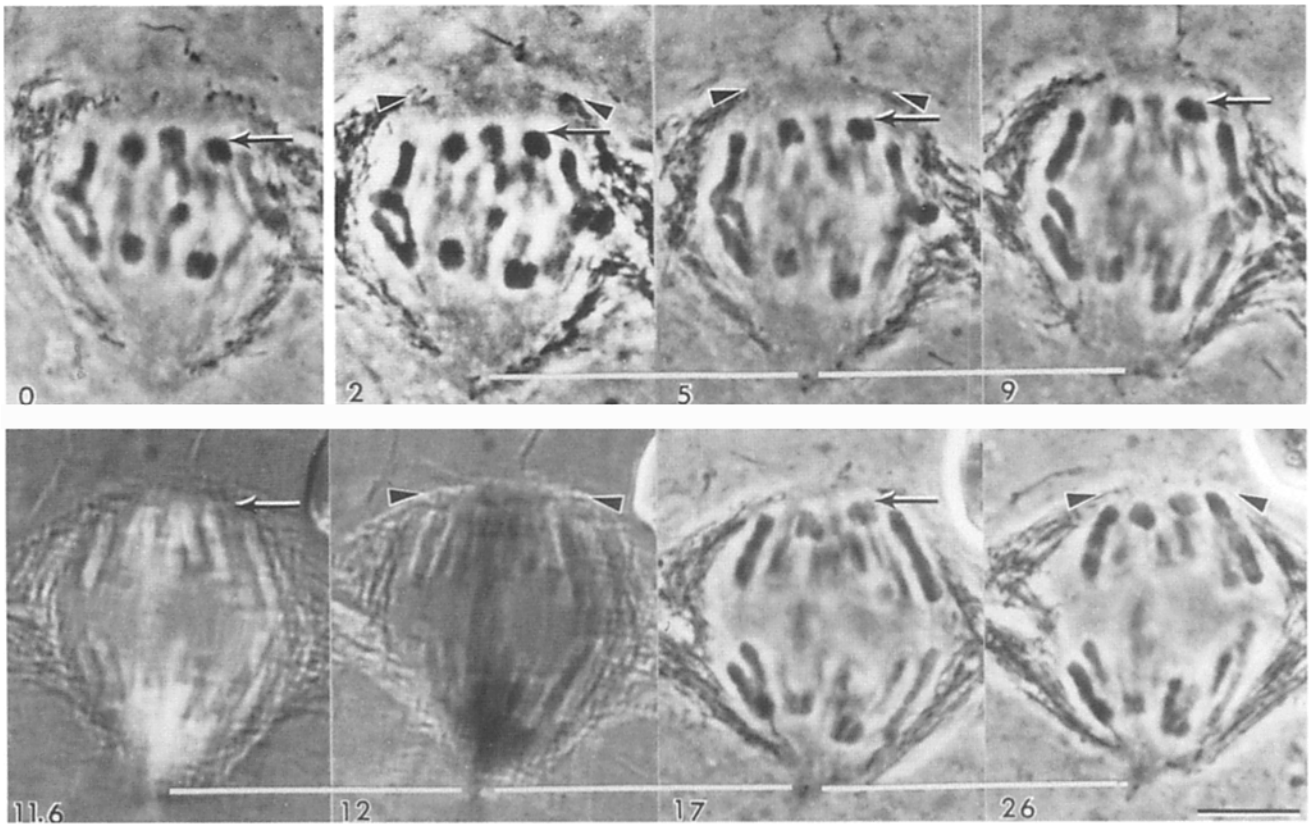


Figure 3. Independent chromosome movement in a cut spindle. The time in minutes is given on each print. The prints were aligned to bring a centriole to the same position vertically, as shown by the white lines connecting centrioles in adjacent prints. The first print (0 min) shows the spindle 1.5 min after the upper part was cut off and swept away. The cut edge is indicated by arrowheads on several prints. The chromosome indicated by the arrow moved to $1\ \mu\text{m}$ of the edge by 17 min. The chromosome to its right moved more slowly at first (0–9 min), but eventually caught up (17–26 min). The 11.6- and 12-min prints show the spindle by polarization microscopy at opposite contrast settings; the other prints are phase contrast views. Bar, $10\ \mu\text{m}$.

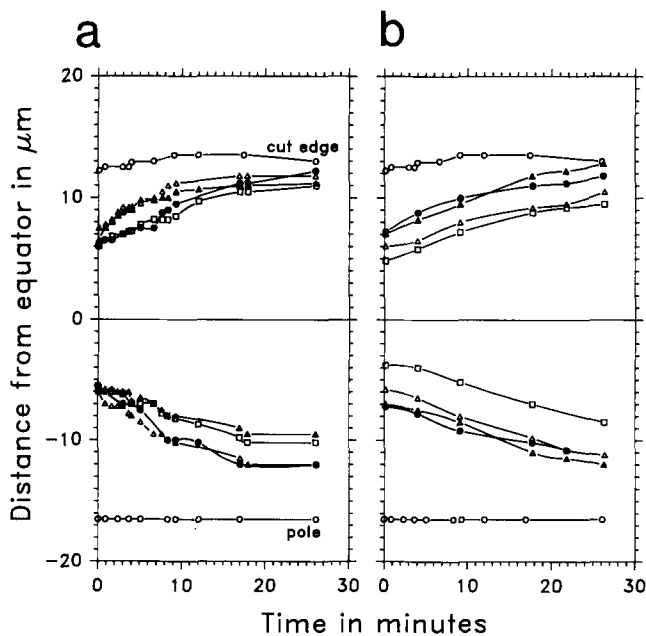


Figure 4. Graphs of chromosome movement for the spindle shown in Fig. 3. Partner chromosomes, one in the cut and one in the uncut half-spindle, are plotted with the same symbol. (a) Movement of four chromosomes visible in the 5-min print in Fig. 3. Symbols for chromosomes as seen in Fig. 3, from left to right: open squares,

both the cut edge and all the chromosomes an equal distance away from the equator. Similarly, microtubule depolymerization at the cut edge would appear to move the cut edge as a unit toward all the chromosomes. There are examples of independent movement of individual chromosomes in every spindle studied. In Fig. 3, compare the chromosome tagged by the arrow with the one to its right. The one to the right moved more slowly at first, until the chromatids separated; by 5 min, it was $1.5\ \mu\text{m}$ behind the one at the arrow. Thereafter, however, the chromosome on the right moved more quickly and eventually caught up with the other chromosome (26 min print). This example and others are evident in Fig. 4 because one line crosses another.

The spindles considered so far were cut sufficiently far from the chromosomes that extensive chromosome movement was possible, for an unequivocal demonstration of its reality. If the spindle is cut closer to the chromosomes, obviously they cannot move very far before nearing the edge, but they arrive there sooner. Since the spindle is gradually deteriorating, an early arrival near the edge gives the motor a

solid triangles, open triangles (the chromosome indicated by the arrow and its partner in the uncut half-spindle), and solid circles. (b) Movement of chromosomes from another focal level plotted with pole and cut edge taken from a. The data points are fewer because this level was less frequently in focus.

Table I. Chromosome Velocity and Extent of Movement

Chromosome	Velocity		Distance moved	
	Average	Range	Average	Range
	$\mu\text{m}/\text{min}$	$\mu\text{m}/\text{min}$	μm	μm
Cut half-spindle	0.27	0.1–0.5	4.1	1.0–6.2
Uncut half	0.33	0.2–0.6	4.6	3.0–6.5
Intact cells	0.69*	0.3–1.0*		

* Calculated from regression coefficients in Table II of Nicklas, 1979; for the range, 95% confidence limits are given.

better chance to show us just how close to the edge it can move chromosomes. Such an experiment is shown in Figs. 5 and 6. Spindle elongation was absent in this spindle and therefore the movement seen is purely chromosome-to-edge or chromosome-to-pole movement. The two chromosomes indicated by the arrows moved smoothly toward the edge, covering distances of 2.5 and 4.0 μm at the respectable velocities of 0.3 and 0.45 $\mu\text{m}/\text{min}$ (values for the chromosome on the left are given first). The chromosomes ended only 0.8 and 0.5 μm from the cut edge, when only a short tuft of birefringence was visible at their kinetochores (10.9- and 11-min prints, Fig. 5). These chromosomes moved as individuals, with different rate and extent, so that the chromosome on the right caught up with the one on the left.

The independent behavior of individual chromosomes is evident from the graph, Fig. 6. Especially in the cut half-

spindle, both the rate and the extent of movement vary. Most chromosomes ended up close to the cut edge, but one did not move edgeward at all. That chromosome (Fig. 6, \square) was tied to its partner by a chromatin bridge and apparently was pulled back toward the equator by its partner in the uncut half-spindle. Another point of interest is whether the movement of partner chromosomes in the two half-spindles is coordinated, so that if one stops moving, its partner also stops (cf. Forer, 1966). In these spindles, movement was not coordinated: chromosomes in the uncut half-spindle kept on moving after their partners in the cut half-spindle stopped (Fig. 6).

An important question is whether kinetochore microtubules shorten in the cut half-spindle as chromosomes move toward the cut edge. If not, the kinetochore microtubule bundles will protrude beyond the edge. No protrusion is evident in Fig. 5 as chromosomes move toward the cut edge (0–11 min), and the kinetochore bundles appear to grow shorter. Protruding microtubules could escape detection by polarization microscopy if their angle changed markedly at the edge (contrast is zero for protein fibers that are parallel to the polarizer/analyzer axis). However, to be invisible the microtubules would have to swing quite uniformly to between ~ 30 and 60° to the spindle axis. Definitive evidence on this point is provided by immunochemical staining of microtubules after chromosome movement. The upper row in Fig. 7 shows one focal level, the second row another level; in each row, the prints are aligned so that the cut edge (marked by lines)

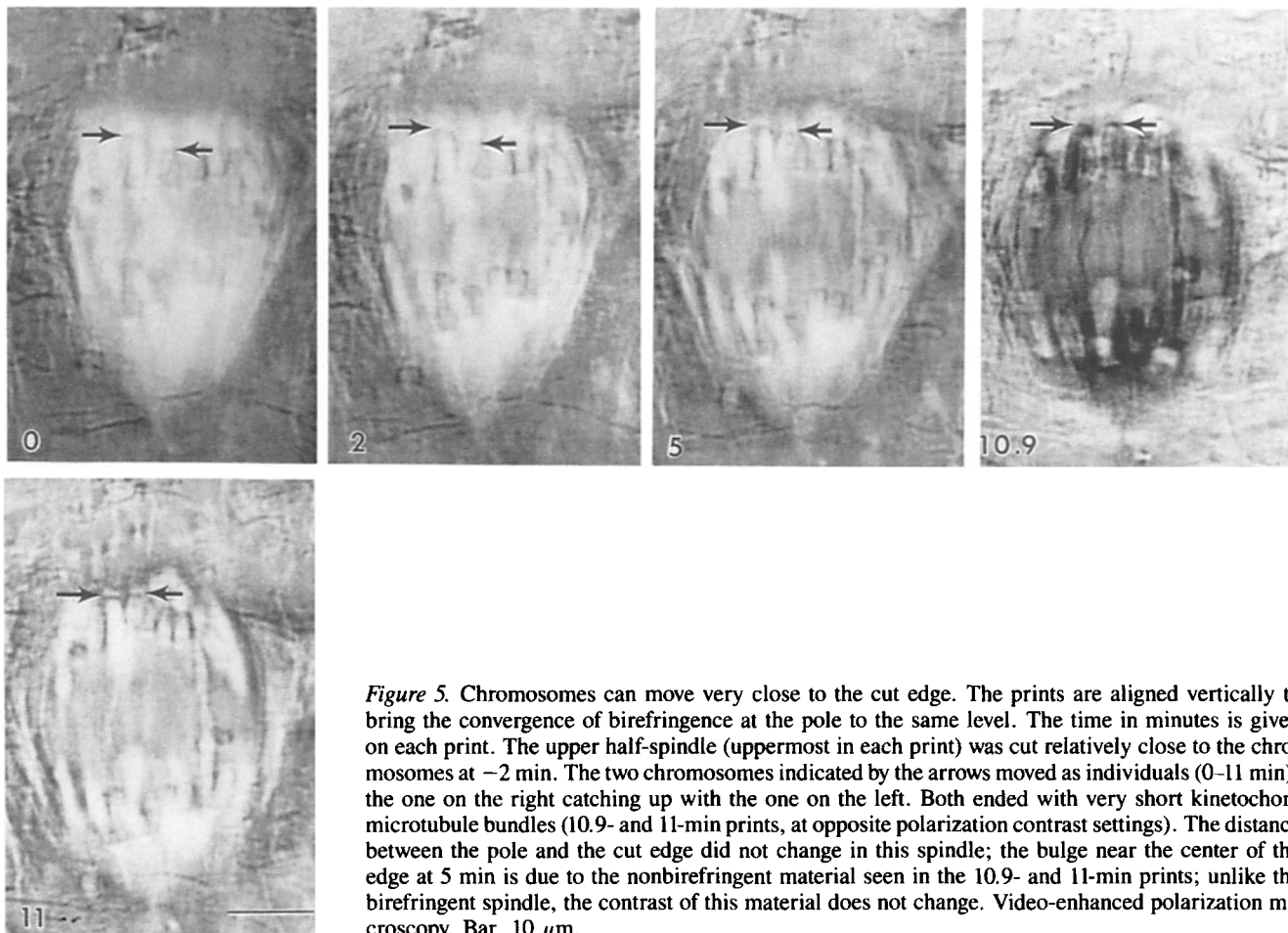


Figure 5. Chromosomes can move very close to the cut edge. The prints are aligned vertically to bring the convergence of birefringence at the pole to the same level. The time in minutes is given on each print. The upper half-spindle (uppermost in each print) was cut relatively close to the chromosomes at -2 min. The two chromosomes indicated by the arrows moved as individuals (0–11 min), the one on the right catching up with the one on the left. Both ended with very short kinetochore microtubule bundles (10.9- and 11-min prints, at opposite polarization contrast settings). The distance between the pole and the cut edge did not change in this spindle; the bulge near the center of the edge at 5 min is due to the nonbirefringent material seen in the 10.9- and 11-min prints; unlike the birefringent spindle, the contrast of this material does not change. Video-enhanced polarization microscopy. Bar, 10 μm .

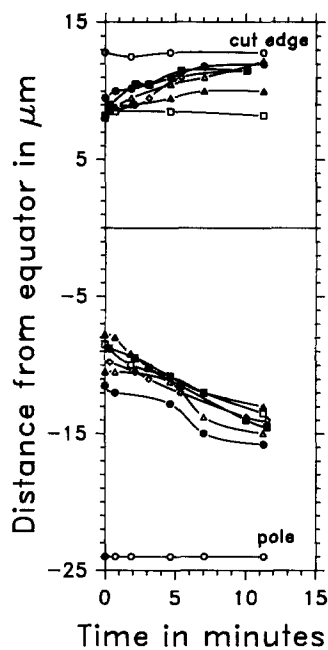


Figure 6. Graph of chromosome movement for the spindle shown in Fig. 5. Partner chromosomes, one in the cut and one in the uncut half-spindle, are plotted with the same symbol. Symbols for the chromosomes visible in Fig. 5, from left to right, starting with the left chromosome indicated by the arrow: solid circles, open triangles, solid triangles, open squares.

is at the same position vertically. The chromosome marked by the arrow in Fig. 7, *a-c* moved 2 μm closer to the edge during the 10.5 min between cutting and fixation. Immunofluorescence microscopy (Fig. 7 *d*) shows that the cut edge has the same general contour after chromosome movement as it had originally. One line in Fig. 7 *d* marks the original position of the cut edge and a second line indicates 2 μm beyond the edge, corresponding to the distance the arrowed chromosome moved. Clearly, the kinetochore microtubules do not protrude beyond the cut edge. The three chromosomes indicated by the arrows in Fig. 7, *e-g* each moved 1.5 μm ; no corresponding protrusion from the cut edge is visible in the immunofluorescence view of the spindle (Fig. 7 *h*). Two additional cells in which chromosomes moved 1-2 μm before fixation for immunostaining also showed no protrusion of microtubules from the cut edge.

Chromosome velocity and the extent of movement from a sample of 25 chromosomes from four spindles are given in Table I. These spindles were cut approximately half way between the chromosomes and one pole so as to allow the chromosomes to move a substantial distance before nearing the cut edge. The velocity was measured in the first 10-12 min after the spindle was cut and was corrected for the small

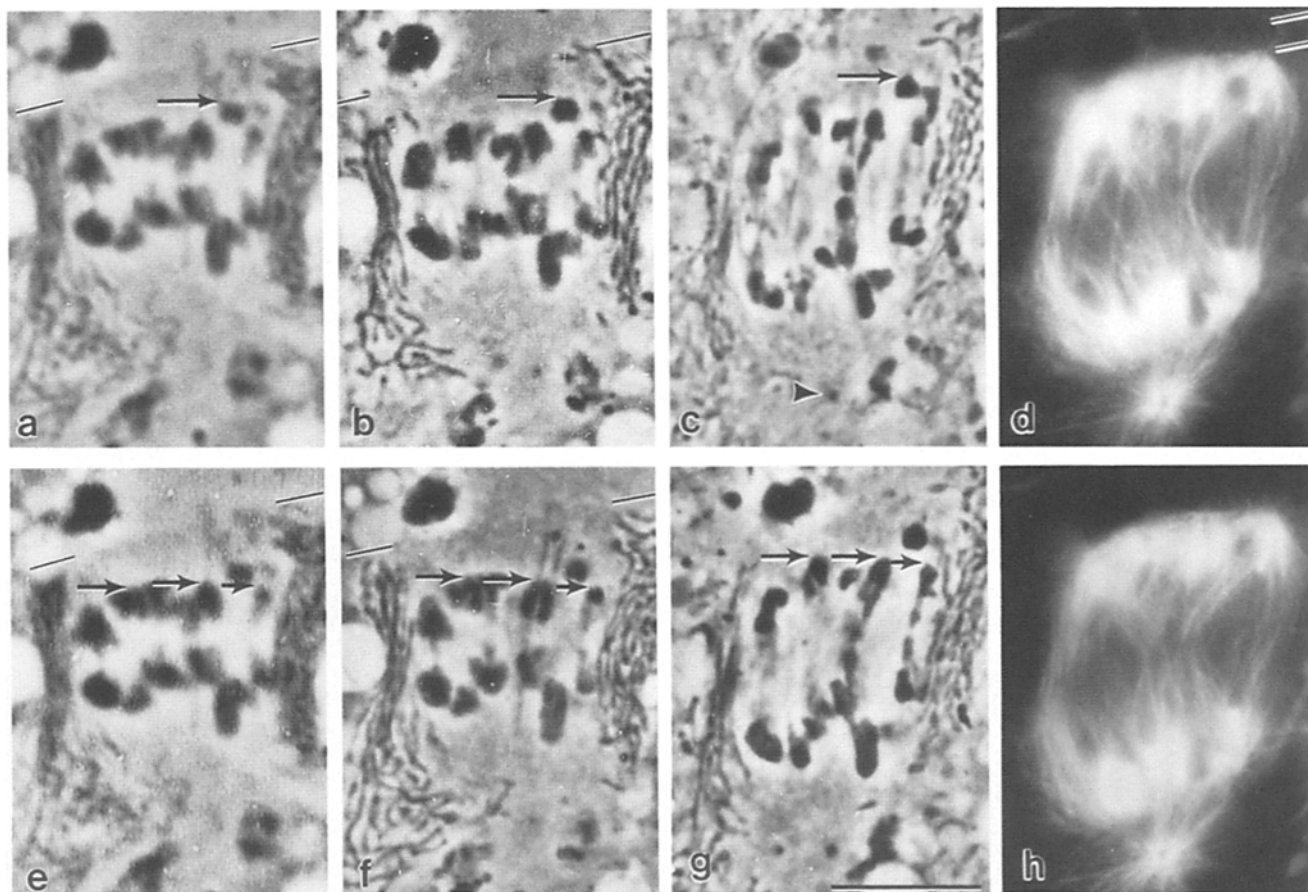


Figure 7. A cut anaphase cricket spindle fixed during chromosome movement, 10.3 min after cutting. Only the main part of the cut spindle is shown; phase contrast (*a-c*, *e-g*) and anti-tubulin immunofluorescence (*d* and *h*) images. The position of the cut edge is marked by lines at the sides of the spindle (*a* and *b*, and *e* and *f*); the edge is at the same vertical position for prints *a-d* and *e-h*. *a-d* show the spindle at one focal level and *e-h* show a second focal level. *a*, just after cutting (the poorer quality is due to the objective used during the operation); *b*, 1 min later; *c*, after fixation. (*d*) The chromosome marked by the arrow in *a-c* moved 2 μm before fixation. The lines in *d* mark off 2 μm from the cut edge, showing how far the kinetochore microtubules would have protruded if microtubule disassembly did not occur as the chromosome moved. (*e-h*) *e* is the same negative as *a*, printed at high contrast to show the cut edge more clearly. (*e-g*) The arrowed chromosomes each moved 1.5 μm . (*h*) No corresponding protrusion of kinetochore microtubules is seen. Bar, 10 μm .

Table II. Approaching the Cut Edge: How Close the Best Performers Came

Final distance kinetochore to edge	Number of chromosomes
3.0 μm or closer	41
2.0 μm or closer	35
1.5 μm or closer	22
1.0 μm or closer	18
0.5 μm or closer	8
0.25 μm	4

amount of spindle elongation found (0.5–1.8 μm). Hence the values in the table are for velocity and extent of movement due solely to chromosome-to-edge or chromosome-to-pole movement. The extent of movement was measured after movement had slowed greatly or ceased, typically ~ 30 min after the spindle was cut.

The average velocity in the cut half of the spindle and in the uncut half are both ~ 0.3 $\mu\text{m}/\text{min}$ and the ranges in velocity overlap (Table I). A comparable velocity of 0.35 $\mu\text{m}/\text{min}$ was found in an uncut spindle from a demembrated cell (Nicklas, 1977, Fig. 5, legend). The average velocity in cut spindles is about half the average found in uncut spindles in intact cells. However, the most quickly moving chromosomes in cut spindles (0.5 and 0.6 $\mu\text{m}/\text{min}$, Table I) move almost as fast as the average in intact cells (0.69 $\mu\text{m}/\text{min}$, Table I). Chromosome velocity did not differ significantly for chromosomes with longer versus shorter kinetochore microtubules. The chromosomes in the cut half-spindles included in Table I had kinetochore microtubule bundles 4–8 μm long immediately after the cut was made. Their average velocity was 0.27 $\mu\text{m}/\text{min}$. In contrast, kinetochore microtubule bundles in the cut half of the spindle in Fig. 5 were only 2–4 μm in length after cutting. The average chromosome velocity in the cut half of that spindle was 0.32 $\mu\text{m}/\text{min}$ (sample of eight chromosomes).

The extent of movement in cut spindles is affected by many variables including when the observations start relative to the beginning of anaphase and how close the cut is to the chromosomes. Hence, a comparison with intact spermatocyte spindles is not meaningful. The point is that chromosomes in both halves of cut spindles can move several micrometers; about as far as chromosomes ever move in the small spindles of many normal cells such as cultured mammalian cells.

Chromosomes in the cut half-spindle move toward the cut edge and eventually stall. How close to the edge they come was determined in a sample of 41 chromosomes from 10 spindles (Table II). The question is how well the chromosomes and spindles can do at their best, and therefore the sample is deliberately biased toward the best performers. The sample includes all the spindles in which at least one chromosome moved to within 1.5 μm of the edge and includes all the in-focus chromosomes in those spindles, provided they moved at least 1.5 μm . Especially noteworthy are the eight chromosomes that moved to 0.5 μm of the edge or closer.

Spindles with Cuts through Both Half-Spindles or through the Interzone

Three spindles were cut between the chromosomes and the pole in both half-spindles, which produces a spindle that lacks both polar regions. The chromosomes continued to move in all three spindles, but the extent was reduced to 1–2 μm toward each cut edge in these doubly abused spindles.

Seven spindles were sliced in half by a cut through the interzone between the separating chromosomes. The aim was to determine if poleward movement is driven by a push from behind; if so, a cut through the interzone might stop movement. An isolated half-spindle resulting from an interzonal cut is shown in Fig. 8. Chromosome movement was not extensive, 2–4 μm , but it is evident that the chromosomes had moved closer to the pole by 8 min (Fig. 8). The chromosomes do not move far in such experiments because the experiment cannot be done until the chromosomes have separated, since chromosomes are difficult to cut cleanly. By that time, there is little poleward movement remaining to be accomplished. Chromosomes also continued to move in the other six examples, which were cut anywhere in the accessible part of the interzone, approximately the central 5–10 μm .

Discussion

Chromosome Movement in Cut Spindles is like Normal Anaphase Movement

Chromosomes continue to move after a spindle in anaphase is cut between the chromosomes and one pole, and the polar part is swept away. The first question is whether the move-

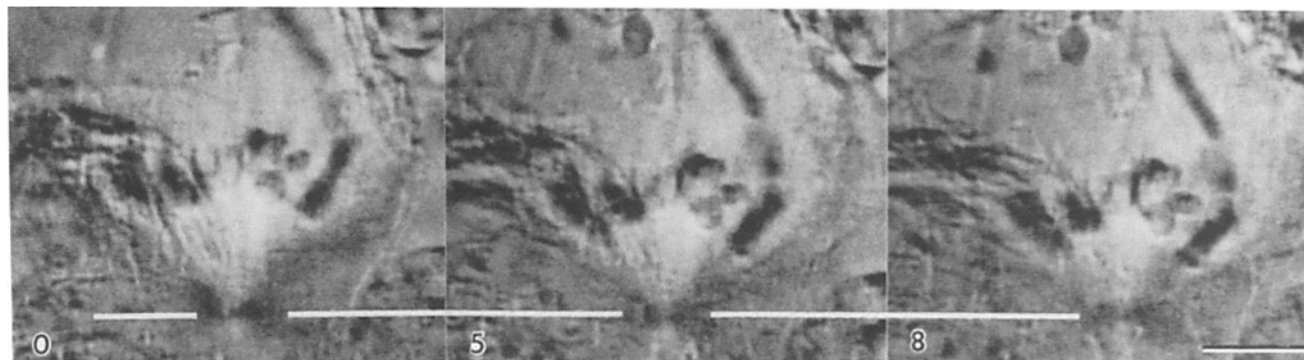


Figure 8. Chromosomes move poleward in an isolated half-spindle. The spindle was cut through the interzone at -3 min and one of the halves was swept away, leaving the solitary half-spindle shown here. Movement of chromosomes leads to a decrease in the size of the birefringent zone. The time in minutes is indicated on each section. Video-enhanced polarization microscopy. Bar, 10 μm .

ment is comparable to normal chromosome-to-pole movement. Chromosomes in cut spindles move smoothly for considerable distances and parallel to the spindle axis, as they do in uncut spindles in normal cells. The only evident difference is the slower average speed of chromosomes in a cut spindle, but the fastest ones move almost as quickly as the average chromosome in a normal cell. In the cut half-spindle, chromosomes move toward the cut edge, just as they move toward the pole in an uncut spindle. Measurements of the distance between the cut edge and the pole in the intact half-spindle eliminate the possibilities (a) that the edge is moving toward the chromosomes (as would be the case if microtubules depolymerize at the cut edge) or (b) that spindle elongation moves chromosomes closer to the edge. In some cut spindles (e.g., Figs. 1 and 5), the pole-to-edge distance does not change: neither depolymerization nor elongation is occurring (unless they precisely balance one another), and chromosomes obviously are moving toward the cut edge. In the other cut spindles, the distance between the edge and the pole increases due to spindle elongation, but this poses no problem: while elongation increases the separation between chromosomes just as in intact spindles, it does not move them closer to the edge (Fig. 2).

The independent movement of individual chromosomes also testifies that movement toward the cut edge is genuine. The chromosomes move at different speeds, one commonly passing another (e.g., Figs. 1 and 2). Such movement cannot be due to any process affecting all chromosomes equally, such as spindle elongation or the depolymerization of microtubules from the cut edge toward the chromosomes.

Hence, chromosomes genuinely move toward the edge in cut half-spindles, and they do so despite the absence of a sizable part of the putative traction fiber.

Implausible Explanations for Movement in Cut Half-Spindles

Motors on the Coverslip. Microtubules can be moved by motors attached to a glass substrate (Vale et al., 1985). It is natural to ask if such motors operate in cut half-spindles, because the microtubules were tightly pressed against the glass coverslip when the spindle was cut and any loose motors from the surrounding demembrated cells have access to the glass. This explanation is ruled out, however. A stereoscopic reconstruction of a cut spindle shows that it returned to about its original thickness after the operation (Nicklas et al., 1989). Hence most microtubules at the cut edge were no longer near the coverslip. Moreover, cut spindles are not attached to the glass, but drift freely around along with other materials. Hence most of the moving chromosomes and microtubules are nowhere near the coverslip, and none remain in close contact with the same patch of glass for very long.

Microtubules beyond the Edge. While some microtubules extend beyond the cut edge, they are too few to account for the movement of all or most chromosomes toward the edge (Nicklas et al., 1989). In addition, most microtubules that extend beyond the edge lie at oblique angles to the spindle axis, and would support movement sideways rather than directly toward the edge. Moreover, these microtubules appear to be unsupported, and free to flop around in the drift of materials around the cut spindle, making them poor candidates to pull on chromosomes (Nicklas et al., 1989).

A Push from the Rear. It has been suggested that chromo-

somes are pushed to the poles from behind, by motors in the interzone between the separating chromosomes (Margolis et al., 1978). This possibility seems unlikely, but has not been decisively eliminated (for review, see Nicklas, 1971). Recently, Hiramoto and Nakano (1988) and Hiramoto and Shôji (1982) reported elegant experiments in intact echinoderm eggs in which chromosomes continued to move poleward in anaphase after the spindle interzone was cut or was partly removed by suction. Unfortunately, structural information is lacking, so how cleanly the interzone was severed or how completely it was removed by suction is not known. An illustration of a sucking experiment (Hiramoto and Shôji, 1982, Fig. 2; Hiramoto and Nakano, 1988, Fig. 10) suggests that the interzone remained intact on one side of the spindle.

I have cut completely through the interzone and moved one half-spindle far from the other one. From observations on cut half-spindles (Nicklas et al., 1989), it is to be expected that all microtubules are severed when a spindle is pressed against the glass by a microneedle. These experiments eliminate any motor situated in the middle of the spindle behind the chromosomes, because chromosome movement continued when that part of the spindle was simply not present (Fig. 8). This leaves only the region immediately behind the chromosomes as a possible site for a pushing motor, a very unlikely site but one that is not yet ruled out.

The more plausible sort of pushing motor is unlikely regardless of its position in the interzone. A motor behind the chromosome could push directly on the kinetochore via microtubules that entered the kinetochore from the rear and terminated there, but such microtubules have not been seen. The alternative is an interzone motor linked to microtubules that extend beyond the chromosome and connect with its kinetochore microtubules (Margolis et al., 1978). This would push kinetochore microtubules and chromosome together toward the cut edge, and therefore it would cause the microtubules to protrude from the cut edge as chromosomes move toward it. That has not been seen, as discussed more fully below.

The Motor Is in or near the Kinetochore

If the motor is not behind the chromosomes nor beyond the cut edge in a cut half-spindle with no polar region, then it must be somewhere between the chromosomes and the edge. In cut half-spindles, eight chromosomes moved to 0.5 μm or less of the apparent edge before stopping (Table II). However, as seen by electron microscopy, the edge sometimes is ragged, with microtubule ends forming a fringe extending 0.5 μm on either side of a line through the middle of the fringe (Nicklas et al., 1989). In the present experiments, the cut edge was viewed by phase contrast or polarization microscopy, and what was identified as the edge is probably near the middle of the fringe of microtubule ends. Hence, when the cut is ragged, microtubules may extend 0.5 μm beyond the apparent edge, and a chromosome scored as coming to 0.5 μm of the edge may actually have been no closer than 1 μm to the true end of some microtubules. Some cuts are not so ragged (Nicklas et al., 1989), but for the present a conservative assessment based on ragged cuts suffices. I conclude that the motor for poleward chromosome movement in anaphase should be sought within 1 μm of the kinetochore.

The stringent limitations this places on a traction fiber applies to models of any sort: the more conventional models

in which kinetochore microtubules are pulled poleward by a microtubule-associated motor or by actin (e.g., Forer, 1988; Margolis et al., 1978; McIntosh et al., 1969), those in which chromosomes are pulled poleward by an elastic traction fiber attached at the kinetochore (Pickett-Heaps, 1986), and those in which microtubules zip together laterally (Bajer, 1973; Fuge et al., 1985). Whatever form of traction fiber is invoked, it must meet a new standard: the fiber must be capable of moving chromosomes when reduced to a nubbin only 1 μm long or less.

Additional results point to the kinetochore itself as the site of the motor. A traction fiber would pull the kinetochore microtubules toward the pole along with the chromosome. Hence, when the chromosome is moving toward a cut edge, the kinetochore microtubules should be extruded from the edge. This has not been seen; instead, the kinetochore microtubule bundle shortens as the chromosome moves toward the edge. It might be argued that microtubules actually are extruded but that the protruding portion quickly depolymerizes and therefore is not seen. That is unlikely because numerous microtubules from other spindles or asters are often seen in the vicinity of the edge (Nicklas et al., 1989): it is not a general disassembly zone. It has been suggested that a surrogate pole forms at the edge (Wolniak, 1988), and if the pole is normally the site of disassembly, then the replacement pole might also function in that capacity. But a surrogate pole would have to form at remarkable speed after the true pole is cut away, since microtubules protruding from the edge have not been seen in the first few minutes after cutting (Fig. 5, 0–11 min). During this time, the kinetochore microtubule bundles certainly appear to shorten, and the extent of shortening matches the extent of movement of each chromosome (Fig. 5).

Since disassembly evidently is occurring, but very likely not at the edge, the kinetochore is the probable site of disassembly as chromosomes move on cut spindles. Adding this to the earlier evidence (Gorbsky et al., 1987, 1988; Mitchison et al., 1986), the case for the kinetochore as a disassembly site in anaphase is very strong.

The failure to find microtubules protruding from the cut edge also strongly suggests that kinetochore microtubules are stationary and do not pull on chromosomes, and hence that the motor is in the kinetochore. A traction fiber is not conclusively ruled out either by the present experiments or by earlier ones involving chromosomes and microtubules *in vitro* (Koshland et al., 1988) or fluorescent microtubules marked by a bleached band (Gorbsky et al., 1987, 1988). Collectively, however, these experiments make a motile kinetochore a most attractive alternative to traction. Notice that the experiments are very different, and the reservations about them are different: the relevance of the *in vitro* experiments may be questioned, the adequacy of microtubule marking by bleaching is not beyond doubt, and alternative explanations can be formulated for why kinetochore microtubules are not extruded at a cut spindle edge. For me, however, it is becoming easier to believe in the kinetochore as the motor than to believe that all of the diverse reservations are valid.

Kinetochore as Motor: Models

The one certain function of the kinetochore is as an attachment site for microtubules. If the kinetochore is also a site for microtubule disassembly and translocation, how is attachment maintained? Models of the sort first proposed by

Margolis and Wilson (1981) offer an attractive solution (for review, see Nicklas, 1988). The structural principle is a kinetochore with an outer plate perforated to form sleeves into which microtubules insert. The termination of microtubules at the inner surface of the outer plate is actually seen in the best-known kinetochores (for review, see Rieder, 1982). Such a model frees the end of each kinetochore microtubule to gain or lose subunits and allows attachment to be maintained by lateral interaction between the microtubule lattice and the wall of the kinetochore sleeve. Based on this theme, two principal classes of motors have been suggested, one powered by an ATPase-microtubule translocator built into the sleeve (e.g., Mitchison, 1987; Salmon, 1989), another by microtubule disassembly without ATP hydrolysis (e.g., Hill, 1985; Koshland et al., 1988). At present, a choice between these mechanisms depends on how seriously one takes the evidence that poleward chromosome movement may not require concurrent ATP hydrolysis (Koshland et al., 1988; Cande, 1982; Spurck and Pickett-Heaps, 1987).

Why Do Chromosomes Move At All on Cut Spindles? The Question of Skeletal Support

After seeing chromosomes move in cut spindles, I thought at first that they might move all the way to the cut edge and fall off, which would be conclusive proof that the motor is in the kinetochore. I now think that is unlikely, for an interesting reason.

Something must bear the load when chromosomes move, so that the chromosomes move toward the poles rather than the poles toward the chromosomes. In intact spindles, cross-linked microtubules form a skeleton, the central spindle, that very likely fixes the poles in position (for review, see Nicklas, 1971; McIntosh, 1985). The linkage of kinetochore microtubules to the central spindle provides a stable anchorage for them and allows chromosome-to-pole movement. However, kinetochore microtubules are firmly cross-linked to those of the rest of the spindle only near the pole (Nicklas et al., 1982). The problem in cut spindles can be appreciated by looking at chromosome movement toward the cut edge in Fig. 5. Suppose that the kinetochore is the motor. As the kinetochore pulls the chromosome upward, it pulls downward on the kinetochore microtubules. If the kinetochore microtubules were not fixed in position near the cut edge, then the kinetochore microtubules would be reeled into the kinetochore, i.e., the ends of the kinetochore microtubule bundles would be pulled toward the chromosomes. Hence the problem: in a spindle in which the whole polar region has been cut away, how do chromosomes move toward the cut edge? The obvious explanation is that weak linkages of some sort connect the kinetochore microtubules near the cut edge to those of the central spindle. One possibility is linkage by embedment in a gel-like matrix. The same micromanipulation experiments that show strong microtubule cross-linkage only near the poles also provide clear evidence for weaker associations of microtubules in the remainder of the half-spindle (Nicklas et al., 1982). Micromanipulation experiments in cut spindles suggest that the matrix may be stickier than normal (unpublished observations), which may bind microtubules together more effectively. Thus, the necessary supporting skeleton for chromosome movement may be an experimental artifact. If so, it is a lucky artifact, since in its absence no chromosome movement might be seen. Another

possibility is a dynamic linkage of spindle microtubules, as suggested by recent observations of lateral associations of microtubules in living cells (Cassimeris et al., 1988).

Whatever it is that binds microtubules together, its effectiveness will decline as chromosomes approach the cut edge. The length of the kinetochore microtubules available for binding to other microtubules or the matrix falls toward zero as chromosomes near the edge (e.g., Fig. 5), and hence chromosome movement should stall some distance short of the edge, as is observed. Chromosomes that have come close to the edge might also be expected to be imperfectly linked to the microtubules driving spindle elongation, and hence not be moved as far as others. This has been observed (Fig. 2). As chromosomes approach the edge, the last bit of kinetochore microtubule length may be reeled into the kinetochore; an attempt to watch this take place is now underway.

The central spindle is more than a static skeleton; in most cells, the central spindle not only provides support, it is also the site of the motors for spindle elongation. Genuine exceptions probably exist in the spindles of some fungi (Aist and Berns, 1981), in which the spindle poles appear to be held in place and pulled apart by astral microtubules associated with cytoplasmic structures and motors: "external support" as opposed to "internal support" by cross-linked spindle microtubules. Without question, however, the isolated central spindle of diatoms can support itself and elongate by internal, not external, motors (Cande and McDonald, 1985). It has been suggested that external support and elongation might be true of spindles in larger eukaryotes as well as fungi (Hiramoto and Nakano, 1988; Kronebush and Borisy, 1982). Clearly, however, two spindle poles and any associated motors are not necessary for the elongation of the cut spindles of demembrated spermatocytes, because elongation occurs in the absence of one pole (Fig. 2). Since the spindles are not stuck to the glass or otherwise held in place, motors associated with the one remaining pole might move the spindle about, but they could not cause elongation. The cut spindle evidently has internal, not external, engines for elongation.

In conclusion, cut spindles provide graphically direct, conclusive evidence that the motor for poleward chromosome movement in anaphase is in the kinetochore or very close to it; the putative traction fiber is all but gone, yet movement continues. While it has not yet been proven that the motor is in the kinetochore itself, recent experiments, including those reported here, make a very strong case for a motile kinetochore.

To me, a kinetochore that participates actively in its own movement is an exciting prospect. However, there is no denying the explanatory power of the traction fiber model (Forer, 1988; Wolniak, 1988), particularly for chromosome movement to the metaphase plate in prometaphase. Two different motors very likely operate in anaphase, one for chromosome-to-pole movement and one for spindle elongation (Cande, 1982; Cohn and Pickett-Heaps, 1982; Lee, 1989). Might there be three motors in mitosis? Might there be a traction fiber in prometaphase, a motile kinetochore in anaphase, and a sliding microtubule motor for anaphase spindle elongation?

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