The Impact of Chromosomes and Centrosomes on Spindle Assembly as Observed in Living Cells

Dahong Zhang and R. Bruce Nicklas

Department of Zoology, Duke University, Durham, North Carolina 27708-1000

Abstract. We analyzed the role that chromosomes, kinetochores, and centrosomes play in spindle assembly in living grasshopper spermatocytes by reconstructing spindles lacking certain components. We used video-enhanced, polarization microscopy to distinguish the effect of each component on spindle microtubule dynamics and we discovered that both chromosomes and centrosomes make potent and very different contributions to the organization of the spindle.

Remarkably, the position of a single chromosome can markedly affect the distribution of microtubules within a spindle or even alter the fate of spindle assembly. In an experimentally constructed spindle having only one chromosome, moving the chromosome to one of the two poles induces a dramatic assembly of microtubules at the nearer pole and a concomitant disassembly at the farther pole. So long as a spindle carries a single chromosome it will persist normally. A spindle will also persist even when all chromosomes are detached and then removed from the cell. If, however, a single chromosome remains in the cell but is detached from the spindle and kept in the cytoplasm, the spindle disassembles.

One might expect the effect of chromosomes on spindle assembly to relate to a property of a specific site on each chromosome, perhaps the kinetochore. We have ruled out that possibility by showing that it is the size of chromosomes rather than the number of kinetochores that matters.

Although chromosomes affect spindle assembly, they cannot organize a spindle in the absence of centrosomes. In contrast, centrosomes can organize a functional bipolar spindle in the absence of chromosomes. If both centrosomes and chromosomes are removed from the cell, the spindle quickly disappears.

In eukaryotes, the assembly of a bipolar spindle is essential for the accurate segregation of chromosomes during cell division. Despite much progress toward understanding the mechanisms of chromosome movement and segregation (reviewed by Salmon, 1989; Wadsworth, 1993), some basic puzzles of spindle assembly remain. One puzzle concerns the role of chromosomes. Are the chromosomes simply inert carriers of genetic information or are they actively involved in spindle assembly? The traditional view is that the interphase unipolar microtubule array is transformed into the mitotic bipolar structure (Bajer and Mole-Bajer, 1969; Vandré et al., 1984) at the onset of mitosis when centrosomes replicate and separate. An increased rate of microtubule turnover at the onset of mitosis is thought to be involved in this structural transformation (Salmon et al., 1984*b*; Saxton et al., 1984; Mitchison et al., 1986). Centrosomes establish the bipolarity of a spindle by acting as organizing centers (Mazia, 1961; Nicklas, 1971), which nucleate the polarized assembly of microtubules (McIntosh, 1983). Chromosomes become attached to the poles when kinetochores capture microtubules emanating from the poles (Rieder and Alexander, 1990; Hayden et al., 1990; Nicklas and Ward, 1994). Once captured, microtubules are more stable than uncaptured polar microtubules (see Mitchison and Kirschner, 1985; Mitchison et al., 1986, for in vitro studies and Nicklas and Kubai, 1985, for in vivo studies). Attachment of microtubules at the kinetochore presumably alters microtubule dynamics yielding a selective stabilization that is thought to be a key factor in generating the bipolar organization of the spindle (Kirschner and Mitchison, 1986).

Although centrosomes and kinetochores are important, they are not the only players. For instance, in early cleavage of echinoderm embryos, centrosomes fail to organize a bipolar spindle in the absence of nuclei (Sluder et al., 1986; references in Sawin and Mitchison, 1991). In vitro, microtubule length can be regulated by factors other than tubulin concentration (Brinkley et al., 1981). The body of evidence suggests that the whole chromosome, not only the kinetochore, may be actively involved in spindle assembly. For instance, the reduction in spindle microtubule mass resulting from chromosome extraction (Marek, 1978) is due to a loss of both kinetochore and nonkinetochore microtu-

Address all correspondence to Dahong Zhang, Dept. of Zoology, Duke University, Box 91000, Durham, NC 27708-1000. Tel.: (919) 613-8195. Fax: (919) 613-8177.

bules (Nicklas and Gordon, 1985). Centrosomes injected into arrested *Xenopus* eggs nucleate microtubule arrays only in the proximity of the nucleus or chromatin (Karsenti et al., 1984). Furthermore, in *Xenopus* egg extracts, the effect of the nucleus on spindle assembly is independent of specific kinetochore–microtubule interactions (Sawin and Mitchison, 1991). In some organisms, chromosomes can organize a bipolar spindle in the apparent absence of centrosomes (Dietz, 1966; Karsenti et al., 1984; Church et al., 1986; Steffen et al., 1986; Theurkauf and Hawley, 1992).

The exact role of chromosomes remains uncertain. In particular, it is not clear whether it is the chromosome as a whole or only the kinetochore that plays the more important role in spindle microtubule assembly. And, in some mitotic cells, chromosomes and kinetochores may play no part at all. For instance, chromosomes by themselves cannot organize a spindle in either echinoderm embryos (Sluder and Rieder, 1985) or newt lung cells (Rieder and Alexander, 1990). Obviously, a comprehensive understanding of the interaction of chromosomes, kinetochores, and centrosomes in spindle formation is needed.

Our experiments were designed to permit study of spindle assembly in living cells so as to distinguish the function of chromosomes from that of kinetochores and centrosomes (Fig. 10 summarizes the experimental designs and results). We dissected normal spindles and reconstructed them to our specifications with any desired combination of centrosomes and chromosomes. We find that when chromosomes are asymmetrically distributed within the spindle, there is a rapid, localized effect on spindle microtubule assembly. The effect is a property of whole chromosomes, and not of kinetochores. In grasshopper spermatocytes chromosomes have a large impact on microtubule assembly, and centrosomes are indispensable for spindle organization. That is, chromosomes and centrosomes act in concert to organize a functional spindle.

Materials and Methods

Materials

Spermatocytes of the grasshopper *Chortophaga australior* (Rehn and Hebard) were cultured as described earlier (Nicklas and Ward, 1994), but using a different chamber that permits micromanipulation (Kiehart, 1982).

Video-enhanced Polarization Microscopy

Cells were observed with a video-enhanced polarization microscope as previously described (Nicklas and Ward, 1994) except that the condenser numerical aperature (NA)[1] was slightly reduced to 1.2 because of the culture chamber. Images were acquired and processed as described earlier (Nicklas and Ward, 1994).

Measurement of Volume-birefringence

Volume-birefringence (BR_{volume}) reflects the total mass of aligned birefringent material. For the grasshopper spindle, this is calculated as follows (Marek, 1978):

$$BR_{volume} = (\pi/12) \times L \times W \times \Gamma_{sp}$$

where $\pi/12$ is the shape constant, L and W are the length and width of the spindle, and Γ_{sp} is the retardation of the spindle. The retardation was measured from unprocessed video images stored on the computer hard disk. The Image 1 system (Universal Imaging Corp., West Chester, PA), was used to measure brightness, and a standard curve of image brightness versus retardation was obtained using mica chips (Salmon et al., 1984*a*). Spindle retardation was determined by calculating the difference in brightness between the background and the spindle ($B_b - B_s$) and then reading the corresponding retardation from the standard curve. Due to the shallow depth of focus given by the high NA lenses employed, the video image of the spindle did not encompass the entire thickness of the spindle. As a consequence, the measured retardation was an underestimate of actual spindle retardation. Ideally, the image used for retardation measurements would be obtained using an objective and a condenser having low NAs so as to include the entire thickness of the spindle in the image. Switching objectives and condensers was not practical in our experiments, however, because the attendant mechanical disturbances might move the micromanipulation needle and kill the cell. Therefore we used optics with high numerical aperture as necessary for high resolution images, and calibrated the system to provide reliable retardation measurements. The true retardation of a given spindle was determined using a Nikon rectified NA 0.65/40× objective with the condenser set to NA 0.4, which ensures that the entire thickness of the spindle is well within the depth of focus of the microscope (Marek, 1978). The same spindle was then imaged with our standard NA 1.2 system, and the apparent retardation was determined. An average correction factor of 1.54 ± 0.08 (actual retardation/apparent retardation at NA 1.2) was obtained from measurements on 30 spindles and was applied to correct the retardation values as measured with the high NA system.

Micromanipulation

Spindles were manipulated with a piezoelectric micromanipulator (Ellis and Begg, 1981) using a glass needle with a tip ~0.1 μm in diameter. Chromosomes were extracted from the cells as described by Marek (1978) and Nicklas and Gordon (1985). Detached chromosomes lack kinetochore microtubules; once detached from the chromosome, kinetochore microtubules quickly disassemble into subunits (Nicklas and Kubai, 1985) that are left behind when the chromosome is extracted from the cell.

Micromanipulation of centrosomes is possible because centrioles and astral microtubules are visible in polarization microscopy. A centrosome was usually detached from the spindle as an entity including some associated microtubules: i.e., as an aster. Once detached, an aster could be moved to a desired region of the cell or even extracted from the cell, much like micromanipulation of a chromosome. Typically, only a negligible length of astral microtubules was seen attached to the centrosome as it was removed from the cell.

Immunofluorescence

Immunofluorescence staining of spindle microtubules and centrosomal material was carried out as previously described (Nicklas et al., 1993).

Results

Manipulation of Chromosomes and Centrosomes

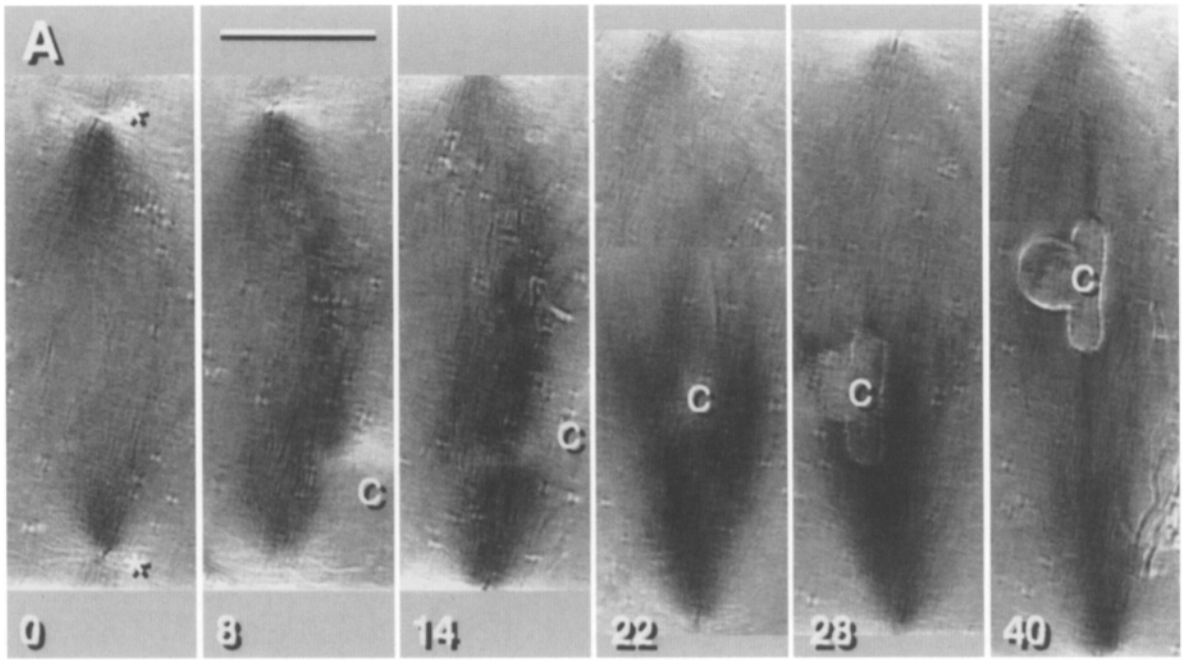
In meiosis I spermatocytes of *Chortophaga australior*, the normal complement of chromosomes consists of 11 bivalents plus the X chromosome. Extracting chromosomes by micromanipulation from the spindle does not impair the health of the cell (Marek, 1978; Nicklas and Gordon, 1985), and cells with a reduced number of chromosomes can undergo a normal anaphase. This is true even when the spindle carries only a single bivalent, or only the X chromosome, or even when it is entirely devoid of chromosomes (Zhang and Nicklas, manuscript in preparation). Similarly, the centrosomes can be removed from spermatocyte spindles at various stages of meiosis without preventing the cell from progressing through the cell cycle.

1. *Abbreviations used in this paper*: BR_{volume}, volume–birefringence; NA, numerical aperture.

Cells deprived of centrosomes or chromosomes or the spindle can still undergo a normal cytokinesis (see below).

Spindle Assembly with a Single Chromosome

A Chromosome within the Spindle. The impact of chromosomes on spindle assembly was revealed by creating a spindle with a single chromosome close to one pole. A typical example chosen from 30 such experiments is shown in Fig. 1 and see Fig. 10 *A1*. During metaphase, all chromosomes but one were removed from the cell; then both centrosomes were detached from the spindle poles and moved into the cytoplasm. Soon, microtubules growing from the freed centrosomes interacted and established a bipolar, chromosome-free spindle (Fig. 1 *A*, 0 min) which was shorter than usual but which had the usual, equal distribution of microtubule birefringence in its two halves (Fig. 1 *B*, 0 min). Placing a single chromosome at one of the poles of the newly formed spindle (Fig. 1 *A*, 8 min) triggered an immediate asymmetric redistribution of microtubules: microtubule concentration increased at that pole and decreased at the other as indicated by changes in volume–birefringence (Fig. 1 *B*, 8–22 min). 14 minutes after the introduction of the chromosome, the microtubule content of the half-spindle containing the chromosome was nearly four times greater than that of the other half-spindle (Fig. 1 *B*, 22 min). As the chromosome congressed to the equator of the newly formed spindle, the normal, symmetrical distribution of microtubules at the two poles was gradually reestablished (Fig. 1, *A* and *B*, 40 min).



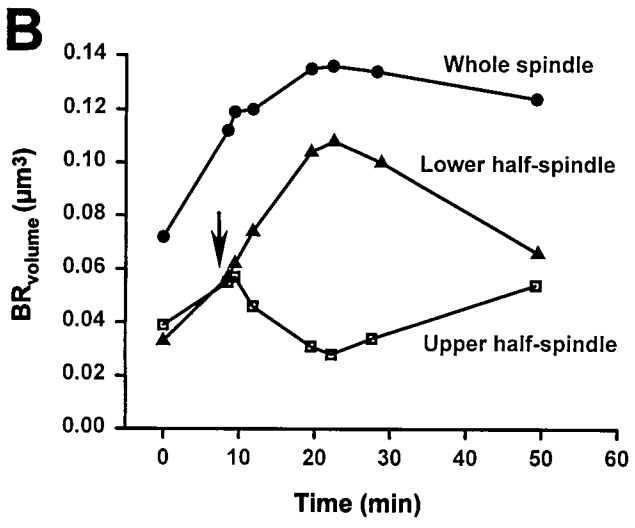
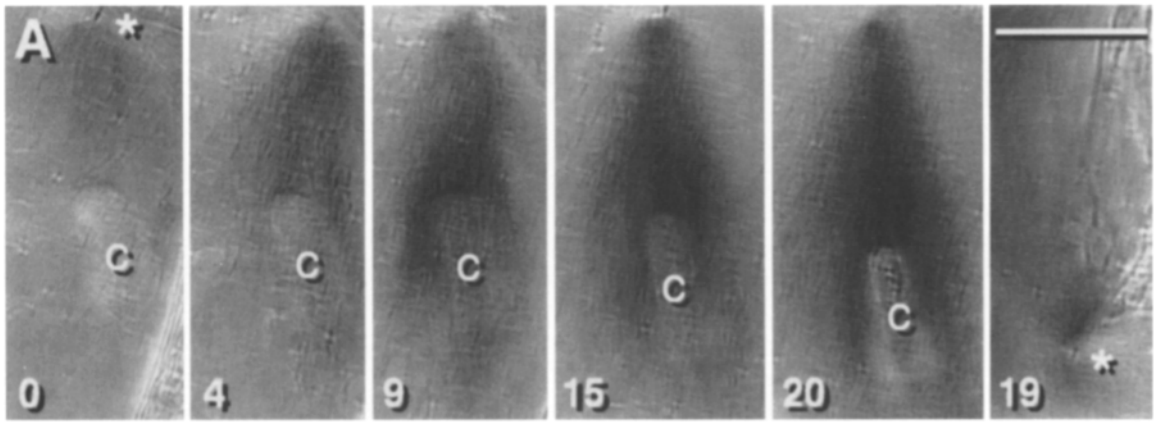


Figure 1. (*A*) The impact of a single chromosome on microtubule assembly in a bipolar spindle. Time is given in minutes on each image. Microtubules are seen as black lines or bundles and in some areas as condensed arrays. Centrosomes (*) were detached from the original spindle and placed in the cytoplasm. Microtubules grew from the freed centrosomes and interacted to form a new, but small, spindle (0 min image). Moving the only chromosome (*c*) in the cell to one of the two spindle poles (8 min) induced a dynamic assembly/disassembly of microtubules in the spindle (14–28 min). Condensed microtubule arrays first appeared near the chromosome (8 min), then across the entire lower half of the spindle (14–28 min). Microtubules close to but not in direct contact with the chromosome were affected (8–28 min). As microtubules at the lower half-spindle assembled, those at the upper half-spindle disassembled (22–28 min). After the chromosome congressed to the equator, the microtubule distribution gradually became more uniform (40 min). The size of the spindle nearly doubled after the chromosome was moved into it (8, 40 min; see also *B*). The 22–40 min images are montages of adjacent video frames. (*B*) The volume-birefringence (BR_{volume}) of the spindle shown in *A*. Dynamic changes of microtubule concentration at the two poles and within the entire spindle began as soon as the chromosome was moved into the spindle (*arrow*). The BR_{volume} of the entire spindle increased initially after the chromosome was introduced, then remained at a relatively steady state regardless of the position of the chromosome. At 22 min, the difference in BR_{volume} between the two half-spindles reached a maximum and reflected a nearly fourfold difference in the concentration of birefringent material in the two half-spindles. Bar, 10 µm.

Vigorous growth in length of the newly formed, chromosome-free spindle was also triggered by introduction of the only chromosome (Fig. 1, *A* and *B*, 8 min onward). At steady state, the length of the spindle increased to normal, and the total mass of spindle microtubules nearly doubled, matching the values determined by Marek (1978) and Nicklas and Gordon (1985) in relation to the number of chromosomes present in the spindle. The increase in microtubule content in the newly formed spindle after the addition of one chromosome can be attributed solely to the impact of the chromosome on microtubule assembly in the half-spindle containing the chromosome; the opposite half-spindle actually experienced a loss in microtubule content (Fig. 1, *A* and *B*, 8–22 min). Along with the growth of the newly formed spindle, the original spindle lacking both chromosomes and centrosomes disassembled (not shown). If the only chromosome remaining in the cell was left in the original spindle, however, the newly formed spindle did not persist; instead, it fused with the original spindle (not shown).

The chromosome's effect is not limited to microtubules in its immediate vicinity (Fig. 1 *A*, 8–22 min). An increase in microtubule content is observed throughout the half-spindle containing only a single chromosome or even when the chromosome remains on the surface of the spindle. The effect first appears on the side of the half-spindle associated with the chromosome and then spreads progressively across the entire half-spindle.

Placing a chromosome near one pole resulted in a spindle that was asymmetrical in thickness. Optical sections of ten experimental cells showed that the half-spindle containing the chromosome had a thickness of 8–10 μm, on



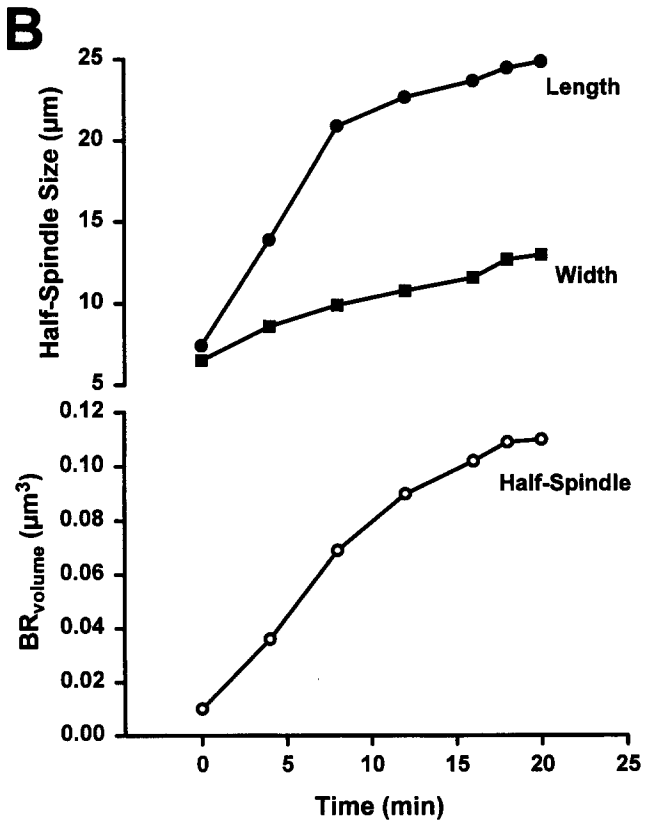


Figure 2. Microtubule assembly at a secluded centrosome induced by a single chromosome. (*A*) Polarization microscopy images. (*B*) Measurements of half-spindle size (above) and volume-birefringence (*below*). After micromanipulation to remove all but a single chromosome from the cell (*A*), one of the centrosomes (*) was detached from a spindle pole and placed in the cytoplasm away from the spindle (0 min). The introduction of the only chromosome (*c*) to the secluded centrosome triggered a linear increase ($time_{1/2}$ = 8 min) in microtubule concentration at that centrosome (*B*, 0–20 min), as well as a massive disassembly of the original spindle (*A*, 19 min image). Bar, 10 μm.

average, while the empty half-spindle was only 2–4 μm thick.

A Chromosome at a "Secluded" Centrosome. The impact of a chromosome on spindle assembly was particularly clear when we observed "secluded" centrosomes; i.e., those that were detached from a spindle containing a single chromosome and moved into the cytoplasm (Fig. 10 *A2*). Fig. 2 shows one of six such experiments. The secluded centrosome lies at the top of the image associated with a few microtubules (Fig. 2 *A*, 0 min). As soon as the only remaining chromosome in the cell was brought nearby from the original spindle, a rapid increase of microtubules at the centrosome was observed (Fig. 2 *A*, and *B*, 0–20 min) along with an equally massive disassembly of microtubules at the centrosome that remained associated with the original chromosome-free spindle (not shown). Within 20 min, a new monopolar spindle had formed (Fig. 2 *A*, 20 min), and the original spindle from which one centrosome and all chromosomes had been removed essentially disappeared (Fig. 2 *A*, 19 min). Measurements of volume–birefringence showed that at equilibrium, the mass of microtubules in the original spindle was unmeasurably small, while the mass at the monopolar spindle with a single chromosome (BR_{volume} = 0.110) was roughly equal to the mass left in the original spindle just after chromosome removal (BR_{volume} = 0.118). Thus, nearly all the microtubules of the original spindle disassembled and presumably reassembled at the secluded centrosome associated with the chromosome. The half-time required for this turnover was ~8 min (Fig. 2 *B*).

In polarization microscopy, the visibility of microtubules is sensitive to the angle of the microtubules relative to the optical system, and irregularly arranged microtubules might have been overlooked in these birefringence measurements. To test whether the observed differences in birefringence might be due to changes in microtubule arrangement, we used anti-tubulin immunofluorescence to render all microtubules equally visible. The effect of a single chromosome, whether at a spindle pole (Fig. 3 *A*) or at a mechanically secluded centrosome (Fig. 3 *B*), was just as obvious as it was with polarization observations (Figs. 1 and 2). Additionally, immunolocalization of spindle microtubules (Fig. 3 *A*) confirms the bipolar structure of the spindles observed in polarization microscopy (Fig. 1).

Chromosomes and Centrosomal Material. Differences in microtubule concentration in half-spindles with or without a chromosome might result from an enhancement of the nucleation capacity of the centrosome associated with the chromosome. This possibility was assessed using antibodies against centrosomal material. Immunofluorescence staining of the centrosomes with either γ-tubulin or MPM-2 antibody failed to reveal any difference in fluorescence intensity between the two poles in manipulated cells such as those shown in Figs. 1 and 2.

Chromosomes and the Spindle's Mechanical Integrity. The half-spindle with a paucity of microtubules, caused by placing the only chromosome at the opposite spindle pole, was mechanically weakened. In each of four spindles tested, little resistance was encountered when the centrosome of a chromosome-free, microtubule-impoverished half-spindle was gently pushed toward the equator. The centrosome could be moved freely until the microtubule-rich region near the chromosome was encountered (Fig. 4). A comparable push at the pole of a normal spindle did not shift the centrosome.

A Chromosome Outside the Spindle. Do chromosomes affect spindle assembly when they are located outside the spindle? We examined this question in three metaphase cells by detaching the last remaining chromosome from the spindle and placing it in the cytoplasm (Fig. 5 *A* and 10 *A3*). As soon as the chromosome was removed, the spindle started to disassemble (Fig. 5 *A*, 6 and 20 min). While

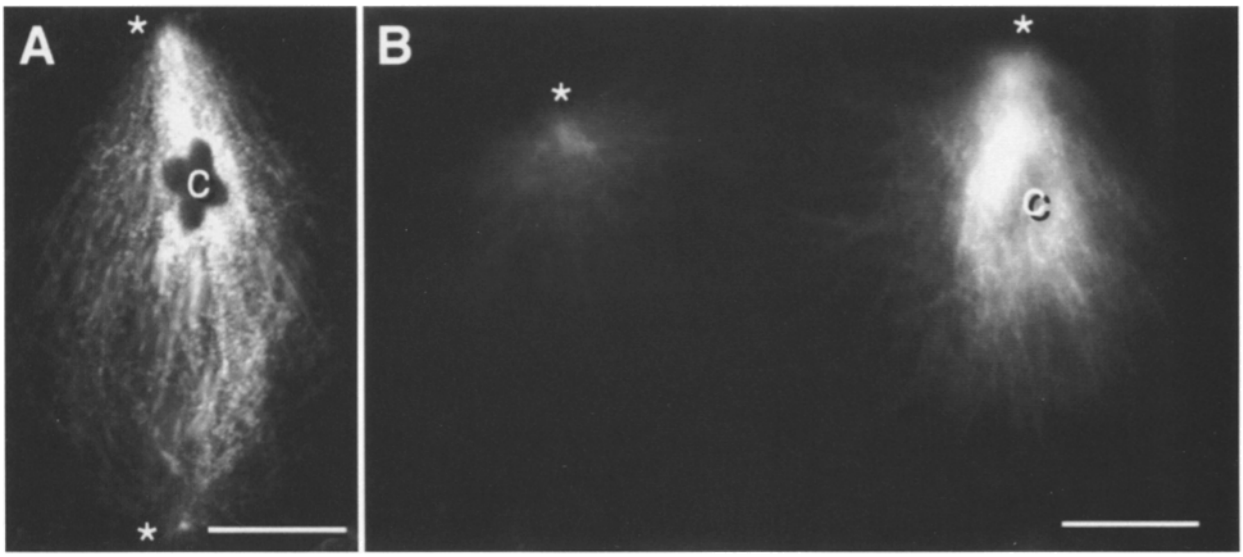


Figure 3. Immunolocalization of spindle microtubules. (*A*) A spindle with a single chromosome at one pole as shown in Fig. 1. (*B*) Two secluded centrosomes (*), one not associated with a chromosome (*left*) and the other associated with a single chromosome (*right*) as shown in Fig. 2. Microtubules are seen as bright fibers or condensed arrays, and the chromosome (*c*) is visible as a darker area in the image. Microtubule assembly observed here is similar to that observed with polarization microscopy. Bars, 10 μm.

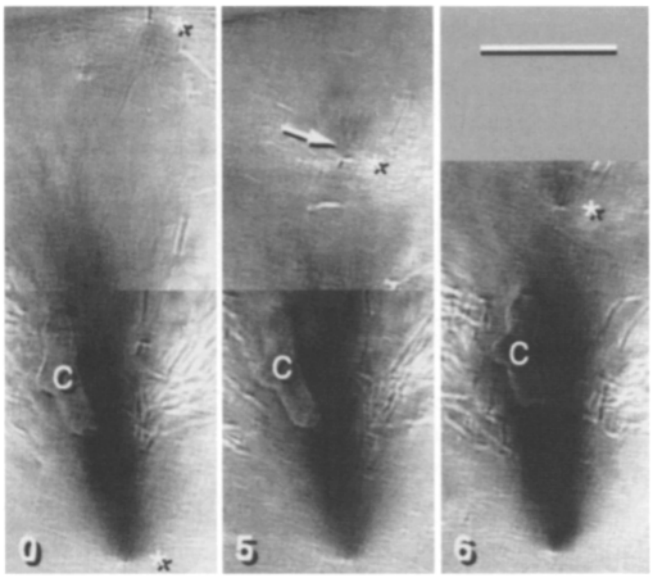


Figure 4. The mechanical integrity of a spindle containing a single chromosome. An asymmetry was produced by placing a chromosome (*c*) near the lower pole (0 min). The upper pole was then easily moved closer to the equator by pushing the centrosome (*) gently with a micromanipulation needle (*arrow*) (5–6 min). The 0–5 min images are montages of adjacent video frames. Bar, 10 μm.

the spindle was dissolving, numerous long astral microtubules appeared, extending from the centrosomes all the way to the cell membrane (Fig. 5 *A*, 18 and 31 min). Evidently, these long microtubules were easily captured by the chromosome in the cytoplasm, and as a result the chromosome moved back to the spindle, leading to the reassembly of the spindle. Then we detached the chromosome again and repositioned it in the cytoplasm. In ~30 min, the length of the spindle decreased to less than half that of its original length (Fig. 5 *D*); the end result was two strong asters lying at opposite sides of a group of mitochondria that had surrounded the spindle before its dissolution (Fig. 5 *A*, 20 min). At this point, although the manipulated cell was actually in late metaphase, it resembled a cell just after nuclear envelope breakdown. In contrast, if a single remaining chromosome was left within the spindle, then the spindle persisted (Fig. 5, *B* and *D*); five such experiments were performed.

The effect of a single chromosome on spindle assembly was observed only when the chromosome was either inside the spindle or some distance away from it. In manipulated cells where the only remaining chromosome was detached from the spindle but left in close proximity to a spindle pole, neither an appreciable enhancement of half-spindle microtubules nor a disassembly of the whole spindle was observed; instead, the chromosome moved back into the spindle (not shown). Although a detached chromosome placed in the cytoplasm affected microtubule assembly of the original spindle, it did not assemble a new spindle around itself (Fig. 5 *A*, 18 min).

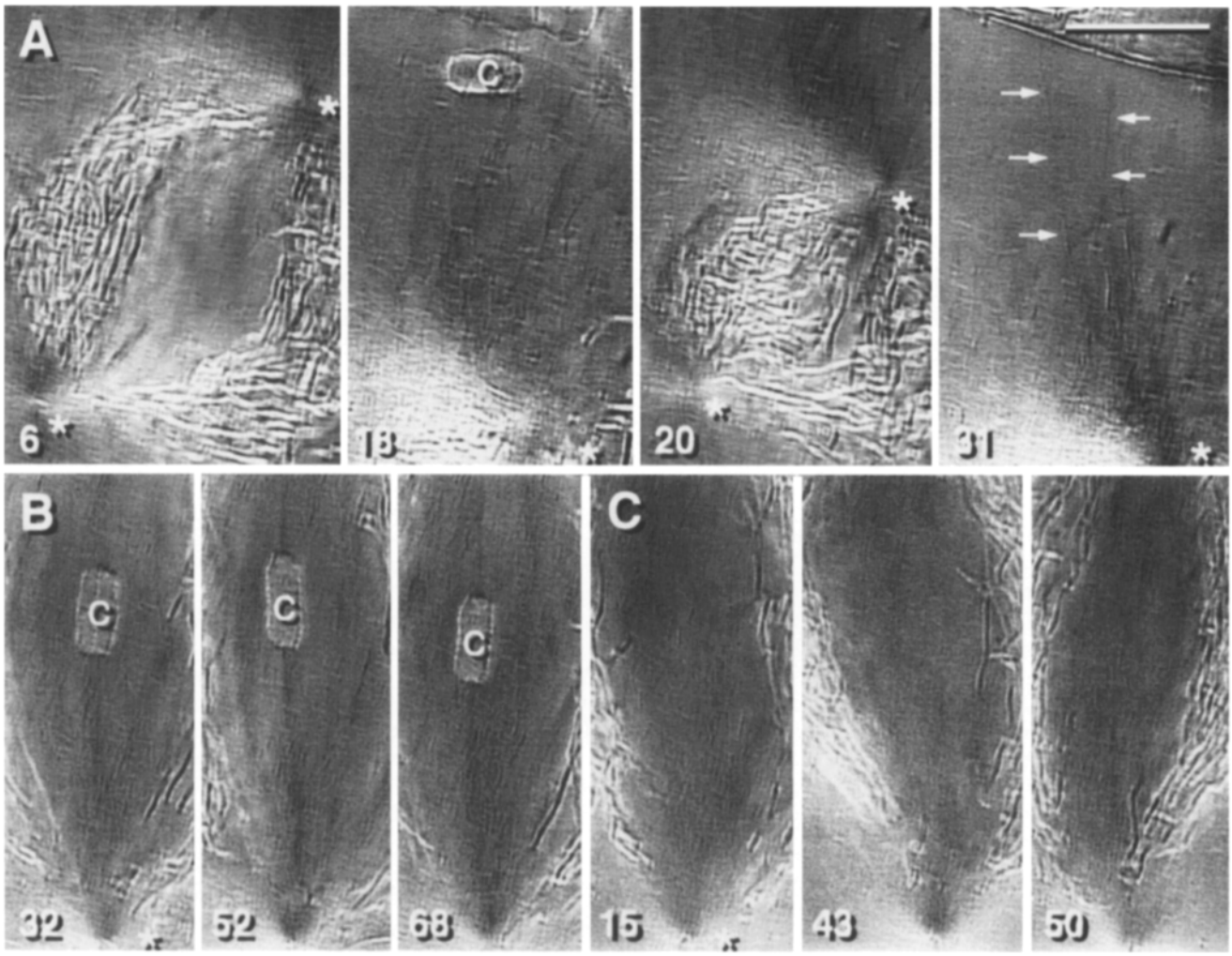
Spindle Assembly and Centrosomes

Spindles without Chromosomes but with Centrosomes. Centrosomes alone can maintain a functional spindle in the complete absence of chromosomes. Fig. 5 *C* shows one of six cells from which all chromosomes were removed during metaphase. The spindle remained intact and its length did not change (Fig. 5 *D*). Measurements of volume-birefringence at equilibrium showed that spindles with no chromosomes contained ~43% (± 0.04) as much aligned birefringent material as the original spindle, a result that agrees well with the extrapolations made by Marek (1978) and Nicklas and Gordon (1985). Cells deprived of all chromosomes remained healthy as shown by their ability to complete anaphase and cytokinesis (Zhang and Nicklas, manuscript in preparation).

Spindles without Centrosomes. The importance of centrosomes in the maintenance of an already-formed spindle was revealed when both centrosomes as well as all chromosomes were extracted from the cell (Fig. 10 *B1*). Fig. 6 presents one of the three cells investigated in which all of the chromosomes were removed from the cell in prometaphase and then the asters were also moved out of the cell. The previously stable spindle soon began to disassemble and then disappeared, its space marked only by a mass of mitochondria that were previously excluded from the spindle (Fig. 6, 25 min). The cell was healthy as indicated by the occurrence of a normal cytokinesis some time later (Fig. 6, 133 min). In this type of experiment, most, if not all, of the centrosomal material was removed from the cell along with the asters. Indirect immunofluorescence staining of centrosomal materials using either MPM-2 or γ-tubulin antibody revealed that: (*a*) detached centrosomes and undetached centrosomes are similar both in size and intensity of fluorescence, and (*b*) no detectable staining is left at the pole after centrosome removal.

Substituting a Chromosome for a Centrosome

Our observations suggest that chromosomes affect the assembly dynamics of microtubules in their vicinity, but they do not supply nucleation sites for microtubule assembly (Fig. 5 *A*, 18 min). To verify this, a centrosome and all chromosomes but one were detached from a prometaphase spindle and removed from the cell; then the remaining chromosome (the X chromosome in this case) was placed in the position formerly occupied by the centrosome (Figs. 7 and 10 *B2*). The presence of the chromosome had a stabilizing effect on the centrosome-free half-spindle: microtubules remained focused at the pole and a bipolar spindle structure was maintained (Fig. 7, 0–39 min). However, the increase in microtubule concentration at the pole with the chromosome was small in comparison with the large increase that occurred when a centrosome was present. Clearly, to serve as an effective centrosome surrogate, a chromosome must be located within the spindle: as soon as the chromosome was detached and moved out of the spindle, even though the chromosome's kinetochore was positioned at the pole, the half-spindle disassembled, and all that persisted was a monopolar spindle focussed on the remaining centrosome (Fig. 7, 58–102 min). In other words, an attached chromosome stabilized a spindle but a detached one did not, even when placed at a pole. Five such experiments were performed with either the X chromosome or the bivalents.



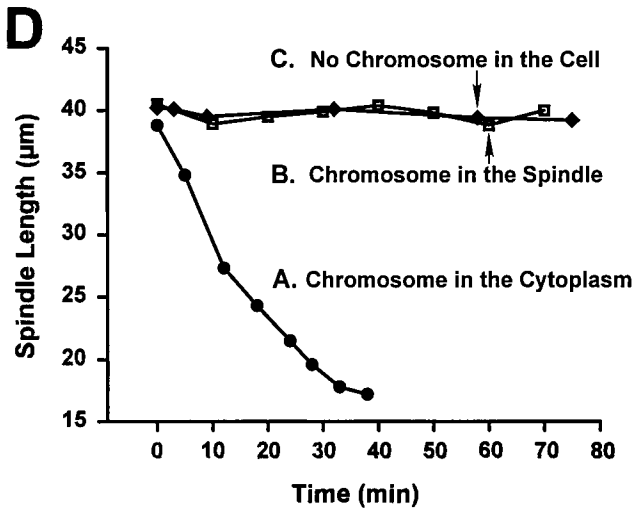


Figure 5. The impact of a single chromosome on the fate of a spindle. (*A*) A spindle rapidly disassembled when the only remaining chromosome (*c*) was removed from the spindle and placed in the cytoplasm (6–20 min). As the spindle disappeared, numerous long microtubules (*arrows*) assembled at the centrosomes (*) (18 and 31 min; only the areas above the upper centrosome are shown). (*B*) A spindle persisted when the only chromosome (*c*) remained within (32–68 min). (*C*) A spindle also persisted if all chromosomes were removed from the cell (15–50 min). (*D*) The kinetics of changes in spindle length in the cells shown in *A–C*. Bars, 10 μm.

Distinguishing Effects of Chromosomes from Effects of Kinetochores

Size of Chromosomes versus Number of Kinetochores. Chromosomes vary greatly in size but kinetochores and other specific chromosomal sites do not (Moens, 1979). This fact can be exploited to determine whether it is a specific site on a chromosome or the whole chromosome that affects microtubule assembly (see Fig. 10 *C1*). Three cells containing one large chromosome and three small ones were produced experimentally. The large chromosome was placed at one pole and three small ones at the other pole in order to create a situation in which one pole interacted

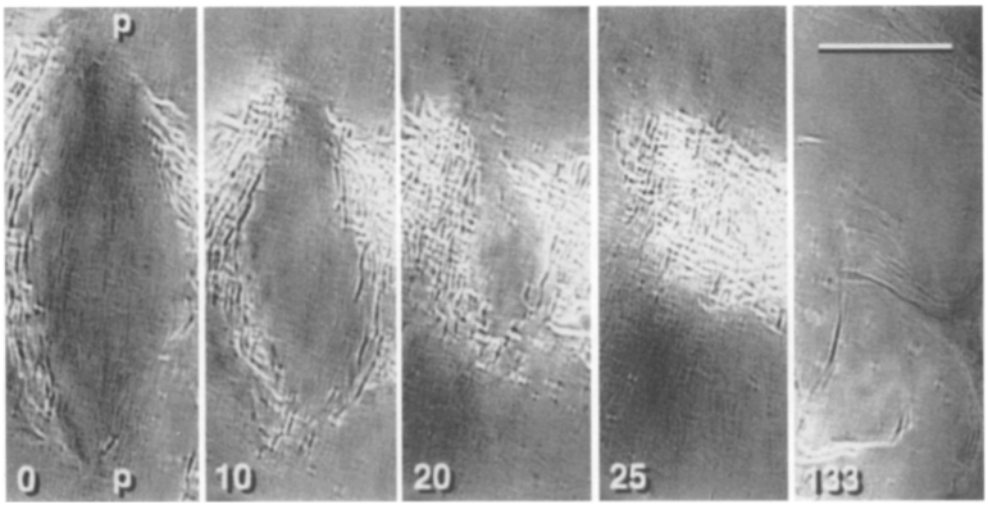


Figure 6. Centrosomes are required for persistence of the spindle. When all chromosomes and both centrosomes were removed from the cell, the spindle rapidly disassembled (0–25 min). A mass of mitochondria then invaded the space originally occupied by the spindle (25 min). Normal cytokinesis occurred even though the spindle was absent (133 min). *P*, spindle poles. Bar, 10 μm.

with a greater number of kinetochores but with less chromosome bulk or volume than the other (Fig. 8, 71 min and Table I). Before chromosome extraction, more microtubules had assembled at the upper pole due to the presence there of the X chromosome (Fig. 8, 0 min and Table I). After the 3:1 chromosome imbalance was created, however, microtubule concentration decreased at the upper pole, the pole associated with three small chromosomes, while it increased at the lower pole (Fig. 8, 69 min). At equilibrium, microtubule concentration at the upper pole was about 30% less than at the lower pole (Table I). This differential in microtubule concentration is proportional to the difference in chromosome volume at the two poles, and not to the number of kinetochores (Table I). Qualitatively similar results were obtained in the two other cells that were similarly manipulated.

Location of Chromosome versus Position of Kinetochore. When all the bivalents are extracted from a cell leaving only the X chromosome, the impact of a kinetochore versus the whole chromosome becomes apparent (Figs. 9 and 10 *C2*). The X chromosome is moderately large compared with the bivalents, and it has a kinetochore at only one

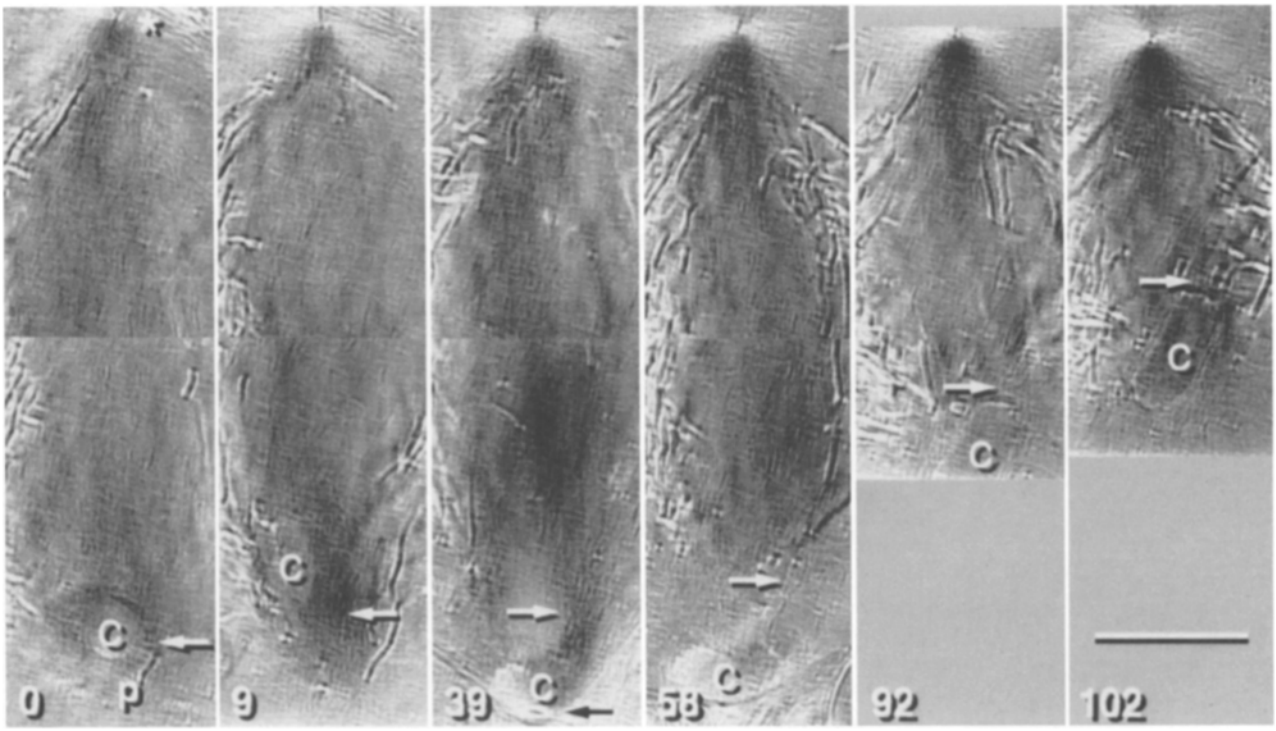


Figure 7. Substitution of a chromosome for a centrosome at the spindle pole. Replacing one centrosome with a chromosome (*c*, X chromosome in this case) did not disturb the integrity of that pole (0–9 min). Microtubules at the lower pole stabilized around the chromosome, with a slight enhancement in concentration (9–39 min). When the chromosome was then detached from the pole (39 min) with a micromanipulation needle (*black arrow*), the pole gradually disassembled even though the kinetochore (*white arrow*) was nearby (58–102 min). The 0–58 min images are montages of adjacent video frames. *P*, spindle pole. Bar, 10 μm.

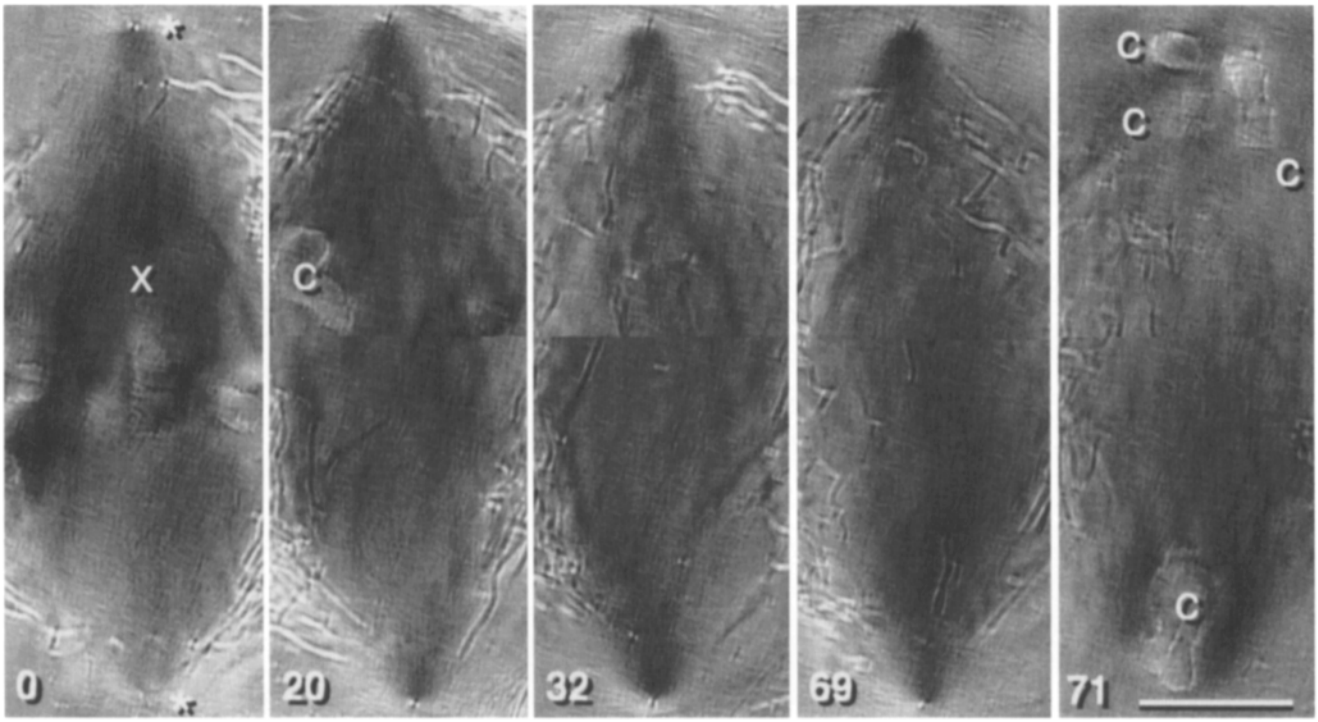


Figure 8. The whole chromosome, not the kinetochore, affects spindle assembly. Before chromosomes were extracted, the concentration of microtubules was higher in the vicinity of the X chromosome, here seen at the upper pole (0 min). When the X and most other chromosomes were removed a relatively even distribution of microtubules was reached (20 min). When one large chromosome (*c*) was moved to the lower pole, and three small ones were moved to the upper pole (32 min; chromosomes are not shown at this focal level, but can be seen on 71 min image), the microtubule distribution became uneven. The concentration of microtubules was greater in the lower half-spindle which was associated with a greater volume of chromosomal material but a smaller number of kinetochores (32–71 min). All images are montages of adjacent video frames. Bar, 10 μm.

end. In the cell in Fig. 9 the X chromosome was first placed near the lower pole, with its kinetochore facing that pole (Fig. 9 *A*, 0 min); spindle birefringence increased in its vicinity (Fig. 9, *A* and *B*, 16 min). The X was then detached from the spindle and moved to the upper pole, but with its kinetochore still pointed toward the lower pole (Fig. 9, *A* and *B*, 28 min). A rapid redistribution in microtubule concentration followed: birefringence increased in the upper half-spindle, where the chromosome arm lay, and decreased at the lower half-spindle (Fig. 9, *A* and *B*, 16–42 min). This altered distribution of birefringence persisted as the chromosome moved toward the lower pole, even though its kinetochore moved progressively closer to the lower pole and a kinetochore fiber connected it to the lower pole (Fig. 9, *A* and *B*, 63–72 min). Measurements of volume-birefringence showed the microtubule concentration in the upper half-spindle to be ~3.5 times higher than in the lower half-spindle (Fig. 9 *B*, 63 min). An even distribution of microtubules was gradually achieved but only after the whole chromosome had reached the equator (Fig. 9 *B*, 90–120 min). Clearly, microtubule concentration in the two half-spindles was affected by the bulk of the chromosome, not the kinetochore. Although the distribution of microtubules between the two half-spindles changed radically depending on the position of the X chromosome, the total volume-birefringence of the whole spindle remained relatively constant throughout the experiment (Fig. 9 *B*, spindle BR_{volume}). Again, note that the microtubules need not contact the chromosome to be affected (Fig. 9 *A*, 63–72 min). Three such experiments were performed.

Discussion

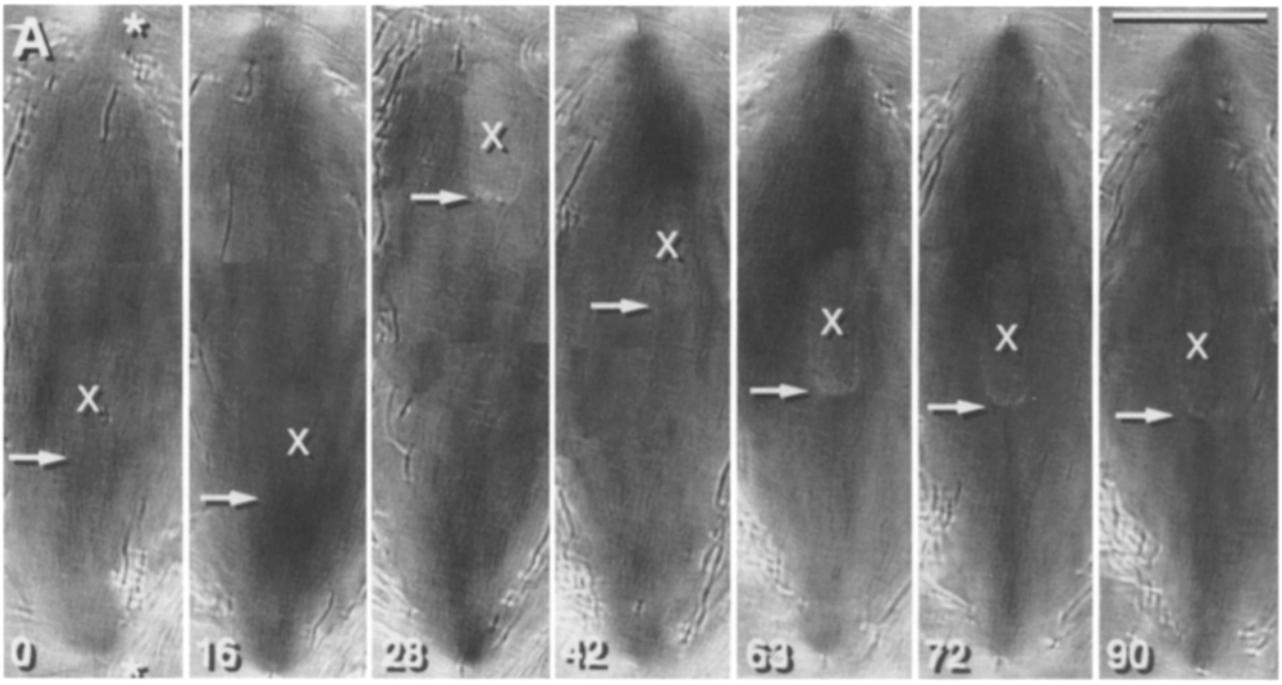
Chromosomes and Spindle Organization

Chromosomes affect the total mass of assembled spindle microtubules as well as the distribution of microtubules within the spindle. Removing all chromosomes reduces the total mass of microtubules to only ~43% of the original mass of spindle microtubules. This agrees remarkably well with estimated values reported by other investigators: measurements for spindles carrying one or a few chromosomes were extrapolated to zero chromosomes, giving values of ~47% based on volume-birefringence (Marek,

Table I. Spindle Pole Volume-Birefringence in Relation to the Size of Chromosomes and the Number of Kinetochores

Pole	Number of chromosomes	Chromosome volume (μm³)	Number of kinetochores	BR_{volume} (μm³)	
				0 min	69 min
Upper*	3	61	6	0.142	0.062
Lower*	1	88	2	0.085	0.089

* As seen in Fig. 8.



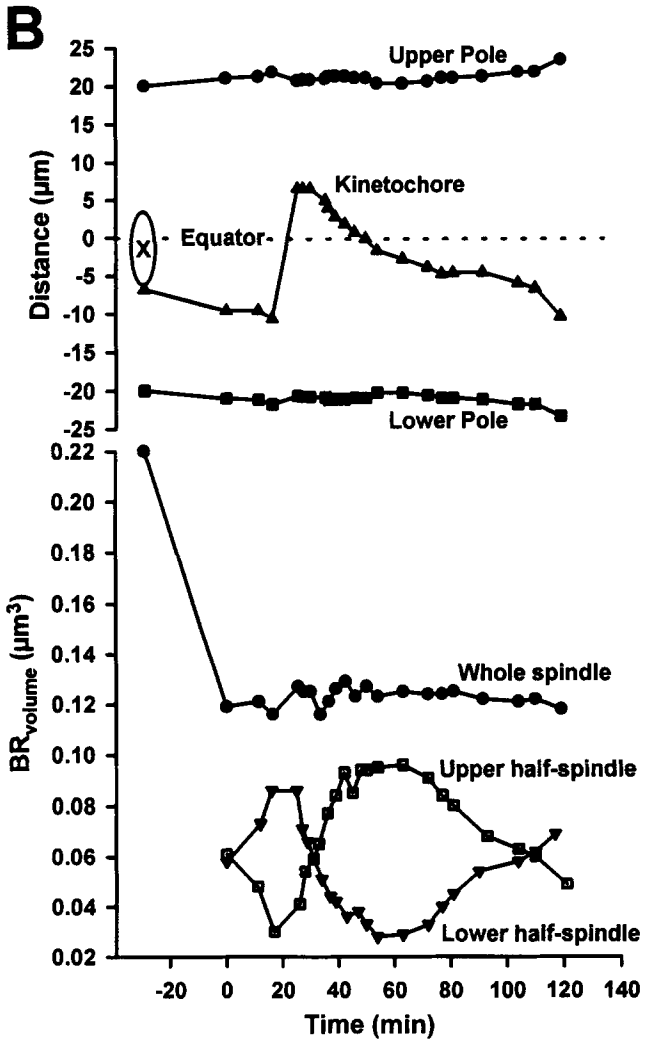


Figure 9. (*A*) The location of the chromosome, not the position of the kinetochore, affects spindle assembly. When the X chromosome was moved to the lower pole and positioned with its kinetochore (*arrow*) facing that pole, microtubule assembly at the lower half-spindle was enhanced (0–16 min). Moving the X chromosome to the upper pole (28 min) triggered a redistribution of microtubules in the spindle, with a greater concentration of microtubules in the upper half-spindle (28–42 min). This occurred even though the kinetochore was still pointed toward the lower pole (28–90 min). The concentration differential persisted even when kinetochore microtubules formed and the kinetochore moved beyond the equator (63–90 min). All images are montages of adjacent video frames. (*B*) Kinetochore position (*above*) and BR_{volume} changes (*below*) within the spindle shown in *A*. At the peak level (63 min), microtubule concentration in the upper half-spindle associated with the bulk of the chromosomal material was ~3.5 times higher than that in the lower half-spindle containing the kinetochore. The BR_{volume} of the spindle as a whole decreased after the bivalents were removed (0 min) but thereafter remained relatively constant regardless of the position of the chromosome. Bar, 10 μm.

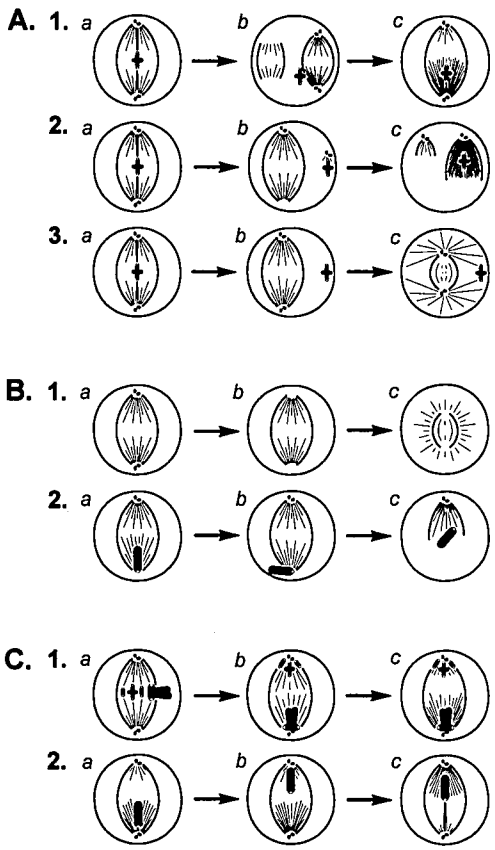


Figure 10. Summary of experimental designs and results. Metaphase cells with a reduced number of chromosomes (shown in black with small open circles indicating the kinetochores) or centrosomes (*paired dots*) are created by micromanipulation. Microtubules are shown as fine lines. (*A*) The impact of a single chromosome on spindle assembly. (*1*) Chromosome enhances spindle assembly. (*a*) Cell with a single chromosome; (*b*) Centrosomes and the chromosome moved to a new region assemble a new spindle (*right*) and cause the original spindle (*left*) to disassemble; (*c*) Chromosome placed at one spindle pole of the newly-formed spindle facilitates microtubule assembly at that pole and disassembly at the other pole. (*2*) Chromosome enhances microtubule assembly of the centrosome. (*a*) Cell with a single chromosome; (*b*) chromosome is placed at a secluded centrosome; (*c*) Assembly of a monopolar spindle (right) at secluded centrosome and disassembly of the original spindle (*left*). (*3*) Chromosome affects the fate of the spindle. (*a*) Cell with a single chromosome; (*b*) Chromosome is moved away from the spindle; (*c*) Spindle disassembles while two large asters reform. (*B*) Centrosomes are indispensable in spindle assembly. (*1*) Centrosomes organize the spindle. (*a*) Spindle persists in the absence of chromosomes; (*b*) Both centrosomes are removed; (*c*) Spindle disassembles. (*2*) Chromosome's kinetochore cannot substitute for nucleation sites of the centrosome. (*a*) Spindle with one centrosome; the other one is replaced by the X chromosome; (*b*) Chromosome's arm is moved out of the spindle with its kinetochore being positioned at the pole; (*c*) The half-spindle with the kinetochore as a surrogate of the centrosome disassembles. (*C*) The chromosomes, not the kinetochores, affect spindle assembly. (*1*) Chromosome mass affects spindle assembly. (*a*) Cell with one large chromosome having a greater total mass than that of the sum of three small ones; (*b*) Large chromosome (two kinetochores) is placed at one pole and three smaller ones (six kinetochores) placed at the other pole; (*c*) More microtubules assemble at the half-spindle with the large chromosome. (*2*) Chromosome arm affects spindle assembly. (*a*) Spindle with the X chromosome (one kinetochore) in the half-spindle with enhanced microtubule assembly; (*b*) X chromosome is moved to the pole with few microtubules with its kinetochore at the equator; (*c*) Microtubules reassemble at the half-spindle with the bulk of the chromosome, not the kinetochore.

1978) and ~40% based on actual measurements of total microtubule length (Nicklas and Gordon, 1985). Our studies yield a further significant insight in that they show that chromosomes affect not only the mass but also the distribution of microtubules within a spindle (see Fig. 10 for a summary). A single chromosome placed at a pole enhances microtubule assembly at that pole and drives disassembly at the other pole, creating a nearly fourfold difference in microtubule mass between the two half-spindles at equilibrium (Figs. 1 and 10 *A1*). If the only chromosome in the cell is removed from the spindle and placed at a secluded centrosome, it causes the assembly of a large monopolar spindle at the centrosome and the disassembly of the original spindle (Figs. 2 and 10 *A2*). When the only chromosome is detached from the spindle and left in the cytoplasm, the spindle gradually disassembles, a surprising outcome given that the spindle would be stable if that chromosome were altogether removed from the cell (Figs. 5 and 10 *A3*). Together, these observations indicate that a single chromosome can affect microtubule distribution in the entire spindle. The total mass of spindle microtubules, however, remains unchanged unless additional chromosomes are added or removed.

What part of the chromosome is responsible for its impact on microtubules? A kinetochore can stabilize microtubules (Mitchison and Kirschner, 1985; Nicklas and Kubai, 1985; Wadsworth, 1993), and therefore it is the obvious candidate. Our results rule out that possibility. It is the size of chromosomes, rather than the number of kinetochores that matters. A single large chromosome with two kinetochores has a greater impact than three smaller ones with six kinetochores (Figs. 8 and 10 *C1*). Moreover, when the X chromosome with its single kinetochore is placed in the spindle so that the major part that lacks a kinetochore is near one pole and the kinetochore itself is closer to the other pole, the half-spindle associated with the bulk of the chromosome acquires ~3.5 times greater microtubule density than the half-spindle containing the kinetochore (Figs. 9 and 10 *C2*). We conclude that the factor or factors that facilitate spindle microtubule assembly are associated with the whole chromosome, not the kinetochore. Similar effects on microtubule assembly have also been seen after injection of nuclei or DNA in *Xenopus* eggs (Karsenti et al., 1984), pointing to chromatin or DNA/protein, rather than kinetochores. Recently, Sawin and Mitchison (1991) demonstrated in vitro that an enhanced formation of microtubule arrays near chromatin does not depend on specific kinetochore–microtubule interactions.

Centrosomes and Spindle Organization

The function of centrosomes in spindle assembly can be appreciated when we eliminate any confounding influence of the chromosomes by removing them from the cell (Fig. 5 *C*). In our material, spindle integrity and bipolarity are maintained for hours in the absence of chromosomes. If, however, the centrosomes are also extracted from the cell, the spindle rapidly disassembles (Figs. 6 and 10 *B1*). More-

over, when detached centrosomes are placed in the cytoplasm, they organize a bipolar spindle (Figs. 1 and 10 *A1*). Taken together, these results show the indispensable role of centrosomes in organizing and maintaining the bipolarity of the spindle in grasshopper spermatocytes. The critical role of centrosomes in nucleating microtubule assembly during spindle formation has been extensively investigated in many cell types (Mazia et al., 1981; Bajer, 1982; McIntosh, 1983; Karsenti and Maro, 1986; Kalt and Schliwa, 1993; Archer and Solomon, 1994). Our findings provide evidence that in spermatocyte meiosis, the essential role of centrosomes in spindle organization may even be independent of the presence of chromosomes. This conclusion is at odds with many previous studies of mitosis in echinoderm embryos. In such materials, centrosomes do not organize a bipolar spindle in the absence of chromosomes (Sluder et al., 1986; references in Sawin and Mitchison, 1991). The differences between these observations and ours on what centrosomes can do by themselves may reflect inherent biological differences between mitotic and meiotic systems (reviewed by Rieder et al., 1993). Alternatively, the differences may reflect variations in experimental design as well as an important general role of nuclear components in spindle assembly. In our experiments, the chromosomes are eliminated from the cells after nuclear envelope breakdown, whereas in the studies of echinoderm embryos, the whole nucleus is completely absent from the start of mitosis. Thus, some factor associated with either chromosomes or nuclear sap may play a critical role in spindle organization. In our system some of the required factor may be left in the cytoplasm after chromosome extraction, but in other materials it could be absent from cells that entered mitosis without nuclei. We have recently obtained evidence that centrosomes in spermatocytes, as in mitotic cells, cannot organize a spindle when the whole nucleus is removed from the cell in prophase (Zhang and Nicklas, manuscript in preparation).

Chromosomes and Centrosomes Together in Spindle Organization

In most cells, including the ones we study, the indispensable role of centrosomes is the nucleation of microtubule assembly (Mazia, 1961; Nicklas, 1971; Mazia et al., 1981; Bajer, 1982; McIntosh, 1983; Karsenti and Maro, 1986; Kalt and Schliwa, 1993; Archer and Solomon, 1994). In our experiments we found that chromosomes can enhance assembly but only when a separate nucleation center, the centrosome, is present. For example, chromosomes placed in the cytoplasm at some distance from a centrosome do not induce microtubule assembly around themselves. Our evidence obtained in meiotic spermatocytes agrees well with the findings in mitotic echinoderm embryos that chromosomes cannot organize a spindle in the absence of centrosomes (Sluder and Rieder, 1985; Rieder and Alexander, 1990). In contrast, it differs from the findings in some meiotic systems. For instance, chromosomes alone are competent to induce spindle formation in crane fly spermatocytes (Dietz, 1966; Steffen et al., 1986), in *Drosophila* spermatocytes (Church et al., 1986) and in *Drosophila* oocytes (Theurkauf and Hawley, 1992). Perhaps in some of these cells, the chromosomes are associated with dispersed centrosomal materials that serve as nucleation sites as found in other exceptional cases (Maro et al., 1985; Sawada and Schatten, 1988; Wilson and Forer, 1989; Casal et al., 1990; Messinger and Albertini, 1991).

It is conceivable that chromosomes promote spindle assembly by enhancing microtubule nucleation at the centrosome. Our preliminary results indicate that this is unlikely. There is good evidence that γ-tubulin is involved in microtubule nucleation by centrosomes (Oakley et al., 1990; Zheng et al., 1991; Stearns et al., 1994). Using immunofluorescence staining, we found that the amount of γ-tubulin associated with a centrosome did not change when a chromosome was placed nearby, although microtubule concentration was greatly enhanced by that chromosome. Furthermore, a chromosome placed close to a centrosome but out of the spindle, did not appreciably affect concentration of organized microtubules in the spindle.

Alternatively, chromosomes might stabilize spindle microtubules nucleated at centrosomes through direct chromatin–microtubule interaction. The stability of the entire microtubule array could then be propagated by crossbridging between microtubules at the surface of the chromosome and those further away. Some of our observations are consistent with this possibility. When the only remaining chromosome is placed at one side of the pole, the effect on microtubule density is initiated from that side and spreads gradually across the half-spindle (Figs. 1 and 10 *A1*). Furthermore, when a chromosome is placed at a pole whose centrosome has been removed, the microtubules at that pole are stabilized and remain in a coherent group, as long as the chromosome is present; but if a chromosome's kinetochore is introduced as a surrogate for the centrosome, the half-spindle disassembles (Figs. 7 and 10 *B2*). Chromatin–microtubule interaction as a way of stabilizing microtubules has also been suggested to account for the chromatin-associated microtubule arrays that are observed in vitro (Sawin and Mitchison, 1991). Crossbridge-like linkages between spindle fiber microtubules have repeatedly been seen in both plant and animal cells (Hepler and Jackson, 1968; McIntosh et al., 1979; Pickett-Heaps et al., 1982; McIntosh, 1983; Saxton and McIntosh, 1987; Masuda et al., 1988), and such cross-bridges may also link chromatin and nonkinetochore microtubules (Fuge, 1990; Fuge and Falke, 1991).

Since microtubules that do not contact a chromosome directly are nonetheless affected by the presence of the chromosome, the involvement of a diffusible factor can be postulated. The diffusible factor might be a protein that stabilizes microtubules, for example, by cross-linking them or by capping their ends (reviewed by Salmon, 1989). Yeo et al. (1994*a*) have identified a new chromosomal protein, RMSA-1, that may well play an essential but as yet unspecified role in spindle assembly. Spindle organization is disrupted when antibodies to RMSA-1 are injected into cells. The protein has been found to be associated with meiotic chromosomes in crane flies (Yeo et al., 1994*b*). A similar protein could well be present in grasshopper spermatocytes.

Chromosomes and the Fate of the Spindle

We find that the presence or absence of a single chromo-

some can determine the fate of a spindle (Fig. 5). So long as a single chromosome is present in the spindle, the spindle persists (Fig. 5 *B*), but as soon as that chromosome is detached and removed to the cytoplasm the spindle rapidly disassembles (Figs. 5 *A* and 10 *A3*). An obvious possible explanation is that the spindle falls apart because it is no longer subject to the stabilizing influence of a chromosome. Paradoxically, however, the spindle persists when the last chromosome is altogether removed from the cell (Fig. 5 *C*), so the simple absence of a chromosome's influence does not suffice as an explanation for spindle disassembly. Whatever the mechanism may be, chromosomes apparently play a more important role in spindle assembly than we previously thought. Chromosomes may be directly involved in spindle assembly or they may simply regulate the process.

It is worth noting that the dissolution of the spindle resulting when the only remaining chromosome is moved to the cytoplasm occurs concomitantly with the formation of large asters. In some instances, microtubules emanating from these asters can be captured by the chromosome, an event that leads to reassembly of the spindle.

What is the normal function of the effect of chromosomes on microtubule assembly or stability? Perhaps this action is an essential component of spindle formation. We produce a situation similar to that after normal nuclear envelope breakdown when we place the last chromosome left in the cell in a chromosome-free spindle (Figs. 1 and 10 *A1*). The increased density of microtubules that results from chromosome introduction may mimic the activation of spindle assembly that occurs upon nuclear envelope breakdown. Indeed, spindle birefringence and microtubule content increase dramatically at this stage in the mitotic process (Roos, 1973; Inoué and Sato, 1967). Karsenti et al. (1984) have found that injected nuclei activate the nearby centrosomes, whereas distant centrosomes remain inactive. Taken together, our results support the proposal (Yeo et al., 1994*a*, *b*) that chromosomes, like centrosomes, make an indispensable contribution to microtubule assembly in normal spindle formation.

We thank Dr. S. Inoué and Nikon, Inc. for their generous help in making our polarization microscopy possible; A. McKibbins and S. Ward for expert technical assistance; Dr. D. Maroni for a critical reading of the manuscript.

This work was supported in part by a Charles W. Hargitt Research Fellowship in Cell Biology from Duke University and by grant GM-13745 from the Institute of General Medical Science, National Institutes of Health.

Received for publication 6 December 1994 and in revised form 13 March 1995.

References

Archer, J., and F. Solomon. 1994. Deconstructing the microtubule-organizing center. *Cell.* 76:589–591.

Bajer, A. S. 1982. Functional autonomy of monopolar spindle and evidence for oscillatory movement in mitosis. *J. Cell Biol.* 93:33–48.

Bajer, A. S., and J. Mole-Bajer. 1969. Formation of spindle fibers, kinetochore orientation and behavior of the nuclear envelope during mitosis in endosperm. *Chromosoma (Berl.).* 27:448–484.

Brinkley, B. R., S. M. Cox, D. A. Pepper, L. Wible, S. L. Brenner, and R. L. Pardue. 1981. Tubulin assembly sites and the organization of cytoplasmic microtubules in cultured mammalian cells. *J. Cell Biol.* 90:554–562.

Casal, J., C. González, and P. Ripoll. 1990. Spindles and centrosomes during male meiosis in Drosophila melanogaster. *Eur. J. Cell Biol.* 51:38–44.

Church, K., R. B. Nicklas, and H.-P. P. Lin. 1986. Micromanipulated bivalents can trigger mini-spindle formation in *Drosophila melanogaster* spermatocyte cytoplasm. *J. Cell Biol.* 103:2765–2773.

Dietz, R. 1966. The dispensability of the centrioles in the spermatocyte divisions of *Pales ferruginea* (Nematocera). *Heredity* (Suppl.). 19:161–166.

Ellis, G. W., and D. A. Begg. 1981. Chromosome micromanipulation studies. *In* Mitosis/Cytokinesis. A. M. Zimmermann, and A. Forer, editors, Academic Press, NY. 155–179.

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

Fuge, H., and D. Falke. 1991. Morphological aspects of chromosome spindle fibers in *Mesostoma* "microtubular fir-tree" structures and microtubule association with kinetochores and chromatin. *Protoplasma.* 160:39–48.

Hayden, J. H., S. S. Bowser, and C. L. Rieder. 1990. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. *J. Cell Biol.* 111:1039–1045.

Hepler, P. K., and W. T. Jackson. 1968. Microtubules and early stages of cell-plate formation in the endosperm of *Haemanthus katherinae* Baker. *J. Cell Biol.* 38:437–446.

Inoué, S., and H. Sato. 1967. Cell motility by labile association of molecules. *J. Gen. Physiol.* 50:259–292.

Kalt, A., and M. Schliwa. 1993. Molecular components of the centrosome. *Trends Cell Biol.* 3:118–128.

Karsenti, E., and B. Maro. 1986. Centrosomes and the spatial distribution of microtubules in animal cells. *Trends Biochem. Sci.* 11:460–463.

Karsenti, E., J. Newport, R. Hubble, and M. Kirschner. 1984. Interconversion of metaphase and interpahse microtubule arrays, as studied by the injection of centrosomes and nuclei into *Xenopus* eggs. *J. Cell Biol.* 98:1730–1745.

Kiehart, D. P. 1982. Microinjection of echinoderm eggs: apparatus and procedures. *Methods Cell Biol.* 25:13–31.

Kirschner, M., and T. Mitchison. 1986. Beyond self-assembly: from microtubules to morphogenesis. *Cell.* 45:329–342.

Marek, L. F. 1978. Control of spindle form and function in grasshopper spermatocytes. *Chromosoma (Berl.).* 68:367–398.

Maro, B., K. K. Howlett, and M. Webb. 1985. Non-spindle microtubule organizing centers in metaphase-arrested mouse oocytes. *J. Cell Biol.* 101:1665–1672.

Masuda, H., K. L. McDonald, and W. Z. Cande. 1988. The mechanism of anaphase spindle elongation: uncoupling of tubulin incorporation and microtubule sliding during in vitro reactivation. *J. Cell Biol.* 107:623–633.

Mazia, D. 1961. Mitosis and the physiology of cell division. *In* The Cell. Vol. 3. Academic Press, Inc., New York. 77–412.

Mazia, D., N. Paweletz, G. Sluder, and E.-M. Finze. 1981. Cooperation of kinetochores and pole in the establishment of monopolar mitotic apparatus. *Proc. Nat. Acad. Sci. USA.* 78:377–381.

McIntosh, J. R. 1983. The centrosome as organizer of the cytoskeleton. *Modern Cell Biol.* 2:115–142.

McIntosh, J. R., K. L. McDonald, M. K. Edwards, and B. M. Ross. 1979. Three-dimensional structure of the central mitotic spindle of Diatoma vulgare. *J. Cell Biol.* 83:428–442.

Messinger, S. M., and D. F. Albertini. 1991. Centrosome and microtubule dynamics during meiotic progression in the mouse oocyte. *J. Cell Sci.* 100:289–298.

Mitchison, T. J., and M. W. Kirschner. 1985. Properties of the kinetochore in vitro. II. Microtubule capture and ATP-dependent translocation. *J. Cell Biol.* 101:766–777.

Mitchison, T., L. Evans, E. Schultze, and M. Kirschner. 1986. Sites of microtubule assembly and disassembly in the mitotic spindle. *Cell.* 45:515–527.

Moens, P. B. 1979. Kinetochore microtubule numbers of different sized chromosomes. *J. Cell Biol.* 83:556–561.

Nicklas, R. B. 1971. Mitosis. *Adv. Cell Biol.* 2:225–297.

Nicklas, R. B., and G. W. Gordon. 1985. The total length of spindle microtubules depends on the number of chromosomes present. *J. Cell Biol.* 100:1–7.

Nicklas, R. B., and D. F. Kubai. 1985. Microtubules, chromosome movement, and reorientation after chromosomes are detached from the spindle by micromanipulation. *Chromosoma (Berl.).* 92:313–324.

Nicklas, R. B., and S. C. Ward. 1994. Elements of error correction in mitosis: microtubule capture, release, and tension. *J. Cell Biol.* 126:1241–1253.

Nicklas, R. B., L. E. Krawitz, and S. C. Ward. 1993. Odd chromosome movement and inaccurate chromosome distribution in mitosis and meiosis after treatment with protein kinase inhibitors. *J. Cell Sci.* 104:961–973.

Oakley, B. R., C. E. Oakley, Y. Yoon, and M. K. Jung. 1990. γ-Tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans. Cell.* 61:1289–1301.

Pickett-Heaps, J. D., D. H. Tippit, and K. R. Porter. 1982. Rethinking Mitosis. *Cell.* 29:729–744.

Rieder, C. L., and S. P. Alexander. 1990. Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. *J. Cell Biol.* 110:81–96.

Rieder, C. L., J. G. Ault, U. Eichenlaub-Ritter, and G. Sluder. 1993. Morphogenesis of the mitotic and meiotic spindle: conclusions obtained from one system are not necessarily applicable to the other. *In* Chromosome Segregation and Aneuploidy. B. K. Vig, editor. NATO ASI Series. Vol. H72. Springer-Verlag, Berlin Heidelberg. 183–197.

Roos, U.-P. 1973. Light and electron microscopy of rat kangaroo cells in mito-

sis. I. Formation and breakdown of the mitotic apparatus. *Chromosoma (Berl.)*. 40:43–82.

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

Salmon, E. D., M. McKeel, and T. Hays. 1984*a*. Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. *J. Cell Biol.* 99:1066–1075.

Salmon, E. D., R. J. Leslie, W. M. Saxton, M. L. Karow, and J. R. McIntosh. 1984*b*. Spindle microtubule dynamics in sea urchin embryos. Analysis using fluorescence-labeled tubulin and measurements of fluorescence redistribution after laser photobleaching. *J. Cell Biol.* 99:2165–2174.

Sawada, T. O., and G. Schatten. 1988. Microtubules in *Ascidian* eggs during meiosis, fertilization, and mitosis. *Cell Motil. Cytoskeleton.* 9:219–230.

Sawin, K. E., and T. J. Mitchison. 1991. Mitotic spindle assembly by two different pathways in vitro. *J. Cell Biol.* 112:925–940.

Saxton, W. M., and J. R. McIntosh. 1987. Interzone microtubule behavior in late anaphase and telophase spindles. *J. Cell Biol.* 105:875–886.

Saxton, W. M., D. L. Stemple, R. J. Leslie, E. D. Salmon, M. Zavortink, and J. R. McIntosh. 1984. Tubulin dynamics in cultured mammalian cells. *J. Cell Biol.* 99:2175–2186.

Sluder, G., and C. L. Rieder. 1985. Experimental separation of pronuclei in fertilized sea urchin eggs: chromosomes do not organize a spindle in the absence of centrosomes. *J. Cell Biol.* 100:897–903.

Sluder, G., F. J. Miller, and C. L. Rieder. 1986. The reproduction of centrosomes: nuclear versus cytoplasmic controls. *J. Cell Biol.* 103:1873–1881.

Steffen, W., H. Fuge, R. Dietz, M. Bastmeyer, and G. Muller. 1986. Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation. *J. Cell Biol.* 102:1679–1687.

Stearns, T., and M. Kirschner. 1994. In vitro reconstitution of centrosome assembly and function: the central role of γ-tubulin. *Cell.* 76:623–637.

Theurkauf, W. E., and R. S. Hawley. 1992. Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* 116:1167–1180.

Vandré, D. D., P. Kronebusch, and G. G. Borisy. 1984. The interphase-mitosis transition: microtubule rearrangements in cultured cells and sea urchin eggs. *In* Molecular Biology of the Cytoskeleton. G. G. Borisy, D. W. Cleveland, and D. B. Murphy, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 3–16.

Wadsworth, P. 1993. Mitosis: spindle assembly and chromosome motion. *Curr. Opin. Cell Biol.* 5:123–128.

Wilson, P. J., and A. Forer. 1989. Acetylated alpha tubulin in spermatogenic cells of the crane fly *Nephrotoma suturalis*: kinetochore microtubules are selectively acetylated. *Cell Motil. Cytoskeleton.* 14:237–250.

Yeo, J.-P., F. Alderuccio, and B.-H. Toh. 1994*a*. A new chromosomal protein essential for mitotic spindle assembly. *Nature (Lond.)*. 367:288–291.

Yeo, J.-P., A. Forer, and B.-H. Toh. 1994*b*. A homologue of human regulator of mitotic spindle assembly protein (RMSA-1) is present in crane fly and associates with meiotic chromosomes. *J. Cell Sci.* 107:1845–1851.

Zheng, Y., M. K. Jung, and B. R. Oakley. 1991. γ-Tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell.* 65:817–823.