The Total Length of Spindle Microtubules Depends on the Number of Chromosomes Present

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ABSTRACT We extracted chromosomes by micromanipulation from *Melanoplus differentialis* spermatocytes, producing metaphase spindles with only one or a few chromosomes instead of the usual complement of 23. Cells with various numbers of chromosomes were prepared for electron microscopy, and spindle microtubule length was measured. A constant increment of microtubule length was lost upon the removal of each chromosome; we estimate that only ~40% of the original length would remain in the total absence of chromosomes. Unexpectedly, kinetochore microtubules were not the only ones affected when chromosomes were removed: nonkinetochore microtubules accounted for a substantial fraction of the total length lost. No compensatory increase in microtubule length outside the spindle was found. Studies by others show that the kinetochore microtubules of extracted chromosomes are left behind in the cell and dissassemble. The resulting increase in subunit concentration would be expected from in vitro studies to drive microtubule assembly until the original total microtubule length was restored, but that did not happen in these living cells.

We conclude that the assembly of a certain, large fraction of microtubule subunits into stable microtubules is dependent on the presence of chromosomes. Possible explanations include (*a*) limits on microtubule length that prevent any net assembly of the subunits released after chromosomes are removed or (*b*) a promotion of microtubule assembly by chromosomes, which therefore is reduced in their absence. Chromosome-dependent regulation of microtubule length may account for some features of normal mitosis.

Microtubules in the mitotic spindle vary in length within definite limits, and controlled changes in length occur as chromosomes move. Cellular control of microtubule length could be very simple, involving only the basic principles of nucleated self-assembly. If preformed initiation sites are present in addition to tubulin, then elongation by the addition of tubulin subunits at the initiation sites may be the predominant reaction. In that case, the initial tubulin concentration would determine the total microtubule length at the steady state, and the number of initiation sites would determine the number of microtubules—the number of pieces into which the total length is split. A defined number of microtubules of a definite average length would result. Bryan (2) showed that this is just what happens in vitro when assembly occurs in the presence of microtubule fragments that act as initiation sites. At constant initial tubulin concentrations, the length of each microtubule is related simply to the number per unit volume of microtubule fragments: if the number of fragments is halved, the microtubules formed are twice as long, on the average (2).

Do the tubulin concentration and the number of initiation sites suffice to explain microtubule length in living cells? Marek (8) made the first attempt at a test. By micromanipulation, he detached chromosomes from the spindle and extracted them from the cell. He then used polarized light microscopy of the living cells to determine the "volume birefringence," a measure of the total aligned fibrous material in the spindle. To consider the results in terms of microtubule length control, Marek had to assume (*a*) that the kinetochore microtubules originally attached to the extracted chromosomes remain behind in the cell and break down into tubulin subunits, and (*b*) that volume birefringence is a reliable measure of the total length of spindle microtubules. Marek made convincing arguments that both assumptions are reasonable but emphasized that final proof was lacking. Given those assumptions, the expectations are clear. If microtubule length in living cells is determined as it is in vitro (2), then volume birefringence should be a constant, independent of chromosome number. Even if many chromosomes are removed, the subunits released from their kinetochore microtubules should

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reassemble onto the remaining spindle microtubules until the original steady-state microtubule content is reestablished. Unexpectedly, nothing of the kind was found. Instead, the steady-state volume birefringence dropped as chromosomes were removed and was linearly related to the number of chromosomes remaining on the spindle (8). This raised the possibility that the total length of spindle microtubules is affected by chromosomes, and not by the tubulin concentration alone, as in vitro.

Direct evidence that microtubule length can be controlled by something other than the tubulin concentration was recently obtained by Brinkley et al. (1). They studied microtubule assembly at centrosomes in lysed interphase cells. In the presence of added tubulin, a number of microtubules grew from each centrosome to a fixed length—increasing the tubulin concentration beyond a certain value was without further effect on microtubule length. Moreover, the final microtubule length attained was very different in lysed 3T3 cells as compared to their virus-transformed counterparts. Brinkley et al. (1) concluded that microtubule number and final length may well be controlled in living cells, as in the lysed cells they studied, by microtubule organizing centers like the centrosome and the surrounding cytoplasm, not by the tubulin concentration.

We have returned to Marek's (8) approach in an attempt to get unambiguous information on microtubule length regulation in unlysed, living cells. Electron microscopic measurements of microtubule length in cells with many and with few chromosomes are reported here. The results clearly demonstrate that chromosomes have a role in the determination of spindle microtubule length: a constant increment of microtubule length is lost with the removal of each chromosome.

MATERIALS AND METHODS

Materials: Spermatocytes from a laboratory colony of the grasshopper *Melanoplus differentialis* (Thomas) were cultured as previously described (10) except that, to minimize evaporation, the testicular follicles were transferred to a culture chamber filled with halocarbon oil before the follicles were cut and the cells were spread on the coverslip.

Micromanipulation: Light microscopy and micromanipulation instruments and procedures were as previously described (12). Natural cell-to-cell variation in spindle organization (birefringence) is prevalent in cultured spermatocytes (8), but should be minimal in nearby cells from the same culture. Therefore, spermatocytes in metaphase I that were near numerous other metaphases were chosen for micromanipulation. When possible, a second, nearby cell was used for a second experiment or as an unmanipulated control. Microtubule length was measured in six cells, a pair of neighboring cells from each of two cultures plus single cells from each of two more cultures.

Bivalents were extracted from the cells as described by Marek (8). Briefly, one or more bivalents were detached from the spindle and were moved away from the spindle, distending the cell membrane. Soon, only a thin strand of tightly appressed membrane connected the chromosome(s) and the cell. The connection then snapped, yielding a mini-cell containing the extracted chromosome(s). The volume of the mini-cell was only slightly greater than that of the chromosome(s). The sequence of detachment and extraction was repeated until the manipulated cell contained the desired number of chromosomes. About one cell in three died after the operation, probably because the membrane failed to seal completely; in these, overcondensed chromosomes and collapsing spindles were soon evident and such cells were rejected as objects for electron microscopy.

The cells were followed in life for at least 30 min after the last operation. This allowed ample time for spindle microtubules to reestablish a steady state. Fixation was further delayed until the cells were in late metaphase in order to reduce variation due to differences between mitotic stages. Late metaphase was judged by completion of chromosome congression and in some cases by the beginning of anaphase in other cells nearby (cells from the same testicular cyst enter anaphase within ~20 min of each other). The time from completion of the last operation to the start of fixation varied from 39 to 66 min for cells in which microtubule length was measured.

Fixation and Electron Microscopy: Cells were prepared for electron microscopy as described previously (reference 11 and references therein). Briefly, fixation was begun by injecting agar-treated glutaraldehyde into the aqueous medium near the target cells. After removal of the oil over the cells by flushing with additional fixative, further processing and flat-embedding were carried out by standard procedures. The cells were serially sectioned in a plane longitudinal to the spindle axis at a thickness of 80–90 nm.

A Zeiss 10A electron microscope (Carl Zeiss, Inc., New York) operated at 80 kV was used to make micrographs of the serial sections at a magnification of × 2,700 or × 3,400 on 70-mm roll film (film type 613, Chemco, Glen Cove, NY). The magnification was determined for each cell from a micrograph of a grating replica.

Microtubule Length Measurements: Microtubule length was measured in tracings prepared from a sample of the serial electron micrographs for each cell. The sample consisted of half of the spindle in every fifth section.

Micrograph negatives were magnified 14 times using an aerial film viewer (11). A transparent polyester plastic sheet was placed over the screen of the viewer and all the microtubule profiles seen in the selected sections were traced on the sheet, along with chromosome outlines and mitochondria. This was repeated for every fifth section through the whole series of 100 or more sections for each cell (if the fifth section in a group was missing or unusable, then a nearby section was traced). Every kinetochore in the cell was represented in approximately the same fraction of the selected sections as in the entire set of sections. This unbiased sample of kinetochores should ensure an unbiased sample of kinetochore microtubules, which are major contributors to the total microtubule length.

The sampled spindle region was the half-spindle that did not contain the X-chromosome, delimited as follows. Mitochondria surrounding the spindle defined the lateral spindle boundary, and the position of the equator was determined from the midpoints of the bivalents (if several were present) or from half the distance between the poles (defined by pericentriolar material or the region of microtubule convergence). The boundaries so defined are in regions of relatively low microtubule density, and hence the measurement of total microtubule length is insensitive to small inconsistencies in boundary placement. The region defined as "spindle" is necessarily somewhat arbitrary, but a smaller region would exclude some undoubted spindle microtubules (peripheral kinetochore microtubules) whereas a larger region would include little additional microtubule length.

The lengths of the lines representing microtubules were measured with an electronic planimeter (model 1224, Numonics, Corp., Landsdale, PA) or a digitizer (Numonics model 1220) interfaced with a microcomputer (model 2600, Vector Graphics, Thousand Oaks, CA). The lengths of the lines in the tracings were divided by the magnification factor to obtain the corresponding microtubule lengths, which then were summed for the sampled spindle region of each cell. For two of the six cells examined, microtubule length was also measured in that half of the half-spindle nearer the pole and in the region outside of the spindle in the half of the cell containing the measured half-spindle.

Errors of Measurement: The error in planimetry per se is very low—<0.2% of the total measured length, as determined directly from measurements of lines of known length.

Foreshortening is a potential source of error, but the error can be shown to be small and can be reduced to a negligible level by a correction factor. Foreshortening can occur when the microtubule axis is not parallel to the plane of sectioning. Then, when electron micrographs are made with the sections at the usual angle of 90° to the electron beam, the projected image of the microtubule is shorter than its true length. The effect of foreshortening is easily calculated for microtubules that traverse the full thickness of the section: the actual length in one section equals the square root of the sum of the squares of the apparent length (as measured on the micrograph) and the section thickness (taken as 85 nm). The effect of foreshortening drops quickly with increasing apparent length. For instance, if the apparent length is 0.5 μm, the actual length is 0.507 μm: only 1% greater. Computer analysis makes it easy to calculate the maximum (see below) foreshortening error for the actual measurements. The data for spindle microtubules (the measured apparent length of each segment in each section) for two cells were sorted into length classes (0–0.1 μm, 0.1–0.2 μm, 0.2–0.3 μm, etc.), and from the number of segments in each class, the apparent total length for that class was calculated. The foreshortening error for that length class was calculated as above and used to obtain a corrected length. This was summed for all length classes to give the corrected total microtubule length for the cell; from this an overall foreshortening factor was obtained by dividing the corrected total by the originally measured total.

The overall maximum foreshortening factor was determined for two cells, one with all 11 bivalents and one with none (only the X-chromosome remained). The two factors were identical to three digits: 1.03. Thus the correction for foreshortening was very small, only 3% of total length, and did not differ for spindles with very different numbers of chromosomes. The calculation gives

a maximum value for foreshortening because it is assumed that all microtubule segments in a section traverse the whole thickness of the section. However, the error due to foreshortening is so small that the exact value used as a correction factor hardly matters; a value of 1.02 was chosen as a reasonable estimate.

Kinetochore Microtubule Counts: The number of kinetochore microtubules at their insertion into the kinetochore was counted as previously described, with an estimated accuracy of ±10% (11).

Two-dimensional Reconstructions: To provide a qualitative view of microtubule distribution, we reconstructed the chromosomes and microtubules in a comparable region (near the interpolar axis) of two cells. The aerial film viewer was used as described above to trace onto a single sheet of plastic the microtubules from 10 sections and the chromosome outlines and the centriole(s) from one of those sections. Because only a survey view was desired, alignment of microtubule ends from adjacent sections was only approximate, and for clarity, the few microtubules that overlapped the chromosome outlines were omitted.

RESULTS

Terminology and Perspective

The portion of the spindle microtubule length that varies with the number of chromosomes present will be designated the "chromosome-dependent spindle," and the remainder, which would be present in the total absence of chromosomes, the "chromosome-independent spindle."

The normal chromosome complement in *Melanoplus differentialis* spermatocytes at division I is 11 bivalents (pairs of homologous chromosomes) plus the X-chromosome, or 23 chromosomes in all. Marek (8) succeeded in reducing the number of bivalents to as few as two, and we have succeeded in reducing the number to zero, leaving only the X-chromosome. Thus, the number of chromosomes can be reduced to only 4% of the number normally present.

Three particular cells will be referred to specifically, and will be identified according to the number of chromosomes present: "cell *23*" is a cell with the full complement of 23 chromosomes, "cell *11*" is one with 11 chromosomes, and "cell *1*" is one of the two cells with only the X-chromosome.

Marek (8) established the healthiness of cells from which chromosomes have been extracted by showing that anaphase is normal in cells with as few as two bivalents plus the X-chromosome. To this we can add the observation of normal anaphase in a cell with only one bivalent plus the X-chromosome and also a normal prometaphase pole-to-pole movement of the X-chromosome in a cell in which it was the only remaining chromosome.

Spindle Microtubule Length

MEASURED VALUES: Microtubule length plotted against the number of chromosomes present is shown in Fig. 1. The six cells examined span the full range from one with the normal chromosome number of 11 in the measured half-spindle (22 plus the X-chromosome in all) to two cells with none (the X-chromosome in the unmeasured half-spindle was the sole remaining chromosome). Obviously, spindle microtubule length was not independent of the number of chromosomes, but instead was greatly reduced when few chromosomes were present. The dashed line in Fig. 1 is from a linear regression analysis. The coefficient of determination is 0.804: 80% of the variation is explained by a linear dependence of microtubule length on the number of chromosomes. Also, a linear relationship is acceptable on an analysis of variance computation ($F_{1,4} = 16.43$, $P = 0.015$): a large and significant fraction of the variance has been explained.

The equation for the regression line shown in Fig. 1 is $Y = 863 + 111.5X$, where Y is the microtubule length (in micrometers) within the sampled region and X is the number of chromosomes. This equation provides quantitative estimates of the partition of spindle microtubule length into a chromosome-independent portion (863 μm) and a chromosome-dependent portion, which is related to the number of chromosomes present (111.5 μm per chromosome).

CALCULATED VALUES FOR THE WHOLE SPINDLE: Constants for the whole spindle (Table I *A*) were calculated from the regression equation estimates just mentioned and used to estimate microtubule length in a spindle with the full complement of 23 chromosomes (Table I *B*).

Two-dimensional Reconstructions

Two-dimensional reconstructions of comparable regions of two cells are shown in Figs. 2 (cell *23*) and 3 (cell *1*). The lower microtubule content in cell *1* is obvious, and so is the major reason for the difference: the spindle containing only the X-chromosome (Fig. 3) is narrower (only about half as wide). In this instance, the spindle with fewer than the usual number of chromosomes is shorter than the control, but that is atypical (8). Quantitative differences aside, however, the

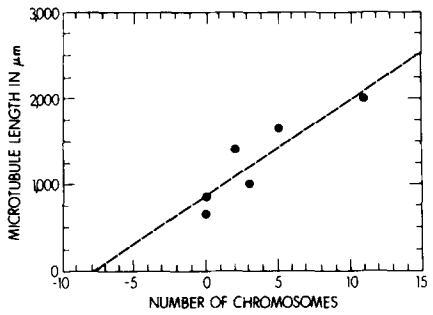


FIGURE 1 Spindle microtubule length as a function of the number of chromosomes on the spindle. The plotted values (filled circles) are from the measured sample of each spindle (every fifth section of the half-spindle lacking the X-chromosome). In consequence, a spindle with the full complement of 23 chromosomes is shown here as having 11—the number in the sampled region. The dashed line is from a linear regression analysis.

TABLE I

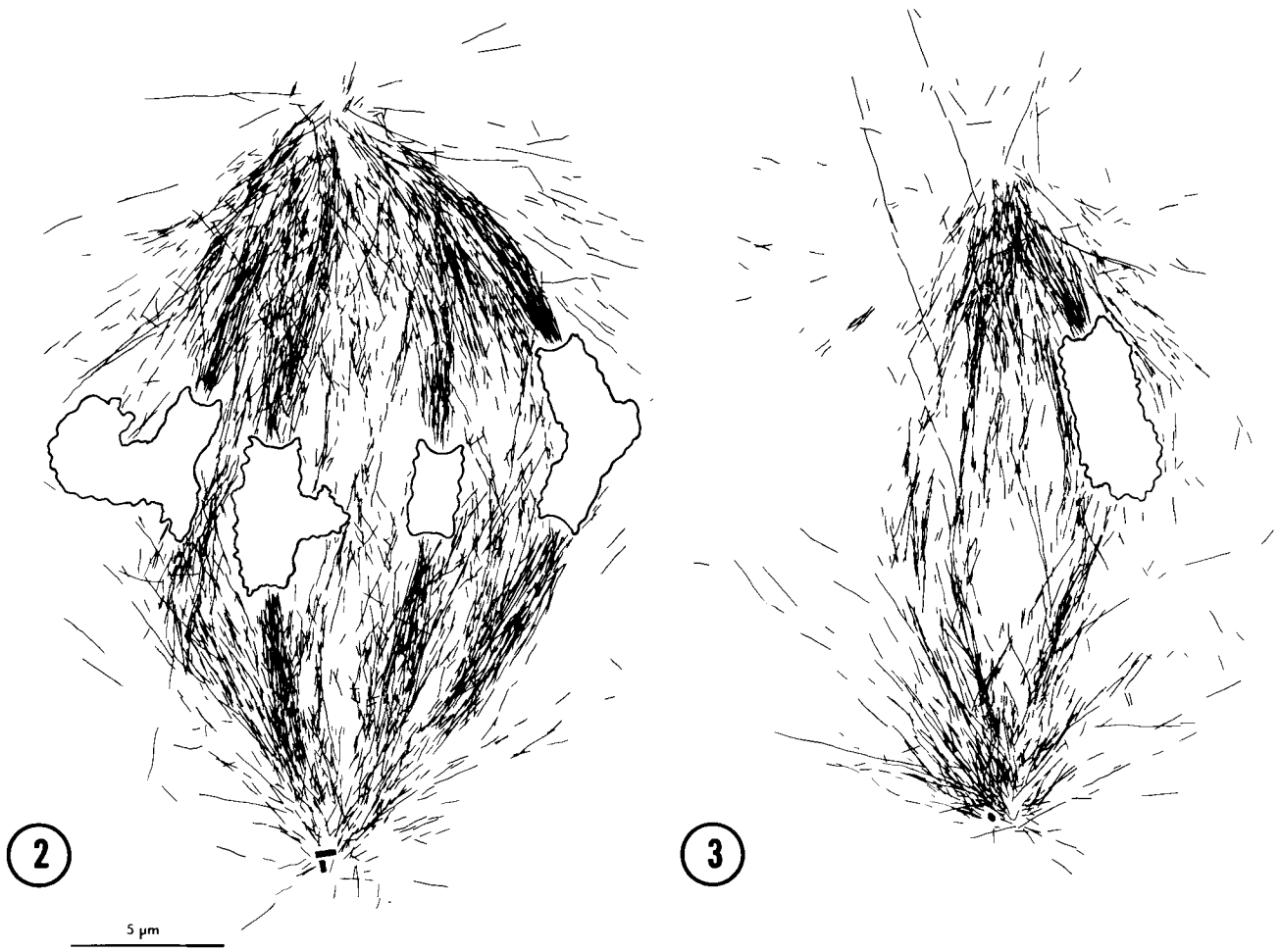
Calculated Values for Microtubule Length in Whole Spindles

	Length of microtubules
	μm
A. Constants	
Chromosome-independent spindle*	8,800
Chromosome-dependent spindle, per chromosome‡	569
B. Values for a cell with 23 chromosomes	
Chromosome-dependent spindle	13,100
Total for whole spindle§	21,900
Chromosome-independent spindle as percent of total	40%

* From the regression equation (see text) value of 863 μm for the sampled region, multiplied by 2 (to include the other half-spindle), by 5 (since only every fifth section was measured), and by 1.02 (to correct for foreshortening).

‡ From the regression equation value of 111.5, multiplied by 5 and by 1.02.

§ 8,800 plus 13,100.



FIGURES 2 and 3 Two-dimensional reconstruction of part of the spindle in a cell containing the full complement of 23 chromosomes (Fig. 2) and a cell containing only the X-chromosome (Fig. 3). Comparable spindle regions are shown in both cells: starting from the level of the centriole(s) at one pole and proceeding 10 sections toward the level of the centriole(s) at the other pole. The reconstructions are reproduced at the same magnification.

spindle containing only the X-chromosome (Fig. 3) is remarkably similar to the spindle with the full chromosome complement (Fig. 2). Thus, in both spindles, most microtubules are roughly parallel to the interpolar axis and to each other, but in both a substantial number are less regularly disposed. An equally important similarity is the paucity of microtubules outside the spindle. To be sure, a few long microtubules extend beyond the spindle in the cell with only one chromosome (Fig. 3), and these have no counterparts, in this sample at least, in the cell with the full complement of chromosomes (Fig. 2). Nevertheless, it is evident that in both cells the microtubules are concentrated in the region between the poles and the rest of the cell has very few.

Additional Measurements of Microtubule Length

For cell *23* and cell *1*, microtubule length has been measured in the region of the half-spindle near the pole and also in the region outside the spindle (Table II *A*).

The microtubule length near the pole as a percentage of the total half-spindle length is similar for the two cells: 40% for cell *23* and 44% for cell *1* (from data in Table II *A*). Thus the distribution of microtubule length near the pole versus near the equator probably changes little if at all as chromosomes are removed.

TABLE II

Microtubule Length Measurements in Two Cells, One with 23 Chromosomes (Cell 23) and One with 1 Chromosome (Cell 1)

	Cell *23*	Cell *1*
	μm	
A. Length of microtubules*		
Entire half-spindle	1,979	848
Poleward half of the half-spindle	784	377
Outside the half-spindle	192	106
B. Length in separated components		
Chromosome-independent spindle: length near the pole as percent of total	44%‡	
Chromosome-dependent spindle: length near the pole as percent of total	36%§	

* Measured values in delimited regions of every fifth section, half-spindle without the X-chromosome.

‡ From values for cell 1, which has no chromosomes in the measured half-spindle: (length near pole/length in entire half-spindle) ×100 = (377/848) × 100 = 44%.

§ From values for cell 23 minus those for cell 1: chromosome-dependent spindle near pole = 784 − 377 = 407 μm; chromosome-dependent portion of entire half-spindle = 1,979 − 848 = 1,131 μm; (407/1131) × 100 = 36%.

A similar comparison can be made for separated components of the spindle: the chromosome-dependent and independent components (Table II *B*). For both components,

~40% of their length is in the poleward half. In other words, both in the absence of chromosomes and in their presence, the equatorial region is richer in microtubule content. These are only rough estimates, of course, since measurements on only two cells are involved.

The microtubule population outside the spindle is small in both cells (Table II *A*). As a percentage of microtubule length within the half-spindle, the length outside is 10% for cell *23* and 12% for Cell *1*. Note particularly that the absolute length of microtubules outside the spindle in the cell with only one chromosome is even less, rather than more, than in the cell with all 23 chromosomes (Table II *A*). Qualitative observations on the other cells fit with these measurements: regardless of chromosome number, few microtubules are seen outside the spindle.

Kinetochore Microtubule Counts

The number of kinetochore microtubules is roughly constant regardless of how many chromosomes were removed from each cell (Table III). Though the sample is small, any significant change in kinetochore microtubule number correlated with the large change in chromosome number is ruled out.

DISCUSSION

Defining the Problem: What is Affected When Chromosomes Are Removed from the Cell?

MICROTUBULE LENGTH AND VOLUME BIREFRINGENCE: An increment of spindle microtubule length was lost for each chromosome removed from the cells. In the total absence of chromosomes, only ~40% of the original microtubule length would remain (Table I). These findings are in good agreement with Marek's (8) results from birefringence measurements. He found an incremental loss of volume birefringence upon chromosome removal and estimated that ~47% would remain in the absence of chromosomes. The microtubule length that would remain in the absence of chromosomes (the "chromosome-independent spindle" in our terminology) can also be estimated from the *x*-axis intercept of the regression line (Fig. 1). On that basis, microtubule length in the chromosome-independent spindle is equivalent to that contributed by eight chromosomes. The corresponding figure for volume birefringence measurements is nine chromosomes (8). Hence the relationship between chromosomes and spindle organization established by Marek's extensive experiments is confirmed in our small sample. What we have added is unequivocal evidence that chromosome removal affects spindle microtubules.

These data also bear on the quantitative relationship between volume birefringence and microtubule length. On the assumption that only microtubules contribute to spindle birefringence, it was calculated that the volume birefringence measured in *Melanoplus* spermatocytes with the full complement of chromosomes corresponds to a total spindle microtubule length of 29,000 μm (8; a recalculation using current values for tubulin dimensions gives a length of 28,000 μm). Our direct electron microscopic measurements of microtubule length yield a value of 22,000 μm for the whole spindle (Table I), >75% of the length expected from birefringence measurements. In fact, the agreement is still better, because the value from birefringence is a maximum (8) and the value from electron microscopy is a minimum, since some microtubule profiles inevitably are missed when the tracings are made from the micrographs. We conclude that our current electron microscopic procedures preserve more than three-quarters of the spindle microtubule length present in living *Melanoplus* spermatocytes. No better quantitative information on spindle microtubule length preservation is available for whole cells; only in isolated spindles has it been demonstrated that the preserved microtubule length is sufficient to account for spindle birefringence (15).

THE FATE OF THE KINETOCHORE MICROTUBULES OF EXTRACTED CHROMOSOMES: When chromosomes are detached from the spindle and then extracted from the cell, are their kinetochore microtubules left behind in the cell? Electron microscopic studies (10; Nicklas R. B., and D. F. Kubai, in preparation) show that chromosome detachment from the spindle is genuine and complete: if the cell is fixed soon after detachment, no microtubules are usually seen at the kinetochore (rare exceptions have one or two short kinetochore microtubules). The possibility that kinetochore microtubules remain associated with the chromosome but are not preserved can be rejected. In a few cells fixed within 30 s after detachment, the former kinetochore microtubules were identified by their number and position near the microtubule-free kinetochore (Nicklas R. B., and D. F. Kubai, in preparation).

So the kinetochore microtubules certainly are left behind. Equally certainly, they soon disassemble into something no longer recognizable as microtubules, since microtubule length is lower in cells from which chromosomes have been extracted. The critical question is whether these microtubules disassemble into assembly-competent subunits, that is, into tubulin that can reassemble into microtubules in appropriate circumstances.

Marek's (8) experiments help answer this question. In some experiments, several chromosomes were temporarily removed from the spindle and moved to the cell periphery, but were not extracted from the cell. Eventually, the chromosomes reattached to the spindle, restoring its original condition. At first, spindle birefringence decreased, almost exactly to the same extent as if the chromosomes had been permanently removed, but as the chromosomes reassociated with the spindle, the birefringence returned to the original level. In another set of experiments that used cells containing two spindles, chromosomes were detached from one spindle and positioned so that they would reattach to the second spindle. Spindle birefringence invariably decreased in the spindle with the smaller number of chromosomes and increased in the spindle with the larger number. As Marek stressed, the significance

TABLE III

Kinetochore Microtubule Counts

		Number of kinetochore microtubules	
	Number of chromosomes	Autosomes (half-bivalents)	X-chromosome
Cell 23	23	43, 23, 41, 38	40
Cell 11	11	38, 39	38
Cell 1	1	—	31
Average		37	36

of these results depends on whether or not the detached chromosomes lose their original kinetochore microtubules. If not, the chromosomes would merely be transporters of kinetochore microtubules, from the spindle to the cell periphery and back again, or from one spindle to another. Electron microscope observations (10) validate the assumption that kinetochore microtubules are lost on detachment. In that case, to quote Marek (8), "... in these experiments, the subunits must remain in the cell and are incorporated into the spindle, but only as a chromosome interacts with the spindle." Thus, the experimental and the electron microscope observations combine to provide compelling evidence not only for the liberation and reincorporation of subunits from the kinetochore microtubules of detached chromosomes, but also for some role of the chromosome in that reincorporation.

MICROTUBULES OUTSIDE THE SPINDLE DO NOT GROW: As chromosomes are removed and microtubule length within the spindle decreases, a compensating growth of microtubules outside the spindle might be expected. This possibility is eliminated by qualitative observations on all the cells and by length measurements in two cells (Table II *A*).

KINETOCHORE MICROTUBULES ARE NOT THE ONLY ONES AFFECTED: It might be expected that only kinetochore microtubules would be lost when a chromosome is removed from the cell, but that is not so. The maximum length of a single kinetochore microtubule is the kinetochore-to-pole distance, which we measured for all 23 chromosomes in one cell (cell *23*) and obtained 10.7 μm as the average. Multiplication by the average number of kinetochore microtubules, 37 (Table III), gives 400 μm as the maximum length attributable to the kinetochore microtubules of one chromosome—too little to account for all 570 μm of the microtubule length associated with the presence or absence of a chromosome (Table I). From these figures, kinetochore microtubules account for no more than 70% of the chromosome-dependent microtubule length, and probably much less (in *Melanoplus* cells in anaphase, many kinetochore microtubules are shorter than the kinetochore-to-pole distance [11]). Note that this conclusion does not depend upon assumptions about microtubule preservation during preparation for electron microscopy since only the preserved number and length are in question: the preserved number of kinetochore microtubules is simply too low to account for the preserved microtubule length in the chromosome-dependent spindle.

It is interesting in itself that the presence or absence of a chromosome affects the existence of so many nonkinetochore microtubules that, unlike kinetochore microtubules, have no direct connection to the chromosome. It is also significant for the interpretations that follow.

Chromosomes and Spindle Microtubule Length: Possible Interpretations

The dependence of microtubule length on the number of chromosomes has now been characterized as follows: when chromosomes are removed from the spindle, kinetochore microtubules and some nonkinetochore microtubules break down into subunits, but no net reassembly of those subunits into stable microtubules occurs in the absence of chromosomes. This is not expected on the simple model of self-assembly that suffices to explain in vitro results (2). On that model, the liberated subunits would drive microtubule assembly until the subunit concentration dropped to the same value as before the chromosomes were removed, and the original total length of microtubules would be restored. In living spermatocytes, on the contrary, the steady-state microtubule length depends somehow on chromosomes, and not on the subunit concentration alone.

In the interpretations that follow, "chromosome" refers to the whole chromosome or to a part, such as the kinetochore. In fact, the active unit is likely to be one particular site on each chromosome rather than the whole, since the number of chromosomes, rather than their size, matters.

How might chromosomes affect microtubule assembly or stability? One possibility is a linkage between chromosomes and factors which limit microtubule growth. For instance, kinetochore microtubules might have both ends capped, one end by the kinetochore, the other by the polar organizer (14). Such caps might prevent further growth once some definite length had been reached, even in the presence of enough tubulin subunits to sustain further elongation. Brinkley et al. (1) suggested a similar capping mechnism as one explanation for the restricted growth of the centrosomal microtubules in their experiments. The rest of the chromosome-dependent microtubules might be stabilized by cross-linkage (16) to stable kinetochore microtubules, and for that reason are present only when chromosomes are present. The remaining problem would be to explain why the subunits that are liberated when chromosomes are removed are not incorporated into the microtubules of the chromosome-independent spindle. Here again, some restriction on growth such as capping molecules would be necessary. Proposals of this sort work by imposing limitations on microtubule subunit exchange, which tends to make assembly irreversible. This is a potential difficulty because somehow the dynamic changes in microtubule length that are the spindle's stock in trade must be accommodated. The need is most obvious for those kinetochore microtubules that span the kinetochore-to-pole distance and must change in length during congression in prometaphase and chromosome-to-pole movement in anaphase.

Another possibility is that chromosomes promote microtubule assembly beyond simply providing binding or nucleation sites for kinetochore microtubules. For instance, suppose that chromosomes have a nucleotide disphosphokinase activity, which is required to regenerate tubulin-GTP and is otherwise in short supply. In that case, each chromosome would represent an increment of nucleotide diphosphokinase activity, and even in the face of the additional tubulin released when chromosomes are removed, the concentration of assembly-competent tubulin would depend on the number of chromosomes still present. This proposal is related to a general model of microtubule initiation by organizing centers (3; see also reference 9 for review and a specific version of De Brabander's model), but an effect on steady-state length regulation was not considered. There is no evidence for the required assembly-promotion activity of chromosomes, but the proposal has two corollaries which may motivate a search for some such activity. First, in the presence of a chromosome, elongation of all the microtubules in its vicinity would be expected, in agreement with the observations: both kinetochore and nonkinetochore microtubules are affected by chromosomes and the bulk of the chromosome-dependent microtubule length is in the vicinity of the chromosomes, not the pole (Table II *B*). Second, the proposal allows, even demands, the dynamic assembly and disassembly of microtubules which is central to our present conception of spindle organization

(for review, see reference 4).

Our results and interpretations are related to those of Karsenti et al. (7), who injected parts of cultured cells into *Xenopus* eggs. They found that injected nuclei organized microtubule arrays and, most remarkably, that proximity to a nucleus made centrosomes active in organizing microtubule arrays in a cytoplasmic environment in which centrosomes by themselves were inactive. These results and ours may or may not have a common explanation. The important point at present is simply that two entirely different experimental approaches that use different materials show that nuclei or chromosomes dramatically affect the extent of microtubule assembly in cells.

Implications for Normal Mitosis

The nuclear activation of microtubule assembly at centrosomes may well be essential for normal spindle formation in many cells (7), and this may also be true for the chromosome-dependent changes in microtubule length reported here. Certainly the impression from both light (5) and electron microscopy (e.g., reference 13) is that after the nuclear envelope breaks down, spindle birefringence and microtubule content increase dramatically just when chromosomes begin to interact with the spindle. Moreover, the number of chromosomes often varies substantially from cell to cell in otherwise normal organisms with large numbers of supernumerary chromosomes (6). Our results imply that spindle organization will automatically adapt to the number of chromosomes present even if, as one would expect, the same quantity of tubulin is present in all cells. (An alternative would be a regulator of tubulin synthesis that is sensitive to the number of chromosomes in each cell.)

Both kinetochore and some nonkinetochore microtubules respond coordinately to the presence or absence of chromosomes. Do the microtubules with a common dependence on chromosomes share a common structural or function role as well? For example, these kinetochore and nonkinetochore microtubules together may form the bundle that extends between the kinetochore of each chromosome and the pole. Further investigation of this point would require the identification of which nonkinetochore microtubules are chromosome-dependent, rather than merely the demonstration that they are responsive to the presence or absence of chromosomes.

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