Measurements of the Force Produced by the Mitotic Spindle in Anaphase

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ABSTRACT The force the spindle exerts on a single moving chromosome in anaphase was measured with a flexible glass needle calibrated in dynes per micron of tip deflection. The needle was used to produce a force on the chromosome, which opposed that produced by the spindle and was measurable from needle tip deflection. The measurements were made in intact grasshopper spermatocytes after proving that the presence of materials such as the cell surface did not interfere.

The results from 12 experiments in seven cells are as follows: Chromosome velocity was not affected until the opposing force reached $\sim 10^{-5}$ dyn, and then fell rapidly with increasing force. The opposing force that caused chromosome velocity to fall to zero—the force that matched the maximum force the spindle could produce—was of order 7×10^{-5} dyn. This directly measured maximum force potential is nearly 10,000 times greater than the calculated value of 10^{-8} dyn for normal chromosome movement, in which only viscous resistance to movement must be overcome.

The spindle's unexpectedly large force potential prompts a fresh look at molecular models for the mitotic motor, at velocity-limiting governors, and at the possibility that force may sometimes affect microtubule length and stability.

Chromosome movement in mitosis is necessarily, if only partly, a problem in mechanics—a matter of forces and of spindle mechanical properties that govern the response to those forces. For this reason, how large a force the spindle can develop is interesting in itself. Moreover, the maximum force potential is a revealing characteristic of any motor and sets some constraints on speculation about the molecular nature of the motor.

Two important features of the forces that move chromosomes in mitosis were established long ago by hydrodynamic analysis, without a single direct measurement. First, the force required for normal chromosome movement is very low, since only the viscous resistance to movement (drag) must be overcome (9, 17). In fact, it is so low, both absolutely and relative to other biological motile systems, that this by itself places significant constraints on acceptable molecular models (11). Second, it was nevertheless clear that the spindle can produce significantly greater force (9, 17), but how much greater could not even be guessed.

Direct measurement of spindle forces was the obvious next step. I have made such measurements, using a flexible, calibrated glass needle (19) to exert a known force on a chromosome in anaphase. The force applied by the needle opposed that produced by the spindle and could be increased until it balanced the spindle's force, and chromosome movement stopped. At that point, the opposing force equaled the maximum force the spindle could develop. The maximum force measured in this way was surprisingly large: several thousand times greater than the force required for normal chromosome movement as calculated from hydrodynamics. This inspires new respect for the spindle's competence as a force producer and prompts a fresh look at molecular models for mitosis in the light of spindle mechanics. It also prompts a look at a possibility raised by the recent work of Hill and Kirschner (5); so large a force may directly affect microtubule length and stability.

MATERIALS AND METHODS

The grasshopper *Melanoplus sanguinipes* (Fabricius), obtained from a laboratory colony, and the cricket *Acheta domestica* (Linnaeus) from Flucker's Cricket Farm, Baton Rouge, LA were used. Unless otherwise noted, all results and conclusions apply to *Melanoplus* spermatocytes; a few experiments on *Acheta* spermatocytes will be noted in passing. The spermatocytes were cultured as previously described (13) except that FC-47 fluorocarbon oil (3M, St. Paul, MN) was used to cover the cells rather than Voltalef 10-S oil. The low viscosity of FC-47 oil resulted in rapid stabilization of tip position after each movement of the flexible force-measuring needle. FC-47 is no longer available, but per-

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fluorotributylamine from PCR Research Chemicals, Inc. (PCR, Inc., Gainesville, FL) is a satisfactory substitute. The cells were cultured at temperatures ranging from 24 to 27.5°C; the variation for any one cell was ±0.5°C.

The glass needles used to measure forces were fabricated and calibrated in a two-step process as described by Yoneda (19). Relatively stiff "reference needles" are calibrated directly by suspending weights from their tips. The more flexible "force-measuring needles" used in experiments on cells are calibrated indirectly by bending them against reference needles and determining the relative stiffness. In the present work, the weights for reference needle calibration were fabricated from wire as described by Yoneda (19), but very thin, 25-μm diam, chromel thermocouple wire (Omega Engineering, Inc., Stamford, CT) was used. Weights as light as 3.9 μg were made, which permitted direct calibration of reference needles as flexible as the stiffer ones used in experiments on cells. I used two reference needles (calibration factors: $2.0–7.1 \times 10^{-5}$ dyn per 1 μm deflection of the tip) to calibrate four force-measuring needles (calibration factors: $0.76–2.5 \times 10^{-5}$ dyn per 1-μm tip deflection).

Multiple calibrations (19) gave the same calibration factor within 6% for each reference needle and within 11% for each force-measuring needle. From these values, the overall calibration accuracy was estimated as $\pm \sqrt{6^2 + 11^2} = \pm 13\%$.

The calibrations were carried out in air, but in the measurements on cells, the needles were partly immersed in FC-47 oil. To be certain that this did not matter, some calibrations were repeated with the needles immersed in FC-47 oil: Exactly the same values were obtained as in air. The viscosity of FC-47 is 270 times greater than air, so naturally a longer time was required for the needle tip to reach stable position after movement. Ample time for stabilization, 10 s or longer, was always allowed, both in calibration and in experiments on cells.

Phase contrast microscopy, micromanipulation of the force-measuring needle, and ciné recording and analysis were carried out as previously described (15), except that Agfa Copex Pan Rapid 16-mm ciné film (Agfa-Gevaert, Inc., Teterboro, NJ) was used. Changes in the position of the needle tip or of a chromosome could be measured to within ± 0.25 μm. Since the force applied to the chromosome corresponded to an average needle tip deflection of 2 μm, the accuracy in determining the deflection was 2 ± 0.25 μm or ± 13%. The change in chromosome position during one experiment was often as little as 0.5 μm, so the accuracy of chromosome velocity determination was often no better than 0.5 ± 0.25 or ± 50%. The accuracy of velocity determination could be increased by maintaining tension on the stretched chromosome for a longer time, so that longer, more accurately measurable, distances would be traveled during each experiment. Longer experiments pose another problem, however. The force is measured only once, at the end of each experiment, and that force must be representative of the force applied during the whole course of the experiment. It is difficult to maintain a constant tension on the stretched chromosome, and even in the present short experiments, variation in tension may well have reduced the validity of the force measurement to the same mediocre but adequate (see Discussion) level attained for velocity determination.

Chromosome velocity varies somewhat from cell to cell, and hence the velocity of the target chromosome was calculated as a percentage of the velocity of an adjacent, unmanipulated control chromosome in the same cell and during the same time interval. The velocity of the adjacent chromosomes was not detectably affected by these experiments: they moved as rapidly as those farther away. For reference, the average absolute velocity for control chromosomes in these cells was 0.73 μm/min (range: 0.52 to 0.93 μm/min).

RESULTS

Plan of the Experiments and Two Examples

The experiment in outline is as follows. The force-measuring needle was lowered through the oil above a cell in anaphase and centered directly over a chromosome at the upper level of the spindle. The aim was to ensure that the needle tip could engage the target chromosome without encountering the bulk of the spindle. The needle tip was then lowered further, and the target chromosome was snagged and stretched by the needle. The flexible needle was bent by the force it applied to the chromosome; that force opposed the force the spindle exerted in attempting to move the chromosome poleward. After some time, the needle was raised slightly. The needle tip then popped free of the chromosome and returned to its unbent state.

Actual experiments are illustrated in Figs. 1 and 2. The 0.0-min print in Fig. 1 shows a cell and the target chromosome (arrowhead) just before an experiment. The chromosome was snagged and stretched slightly, as shown in the 1.1-min print (the tip of the needle is visible as a dark line just to the left of the arrow). Later, the needle was disengaged from the chromosome; the needle tip is in focus in the 1.9-min print (just to the right of the arrow) and the chromosome is in focus in the 2.0-min print. Arrows on the 1.1- and 1.9-min prints indicate the tip position when the needle was bent while applying force (1.1 min) and after it straightened upon release from the chromosome (1.9 min). The vertical distance between the shafts of the arrows indicates the extent of tip displacement, measured as 1.5 μm. The needle calibration factor was 1.2×10^{-5} dyn per 1-μm deflection of the tip, and therefore the force opposing chromosome movement was 1.8×10^{-5} dyn. The movement of the target chromosome was only slightly affected by this opposing force: Lagging of the target chromosome relative to the adjacent control chromosome may just be discernible in comparing the 2.0- and 0.0-min prints. The measured velocity of the target chromosome was 80% of that of the adjacent control chromosome.

Fig. 2 shows an experiment in a second cell, before (0.0-min print), during (0.4 min), and after the experiment (0.8 and 1.0 min). Here, a greater force was applied than in the first cell simply by moving the needle further toward the lower pole after snagging the chromosome. Thus, the chromosome was stretched more markedly (0.4-min print), and the needle tip deflection (the vertical distance between the shafts of the

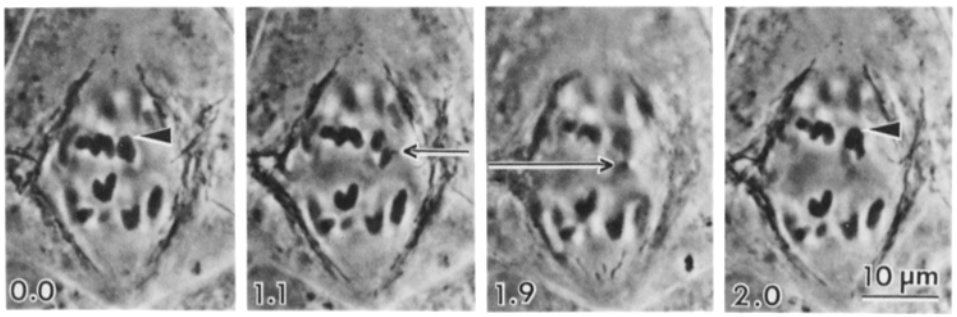

FIGURE 1 Prints from the ciné record of a force measurement experiment. The time in minutes is given on each print. A cell in anaphase is shown, with the kinetochoric end of a target chromosome marked by an arrowhead on the 0.0- and 2.0-min prints. The target chromosome is seen before the experiment (0.0 min), while slightly stretched (1.1 min), and after release (2.0 min). The tip of the flexible, calibrated needle used to stretch the chromosome is visible as a short, dark line at the arrow on the 1.1 and 1.9 min prints. The chromosome was stretched for 1.5 min by a force from the needle applied away from the pole toward which the chromosome was moving. The needle, bent by the force, straightened and sprang back toward the opposite pole upon release from the chromosome (compare the needle position, marked by the shaft of the arrow, during stretching, 1.1-min print, and after release, 1.9 min). From the deflection of the needle, the force opposing chromosome movement was calculated as 1.8×10^{-5} dyn (see text), which reduced the velocity of the target chromosome to 80% of the velocity of an adjacent control chromosome. × 980.

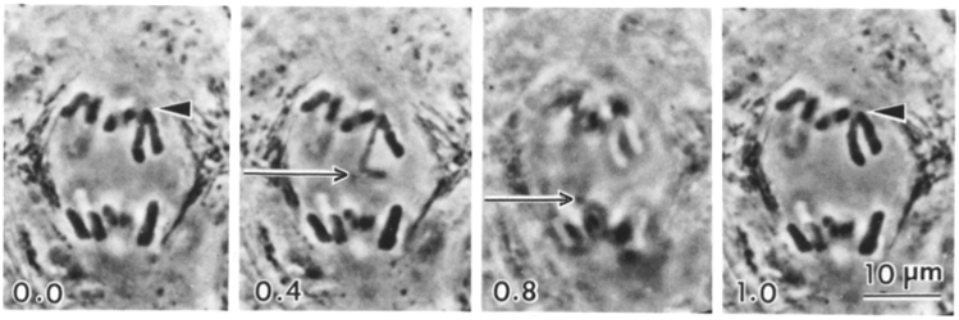

FIGURE 2 Prints from the ciné record of another force measurement experiment, arranged and labeled as in Fig. 1. Compared with the experiment in Fig. 1, the chromosome in this instance was more greatly stretched: compare the target chromosome before and after the experiment (arrowheads, 0.0- and 1.0-min prints) with its configuration while being stretched (0.4-min print; the chromosome was under tension for 0.53 min). A larger deflection of the needle tip is also observed: compare the needle tip position, marked by the arrow, during stretching (0.4 min) and after release (0.8 min). (In the 0.4 min print, the beam tip is slightly out of focus and is just visible as a tiny black dot to the right of the arrow.) The greater tip deflection corresponds to greater force opposing movement, 4.8×10^{-5} dyn, and chromosome velocity was reduced to half the normal rate during this experiment. × 980.

arrows, 0.4- and 0.8-min prints) was greater. The tip deflection was 4 μm and the same needle was used as on the first cell, so a force of 4.8×10^{-5} dyn was applied. At that opposing force, the velocity of the target chromosome was reduced to 50% of the control chromosome.

Chromosomal Elastic Modulus Measurements: Force Measurements Can Be Made in Intact Cells

The experiments were done on intact cells, and hence other materials (particularly the cell surface) were stretched whenever a chromosome was stretched. The force measurements are reliable only if the force required to stretch chromosomes is very much greater than that required to stretch the other materials the needle must penetrate before reaching the chromosome. I originally thought that the effect of these materials would make necessary the use of lysed cells, in which the cell surface is absent. Fortunately, it is possible to demonstrate directly that intact cells can, in fact, be used.

The demonstration has a simple rationale. During anaphase of the first meiotic division, the two chromatids comprising each half-divalent separate except at the kinetochore, so either two chromatids (before separation) or only one (after separation) can be stretched with the calibrated needle (in the experiments shown in Figs. 1 and 2, for instance, only one chromatid was stretched). Thus, the force required to stretch two chromatids can be compared with that required to stretch only one. A 2:1 ratio of the force required will be found only if the force applied by the needle is resisted mainly by chromosomal stiffness. Conversely, the stiffness of a chromosome might be small relative to the stiffness of other materials that are stretched by the needle at the same time as the chromosome is stretched. In this case, the total force required would not be noticeably affected by chromatid number, since the same force would be required to stretch nonchromosomal materials regardless of the number of chromatids stretched. Therefore, the ratio of forces for stretching two versus one chromatids would be close to 1:1 than to 2:1.

The force required to stretch one or two chromatids was determined in 34 experiments in seven intact spermatocytes at late metaphase or anaphase of the first meiotic division. Naturally, the original length and the extent to which chromatids were stretched varied from experiment to experiment, so a standardized force, (the force required for unit increase in length) must be computed. The standardized force is given by the left side of the following equation (simply a rearranged version of the standard relationship between stress and strain for a Hookian elastic body under tension):

$$F\frac{l_0}{\Delta l} = EA, \qquad (1)$$

where F is force, l_0 is the original length of the stretched portion of the chromosome, Δl is the change in length, E is Young's modulus, and A is chromosomal cross-sectional area. If chromosomal elasticity determines the force required, then $F(l_0/\Delta l)$ should be a constant for all experiments in which one chromatid was stretched and a constant twice as great when two were stretched (because the cross-sectional area, A, is doubled).

The results are shown in Fig. 3. The values for standardized force obviously vary considerably within each class (one versus two chromatids), chiefly because discriminating l_0 is difficult and inexact. In fact, the highest value for each class (12×10^{-5} for one chromatid and 29×10^{-5} for two) differs so greatly from the mean that both have been eliminated from further consideration as "gross errors" or "blunders" on Dixon's objective, statistical test (the "r-ratio" test, [1]). Their exclusion does not affect the conclusions that follow (if these two values were included, the two chromatid/one chromatid force-ratio would actually be increased slightly). From here on, then, the sample size is reduced to 32.

The variation within each class does not obscure the effect of chromatid number: the mean standardized force necessary to stretch two chromatids was 7.5×10^{-5} dyn whereas for one chromatid it was 3.2×10^{-5} dyn. The ratio of these forces is 2.3, very close to the value (2.0) expected if materials other than the chromosome made a negligible contribution to the force required. Statistical analysis confirms this: The null hypothesis of no difference between the one and two chromatid classes is rejected at the 99.98% confidence level ($t = 4.73$, $df = 30$). Also, the difference between the means for the two classes is 4.3×10^{-5}, with a 95% confidence interval of

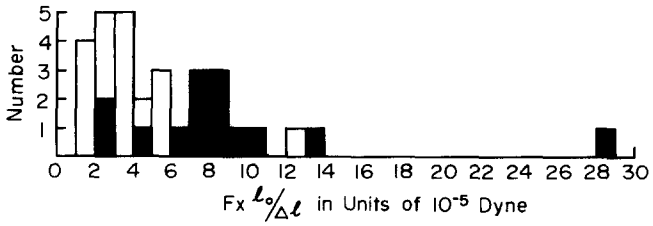

FIGURE 3 Histogram showing the force required for unit extension of one chromatid (open bars) or two chromatids (black bars).

2.7 to 5.9 × 10^{-5}. These results on spermatocytes definitely should not be regarded as typical of all cells: unpublished micromanipulation experiments suggest that insect spermatocytes have an exceptionally flexible cell surface region, particularly compared with cultured mammalian cells.

This analysis assumes that chromosomal elasticity is linear (Hookian), and the ratio near two suggests that indeed it is, within the limit of stretch imposed in these experiments (up to a threefold increase in length). Young's modulus of elasticity is readily calculated from the equation given above, using the mean values for $F(l_0/\Delta l)$ and a measured value of 1.0 μm for the diameter of a chromatid. An average value of 4.3 × 10^3 dyn/cm^2 is obtained for the Young's modulus of *Melanoplus* chromosomes in metaphase and anaphase of the first meiotic division.

Opposing Force and Chromosome Velocity

Force and velocity measurements were made during the first two-thirds of anaphase I, when velocity was nearly constant and before much if any spindle elongation had occurred. Thus the measurements mainly or exclusively reflect the force developed for chromosome-to-pole movement, not for spindle elongation.

The result of applying a known force to a chromosome in anaphase is shown in Fig. 4 for 12 experiments in seven cells, including those shown in Figs. 1 and 2. The evident variation from experiment to experiment does not obscure the most significant points: an opposing force of 10^{-5} dyn had little or no effect on chromosome velocity relative to an adjacent control chromosome, but increasing the force beyond this critical point caused a sharp decrease in velocity and by 5 × 10^{-5} dyn the velocity was only one-half to one-tenth that of the control.

The dashed line in Fig. 4 is from a linear regression analysis of velocity on force. The coefficient of determination is 0.551: 55% of the variation is explained by a linear dependence of velocity on force. Also, a linear relationship is acceptable on an analysis of variance computation ($F_{1,10}$ = 12.27, P < 0.0057): a large and significant fraction of the variance has been explained. The remaining, unexplained variation is due to an unanalyzable mixture of biological variation, errors of measurement, and possibly, some nonlinearity in the relationship between force and velocity. More numerous and more precise measurements would be necessary to establish the exact shape of the force-velocity curve. For the present, it is enough to conclude that the relationship is approximately linear in the 10^{-5} dyn region. More important, the regression analysis is the best way to pool all the data to yield reliable estimates of the impact of opposing force on velocity. The force at which velocity falls to one-half that of the control is the most reliable statistical measure; that force is 3.7 × 10^{-5} dyn, with a 95% confidence interval of ± 30%: 2.6–4.8 × 10^{-5} dyn.

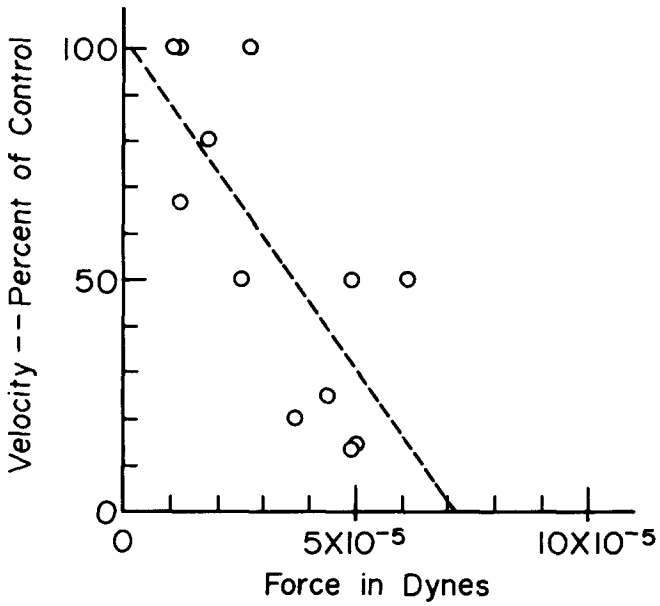

FIGURE 4 Chromosome velocity in anaphase as a function of the force opposing movement. For each experiment, the force applied to one chromosome is plotted against its velocity relative to an adjacent chromosome (open circles). The dashed line is from a linear regression analysis.

As noted in passing above, the scatter of points in Fig. 4 is due in part to biological variation from cell-to-cell. This is best illustrated by measurements on two cells shown in Table I. In both cells, velocity decreased by about half when the force was about doubled, but the absolute values are very different: in cell two, two or three times as great a force as in cell one was required for substantial reduction in velocity.

Three force versus velocity measurements in an intact *Acheta* spermatocyte are worth noting in passing. Chromosome velocity was reduced to 67–80% of the control at opposing forces of 2.3–4.5 × 10^{-5} dyn and fell to or near zero at a force of 5.4 × 10^{-5} dyn. Thus, values similar to those for *Melanoplus* have been obtained in another material.

DISCUSSION

Reliability of the Measurements

The experimental design will first be examined for any flaws and then the accuracy of the measurements will be considered.

The assumption behind the experimental design is that the measured force applied to a stretched chromosome does in fact oppose the force exerted by the spindle. More precisely, the total force from the bent needle must be transmitted undiminished from the point of application to the kinetochore, where the spindle forces act. Two requirements must be met for this to be true. (*a*) There must be no dissipation of energy either from viscous flow in the cell or the oil above it or from plastic deformation of the chromosome. Viscous flow ceases when the needle tip reaches a stable position after each displacement. Since ample time was allowed for stabilization, viscosity did not affect the measurements. Plastic deformation is not occurring because chromosomes recover their original length after stretching, not only in the present experiments but also after far greater elongations (15). Simply because the chromosome is elastic rather than plastic, at any constant length it will transmit a constant force from needle

TABLE I

Biological Variation in the Force Required to Affect Velocity

Cell no.	Opposing force	Velocity
	dyn	*% of control*
1	2.5 × 10^{-5}	50
	4.4 × 10^{-5}	25
2	2.7 × 10^{-5}	100
	6.1 × 10^{-5}	50

to kinetochore as perfectly as a far more rigid elastic body, a steel beam, say. (*b*) All, or very nearly all, the force from the bent needle must be applied only to the chromosome. Inevitably, in experiments on intact cells, materials other than the chromosome are also stretched by the needle along with the chromosome. The stiffness of these other materials must be negligible relative to chromosomal stiffness for the measurements to be valid. This has been proven by demonstrating that the force required to stretch two chromatids was approximately twice as great as that required to stretch only one (the rationale for this test is given in Results). The importance of this demonstration extends beyond showing that the stiffness of materials other than the chromosome was negligible. It is an equally direct proof that the factors already considered, viscosity and plastic deformation, also did not affect the measurements. Thus, the force measurement scheme is validated under the actual conditions of measurement in intact cells.

The accuracy of the measurements can now be considered. A sensible goal is measurements accurate to ± 50% or so, because in view of other uncertainties mentioned below, we have no present use for more accurate measurements. That goal is met for the velocity measurements and the calibration of the force-measuring needles (see Materials and Methods). The desired accuracy is confirmed for force measurements in cells by the ability to distinguish the force required to stretch two chromatids from that required to stretch only one. Finally, even in the face of biological variation (Table I), the pooled force versus velocity data yield a value for the opposing force at which velocity falls to half the normal rate with 95% confidence limits of ± 30%.

In summary, the major conclusion is well justified: 10^{-5} dyn is the decade of opposing force in which velocity falls from nearly normal to zero. Where specific values are needed, extrapolations from the regression line in Fig. 4 will be used as order of magnitude estimates: 2×10^{-6} dyn for the opposing force at which velocity is first affected and 7×10^{-5} dyn for the force at which velocity falls to zero. These values are for force per chromosome and, as already noted, reflect mainly or exclusively the force developed in chromosome-to-pole movement, not in spindle elongation.

Implications

The maximum force the spindle can develop equals the opposing force at which chromosome movement halts, $\sim 7 \times 10^{-5}$ dyn per chromosome. As expected, this is greater than the force required for normal chromosome movement, calculated as $\sim 10^{-8}$ dyn (9, 17). The surprise is how much greater—almost 10,000 times—and this is more apt to be an underestimate than an overestimate. As the opposing force is increased, chromosome movement may cease because the skeleton can sustain no greater load, rather than because the ultimate capacity of the motors has been reached.[1]

The spindle's full capacity as a force producer sometimes comes into play in natural circumstances, most obviously when chromosomes fail to separate properly in anaphase and are stretched by the spindle. In that circumstance, anyway, the adaptive significance of the capacity to produce relatively great force is not obvious and may be nil, since it may lead to chromosome breakage and genetically defective cells. Still, the capacity exists, whatever its significance may be, and the motor postulated in molecular models must be capable of generating the measured maximum force.

FORCE AS A TEST OF MOLECULAR MODELS: Three of the many molecular models for mitotic force production permit an expected value for the force to be calculated (for a review of these and other models, see 8). That virtue is the reason for considering them here, and whether or not they are attractive on other grounds is ignored. The three models allow computation of the maximum force developed per microtubule (see Table II, footnotes). For comparison, an estimate of the actual value for the spindle was obtained by dividing the estimated maximum force per chromosome, 7×10^{-5} dyn, by 15, a microtubule number estimate based on electron microscopic observations. The number of microtubules directly involved in the movement of one chromosome is the appropriate number to use, and the choice depends in part on details not yet specified for most models. A value adequate for present purposes is the number of kinetochore microtubules that span the kinetochore-to-pole distance. The average number of kinetochore microtubules per chromosome in another species of *Melanoplus* is 45, but only about a third of these link kinetochore and pole either directly, as a single, long microtubule, or via linkage to a second microtubule (14). The resulting estimate of 15 effective microtubules per chromosome is certainly plausible for models postulating shearing between kinetochore and nonkinetochore microtubules near the pole (where they come sufficiently close together; [14]). For other models, the estimate seems reasonable within a factor of three: 5–45 microtubules. For instance, on models in which kinetochore microtubules themselves produce the force, short kinetochore microtubules might also contribute if mechanically appropriate conditions exist. If so, the effective number would be ~45, the average total number of kinetochore microtubules. In sum, 15 as a value for the effective number of microtubules is probably as reliable as the values for maximum force or the calculations from the models.

Values for force per microtubule are given in Table II. The estimated actual value falls exactly between those expected from the models: dynein as in flagella yields five times more force than expected and treadmilling or assembly five times less. Alternatively, the computation for models can be expressed in terms of the number of microtubules needed to

[1] A skeletal rather than a motor limit might be expected if the opposing force slows adjacent chromosomes as well as the target chromosome (14). Movement of adjacent chromosomes was not detectably affected in the present, short experiments, but has been seen in earlier experiments of longer duration. For instance, in cell number one of reference 14, both the velocity and microtubules of an adjacent chromosome were affected at a force now estimated at 8×10^{-5} dyn (from the present determination of the force required to stretch *Melanoplus* chromosomes); this is not significantly different from the highest force applied in the present experiments. Conversely, the factor of 10,000 would be an overestimate if the force required for normal chromosome movement were underestimated. Drag due to kinetochore microtubules was ignored in the earlier calculation, so to be safe a new calculation has been made, using the relationships given earlier (9). The revised estimate is 4×10^{-8} dyn for the total drag force on a single large *Melanoplus* chromosome in anaphase, including 50 microtubules each 5 μm long (microtubule estimates from data in 14; other conditions: velocity, 0.5 μm/min; viscosity, 1 poise). The revised estimate would give a 2,000-fold difference between the force normally required and the maximum capability. This is surely a minimal value.

TABLE II

Comparisons between the Spindle and Molecular Models

		Maximum force per kinetochore microtubule	Required no. of microtubules per chromosome
		dyn	
Estimated actual values	Spindle	5×10^{-6}	15
Expectations from models	Dynein*	25×10^{-6}	3
	Treadmilling‡	1.3×10^{-6}	54
	Assembly-disassembly§	0.8×10^{-6}	88

* Properties as in flagella. The measured maximum force per dynein arm, 10^{-7} dyn (7), was multiplied by the observed number of dynein arms per micron of flagellar doublet length, 83 (e.g., 18), and by the estimated effective length of a spindle microtubule, 3 μm (only in the 3 μm nearest the pole might kinetochore microtubules come within a dynein-bridging distance of other microtubules [14]).

‡ The value given by Hill and Kirschner (5) was used. That value comes from a thermodynamic/kinetic analysis, with estimated values for several variables.

§ The force per microtubule was calculated as described earlier (10), using a value of −0.7 kcal/mol for the free energy change for microtubule assembly (6). Note that for a given free energy change, this calculation gives absolutely the maximum possible force that could be generated—100% efficiency in the conversion of free energy to work is assumed.

account for the directly measured force per chromosome (Table II). Only three microtubules would be required if dynein, organized as in flagella, were the force producer, whereas 50–90 would be required on treadmilling or assembly models. Yet, despite the discordance, none of these models can be rejected, since the calculations easily could be off by a factor of five. It bears emphasis that this imprecision is due at least as much to lack of quantitative specificity in all present models for mitosis as to imprecision in the force measurements. After the models have been improved, refinement of the force measurements will be worthwhile.

The present rough test does not rule out either relatively weak (treadmilling, assembly) or relatively strong (dynein) motors. Nevertheless, intrinsically weak motors now appear to be at a disadvantage, given the unexpectedly large force the spindle can develop. Thus, treadmilling or assembly, for instance, must be pushed to their apparent limits, whereas for an intrinsically more powerful motor such as dynein, a minor, entirely plausible change in the model tested would bring it into exact accord with expectations from force measurements (e.g., fewer dynein arms per unit length of microtubule than in flagella).

THE RELATIONSHIP BETWEEN FORCE AND VELOCITY: The velocity of chromosome movement stays constant over a remarkable 100-fold range in the opposing force—from $\sim 10^{-8}$ dyn in normal chromosome movement to between 10^{-6} and 10^{-5} dyn when the opposing force is increased by stretching a chromosome. This portion of the force-velocity relationship seems very different from that for muscle (e.g., reference 2) or treadmilling (5), even allowing for possible differences in the accuracy or range of measurements. In any case, the relationship observed must be explained on any acceptable model for mitosis. The problem is clearly posed by considering dynein, chosen only to provide a concrete example. The directly measured maximum force produced by a single dynein arm is 10^{-7} dyn (7). Therefore, several hundred dyneins would be required to produce the maximum force that can be exerted per chromosome, yet at the force required for normal chromosome movement, only one-tenth the output of a single dynein would suffice! Thus, granted a sufficient force-producing capability to generate a force of 7×10^{-5} dyn, why don't chromosomes move far faster than the observed rate in normal movement, when only 10^{-8} dyn is required? A possible answer is that chromosome velocity is controlled by a governor, a velocity-limiting device or process. Kinetochore microtubules are the obvious choice for the governor, their length determining chromosome position and their rate of depolymerization determining the maximum rate of poleward movement, regardless of how hard the motors push or pull on them (3, 11). This conception was originally introduced as a plausible way in which one class of candidate motors (relatively powerful ones such as myosin or dynein) could be held in check (3, 11). The argument favoring a governor of some sort is extended to all models for mitosis by the present results. Any motor powerful enough to explain the spindle's ultimate capacity as a force producer would, if unchecked, move chromosomes too rapidly under normal circumstances.

Alternatively, the motor itself may limit the velocity and a separate governor may not be necessary, as a reviewer of this report suggested. In motile systems of the actin-myosin and microtubule-dynein class, the observed velocity is a result of many motors, each contributing a small increment of displacement in repetitive mechanochemical cycles. When the force opposing movement is low, the velocity is constant because it is determined by intrinsic properties of the motors alone (the duration of each mechanochemical cycle, and the length of the displacement increment). The velocity does not decrease until the opposing force is great enough to affect the operation of the motors. Consider microtubule sliding in trypsin-treated flagellar axonemes, for instance. The velocity remains constant as sliding continues, even though the zone of microtubule overlap decreases and therefore so does the number of effective dynein motors (16). Thus in this situation, the opposing force per motor increases greatly without any effect on velocity. The question, of course, is how large the force opposing movement can become before velocity is affected. To explain spindle mechanics without invoking a governor, the motors must be unaffected until the opposing force rises to over 10^{-6} dyn per chromosome. Is that likely? I see no way of knowing at present, and therefore make no choice between intrinsic (motor) and extrinsic (governor) constraints on velocity. One or the other, however, is necessary to explain the facts.

FORCE AND MICROTUBULE ASSEMBLY: Hill and Kirschner's remarkable investigation (5) shows that a force of 10^{-6} dyn per microtubule should directly and significantly affect microtubule assembly. This can be illustrated in two ways. First, it is central to Hill and Kirschner's treatment that the total thermodynamic force driving assembly is the ordinary free energy change plus the thermodynamic equivalent of the mechanical force, if any, on the microtubules. From their equation 84, the thermodynamic equivalent of a force of 10^{-6} dyn/microtubule is 0.9 kcal/mol, about the same as the chemical free energy change for protein self-association. Hence, adding a tension force (which favors assembly) of 10^{-6} dyn/microtubule should about double the total thermodynamic force driving assembly and the stability and length of microtubules under tension should increase. Conversely, microtubules under an equivalent compressive force will be less

stable and will tend to shorten (see reference 5, pages 50–51 for an intuitively reasonable explanation of the effects of tension and compression). Second, the effect of mechanical force on the critical concentration of tubulin required for assembly can be calculated. Using Hill and Kirschner's (5) equation 89 and their value of 1.75 μM for the steady state or critical concentration in the absence of mechanical force, a tension force of 10^{-6} dyn would decrease the critical concentration by more than a factor of four, to 0.39 μM. Thus, as the free tubulin concentration falls owing to assembly into microtubules, in the absence of force, microtubule elongation would cease when the tubulin concentration reached 1.75 μM, whereas for microtubules under tension, elongation would continue until the tubulin concentration was four times lower (or until something else intervened).

So a force per microtubule of 10^{-6} dyn should affect assembly significantly. As already seen, the spindle can produce forces in just that vicinity: at a total force per chromosome of 1.5×10^{-5} dyn, each of 15 kinetochore microtubules would be subjected to a tension force of 10^{-6} dyn. The sole assumption here is that only $\sim$15 microtubules per chromosome have kinetochore-to-pole continuity, and therefore are responsible for chromosome attachment to the spindle. Hence, whatever the force on the chromosome, those 15 microtubules must bear it.

Thus, the intriguing possibility is raised that spindle function (force production) may directly affect spindle structure (microtubule length and stability) in some circumstances. Two features of chromosome behavior in prometaphase illustrate why exactly this type of regulation is of interest: Movement to the equator depends on microtubule length adjustment, apparently in response to mitotic forces (4, 12), and the stability of kinetochore-to-pole attachments depends on tension (for review, see 10). It would indeed be satisfying if either mystery or both were explained simply by a direct effect of force on microtubule assembly thermodynamics.

In conclusion, the results (*a*) do not much delimit acceptable models for the motor, but (*b*) show that either a governor or the motor must limit chromosome velocity, and (*c*) suggest that in some circumstances the force developed might directly affect microtubule length and stability.

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REFERENCES

1. Dixon, W. J., and F. J. Massey. 1957. Introduction to Statistical Analysis. 2nd edition. McGraw-Hill, New York.
2. Edman, K. A. P., and J. C. Hwang. 1977. The force-velocity relationship in vertebrate muscle fibers at varied tonicity of the extracellular medium. *J. Physiol.* 269:255–272.
3. Forer, A. 1974. Possible roles of microtubules and actin-like filaments during cell-division. *In* Cell Cycle Controls. G. M. Padilla, I. L. Cameron, and A. M. Zimmerman, editors. Academic Press, Inc., New York. 319–336.
4. Hays, T. S., D. Wise, and E. D. Salmon. 1982. Traction force on a kinetochore at metaphase acts as a linear function of kinetochore fiber length. *J. Cell Biol.* 93:374–382.
5. Hill, T. L., and M. W. Kirschner. 1982. Bioenergetics and kinetics of microtubule and actin filament assembly-disassembly. *Intern. Rev. Cytol.* 78:1–125.
6. Inoué, S., and H. Ritter. 1975. Dynamics of mitotic spindle organization and function. *In* Molecules and Cell Movement. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 3–30.
7. Kamimura, S., and K. Takahashi. 1981. Direct measurement of the force of microtubule sliding in flagella. *Nature (Lond.).* 293:566–568.
8. McIntosh, J. R. 1979. Cell division. *In* Microtubules. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., London. 381–441.
9. Nicklas, R. B. 1965. Chromosome velocity during mitosis as a function of chromosome size and position. *J. Cell Biol.* 25 (1; pt. 2):119–135.
10. Nicklas, R. B. 1971. Mitosis. *In* Advances in Cell Biology Volume 2. D. M. Prescott, L. Goldstein, and E. McConkey, editors. Appleton-Century-Crofts, New York. 225–297.
11. Nicklas, R. B. 1975. Chromosome movement: current models and experiments on living cells. *In* Molecules and Cell Movement. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 97–117.
12. Nicklas, R. B. 1977. Chromosome movement: facts and hypotheses. *In* Mitosis: Facts and Questions. M. Little, N. Paweletz, C. Petzelt, H. Ponstingl, D. Schroeter, and H.-P. Zimmermann, editors. Springer-Verlag, Berlin. 150–155.
13. Nicklas, R. B., B. R. Brinkley, D. A. Pepper, D. F. Kubai, and G. K. Rickards. 1979. Electron microscopy of spermatocytes previously studied in life: Methods and some observations on micromanipulated chromosomes. *J. Cell Sci.* 35:87–104.
14. Nicklas, R. B., D. F. Kubai, and T. S. Hays. 1982. Spindle microtubules and their mechanical associations after micromanipulation in anaphase. *J. Cell Biol.* 95:91–104.
15. Nicklas, R. B., and C. A. Staehly. 1967. Chromosome micromanipulation. I. The mechanics of chromosome attachment to the spindle. *Chromosoma (Berl.).* 21:1–16.
16. Takahashi, K., C. Shingyoji, and S. Kamimura. 1982. Microtubule sliding in reactivated flagella. *In* Prokaryotic and Eukaryotic Flagella (Thirty-Fifth Symp., Society for Experimental Biology). Cambridge University Press, Cambridge. 159–177.
17. Taylor, E. W. 1965. Brownian and saltatory movements of cytoplasmic granules and the movement of anaphase chromosomes. *In* Proceedings of the Fourth International Congress on Rheology, Part 4, Symposium: on Biorheology. A. L. Copley, editor. Interscience, New York. 175–191.
18. Warner, F. D. 1979. Cilia and flagella: microtubule sliding and regulated motion. *In* Microtubules. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., London. 359–380.
19. Yoneda, M. 1960. Force exerted by a single cilium of *Mytilus edulis*. *J. Exp. Biol.* 37:461–468.