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Regulation of Chromosome Speeds in Mitosis

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Abstract—When chromosomes are being separated in preparation for cell division, their motions are slow (~16 nm/s) relative to the speed at which many motor enzymes can move their cellular cargoes (160–1000 nm/s and sometimes even faster) and at which microtubules depolymerize (~200 nm/s). Indeed, anaphase chromosome speeds are so slow that viscous drag puts little load on the mechanisms that generate the relevant forces (Nicklas, *Adv. Cell Biol.* 2:225, 1971). Available evidence suggests that chromosome speed is due to some form of regulation. For example, big and little chromosomes move at about the same speed, chromosomes that have farther to go move faster than others, and chromosome speed is affected by both temperature and an experimentally applied load. In this essay we review data on these phenomena and present our ideas about likely properties of the mechanisms that regulate chromosome speed.

Keywords—Mitosis, Cell division, Chromosome motion, Force–velocity relation.

THE MITOTIC SPINDLE AND THE MOTIONS OF CHROMOSOMES

Cell division is essential to life. In eukaryotes, segregation of duplicated chromosomes into the daughter cells is performed by the "mitotic spindle," a cellular machine made from microtubules (MTs) and many associated proteins. Key steps in this process include the attachment of MTs to chromosomes, the MT-driven alignment of chromosomes at the midplane of the spindle, and the symmetric separation of chromosomes in "anaphase." The spindle MTs are organized in a bipolar arrangement (Fig. 1). Our focus in this paper is on the speeds at which spindle-attached chromosomes move during mitosis, particularly the motion of the duplicated chromosomes to the separate spindle poles, which enable the formation of the two daughter cell nuclei.

Chromosomes range in size and shape from almost spherical objects, ~0.25 μm in diameter, to elongate double cylinders (attached to each other in one region) that are ~1 μm in diameter and as much as 10 μm long. Thus, describing their speed requires some definitions and details. When cells enter the division process, their DNA has already duplicated, so each mitotic chromosome is composed of two genetically identical parts called "chromatids"; these are bound together at a single site called the "centromere." Each chromatid includes one specialized region called a "kinetochore" located at its centromere. Kinetochores are the principal sites of interaction between a chromosome and the MTs of the mitotic spindle. Kinetochores are composed of ~60–100 different types of proteins, including motor enzymes, non-motor proteins that interact with MTs, fibrous proteins that couple these MT-interaction sites with the underlying DNA, and regulatory proteins, such as protein kinases and phosphatases.[8,11]

In addition to their attachment to kinetochores, spindle MTs can also push on the arms of a chromosome, using either comparatively slow and non-processive motors from the kinesin super-family that bind to chromatin[57] or simply through MT polymerization dynamics. Microtubules are linear polymers assembled from the protein tubulin, and their polymerization can exert a force. These forces are, however, weak. The relevant motors, called "chromo-kinesins," produce only ~1 pN per MT, and their speed is ~150 nm/s,[6] so the majority of a chromosome's movements are defined by the MTs that bind to kinetochores; these are called "KMTs." In big cells there is a bundle of KMTs that accumulates on each kinetochore during early mitosis, making a "K-fiber." In the cells of higher eukaryotes, such bundles contain 5–75 MTs, so they are visible in the light microscope. In some small cells, budding yeast (*Saccharomyces cerevisiae*) for example, there is only 1 KMT/kinetochore.

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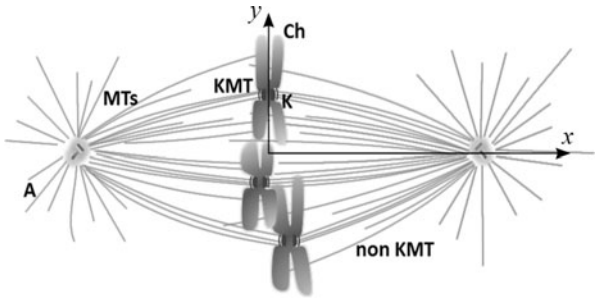

FIGURE 1. Diagram of a mitotic spindle, as might be found in an animal cell. Ch labels one of three chromosomes, which are each composed of two chromatids, thanks to previous DNA replication. K labels a kinetochore and KMT labels a bundle of kinetochore-associated MTs. MTs indicates other spindles MTs, some of which are called nonKMTs, and A indicates MTs that project out from the spindle, called astral MTs. Coordinates *x* and *y* indicate Cartesian axes relative to the center of the spindle for descriptions of motion. Most chromosome motion is approximately along the *x* axis. Redrawn from McIntosh *et al.*[29]

Descriptions of chromosome motion commonly refer to motions of one or both kinetochores. They use either one of the spindle poles or a point midway between the poles as the origin of a Cartesian frame of reference; one axis of this frame is parallel to the pole-to-pole axis (Fig. 1). The majority of chromosome motions are approximately parallel to this axis, which is also approximately parallel to the bulk of the spindle MTs in any region of the spindle. Thus, chromosome motions can generally be thought of as along MTs or in association with a MT end.

Chromosome motion in anaphase is usually at ~1 μm/min (~16 nm/s), though some cells beat this by as much as a factor of 5. At these speeds, the viscous drag on a chromosome of average size is only a fraction of a pN, even assuming that the viscosity of cytoplasm is substantially greater than that of water.[34,54] To estimate the drag force, note that the slow motions and small length scales mean that the Reynolds number is very low ($<10^{-7}$), so viscous effects dominate. In this overdamped limit, the magnitude of the drag force opposing an object's motion is proportional to the speed of its motion through the fluid: $F = \zeta v$. The drag coefficient can be estimated assuming a fluid of viscosity η and treating the chromosome as a cylinder of length L and aspect ratio $a = L/D$ that is dragged through the fluid perpendicular to its long axis. In this case[14]

$$\zeta \approx \frac{4\pi\eta L}{\ln a}. \qquad (1)$$

For a mitotic chromosome, the largest possible drag coefficient would occur for the largest length, $L \approx 10$ μm, and then $a \approx 10$. Even for a large viscosity of 10 Poise (1000 times that of water, consistent with the high end of measured estimates of cytoplasmic viscosity[10]), we estimate a drag coefficient of $\zeta \approx 5.5 \times 10^{-2}$ g/s. The resulting force required to drag the chromosome at a speed of 16 nm/s would be $F \approx 0.9$ pN.

Earlier in mitosis, however, when chromosomes make their initial contact with spindle fibers, motions are commonly ~10-fold faster than in anaphase.[44] These motions approximate the speeds of granules and vesicles that move during interphase in the same cells.[40] This similarity has led to the view that early chromosome motions are caused by the same motors that move granules. The presence of motor enzymes at the kinetochores (e.g., cytoplasmic dynein and two or more different kinesins) is consistent with this view. Structural studies at sufficient resolution have also determined that early chromosome motions commonly occur over the surface of MTs, similar to vesicle transport,[26,43] so this view is likely to be correct. Since the motions are at normal motor speeds, there does not seem to be additional regulation of their speeds.

There is, however, a lack of understanding of the factors that control the directions and magnitudes of pre-anaphase chromosome motions. If one kinetochore interacts with the spindle, it will commonly pull the whole chromosome in the direction of the nearest spindle pole. Sometimes both kinetochores will interact with spindle MTs that emanate from the same spindle pole, again pulling the whole chromosome immediately poleward. However, proper chromosome attachment to the spindle requires that sister kinetochores interact with MTs that emanate from sister poles (Fig. 1), and in this case a chromosome's two kinetochores are pulled in opposite directions. One would then imagine that the net force exerted by the spindle would be zero, at least on average, and indeed the DNA at the centromere is stretched, suggesting the action of opposing forces on the centromere.[50] At this time, though, chromosomes in most cells are not static; they oscillate back and forth, moving approximately parallel to the spindle axis.[41] Chromosome speed during these oscillations is about the same in both directions, but the durations of the movements are not, leading to a bias in net movement that gradually and irregularly moves the chromosomes to the spindle equator, forming the "metaphase plate" of grouped chromosomes. How chromosome position is sensed and thus the motion directed toward the metaphase plate is an important, unsolved problem that will not be considered further here.

Several workers have been able to place visible marks on K-fibers, such as spots of either bright or dim fluorescence, using a labeled form of tubulin.[17,33] Time series images of kinetochores relative to these marks and to the spindle poles have allowed a more precise description of chromosome motion relative to

spindle MTs than is possible simply with images of the chromosomes themselves. The results show that mitotic cells from different species have quite different patterns of chromosome motion relative to K-fibers, both in metaphase and anaphase. In many metaphase cells, when the chromosomes are on average at the spindle midplane, motion of the K-fibers occurs away from the kinetochores, a "flux" that requires the addition of tubulin subunits to the KMTs at or near the kinetochores and a depolymerization of these MTs at or near the spindle pole.[23] This MT flux is generally slow (~16 nm/s) and of constant speed. In very big spindles, like the 40-μm-long spindles formed in fertilized frog eggs, the speed of flux in non-KMTs varies with position in the spindle,[24] suggesting that the MTs that comprise these spindles are short relative to the spindle's length, and that tubulin comes on and off MT ends scattered throughout the spindle.[60]

In cells from vertebrates and many other species, anaphase motion of chromosomes to the spindle poles, called "anaphase-A," begins with a depolymerization of the K-fiber at the kinetochore.[39] As the the chromosomes near the spindle poles, however, this motion slows, and continued anaphase-A results from K-fiber depolymerization at the spindle pole.[23] In yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), however, there is no indication of tubulin depolymerization near the spindle pole at any time.[25] In a nematode worm (*Caenorhabditis elegans*), most of anaphase chromosome motion occurs with K-fibers of approximately constant length (no anaphase-A), but the spindle poles move apart, increasing the separation between sister chromosomes through an elongation of the spindle; this is commonly called "anaphase-B."[21] In the sperm-forming cells of at least two insects, marks on K-fibers show KMT flux toward the spindle poles in both metaphase and anaphase, so here anaphase chromosome separation relies entirely on depolymerization of the K-fiber at the spindle poles,[9,22] followed by spindle elongation. In short, the kinematics of chromosomes relative to their K-fibers are complex and show variability with both time in mitosis and the species of cell studied. We conclude that the motions of kinetochores relative to the KMTs to which they bind are controlled by the cell.

Most spindles show both an anaphase-A and an anaphase-B, though the relative contribution of these two motions to chromosome separation is variable between species. Commonly, chromosome segregation begins with anaphase-A, and -B begins just as -A is finishing. Anaphase-B can be extensive, depending on the size of the spindle relative to the size of the cell; in some elongate fungi, the spindle at the end of chromosome motion is more than ten times the length of the spindle at metaphase, but in most cells anaphase-B simply doubles the length of the metaphase spindle.

CHROMOSOME DYNAMICS

By the time of metaphase, most if not all anaphase chromosomes are associated through their kinetochores with the ends of one or more MTs. For this reason the slower motions of anaphase have been thought to be regulated by MT dynamics.[28,35] One school of thought has suggested that considerable work must be done by the kinetochore and/or spindle pole to take KMTs apart, particularly since many spindle MTs that are not kinetochore-associated elongate during anaphase at the time that KMTs are shortening. Thus, an apparently attractive model for the control of chromosome speed is that in early mitosis chromosomes move over MT walls at rates defined by the kinetics of kinetochore-associated motor enzymes, and subsequent chromosome slowing is defined by the need to depolymerize KMTs for chromosomes to approach the poles.

More recent studies on chromosome dynamics have, however, made this model more complex. In two distantly related yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) it has been possible to delete all kinetochore-associated motor enzymes that move toward the spindle pole, yet chromosome-to-pole motions continued at normal speeds.[18,53] These results have been interpreted to suggest that the forces for anaphase-A come not from motor enzymes but from MTs themselves. Indeed, several studies of the interactions between kinetochore proteins and MTs *in vitro* have shown that both motors and some non-motor MT-associated proteins can follow the end of a shortening MT in an ATP-independent manner, with force generated from the energy stored in the MT lattice as tubulin depolymerizes.[31] The most recent of these studies described as much as 30 pN derived from the shortening of a single MT,[56] so this mode of force generation can easily move the chromosomes, even if their arms have become entangled and the load the spindle must carry far exceeds that imposed by viscous drag.

The observation that motors are dispensable for anaphase-A poses a problem for understanding the regulation of mitotic speeds: if MT dynamics are generating mitotic forces, can they also be resisting motion, so they regulate speed? Since MT depolymerization shows typical speeds of ~200 nm/s, it seems that the control of mitotic chromosome motions must involve additional factors or processes that serve to regulate the rate of MT shortening. Whatever this control mechanism might be, it displays several

subtleties of behavior that make it quite intriguing, as described below.

A second aspect of mitotic chromosome motion is the speed imparted by a kinetochore's attachment to a spindle pole. Poles move apart, both during spindle formation and during Anaphase-B. In some cells spindle elongation is driven by motor activity near the spindle's midplane, where antiparallel spindle MTs interdigitate (Fig. 2).[7] In other cells the force for spindle elongation comes from motors located near the cell's cortex, which can pull on the astral MTs that project away from the spindle and interact with the cell cortex.[1] In the latter case, the direction and speed of motion are controlled by the sliding of the interdigitating MTs near the anaphase spindle midplane; these both slow elongation[46] and assure that the two poles are moving in opposite directions. Both of these motor drives for spindle elongation are coupled to MT dynamics, because the sliding apart of the interdigitating MTs of the interpolar spindle is accompanied by a lengthening of these MTs through addition of tubulin at their pole-distal ends.[47] Anaphase B usually occurs at about the rate of chromosome motion (1 μm/min or ~16 nm/s). Again, we see MT dynamics as a potential regulator for mitotic motions, with the driving force for the motion coming from spindle motors.

EXPERIMENTAL PERTURBATIONS OF CHROMOSOME RATES

Schapp and Forer have performed a detailed study of the temperature dependence of anaphase rates in two insects.[48] Their paper also assembles data from previous studies on the same issue in plant and sea urchin cells. In addition, they measured the effect of temperature on the Brownian motion of intracellular particles, as a reporter for cytoplasmic viscosity, and concluded that the 2–3-fold increase in chromosome speed for a 10 °C change in temperature is a bigger effect than can be explained by physical changes in the medium through which chromosomes move. From all these data one can say with confidence that chromosomes move faster at higher temperatures, and the change is big enough to imply a rather high activation energy, about 63–105 kJ/mol, or 36–60 k_BT at 23 °C, assuming that the same reaction is rate-limiting at all temperatures used.[48] These numbers imply a rate-limiting step whose activation energy will be overcome only occasionally, a likely feature for so slow a motion as anaphase chromosome movement. In this context it is noteworthy that analogous measurements of speed as a function of temperature have recently been made for a cardiac myosin. Here a 10 °C change in temperature produced a change in K_{cat} of about a factor of 3,[45] so large activation energies are found even with a much simpler mechanochemical system, here represented by a purified enzyme. The implications of these data for regulation of anaphase are that speed control involves a process with a big activation energy, but it doesn't tell us what that process is.

Nicklas designed a clever set of experiments to ask where within the spindle the temperature-dependent rate of chromosome motion was set.[36] He put grasshopper spermatocytes onto the stage of an inverted microscope in a cool room (~11 °C). A minute nichrome wire carrying a controlled electric current was used to add heat to different regions of dividing cells. His evidence demonstrated temperature gradients within cells treated this way: spindle birefringence increases with temperature, and by viewing his cells through crossed polarizing filters, he saw that birefringence was higher on the warmed side of the cell. He confirmed that when the whole cell was warmed, chromosomes moved faster, as in the work from the Forer lab. In cells with a temperature gradient along the spindle axis, the chromosomes on the two sides of the spindle always moved at the same speed; their rate was defined by the temperature at the mid-region of the spindle, where MTs that do not associate with kinetochores interdigitate with their counterparts from the opposite pole (Fig. 2). These results in sum tell us

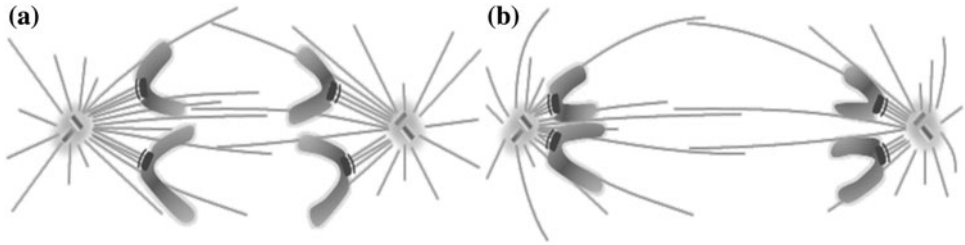

FIGURE 2. Diagrams of chromosomes segregating in anaphase. (a) Chromosomes partially separated, thanks to a decrease in the distance between kinetochores and poles (anaphase-A). The nonKMTs long enough to extend into the opposite half-spindle interdigitate with their counterparts from the opposite pole. (b) Continued chromosome separation thanks to a further decrease in the kinetochore-pole distance and an increase in the separation between the poles (anaphase-B). This motion is usually accompanied by both growth and sliding of the interdigitating MTs. Redrawn from McIntosh *et al.*[29]

that the factors regulating chromosome speed are positively regulated by temperature. They also suggest that at least in insect spermatocytes the rate of MT sliding in the spindle's midzone may contribute to the rate of anaphase-A, but they don't help us understand what the regulatory factors are.

Nicklas also experimented with the effects of added loads on the speed of anaphase chromosomes. He took advantage of the robust membrane around insect spermatocytes and used a mechanically calibrated glass micro-needle to deform the membrane far enough to push on an anaphase chromosome and resist its poleward motion.[37] (This is a little like using one's fingers to manipulate an object inside a water balloon.) As the retarding force was increased, chromosome speed decreased approximately linearly with increasing load, stalling at an estimated added load of ~700 pN/kinetochore. These results imply that chromosome speed in anaphase is not simply load-limited, but that opposing force can induce significant slowing. However, determining what these experiments say about the force generated per KMT is not easy.

Nicklas and colleagues had studied K-fibers in the spermatocytes of a related species by electron microscopy.[38] These structural data suggested that there were about 45 KMTs/kinetochore, but that only ~15 of these made a connection from kinetochore to pole. Nicklas therefore used the latter number to estimate the force each KMT could generate when under maximal load: ~40 pN/MT. It must be added that a recent study that used laser trapping with infrared light to slow or arrest chromosome motion in spermatocytes of crane flies found that only 6–10 pN per kinetochore was sufficient to stop anaphase-A, a factor of ~100 less than observed by Nicklas.[16] The reasons for this discrepancy are not obvious, but the observations mean that the forces developed by a spindle in anaphase are not known for certain. In our opinion, there may be issues of photo-damage from the laser beam in the latter experiments, and Nicklas' observations are probably a better representation of true spindle forces. Regardless of this view, the kinematics reviewed above show that whatever the magnitude of spindle forces, the rates of resulting chromosome motions are regulated.

Two other genre of experiment have probed the regulation of anaphase speed: studies of spindles in lysed cells, where conditions can be modified by the observer, and the use of mutants to alter proteins believed to be important for chromosome motion. Spindles grown in cell-free extracts of frog egg cytoplasm have confirmed that ATP is required for spindle elongation,[12] but no work has yet been done to modulate conditions and observe their effects on chromosome speed. When cultured cells have been lysed during early anaphase with buffers that stabilized the spindle, additional chromosome-to-pole motion requires ATP and the addition of Ca^{++}, an agent known to promote MT depolymerization *in vitro*.[51] These data suggest that MT depolymerization is necessary to allow chromosomes to move poleward, but neither of these studies has informed us about the mechanisms that regulate mitotic speeds. Likewise, the many mutations that have been made in genes important for spindle function have not yet turned up anything to illuminate the regulation of anaphase rates.

INTERPRETATIONS AND MODELS OF THE DATA

Once kinetochores attach to the pole-distal (plus) ends of KMTs, chromosome motion is necessarily coupled to tubulin dynamics. In yeasts, the data tell us that pole-directed motor enzymes are not needed for chromosome-to-pole motion, but analogous data from higher cells are not yet available. It appears that MT depolymerization at the kinetochore is a driving force for anaphase-A, at least in yeasts, but spindle kinematics in other cells show that at least some of anaphase-A is associated with tubulin depolymerization at the spindle pole. In insect spermatocytes, the pole is the only site of KMT depolymerization. Both kinetochores and poles are sites where kinesin 13s are localized[42]; these are enzymes that use the energy of ATP hydrolysis to promote cycles of tubulin depolymerization and MT shortening.[13] Kinesin 8s, which also promote MT shortening[20,55] are commonly found at kinetochores,[52,58] even in yeasts, which lack kinesin 13s. Thus, even if tubulin dynamics are driving chromosome motion, motors may be affecting and controlling the rate of MT depolymerization. Thus, the rate of anaphase-A may be motor-regulated, even if the energy that drives it comes ultimately from MT depolymerization.

A structural fact of some relevance has emerged from detailed study of KMT end shape in several species. In cells from a mammal (*Potorus tridactylus* kidney [PtK1] cells),[27] a nematode (*Caenorhabditis elegans* embryonic blastomeres), two yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), and a green alga (*Chlamydomonas reinhardtii*)[30] KMTs are flared at their kinetochore-proximal end, both in metaphase and anaphase. When chromosomes are moving poleward, the length of the flares on KMTs are distinctly longer than in metaphase, suggesting that the rate-limiting process in KMT shortening is not the loss of tubulin from the MT ends but the process by which adjacent strands of tubulin in the MT wall split apart.

These "lateral" bonds between polymerized tubulins might themselves be regulated, but control seems more likely to be exercised through MT-associated proteins, which bind polymerized tubulins and help to hold adjacent "protofilaments" together. The regulation of these "staples" by a post-translational modification, such as phosphorylation, is a way in which cells might control protofilament splitting and thus the rate of KMT depolymerization. Such a mechanism, either working in parallel with a kinesin 13 or on its own, could govern rates of MT shortening and thus chromosome motion in ways that are compatible with all the data discussed above.

Molecular Friction as a Regulator of Chromosome Speed: A Model Based on Yeast Proteins Studied In Vitro

Recent work on yeast kinetochore proteins interacting with MTs *in vitro* casts some light on the regulation of depolymerization rates for MT plus ends. The yeast kinetochore protein complex called Dam1 (a.k.a. DASH) is assembled from 10 different proteins; once formed, this complex can assemble into rings around MTs.[32,59] The presence of such a ring slows the rate of tubulin depolymerization.[19] Moreover, when a force opposing motion is applied to the ring, e.g., by attaching it to a glass sphere that can be captured in a laser trap, the depolymerization of the surrounded MT can generate up to 30 pN of force/MT with an exponential dependence of the speed on opposing force.[56] The data fit nicely to a model that uses two exponential decay constants: one for the fast motions that occur under low load, and one for the slower motions that occur when the load is higher (Fig. 3). Intriguingly, the force–velocity curve from this work based on purified components is quite similar to equivalent data obtained with isolated yeast kinetochores, whose chemical composition is not explicitly known, but is sure to be more complex than the simple system.[2]

The properties of interactions between a MT wall and oligomers of the Dam1 complex have been assessed by measuring the one-dimensional diffusion of fluorescent Dam1 associated with a MT wall. The diffusion rate decreases exponentially with the degree of Dam1 oligomerization. When as many as nine subunits are in a single complex, its bonds with the MT wall are so strong that diffusion becomes unmeasurably slow; an oligomer containing the ~16 subunits necessary to form a complete Dam1 ring probably diffuses at a negligible rate. The measured one-dimensional diffusion coefficient for smaller complexes corresponds to an activation energy for Dam1 movement over the MT wall of $8.7 \pm 0.7\ k_B T$/Dam1–tubulin interaction, so for this ring to move with the shortening MT end, it must undergo a "forced walk"; it is pushed along by the depolymerizing MT.[19] It seems intuitively plausible that such a device could serve as a regulator for the speed for anaphase-A.

Here we consider a model for chromosome speed regulation based upon the molecular friction provided by the Dam1 ring. Consider a chromosome connected to a single depolymerizing MT by N connectors per MT, where the maximal value of N is 13, the number of protofilaments in a MT. As each connector moves along the MT, we assume that it experiences molecular friction, i.e., each connector has hopping rates forward and backward that are force dependent: the forward

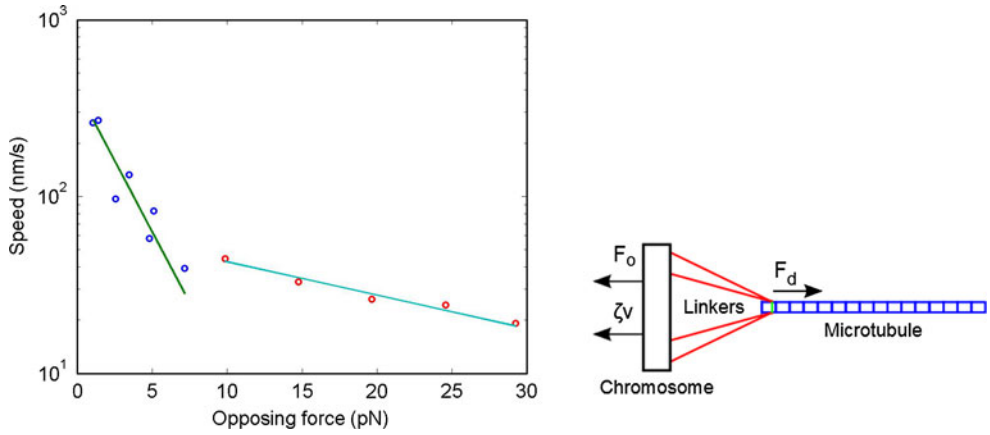

FIGURE 3. Left, force–velocity relation for the molecular-friction model in the high and low force regimes. We plotted (points) and fit (lines) to the data of Volkov *et al.*[56] and Akiyoshi *et al.*[2] in the two force regimes. The fits shown here used F_d = 30 pN. In the high-speed (low-opposing-force) regime, we estimate $k_{hs} \approx 7.9 \times 10^{-4}$/s and $N_{hs} \approx 2.7$, and in the low-speed (high-opposing-force) regime we estimate $k_{ls} \approx 2.2$/s and $N_{ls} \approx 23$. Right, schematic of the molecular-friction model. A depolymerizing microtubule exerts a force F_d shared by N independent linkers which connect to the chromosome. The chromosome experiences a drag force ζv and possible additional opposing force F_o.

hopping rate (in the positive x direction, which is toward the MT minus end) is $k_+(F)$ and the backward hopping rate is $k_-(F)$. The simplest model of molecular friction is that the hopping rates are exponentially force dependent[5]:

$$k_\pm = k_o e^{\pm\beta F(\frac{\delta}{2}\pm\epsilon)}, \tag{2}$$

where k_o is the hopping rate in the absence of force, $\beta = 1/(k_B T) \approx 0.25/(\text{pN nm})$ is the inverse temperature in energy units, F is the net force on the connector attachment, δ is the spacing between binding sites along the MT, assumed to be the 8-nm MT lattice spacing, and ϵ is a parameter that characterizes the asymmetry of the molecular binding potential as the connector moves along the MT. Typically $\epsilon \ll \delta$ and this parameter can be neglected; we will neglect it for our initial estimates.

The net force F is determined by the force exerted by MT depolymerization, the drag force on the chromosome, and any applied force opposing depolymerization. The depolymerization force exerted by the MT is F_d. Each connector is assumed to act as a spring, and is stretched so that it exerts a spring force F_s in the negative x direction on the MT and the same magnitude of force in the positive x direction on the chromosome (Fig. 3). The chromosome experiences a drag force ζv in the negative x direction as well as a possible opposing force F_o. We assume that the chromosome moves at constant speed, so the net force on it is zero, and that each connector exerts the same spring force. Thus, the sum of the spring forces balances the drag force, and $NF_s = \zeta v + F_o$. Each connector attached to the MT experiences a net force $F = (F_d - \zeta v - F_o)/N$.

The net speed of motion is then

$$v = \delta(k_+ - k_-), \tag{3}$$

$$v = \delta k_o \left[e^{\beta F(\delta/2+\epsilon)} - e^{-\beta F(\delta/2-\epsilon)} \right], \tag{4}$$

$$v \approx \delta k_o \exp \frac{\beta\delta(F_d - F_o)}{2N}, \tag{5}$$

where in the last line we have neglected the asymmetry parameter of the potential, the drag force (assuming that $\zeta v \ll F_d$), and the smaller exponential term.

What is the best numerical value of the depolymerization force F_d? The experiments of Volkov *et al.* measured an average depolymerization force of 9 pN and a maximum of 30 pN. In this work, high force values depended on the use of a force-clamp, applied as the MT shortened over about 1 μm, in a complex experimental setup for which not every trial is optimal. This suggests that the maximal value is more likely to represent the force that a depolymerizing MT can actually generate than the mean, whose value is biased down by less successful experiments. However, we also note that the change in slope of the force–velocity relation occurs at approximately 9 pN, suggesting the possibility of a force-dependent change in the nature of the motion. Therefore we studied the effects of using either 9 or 30 pN as the value of F_d.

We can estimate k_o from the diffusion measurements of Volkov *et al.*[56] They found an estimated diffusion coefficient for the whole ring in the range 10^{-8}–10^{-6} $\mu m^2/s$ = 0.01–1 nm^2/s. Therefore, the typical time to hop a distance δ = 8 nm is $\tau = \delta^2/(2D)$ with values in the range 32−3200 s. This gives a very slow hopping rate constant in the range $k_o = 3.1 \times 10^{-4}$–10^{-2}/s. With this slow rate constant, F_d = 30 pN, and N = 13, Eq. (5) gives a zero-force speed of 0.025−2.5 nm/s, quite different from the 300 nm/s measured by Volkov *et al.* However, if we use this rate constant and N = 3, we estimate a zero-force speed of 55−5500 nm/s, a range which includes the measurement of Volkov *et al.* This suggests that a molecular-friction model could be consistent with the data of Volkov *et al.*, if the effective number of attachments is significantly less than 13. This situation is in fact likely, given that the geometry of a planar ring with ~16 subunits is incommensurate with a MT lattice comprising 13 protofilaments, a three-start left-handed helical arrangement of the α- and β-tubulin subunits, and a seam (the likely structure of MTs *in vivo*). Indeed, observations of microbeads coupled to Dam1 complexes and bound to shortening MTs have displayed a wobbling that suggests irregular motion as Dam1 oligomers are pushed by bending protofilaments over the MT lattice.[19]

The observation of Volkov *et al.* that the exponential decay constant of the force–velocity relation changes at around 9 pN opposing force could perhaps occur because the value of N (the number of independently moving attachments) is different in the two force regimes. Motivated by these observations, we fit our model to the pooled data of Volkov *et al.*[56] and Akiyoshi *et al.*,[2] allowing k_o and N to be free parameters separately fit in the two speed regimes with F_d = 30 pN. From these fits we estimate $k_{hs} \approx 7.9 \times 10^{-4}$/s and $N_{hs} \approx 2.7$ in the high-speed (low-opposing-force) regime, and $k_{ls} \approx 2.2$/s and $N_{ls} \approx 23$ in the low-speed (high-opposing-force) regime (Fig. 3). The low value for N when the ring is moving quickly is plausible, because the short dwell time combined with ring wobble may reduce the likelihood that a good bond can form. When the opposing force is higher and MT depolymerization is slowed, there may now be sufficient time for more bonds to form, albeit, the value 23 is implausibly high, given the presumed geometry of the moving ring and the well-established geometry of a

MT. However, 23 and 16 are not greatly different, given the uncertainty in some of the experimental values used here as constraints. An alternative interpretation of the data is that perhaps in the fast motion at low force the ring has not yet fully assembled around the MT, and is partially bound to the lateral MT wall; the slow motion at high force could then correspond to a fully assembled ring. This mechanism could explain the variation in N in the two regimes.

This comparison of the model to the data yields several results that are somewhat counter-intuitive and reduce the likelihood that frictional resistance to ring movement is the sole regulator of chromosome speed in yeasts. In the high-speed regime, if we assume $F_d = 30$ pN, then the value of k_{hs} we estimate is consistent with the diffusion data of Volkov *et al.*, but the effective number of attachments is surprisingly low. If a device is working as a regulator of speed, one might expect that the number of attachments would be high in the high-speed regime in order to slow the motion down. Instead, if this molecular-friction model is correct, then in the low-speed regime the hopping rate unexpectedly increases to be four orders of magnitude higher than in the high-speed case, and at least 2 orders of magnitude higher than is consistent with the Dam1 diffusion measurements. Moreover, in the low-speed regime N_{ls} also increases by an order of magnitude from that in the high-speed regime. In addition, we note that the maximal speed measured in the experiments is ~300 nm/s, a factor of 2–5 larger than occurs in yeast cells. This suggests either that a model of this type may not be applicable to the data, or that the Dam1 ring alone does not constitute the regulator of mitotic speeds in yeasts. As stated above, additional MT-associated proteins may be serving to regulate the rate at which KMT protofilaments split apart, facilitating tubulin depolymerization.

We also performed the fit of the molecular-friction model using the average force of 9 pN as the value of the depolymerization force F_d. In this case, it is not possible to fit the data in the low-speed regime where the applied force is >9 pN, because in that regime the molecular-friction model would predict no or backward motion. In the high-speed regime, using $F_d = 9$ pN leads to the estimates $N_{hs} \approx 2.7$ (as above), and $k_{hs} \approx 1.8$/s. It is striking that this hopping rate constant is approximately equal to the value $k_{ls} \approx 2.2$/s found in the low-speed regime with $F_d = 30$ pN. This suggests an alternate interpretation of the model: perhaps the maximum depolymerization force exerted by the MT changes with the interacting state of the ring. We note however that the hopping rate constant found in this way is 2–4 orders of magnitude larger than that estimated for the full ring by Volkov *et al.*

We also considered the possibility that the step size δ could be smaller than the 8-nm tubulin-dimer spacing, either because the connector, like the Ndc80 complex, interacts in a similar way with the α- and β-tubulin monomers,[3] leading to a step size of 4 nm, or because binding interactions between the connector and the disordered C-terminal tails of tubulin lead to an effectively smaller step size.[59] Because in our model the speed of motion is proportional to δk_o, any decrease in the step size δ requires an increase in the hopping rate k_o by the same factor to keep the same speed. Therefore this model can accommodate various step sizes with corresponding changes in the hopping rate.

If this model is a reasonable physical description of the experiments then the comparison of model and data does suggest the possibility that the nature of the Dam1 ring's MT attachment and motion could change with opposing force. Physically, such a change in effective number of attachments could result from a change in the angles accessible to the ring. Perhaps at low opposing force, where the ring is moving at high speed, the ring is aligned almost perpendicular to the MT axis and motion requires the ring to move as a rigid unit, permitting fewer attachments with the underlying lattice. When the opposing force is higher, and the ring is moving more slowly—more approaching the conditions of free ring diffusion—the ring can tilt, wobble, and deform, so its individual subunits behave more independently.

A Model for the Interaction Between a Connector and a Microtubule

The model we have proposed above is based on *in vitro* experiments on Dam1 rings and isolated yeast kinetochores attached to depolymerizing MTs. This mechanism is unlikely to apply for a wide range of organisms, because the genes that encode proteins similar to Dam1 have not been found outside the fungi. There might, nonetheless, be an alternative device that functions in a manner analogous to the Dam1 complex. Currently, the best candidate is the Ska complex, which binds more tightly to the bent protofilaments of tubulin characteristic of a MT end than to the MT wall.[49] When Ska is MT-associated, it also binds near the N-terminus of Ndc80/Nuf2, the MT-binding end of the ubiquitous Ndc80 complex. Again, the tight binding of a MT-interacting complex might serve as the governor to restrain both anaphase-A and the rate of KMT depolymerization.

In higher organisms, the force–velocity relation of chromosome motion may be different than the exponential form discussed above. Nicklas measured an approximately linear force–velocity relation for chromosomes

moving in insect cells,[37] where multiple MTs interact with each kinetochore. Our simple molecular-friction model is not able to lead to a linear force–velocity relation, even if multiple MTs interact with the chromosome. To see this, suppose that we change our model so that we now have M separate MTs, each with N connectors as above. For simplicity, assume that the connecting springs are all of approximately the same length; the net force on each connector is now $F = (F_d - \zeta v - F_o)/(NM)$. The only change is that the forces have all been divided by M. The form of the force–velocity relation in Eq. (5) above is unchanged except for this rescaling of the force, and the force–velocity relation remains exponential. This suggests that another physical mechanism is necessary to explain the linear force–velocity relation measured by Nicklas.

Another plausible mechanism for the regulation of speed is a more direct physical interaction between the connector and the MT. The model considered so far treats tubulin depolymerization as applying a constant force to the connecting molecule. A more sophisticated model assumes that the MT end has a fluctuating position that can interact in various ways with any attached molecule. The motion of the attached molecule is then determined by the fluctuations of the MT end and of the connector, as well as their interaction.

Suppose that the end of the MT is at site n (measured relative to some fixed lab reference) and the depolymerization rate is p and the polymerization rate is q. This corresponds to the end of the MT undergoing a "hop" forward (toward the minus end) at rate p and backward at rate q (Fig. 4). The connector is at site m and tends to hop forward at rate k_+ and backward at rate k_-. When the MT end and the connector are close to each other, they interact. We describe the interaction by a potential $U(m - n)$ which depends only on the difference $j = m - n$. For large separations $j \gg 1$ the end and the connector have no effect on each other, so the coupling potential tends to zero. However, when j is small the coupling potential changes the motion of both the MT end and the connector: $U > 0$ inhibits the forward (depolymerizing) motion of the MT end and increases the hopping to the right of the connector.

Detailed balance determines how the coupling potential changes the connector hopping rates. If the connector hops left ($m \rightarrow m - 1$), the interaction energy changes from $U(j)$ to $U(j - 1)$. The ratio of $k_-(j)$ to $k_+(j - 1)$ therefore satisfies

$$\frac{k_-(j)}{k_+(j-1)} = \frac{k_-}{k_+} e^{-[U(j-1)-U(j)]}, \tag{6}$$

where k_- and k_+ are the rates in the limit $j \rightarrow \infty$ and for simplicity we are using units where $k_B T = 1$. The potential also influences MT depolymerization in a similar way:

$$\frac{p_j}{q_{j-1}} = \frac{p}{q} e^{-[U(j-1)-U(j)]}. \tag{7}$$

Eqns. (6) and (7) determine only the ratios of the rates. The energy barrier determines how the individual rates change. The effect of the energy barrier on the rates can be represented by a coefficient $0 < f < 1$:

$$\begin{aligned} k_+(j) &= k_+ e^{-f[U(j-1)-U(j)]}, \\ k_-(j-1) &= k_- e^{-(f-1)[U(j-1)-U(j)]}, \\ q_{j-1} &= q e^{-f[U(j-1)-U(j)]}, \\ p_j &= p e^{-(f-1)[U(j-1)-U(j)]}. \end{aligned} \tag{8}$$

Smaller values of f imply that the potential changes predominantly the backward rates, while for large f the interaction primarily affects the forward rates. Note that f is the same for MT end motion and connector hopping since both phenomena involve the same barrier. Further, we emphasize that $f = 0$ and $f = 1$ are physically unrealistic and singular limits.

We do not know *a priori* the shape of the coupling potential $U(j)$. We therefore study two simple forms of $U(m - n)$, subject to the requirements (*i*) $U \rightarrow 0$ for $m \gg n$ (when the MT end and the connector are far

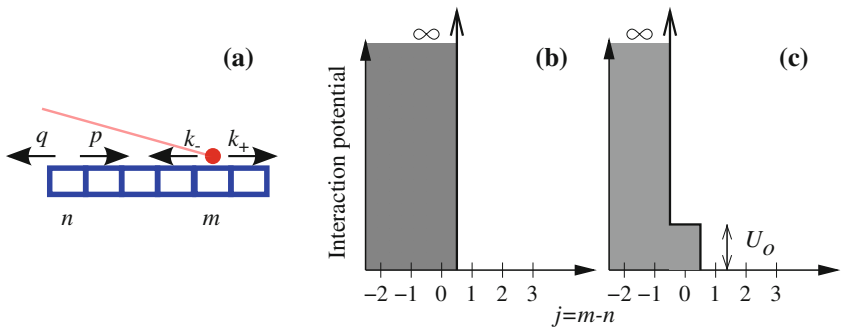

FIGURE 4. (a) Schematic of the interacting hopping model. The plus end of the microtubule is at site *n* and can "hop" forward (toward the minus end) at rate *p* and backward at rate *q*. The connector is at site *m* and hops forward at rate k_+ and backward at rate k_-. (b, c) Coupling potentials between the microtubule end and connector. (b) Hard-wall potential. (c) A potential with a step.

apart, there is no interaction) and (*ii*) $U \to \infty$ for $n \gg m$. The motion can then be solved by solving the master equation for the probability of finding the system at separation j and midpoint position l, following previous work.[4]

The simplest form of interaction is a hard-wall interaction (Fig. 4b), for which it is assumed that some point on the MT wall acts as a hard wall which blocks backward motion of the attachment point. For simplicity, we will consider the limit $q \to 0$, i.e., we neglect fluctuations that repolymerize the MT. We suppose that the attached molecule hops at some speed $k_{\pm}(F_o) = k_o e^{\mp \gamma F_o}$ as above, where F_o is an opposing force and γ is the force sensitivity of the connector hopping rates, for simplicity assumed the same for forward and backward hops. The speed of motion is then

$$v_{hw} = \frac{k_o e^{-\gamma F_o}}{k_o e^{\gamma F_o} + p} \delta p = c v_d \tag{9}$$

To simplify the notation to come, we define $c(F_o) = k_+(F_o)/(k_-(F_o) + p)$. This has an exponential dependence on opposing force (Fig. 5).

A more complex form of interaction is to assume a direct physical interaction between the end of the MT and the attached molecule. A simple form this could take is to assume a potential "step" of height U_o attached to the steric hard wall discussed just above (Fig. 4c). This is assuming a short-range repulsive interaction between that portion of the MT end and the attached molecule; this could easily occur if for example the attachment point could still bind to a slightly curved protofilament, but with a reduced binding affinity making it energetically unfavorable to bind in this region. This form of the interaction alters the motion of the attached molecule, leading to a speed v_1. The result is that

$$v_1 = \frac{c + (1-c)e^{-fUo}}{c + (1-c)e^{-Uo}} c v_d. \tag{10}$$

While this force–velocity relation is never truly linear, in some parameter regimes it can appear quite close to linear. (See Fig. 5.) We chose the MT depolymerization parameters assuming that this point on the MT would, if free, move at a speed $v_d = 25$ nm/s (an estimated depolymerization rate of MTs *in vitro*); then $p = v_d/\delta = 3$/s. We chose the other parameters to qualitatively match the data of Nicklas[37]: the maximum speed of chromosome motion is ~15 nm/s, and the maximum force per MT is ~30 pN.

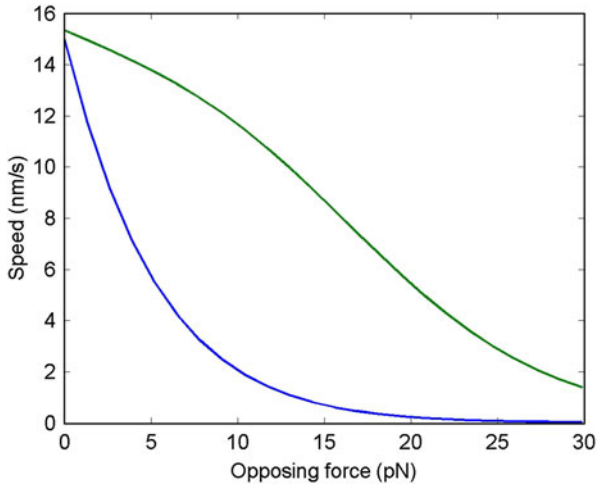

FIGURE 5. Force-velocity relation for the interaction model, showing predictions for a hard-wall (blue) vs. short-range repulsive (green) potential. The zero-force microtubule depolymerization parameters shared by both curves are p = 3/s and δ = 8 nm. The other parameters were selected to qualitatively match the data of Nicklas[37]: the maximum speed is ~15 nm/s, and the maximum force per microtubule is ~30 pN. For the hard-wall potential curve, γ = 1.5/pN and k_o = 4.7/s. For the short-range repulsive potential curve, γ = 2.25/pN, k_o = 0.38/s, U_o = 5 k_BT, and f = 0.1.

Spindle Interzone Effects

The insect cells in which KMTs continue to polymerize at the kinetochores while the chromosomes are moving poleward are an especially interesting case, because the temperature dependence of anaphase speed is defined by the temperature of the zone between the separating chromosome, implying that anaphase-A is being driven from behind and permitted by polar depolymerization of KMTs. Micromanipulation experiments in which K-fibers were cut with a microneedle at various distances from the pole support this idea, because a chromosome will continue to move poleward, so long as >1 μm of K-fiber is left attached to the kinetochore (Fig. 6).[39] These data support a view that pushing forces from the interzone combine with the regulation of tubulin dynamics at both kinetochore and pole to define the speed of anaphase-A. In

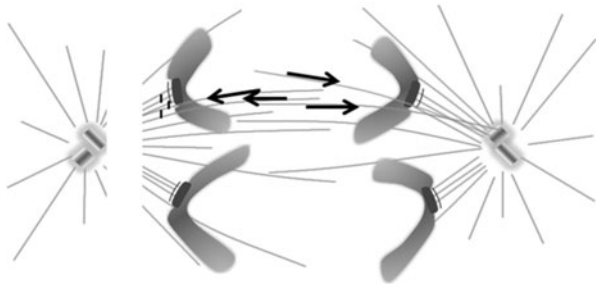

FIGURE 6. During anaphase, sliding forces between antiparallel MTs in the interzone (black arrows) might couple to KMTs (short, black struts), transmitting forces that would push the chromosome poleward, even if the connection between KMTs and pole were broken. Redrawn from McIntosh *et al.*[29]

this view, the force acting at the chromosome is a consequence of drag at the kinetochore as the K-fiber is pushed poleward from the interzone.[15] In this scenario chromosome speed is defined by the nature of this process, perhaps both by effects of molecular friction as discussed above and by effects of motors pushing apart the interzone. These effects would be interesting to consider in future modeling work.

CONCLUSION

We have reviewed the observations and data on the speed of chromosome movements in mitosis. Chromosome movements during anaphase-A are believed to be due to the depolymerization of MTs (which has typical speeds ~200 nm/s) and the action of motor proteins (which have typical speeds ~160–1000 nm/s), yet the speed of chromosome movements are usually only ~16 nm/s. The slow movements mean that typical drag forces on chromosomes are low, suggesting that some other biological factors control the speeds of anaphase chromosome movements.

Experiments measuring the force–velocity relationship of depolymerizing MTs encircled by a Dam1 ring or a yeast kinetochore coupled to a bead by connectors find an exponential force–velocity relation with two different regimes. We considered a molecular-friction model for this motion, and suggest that the difference in the two force regimes could be explained by a change in the effective number of bead-MT attachments with opposing force. It is likely that a molecular device like a Dam1 ring does impose some regulation on the speed of KMT depolymerization, and thus of chromosome motion, but a close look at this process suggests that this kind of molecular friction is unlikely to explain all aspects of chromosome speed regulation. For example, a molecular-friction model is not able to explain the linear force–velocity relation for chromosome motion measured in cells. A model of the coupled MT depolymerization and connector motion positing a short-range repulsive interaction between MT end and connector can in some cases lead to an approximately linear force–velocity relation, and may be a component of the physiological processes that regulate chromosome motion.

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REFERENCES

[1]Aist, J. R., C. J. Bayles, W. Tao, and M. W. Berns. Direct experimental evidence for the existence, structural basis and function of astral forces during anaphase b in vivo. *J. Cell Sci.* 100(2):279–288, 1991. http://jcs.biologists.org/content/100/2/279.

[2]Akiyoshi, B., K. K. Sarangapani, A. F. Powers, C. R. Nelson, S. L. Reichow, H. Arellano-Santoyo, T. Gonen, J. A. Ranish, C. L. Asbury, and S. Biggins. Tension directly stabilizes reconstituted kinetochore–microtubule attachments. *Nature* 468(7323):576–579, 2010. http://www.nature.com/nature/journal/v468/n7323/full/nature09594.html.

[3]Alushin, G. M., V. H. Ramey, S. Pasqualato, D. A. Ball, N. Grigorieff, A. Musacchio, and E. Nogales. The ndc80 kinetochore complex forms oligomeric arrays along microtubules. *Nature* 467(7317):805–810, 2010. http://www.nature.com/nature/journal/v467/n7317/abs/nature09423.html.

[4]Betterton, M. D., and F. Jülicher. Opening of nucleic-acid double strands by helicases: active versus passive opening. *Phys. Rev. E* 71(1): 011904, 2005. http://link.aps.org/doi/10.1103/PhysRevE.71.011904.

[5]Bormuth, V., V. Varga, J. Howard, and E. Schaffer. Protein friction limits diffusive and directed movements of kinesin motors on microtubules. *Science* 325(5942):870–873, 2009. http://www.sciencemag.org/cgi/doi/10.1126/science.1174923.

[6]Brouhard, G. J., and A. J. Hunt. Microtubule movements on the arms of mitotic chromosomes: polar ejection forces quantified in vitro. *Proc. Natl Acad. Sci. U.S.A.* 102(39):13903–13908, 2005. http://www.pnas.org/content/102/39/13903.

[7]Brust-Mascher, I., and J. M. Scholey. Mitotic spindle dynamics in *Drosophila*. In: International Review of Cytology, Vol. 259, edited by K. W. Jeon. San Diego: Academic Press, 2007, pp. 139–172. http://www.sciencedirect.com/science/article/pii/S0074769606590047.

[8]Cheeseman, I. M., and A. Desai. Molecular architecture of the kinetochore–microtubule interface. *Nat. Rev. Mol. Cell Biol.* 9(1):33–46, 2008. http://www.nature.com/nrm/journal/v9/n1/full/nrm2310.html.

[9]Chen, W., and D. Zhang. Kinetochore fibre dynamics outside the context of the spindle during anaphase. *Nat. Cell Biol.* 6(3):227–231, 2004. http://www.nature.com/ncb/journal/v6/n3/abs/ncb1104.html.

[10]Daniels, B. R., B. C. Masi, and D. Wirtz. Probing single-cell micromechanics in vivo: the microrheology of *C. elegans* developing embryos. *Biophys. J.* 90(12):4712–4719, 2006. http://www.sciencedirect.com/science/article/pii/S0006349506726467.

[11]DeLuca, J. G., and A. Musacchio. Structural organization of the kinetochore–microtubule interface. *Curr. Opin. Cell Biol.* 24(1):48–56, 2012. http://www.sciencedirect.com/science/article/pii/S0955067411001499.

[12]Desai, A., P. S. Maddox, T. J. Mitchison, and E. D. Salmon. Anaphase a chromosome movement and poleward spindle microtubule flux occur at similar rates in xenopus extract spindles. *J. Cell Biol.* 141(3):703–713, 1998. http://jcb.rupress.org/content/141/3/703.

[13]Desai, A., and T. J. Mitchison. A new role for motor proteins as couplers to depolymerizing microtubules. *J. Cell Biol.* 128(1/2):1–4, 1995. http://www.jstor.org/stable/1616720.

[14]Doi, M., and S. F. Edwards. The Theory of Polymer Dynamics, Vol. 73. New York: Oxford University Press, 1988.

[15]Dumont, S., and T. Mitchison. Mechanical forces in mitosis. In: Comprehensive Biophysics, Vol. 4, edited by E. Egelman. Amsterdam: Elsevier.
[16]Ferraro-Gideon, J., R. Sheykhani, Q. Zhu, M. L. Duquette, M. W. Berns, and A. Forer. Measurements of forces produced by the mitotic spindle using optical tweezers. *Mol. Biol. Cell*. 24(9):1375–1386. http://www.molbiolcell.org/content/early/2013/03/11/mbc.E12-12-0901.
[17]Gorbsky, G. J., P. J. Sammak, and G. G. Borisy. Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends. *J. Cell Biol*. 104(1):9–18, 1987. http://jcb.rupress.org/content/104/1/9.
[18]Grishchuk, E. L., and J. R. McIntosh. Microtubule depolymerization can drive poleward chromosome motion in fission yeast. *EMBO J*. 25(20):4888–4896, 2006.
[19]Grishchuk, E. L., I. S. Spiridonov, V. A. Volkov, A. Efremov, S. Westermann, D. Drubin, G. Barnes, F. I. Ataullakhanov, and J. R. McIntosh. Different assemblies of the DAM1 complex follow shortening microtubules by distinct mechanisms. *Proc. Natl Acad. Sci. U.S.A*. 105(19):6918–6923, 2008. http://www.pnas.org/content/105/19/6918.
[20]Gupta, M. L., P. Carvalho, D. M. Roof, and D. Pellman. Plus end-specific depolymerase activity of kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. *Nat. Cell Biol*. 8(9):913–923, 2006. http://www.nature.com/ncb/journal/v8/n9/full/ncb1457.html.
[21]Labbé, J., E. K. McCarthy, and B. Goldstein. The forces that position a mitotic spindle asymmetrically are tethered until after the time of spindle assembly. *J. Cell Biol*. 167(2):245–256, 2004. http://jcb.rupress.org/content/167/2/245.
[22]LaFountain, J. R., C. S. Cohan, A. J. Siegel, and D. J. LaFountain. Direct visualization of microtubule flux during metaphase and anaphase in Crane-Fly spermatocytes. *Mol. Biol. Cell* 15(12):5724–5732, 2004. http://www.molbiolcell.org/content/15/12/5724.
[23]Maddox, P., A. Desai, K. Oegema, T. J. Mitchison, and E. Salmon. Poleward microtubule flux is a major component of spindle dynamics and anaphase a in mitotic Drosophila embryos. *Curr. Biol*. 12(19):1670–1674, 2002. http://www.sciencedirect.com/science/article/pii/S0960982202011831.
[24]Maddox, P., A. Straight, P. Coughlin, T. J. Mitchison, and E. D. Salmon. Direct observation of microtubule dynamics at kinetochores in xenopus extract spindles implications for spindle mechanics. *J. Cell Biol*. 162(3):377–382, 2003. http://jcb.rupress.org/content/162/3/377.
[25]Maddox, P. S., K. S. Bloom, and E. D. Salmon. The polarity and dynamics of microtubule assembly in the budding yeast saccharomyces cerevisiae. *Nat. Cell Biol*. 2(1):36, 2000. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2879060/.
[26]Magidson, V., C. B. O'Connell, J. Lončarek, R. Paul, A. Mogilner, and A. Khodjakov. The spatial arrangement of chromosomes during prometaphase facilitates spindle assembly. *Cell* 146(4):555–567, 2011. http://www.sciencedirect.com/science/article/pii/S0092867411007732.
[27]McIntosh, J. R., E. L. Grishchuk, M. K. Morphew, A. K. Efremov, K. Zhudenkov, V. A. Volkov, I. M. Cheeseman, A. Desai, D. N. Mastronarde, and F. I. Ataullakhanov. Fibrils connect microtubule tips with kinetochores: a mechanism to couple tubulin dynamics to chromosome motion. *Cell* 135(2):322–333, 2008. http://www.sciencedirect.com/science/article/pii/S0092867408011197.
[28]Mcintosh, J. R., P. K. Hepler, and D. G. V. Wie. Model for mitosis. *Nature* 224(5220):659–663, 1969. http://www.nature.com/nature/journal/v224/n5220/abs/224659a0.html.
[29]McIntosh, J. R., M. I. Molodtsov, and F. I. Ataullakhanov. Biophysics of mitosis. *Q. Rev. Biophys*. 45(2):147–207, 2012.
[30]McIntosh, J. R., E. O'Toole, K. Zhudenkov, M. Morphew, C. Schwartz, F. I. Ataullakhanov, and E. L. Grishchuk. Conserved and divergent features of kinetochores and spindle microtubule ends from five species. *J. Cell Biol*. 200(4):459–474, 2013. http://jcb.rupress.org/content/200/4/459.
[31]McIntosh, J. R., V. Volkov, F. I. Ataullakhanov, and E. L. Grishchuk. Tubulin depolymerization may be an ancient biological motor. *J. Cell Sci*. 123(20):3425–3434, 2010. http://jcs.biologists.org/content/123/20/3425.
[32]Miranda, J. L., P. D. Wulf, P. K. Sorger, and S. C. Harrison. The yeast DASH complex forms closed rings on microtubules. *Nat. Struct. Mol. Biol*. 12(2):138–143, 2005. http://www.nature.com/nsmb/journal/v12/n2/abs/nsmb896.html.
[33]Mitchison, T. J. Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol*. 109(2):637–652, 1989. http://jcb.rupress.org/content/109/2/637.
[34]Nicklas, R. B. Chromosome velocity during mitosis as a function of chromosome size and position. *J. Cell Biol*. 25(1):19–135, 1965. http://jcb.rupress.org/content/25/1/119.
[35]Nicklas, R. B. Mitosis. *Adv. Cell Biol*. 2:225, 1971. http://europepmc.org/abstract/MED/5005502.
[36]Nicklas, R. B. Chromosome movement and spindle birefringence in locally heated cells: interaction versus local control. *Chromosoma* 74(1):1–37, 1979. http://link.springer.com/article/10.1007/BF00344480.
[37]Nicklas, R. B. Measurements of the force produced by the mitotic spindle in anaphase. *J. Cell Biol*. 97(2):542–548, 1983. http://jcb.rupress.org/content/97/2/542.
[38]Nicklas, R. B., and G. W. Gordon. The total length of spindle microtubules depends on the number of chromosomes present. *J. Cell Biol*. 100(1):1–7, 1985. http://jcb.rupress.org/content/100/1/1.
[39]Nicklas, R. B., G. M. Lee, C. L. Rieder, and G. Rupp. Mechanically cut mitotic spindles: clean cuts and stable microtubules. *J. Cell Sci*. 94(3):415–423, 1989. http://jcs.biologists.org/content/94/3/415.
[40]Rebhun, L. I. Structural aspects of saltatory particle movement. *J. Gen. Physiol*. 50(6):223–239, 1967. http://jgp.rupress.org/content/50/6/223.
[41]Rieder, C. L., E. A. Davison, L. C. Jensen, L. Cassimeris, and E. D. Salmon. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. *J. Cell Biol*. 103(2):581–591, 1986. http://jcb.rupress.org/content/103/2/581.
[42]Rogers, G. C., S. L. Rogers, T. A. Schwimmer, S. C. Ems-McClung, C. E. Walczak, R. D. Vale, J. M. Scholey, and D. J. Sharp. Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature* 427(6972):364–370, 2004. http://www.nature.com/nature/journal/v427/n6972/abs/nature02256.html.
[43]Roos, U. Light and electron microscopy of rat kangaroo cells in mitosis. *Chromosoma* 40(1):43–82, 1973. http://link.springer.com/article/10.1007/BF00319836.
[44]Roos, U. Light and electron microscopy of rat kangaroo cells in mitosis. *Chromosoma* 54(4):363–385, 1976. http://europepmc.org/abstract/MED/1253643.
[45]Ruth, F., R. Sommese, J. Sung, S. Nag, S. Sutton, J. Deacon, E. Choe, L. Leinwand K. Ruppel, and J. Spudich. Molecular consequences of the R453C hypertrophic cardiomyopathy

mutation on human β-cardiac myosin motor function. *Proc. Natl Acad. Sci. U.S.A.* 110(31):12607–12612.

[46]Saunders, A. M., J. Powers, S. Strome, and W. M. Saxton. Kinesin-5 acts as a brake in anaphase spindle elongation. *Curr. Biol.* 17(12):R453–R454, 2007. http://www.sciencedirect.com/science/article/pii/S0960982207013334.

[47]Saxton, W. M., and J. R. McIntosh. Interzone microtubule behavior in late anaphase and telophase spindles. *J. Cell Biol.* 105(2):875–886, 1987. http://jcb.rupress.org/content/105/2/875.

[48]Schaap, C. J., and A. Forer. Temperature effects on anaphase chromosome movement in the spermatocytes of two species of crane flies (*Nephrotoma suturalis loew* and *Nephrotoma ferruginea fabricius*). *J. Cell Sci.* 39(1):29–52, 1979. http://jcs.biologists.org/content/39/1/29.

[49]Schmidt, J. C., H. Arthanari, A. Boeszoermenyi, N. M. Dashkevich, E. M. Wilson-Kubalek, N. Monnier, M. Markus, M. Oberer, R. A. Milligan, M. Bathe, G. Wagner, E. L. Grishchuk, and I. M. Cheeseman. The Kinetochore-Bound ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. *Dev. Cell* 23(5):968–980, 2012. http://www.sciencedirect.com/science/article/pii/S1534580712004236.

[50]Skibbens, R. V., V. P. Skeen, and E. D. Salmon. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* 122(4):859–875, 1993. URL http://jcb.rupress.org/content/122/4/859.

[51]Spurck, T. P., and J. D. Pickett-Heaps. On the mechanism of anaphase a: evidence that ATP is needed for microtubule disassembly and not generation of polewards force. *J. Cell Biol.* 105(4):1691–1705, 1987. http://jcb.rupress.org/content/105/4/1691.

[52]Stumpff, J., and L. Wordeman. Chromosome congression: the Kinesin-8-Step path to alignment. *Curr. Biol.* 17(9):R326–R328, 2007. http://www.sciencedirect.com/science/article/pii/S0960982207011141.

[53]Tanaka, K., E. Kitamura, Y. Kitamura, and T. U. Tanaka. Molecular mechanisms of microtubule-dependent kinetochore transport toward spindle poles. *J. Cell Biol.* 178(2):269–281, 2007. http://jcb.rupress.org/content/178/2/269.

[54]Taylor, E. W. Some comments on the mechanism of mitosis. *Soc. Gen. Physiol.* 30:1, 1975. http://www.ncbi.nlm.nih.gov/pubmed/1188414.

[55]Varga, V., C. Leduc, V. Bormuth, S. Diez, and J. Howard. Kinesin-8 motors act cooperatively to mediate length-dependent microtubule depolymerization. *Cell* 138(6):1174–1183, 2009. http://linkinghub.elsevier.com/retrieve/pii/S009286740900912X.

[56]Volkov, V. A., A. V. Zaytsev, N. Gudimchuk, P. M. Grissom, A. L. Gintsburg, F. I. Ataullakhanov, J. R. McIntosh, and E. L. Grishchuk. Long tethers provide high-force coupling of the dam1 ring to shortening microtubules. *Proc. Natl. Acad. Sci. U.S.A.* 110(19):7708–7713, 2013. http://www.pnas.org/content/early/2013/04/18/1305821110.

[57]Wang, S. Z., and R. Adler. Chromokinesin: a DNA-binding, kinesin-like nuclear protein. *J. Cell Biol.* 128(5):761–768, 1995. http://jcb.rupress.org/content/128/5/761.

[58]West, R. R., T. Malmstrom, and J. R. McIntosh. Kinesins klp5+ and klp6+ are required for normal chromosome movement in mitosis. *J. Cell Sci.* 115(5):931–940, 2002. http://jcs.biologists.org/content/115/5/931.short.

[59]Westermann, S., H. Wang, A. Avila-Sakar, D. G. Drubin, E. Nogales, and G. Barnes. The dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends. *Nature* 440(7083):565–569, 2006. http://www.nature.com/nature/journal/v440/n7083/full/nature04409.html.

[60]Yang, G., L. A. Cameron, P. S. Maddox, E. D. Salmon, and G. Danuser. Regional variation of microtubule flux reveals microtubule organization in the metaphase meiotic spindle. *J. Cell Biol.* 182(4):631–639, 2008. http://jcb.rupress.org/content/182/4/631.