The mechanics of microtubule networks in cell division

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The primary goal of a dividing somatic cell is to accurately and equally segregate its genome into two new daughter cells. In eukaryotes, this process is performed by a self-organized structure called the mitotic spindle. It has long been appreciated that mechanical forces must be applied to chromosomes. At the same time, the network of microtubules in the spindle must be able to apply and sustain large forces to maintain spindle integrity. Here we consider recent efforts to measure forces generated within microtubule networks by ensembles of key proteins. New findings, such as length-dependent force generation, protein clustering by asymmetric friction, and entropic expansion forces will help advance models of force generation needed for spindle function and maintaining integrity.

Introduction

During eukaryotic cell division, the mitotic spindle mediates the separation of chromosomes into the two daughter cells (Gadde and Heald, 2004; Kapoor, 2017). Microtubules within the spindle are organized in a dense array, with their positions, orientations, lengths, regions of overlap, and nucleation sites regulated by motor and nonmotor proteins. During the division process, mechanical forces are necessary to separate the chromosomes, as demonstrated by the pioneering work of Nicklas et al. (1982). Using glass microneedles to directly interact with the chromosomes of dividing grasshopper spermatocytes, the researchers showed that chromosomes in anaphase can experience significant forces. Although calculations based on the observed size and speed of micron-sized chromosomes moving through a viscous environment suggest that it would require only ∼0.1 pN of force to move chromosomes, Nicklas showed that the spindle machinery is capable of exerting forces of up to 700 pN before chromosome motion was stalled (Nicklas, 1983, 1988). This value is many hundreds of times larger than the maximum force typically generated by a single motor protein. This remarkable result suggests that the spindle can produce significantly more mechanical work by exerting forces on the micron-length scale than is minimally required to move chromosomes.

However, the results from this series of experiments also give rise to an important question: If large forces on chromosomes are being applied from the center of the bipolar spindle outward, toward the poles, how does a spindle maintain its structural integrity and not simply collapse under this tension? One plausible answer is that the network of microtubules that fill the spindle but do not interact directly with kinetochores are generating opposing forces, resulting in a force balance across the whole of the bipolar spindle network. These forces could allow the spindle to operate in a steady state, maintaining its structural integrity but also allowing for chromosome motions through the dense microtubule network. Here, we highlight findings from recent studies that are likely to be important for spindle organization and function, including overlap length–dependent pushing and braking forces, protein friction and autonomous clustering of protein ensembles by frictional asymmetry, and entropic forces generated by diffusible cross-linkers. Together, these findings point toward the development of a more refined map of forces in the spindle and will motivate cell biological experiments that directly test these mechanical principles.

The organization of different subsets of spindle microtubules

Microtubules, which form the structural framework of the spindle, can be broadly classified into three distinct categories (Fig. 1 A). First are the kinetochore microtubules, often referred to as k-fibers, which make direct contact at their plus ends with the kinetochores. In many higher-eukaryotic spindles, parallel bundles of multiple microtubules form k-fibers that make contact with the kinetochore on each sister chromatid. For example, in Ptk1 (*Potorous tridactylus*) epithelial cells, which remain relatively flat during mitosis and have been extensively used as a model system, ∼25 microtubules contact each kinetochore at metaphase (McEwen et al., 1997). Second are the astral microtubules that originate predominantly from the centrosome and extend toward the cell cortex. Astral microtubules are involved in chromosome capture, and their interactions with the cortex can help to position the spindle within the cell (Hayden et al., 1990; Grill et al., 2003). Finally, interpolar microtubules are densely packed within the volume between the two spindle poles (Mastronarde et al., 1993).

Interpolar microtubules have much shorter lifetimes than kinetochore microtubules, with reported half-life values of $t_{1/2}$ = ∼20 s, compared with minutes for k-fibers (Saxton et al., 1984). Interpolar microtubules are likely to be cross-linked at overlaps throughout the spindle and are predominantly found in an antiparallel arrangement near the spindle equator and a parallel arrangement at spindle poles where they are clustered and tightly focused. In certain systems, such as *Xenopus laevis*

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Abbreviation used: MAP, microtubule-associated protein.

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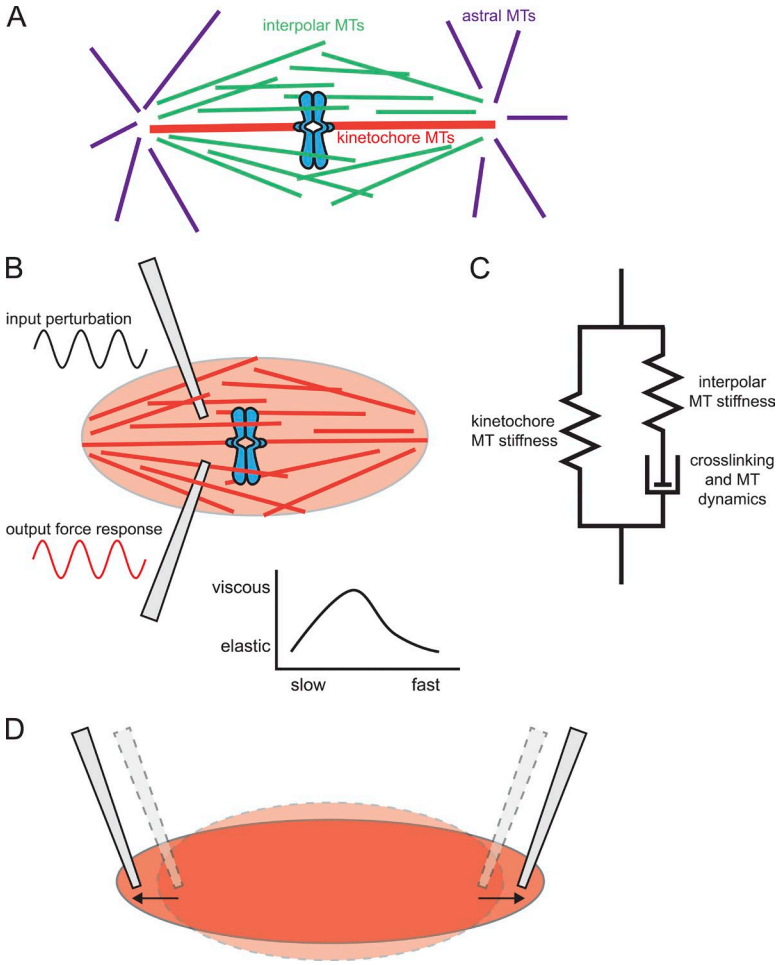

Figure 1. **Mechanics of the spindle microtubule network.** (A) Schematic depicting the three major classes of microtubules of the spindle. K-fibers (red), interpolar microtubules (green), and astral microtubules (purple) are shown. (B) Calibrated microneedles inserted into a spindle are used to apply mechanical perturbations perpendicular to the long axis of the spindle. At fast (seconds) and slow (minutes) timescales, the spindle is more elastic, whereas at intermediate (tens of seconds) timescales, the spindle is more viscous. (C) A Zener-type model of a viscoelastic solid, which includes elastic spring-like elements linked to k-fiber and interpolar microtubule stiffness and viscous damping terms linked to cross-linking dynamics, describes the measured mechanical properties of the spindle. (D) Microneedles inserted near the spindle poles allow for the application of force along the spindle's long axis.

egg extract, interpolar microtubules constitute more than 90% of the spindle microtubule population (Dumont and Mitchison, 2009). Considering this dense arrangement of microtubules that are spaced a mean distance of 30–50 nm apart (Mastronarde et al., 1993), a paradox seems to emerge; this filament network must be able to maintain its structural integrity to both generate and withstand large forces, but it must also be able to accommodate the motion of micron-sized chromosomes throughout its dense interior. This suggests a role for both forces that push microtubules and viscous forces that allow for microtubule sliding and spindle network remodeling. Understanding the magnitudes, localizations, and orientations of these different types of forces in the form of a force map will prove invaluable in explaining how spindles perform the mechanical task of chromosome segregation.

Classification of forces within the spindle microtubule network

The kinds of forces produced within a spindle can be classified as either active forces that use chemical energy and passive forces, such as friction, that dissipate energy.

Active forces. Active forces are predominantly generated by microtubule assembly and disassembly dynamics and by motor proteins. Microtubule filaments grow when GTP-bound tubulin dimers are added to the end of a filament and shrink when GDP-bound dimers are lost into solution. The addition or removal of tubulin dimers results in a gain or release of free energy on the order of 10 kT, and this energy can be converted into mechanical work (Hill and Kirschner, 1982). Single dynamic microtubules can also produce pushing forces in the range of 2–5 pN when growing against a calibrated load (Dogterom and Yurke, 1997), and bundled microtubules can produce tens of piconewtons of force (Laan et al., 2008).

Motor proteins use the free energy released via the hydrolysis of ATP to engage in directional motion along microtubule tracks, producing forces that allow for the displacement of cargos or the relative sliding of microtubules. Mitotic motor proteins such as kinesin-5 (Valentine et al., 2006; Korneev et al., 2007), dynein (Gennerich et al., 2007), and kinesin-8 (Jannasch et al., 2013) have been examined in single-molecule force spectroscopy assays, revealing the force-dependent stepping behaviors of each under load. Single molecules of these motor proteins can generate maximum forces in the 1- to 10-pN range. The rate and directionality of stepping, the lifetime of protein–microtubule interactions, and the processivity of the protein can all be modulated when load is applied. Despite our advanced understanding of single particle behavior, the mechanical outputs of motor protein ensembles such as those found within the spindle remain poorly understood. Although it is possible that force generation within microtubule bundles simply scales with motor protein number, previous studies have shown that multiple motor proteins transporting cargo may not be able to generate sustained additive forces (Jamison et al., 2010). Force generation by motor protein ensembles within cross-linked filaments may therefore be quite complex, and direct measurements are required.

Passive forces. Less studied, but no less important, are the so-called passive forces, which include elastic forces, viscous drags, or frictional resistance. Elastic terms include stretching forces such as those applied across sister kinetochores and the bending of stiff microtubule filaments or bundles. The bending modulus of single microtubules has been determined (Gittes et al., 1993), and theoretical calculations of the stiffness of microtubule bundles have been made (Rubinstein et al., 2009). However, as microtubules may be bundled by different types of proteins with different cross-linking densities, their mechanics can be challenging to estimate. For example, if relative filament sliding occurs, the bundle stiffness likely scales with number of individual microtubules in the bundle. In contrast, for tightly cross-linked bundles that cannot undergo relative sliding, the stiffness would scale with the square of the microtubule number. Understanding properties such as resistance to filament sliding by the relevant cross-linking proteins is therefore critical in describing the mechanics of microtubule networks.

Resistive terms can arise when protein binding is disrupted; for example, nonmotor microtubule-associated proteins (MAPs) that cross-link multiple microtubules may generate frictional resistance to the relative sliding of these microtubules. On the molecular level, it is likely that resistive and frictional forces arise when noncovalent bonds between proteins are broken during the relative motions of various spindle factors. One might expect that the magnitude of these forces increases with velocity, similar to the macroscopic Stokes drag force against an object moving through viscous media, where the resistive force on a spherical object is directly proportional to the speed at which it moves.

Time scales of forces. The spindle can be thought of as a complex viscoelastic material built from ordered arrays of dynamic polymers. Even for simple materials, mechanical responses can depend on whether forces are applied quickly or slowly, as illustrated by the traditional classroom demonstration using a mixture of cornstarch and water. This viscoelastic material behaves as a solid when rapid stirring is attempted, but adopts a more liquid-like behavior when stirring forces are applied slowly. At the molecular level, individual motor proteins are capable of moving at rates ranging from tens to hundreds of nanometers per second (Sharp et al., 2000). Within cells, microtubule plus ends grow at rates on the order of several hundred nanometers per second (Rusan et al., 2001). Therefore, both motor proteins and microtubule dynamics can exert forces rapidly, with motor stepping and tubulin addition events typically occurring many of times per second at the nanometer scale. Frictional forces are similarly likely to arise at similar fast time scales, as proteins interact with sliding microtubules or dynamic filament ends. Elastic forces, such as k-fiber bending or kinetochore stretching across paired chromosomes, are more likely to persist on the order of minutes during metaphase. Therefore, when formulating a spindle force map, one must account for not only active and passive forces generated by different individual components within the spindle, but also the timescale on which these forces act.

Mechanical measurements of the entire spindle microtubule network

How might we begin to understand the micromechanics of the metaphase spindle? Spindles assembled in meiotic extract from *Xenopus laevis* eggs have proven to be an especially powerful system to begin to address this question, as these spindles are not constrained within cellular membranes, and therefore direct access to the spindle can be achieved with both chemical and mechanical probes. Using calibrated microneedles, the time scale–dependent viscoelastic properties of the metaphase spindle have been directly examined (Shimamoto et al., 2011). In brief, two glass needles were first coated with silicone to suppress specific binding interactions with individual spindle components (Fig. 1 B). The needles were inserted into the spindle near the metaphase plate, and perturbations were generated perpendicular to the long axis of the spindle by oscillating one needle, while the second served as a force-calibrated readout. At slow (minutes) and fast (seconds) time scales, the spindle responded as an elastic material, stretching in response to micrometer-scale motions. At intermediate time scales (tens of seconds), the spindle exhibited viscous properties, behaving more like a liquid. These time scale–dependent mechanical properties can be well described as a Zener-type viscoelastic solid (Fig. 1 C). This model consists of two springs and a dashpot. One of the spring-like elements, in which a stretching force produces a specific amount of displacement, may be linked to the bending stiffness of interpolar microtubules. In series with this spring is a dashpot element that acts as a viscous frictional damper that resists motion and may be linked to the lifetimes and strengths of microtubule cross-links. These two elements together are then in parallel with a stiffer spring-like element that likely corresponds to k-fiber bending. The relative contributions of spindle components were determined by biochemical disruption of specific populations of microtubules or mitotic proteins and direct mechanical measurement of the perturbed spindle.

In a different set of experiments, the application of compressive forces with microneedles at the spindle equator reduced the natural width of the spindle by ∼10% and resulted in a compensatory change in spindle length, again consistent with the idea that the spindle behaves like a viscoelastic solid (Itabashi et al., 2009). Applying forces directly at the spindle poles and stretching outward along the long axis reveals that the spindle is able to generate a restoring force that persists for many minutes (Takagi et al., 2014). During this stretching, spindle volume and tubulin density were conserved, as determined by fluorescence microscopy (Fig. 1 D). These experiments revealed the time-dependent viscoelastic properties of the spindle microtubule network, with dynamic interpolar microtubules, long-lived and stiff kinetochore fibers, and protein-mediated microtubule cross-linking, all contributing to the organization of a robust network that can remodel itself to accommodate chromosome motions. Recent biophysical studies have begun to elucidate new properties that describe these spindle mechanics and help to refine the map of forces across the spindle network.

New insights from studies of cross-linked microtubule pairs

The use of reconstituted microtubule networks by small ensembles of purified proteins has proven useful in measuring the mechanics of microtubule network assembly. From these studies, new principles and concepts about force production are beginning to emerge.

Active forces generated within microtubule networks. Kinesin-5 was the first motor protein shown to slide microtubules apart by walking on each filament it cross-links (Kapitein et al., 2005). Kinesin-5 functions as a homotetramer and adopts a bipolar structure, with pairs of N terminus motor domains located at opposite ends of a coiled-

coil tetramerization domain (Kashina et al., 1996; Scholey et al., 2014). Processive directional motion is triggered by cross-linking, and microtubule sliding is enhanced by a nonmotor microtubule-binding domain at the C terminus (Weinger et al., 2011). This ability to push apart antiparallel microtubules is linked to the various cellular functions of kinesin-5, which include establishing spindle bipolarity and maintenance, as well as contributing to the poleward flux of microtubules in metaphase (Ferenz et al., 2010).

The sliding of microtubule pairs has also been demonstrated for two minus end motor proteins. Kinesin-14 (Ncd) is a microtubule minus end–directed kinesin, and it contains a C terminus motor domain and an N terminus nonmotor microtubule-binding domain. These domains also allow kinesin-14 to bundle and slide antiparallel microtubules (Fink et al., 2009); in particular, its processivity along bundled microtubules is greatly enhanced by its N terminus domain. Furthermore, the yeast kinesin-14 homologue Klp2 has been shown to assemble bundles of parallel microtubules (Braun et al., 2009). Another important minus end–directed motor protein, cytoplasmic dynein, has been shown to slide apart two microtubules in a reconstituted microtubule bundle assay (Tanenbaum et al., 2013).

It has been assumed that forces are generated as a result of these motor proteins driving relative microtubule sliding, but this was not directly measured until recent experimental tests using kinesin-5 (Shimamoto et al., 2015). Traditionally, in single-molecule assays, individual proteins of interest were directly conjugated to the trapping bead, which could then be brought into contact with the microtubule substrate. However, to measure the force generated within sliding microtubule pairs cross-linked by an ensemble of kinesin-5 molecules, it would be advantageous to attach the bead directly to one of the microtubules while the second microtubule is immobilized on a surface. Additionally, a method for determining the number of kinesin-5 molecules in the ensemble and the length of overlap between the two microtubules would be required. These criteria have recently been met, and the force production by kinesin-5 ensembles acting within cross-linked microtubule pairs has now been directly monitored, revealing that the magnitudes of both pushing and braking forces scale in proportion to the length of microtubule overlap (Shimamoto et al., 2015).

Previous analyses of cargos carried by multiple kinesin-1 molecules suggest that the force may not persistently scale as more motor proteins engage the microtubule surface (Jamison et al., 2012). Indeed, cross-talk between two or more kinesin molecules may lead to the rapid detachment of one or more of the proteins as the stepping behavior of one kinesin interferes with the mechanics of adjacent kinesins (Furuta et al., 2013). Within these types of systems, if one motor protein steps, a strain is induced along the protein that propagates to other proteins in the ensemble. If the microtubule binding of these other molecules is strongly sensitive to force, this strain will accelerate the unbinding of motor proteins, and therefore persistent force cannot build up. In contrast, during the sliding of antiparallel microtubules by kinesin-5, the magnitude of the sliding force that can be generated scales with both the length of the overlap between antiparallel microtubules and the number of motor proteins that are engaged in cross-linking (Shimamoto et al., 2015; Fig. 2, A and B). This result suggests that kinesin-5 molecules do not interfere with, but rather mainly slow down, the stepping of adjacent molecules in a force-dependent manner without inducing microtubule unbinding or loss of cross-linking. Moreover, it

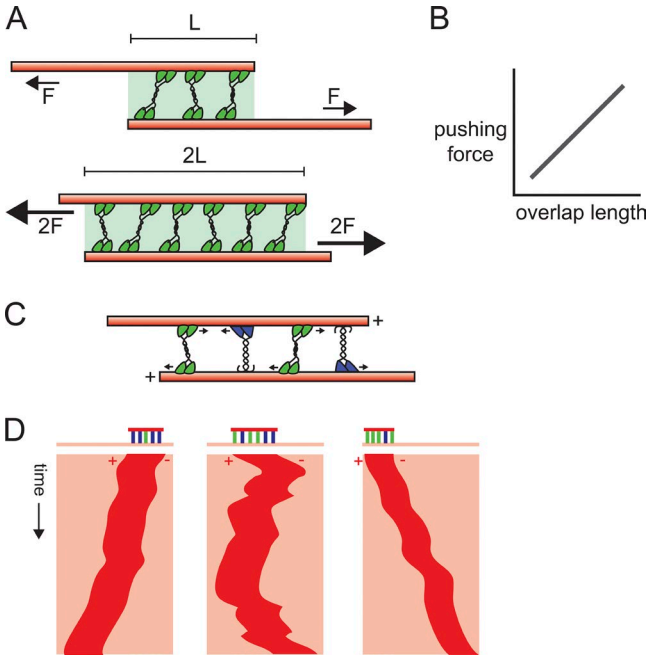

Figure 2. **Motor proteins within overlapping filaments.** (A) Microtubules cross-linked by kinesin-5 with different overlap lengths. Longer overlaps can recruit more motor protein molecules, resulting in an increase in relative microtubule sliding forces. (B) Force generation by kinesin-5 ensembles scales with the length of microtubule overlap. (C) Microtubules can be cross-linked by motor proteins with different directional preferences. (D) When both kinesin-5 and kinesin-14 cross-link microtubule pairs at different ratios, directional microtubule sliding or fluctuations without a preferred directed motion are observed. A stable balance point with no relative microtubule motion cannot be achieved with motor proteins alone.

was shown that when the microtubules are moving at velocities faster than the intrinsic kinesin-5 stepping rate, an ensemble of kinesin-5 proteins generates a resistive braking force, which, remarkably, also scales with the length of microtubule overlap (Shimamoto et al., 2015).

Parallel microtubule overlaps cross-linked by ensembles of kinesin-5 were also examined (Shimamoto et al., 2015). Rather than directional microtubule sliding, back-and-forth force fluctuations with amplitudes of ∼2 pN were observed. Interestingly, the magnitude of these force fluctuations did not depend on overlap length. However, length- and velocity-dependent braking forces against microtubule sliding were measured for parallel overlaps. Taking together the observations for both antiparallel and parallel cross-linking geometries, we concluded that kinesin-5 ensembles can serve as a regulator of microtubule sliding velocity within the spindle, producing larger forces when antiparallel overlaps are long, smaller forces when antiparallel overlaps are short, and braking forces against the relative sliding of antiparallel microtubules moving faster than the natural rate of kinesin-5 stepping and parallel microtubules moving across a range of velocities measured.

Motor proteins with opposing directional preferences can occupy the same overlapping region within microtubule bundles in spindles (Fig. 2 C). In such situations, an antagonistic behavior can emerge; for example, if one class of protein slides plus ends of microtubules apart whereas the other slides minus ends apart. Examination of microtubule bundles cross-linked by both kinesin-5 and kinesin-14 in vitro revealed that a stable force balance within this minimal system could

not be achieved (Fig. 2 D). At similar fractional amounts of the two proteins, an unstable fluctuation microtubule motion was observed; titrating more of one motor protein versus the other resulted in directional motion (Hentrich and Surrey, 2010). The addition of an artificial microtubule cross-linking construct that lacked motor activity served to stabilize this system and significantly reduced the magnitude of unstable fluctuations. This combination of active force generators and passive brake-like elements acting within the same microtubule network could produce stable overlaps that persisted for minutes. In light of these observations, it is clear that a careful characterization is needed of passive microtubule cross-linkers under controlled loads, at different timescales, and with consideration of microtubule orientation.

Passive forces generated within microtubule networks. Inspired by studies of molecular friction generated by the kinesin-8 motor protein Kip3 (Bormuth et al., 2009), we have shown that single molecules of three different nonmotor MAPs that have important mitotic functions generate frictional resistance when dragged along the lattice (Forth et al., 2014). Examined in these assays were NuMA, a large protein that bundles microtubules and localizes to the metaphase spindle pole; EB1, a plus end–binding protein that tracks the growing tips of microtubules; and PRC1, a homodimeric protein in the MAP65 family that preferentially cross-links antiparallel microtubules while localizing to the spindle midzone in anaphase. We demonstrated that these proteins all produce frictional resistance whose magnitude depends nonlinearly on the speed at which the protein was dragged across the microtubule lattice (Fig. 3 A). Additionally, differences in the microtubule binding structural motifs for each of the proteins examined likely resulted in different strengths of frictional interactions. Might it be possible for a single molecule to generate enough friction to modulate the speed of a motor protein? When moving at velocities of 1 µm/s, which is comparable to the speed at which dynein moves, each of the nonmotor MAPs generated frictional forces in the range of 0.1–0.2 pN (Forth et al., 2014). Although this may suggest that a single MAP–microtubule interaction would not provide substantial resistance against motor protein–generated forces, it could be the case that clustered ensembles of such molecules would result in a scaling of the total resistive force. For example, a cluster of only 10 MAPs interacting with a single microtubule could provide piconewtons of resistive load, which would be enough to reduce or even stall the stepping velocity of a motor protein. Indeed, the yeast MAP65, Ase1, can prevent sliding microtubules that are driven by kinesin-14 from completely falling apart (Braun et al., 2011). These results suggest that proteins that bind microtubules may have the ability to act as brakes, resisting the motions of filaments within a dense network such as the spindle.

Interestingly, diverse frictional asymmetries were also observed across these studies (Fig. 3 B). For example, both Kip3 and EB1 produced less frictional resistance when dragged toward microtubule plus ends (Bormuth et al., 2009; Forth et al., 2014). In contrast, NuMA produced less frictional resistance when moving toward minus ends, consistent with its strong localization at spindle poles where minus ends predominantly cluster (Forth et al., 2014). Surprisingly, PRC1 exhibited no directional preference, generating equivalent frictional forces regardless of microtubule polarity.

Might the property of frictional asymmetry be harnessed within active microtubule networks by the cell? Computer simulations predict, and experiments directly verify, that when a dimeric NuMA construct cross-links microtubules in the parallel geometry and those microtubules are then perturbed by external forces, NuMA can autonomously move toward the microtubule minus ends (Forth et al., 2014; Fig. 3 C). By taking advantage of this intrinsic mechanical anisotropy, it is possible that within an actively fluctuating microtubule network, directional motion of certain MAPs can be achieved without the requirement that a motor protein directly transport the molecule. Such fluctuations could result from the competition between plus end– and minus end–directed motor proteins that cross-link the microtubule pair, as described earlier (Hentrich and Surrey, 2010). Because frictional asymmetry likely arises from the directional dependence of force-induced bond breaking at the atomic level, it may also be the case that this type of autonomous directed motion occurs within other active polymer networks in the cell.

In addition to producing frictional resistance and acting as a brake against motion, a fascinating new mechanism of force generation was recently observed for Ase1, the yeast MAP65. Experiments suggest that Ase1 cross-linking two microtubules may behave as a compressible gas, generating entropic forces that increase as diffusible molecules of Ase1 are compacted into smaller and smaller overlap regions (Fig. 3 D; Lansky et al., 2015). These nonmotor cross-linking proteins undergo ther-

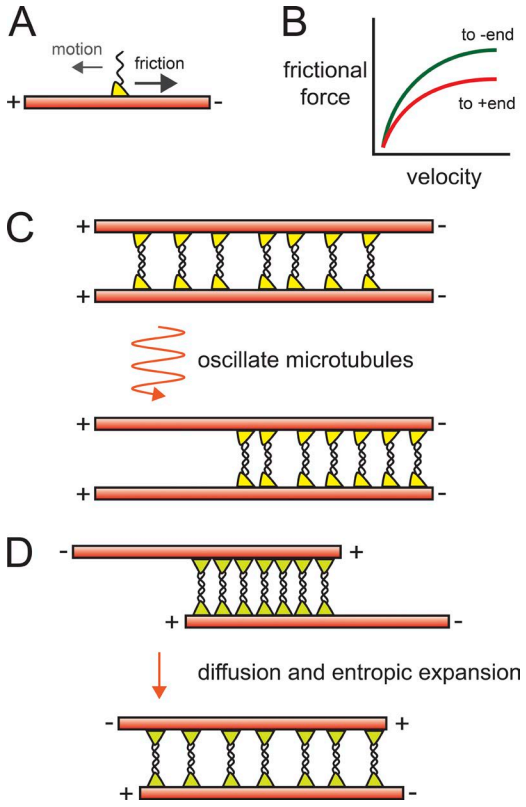

Figure 3. **Nonmotor proteins within overlapping filaments.** (A) Nonmotor MAPs can generate frictional resistance when moving along the lattice surface. (B) Some proteins, such as NuMA, EB1, and Kip3, have been shown to exhibit asymmetric friction, where moving toward one end of the microtubule results in increased resistance compared with motion in the opposite direction. (C) Cross-linking proteins whose microtubule binding domains possess frictional asymmetry can move directionally within fluctuating microtubule bundles. (D) Cross-linking proteins undergo diffusion, which can result in an entropic force that slides microtubules and opposes reduction in overlap lengths.

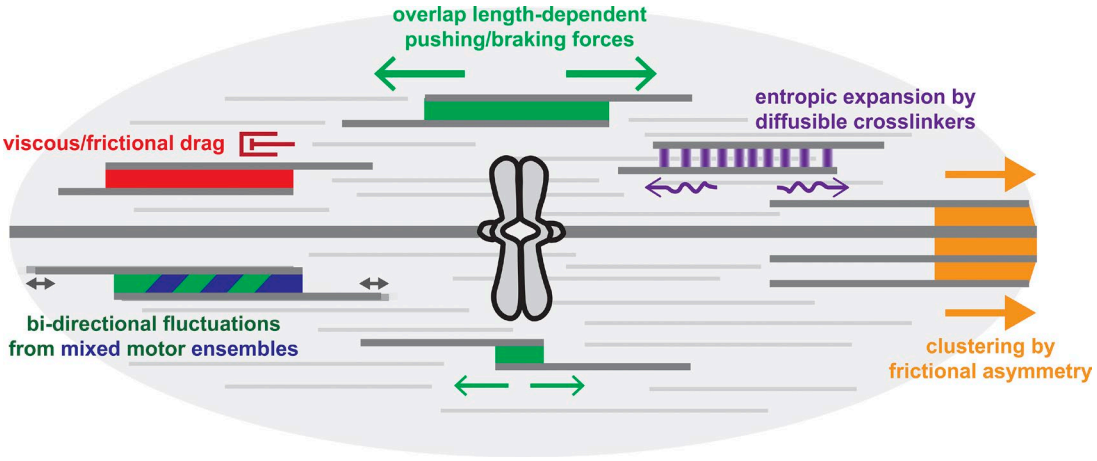

Figure 4. **Proposed model of spindle force map.** Sources of force production within the dense spindle microtubule network include overlap length–dependent pushing and braking forces (green), viscous frictional drag (red), entropic expansion by diffusible cross-linkers (purple), protein clustering by frictional asymmetry (orange), and fluctuations arising from mixtures of plus end– and minus end–directed motor proteins (blue/green).

mally driven diffusion along the lattice of each of the microtubule surfaces they contact. If the number of available binding sites is low relative to the population of Ase1 molecules, the system will undergo expansion to increase the number of binding sites, thereby maximizing the number of possible microscopic states. This is readily achieved in this one-dimensional system by microtubule sliding to increase overlap length. In an elegant series of experiments (Lansky et al., 2015), the magnitude of this entropic force was determined to be on the order of several piconewtons when overlaps were well populated with Ase1 molecules, suggesting that this force might be sufficient to stall weak motor protein motions. This entropic force could also operate in conjunction with the frictional forces, providing substantial resistance to the sliding apart of microtubule overlaps.

Conclusion and perspectives

We are now beginning to understand how forces generated or experienced by individual proteins on the subsecond timescale work in concert to give rise to micrometer-scale outputs that can persist for minutes. For example, we now know that micrometer-scale forces can scale with motor protein number, that friction can be harnessed within active filament networks to move proteins, and that diffusible cross-linkers can generate entropic forces within microtubule bundles. Together with studies using spindles assembled in extract, these findings are shedding light on the biophysical principles that underlie force generation by ensembles of proteins that cross-link microtubules, and we are now beginning to fill in key features of a spindle force map (Fig. 4).

There remain several important outstanding challenges. First, it will be important to understand how systems of increasing complexity lead to diverse emergent behaviors; for example, by studying the generation of force by active and passive cross-linkers that occupy the same region of overlap within a microtubule pair. It will also be useful to probe the generation of forces within pairs of dynamic filaments to understand the interplay between cross-linking proteins, polymerization forces, and the regulators of microtubule dynamics that act near the growing tips of filaments. Second, it will be important to determine whether the principles described here are indeed used in dividing cells. For example, in vivo tests of overlap length–dependent pushing force generation may involve expressing kinesin-5 constructs that produce forces with a different length dependence and observing the corresponding mitotic phenotype. Simple loss-of-function analyses are not likely to inform on this micrometer-sized length measurement by this essential nanometer-sized protein. Likewise, altering the microtubule-binding properties of key proteins and analyzing changes in their localization in dividing cells could reveal how clustering by frictional asymmetry contributes to proper cellular function. Finally, many mitotic proteins are regulated by posttranslational modifications. The next major steps will be to dissect how these biochemical inputs intersect with the mechanics of cross-linking proteins. The assays, tools, and methods that have been developed make this an exciting time to be studying cell division. Findings from these studies are likely to uncover general principles that should be applicable to other complex and dynamic cell biology.

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References

Bormuth, V., V. Varga, J. Howard, and E. Schäffer. 2009. Protein friction limits diffusive and directed movements of kinesin motors on microtubules. *Science.* 325:870–873. http://dx.doi.org/10.1126/science.1174923

Braun, M., D.R. Drummond, R.A. Cross, and A.D. McAinsh. 2009. The kinesin-14 Klp2 organizes microtubules into parallel bundles by an ATP-dependent sorting mechanism. *Nat. Cell Biol.* 11:724–730. http://dx.doi.org/10.1038/ncb1878

Braun, M., Z. Lansky, G. Fink, F. Ruhnow, S. Diez, and M.E. Janson. 2011. Adaptive braking by Ase1 prevents overlapping microtubules from sliding completely apart. *Nat. Cell Biol.* 13:1259–1264. http://dx.doi.org/10.1038/ncb2323

Dogterom, M., and B. Yurke. 1997. Measurement of the force-velocity relation for growing microtubules. *Science.* 278:856–860. http://dx.doi.org/10.1126/science.278.5339.856

Dumont, S., and T.J. Mitchison. 2009. Force and length in the mitotic spindle. *Curr. Biol.* 19:R749–R761. http://dx.doi.org/10.1016/j.cub.2009.07.028

Ferenz, N.P., A. Gable, and P. Wadsworth. 2010. Mitotic functions of kinesin-5. *Semin. Cell Dev. Biol.* 21:255–259. http://dx.doi.org/10.1016/j.semcdb.2010.01.019

Fink, G., L. Hajdo, K.J. Skowronek, C. Reuther, A.A. Kasprzak, and S. Diez. 2009. The mitotic kinesin-14 Ncd drives directional microtubule-microtubule sliding. *Nat. Cell Biol.* 11:717–723. http://dx.doi.org/10.1038/ncb1877

Forth, S., K.-C. Hsia, Y. Shimamoto, and T.M. Kapoor. 2014. Asymmetric friction of nonmotor MAPs can lead to their directional motion in active microtubule networks. *Cell.* 157:420–432. http://dx.doi.org/10.1016/j.cell.2014.02.018

Furuta, K., A. Furuta, Y.Y. Toyoshima, M. Amino, K. Oiwa, and H. Kojima. 2013. Measuring collective transport by defined numbers of processive and nonprocessive kinesin motors. *Proc. Natl. Acad. Sci. USA.* 110:501–506. http://dx.doi.org/10.1073/pnas.1201390110

Gadde, S., and R. Heald. 2004. Mechanisms and molecules of the mitotic spindle. *Curr. Biol.* 14:R797–R805. http://dx.doi.org/10.1016/j.cub.2004.09.021

Gennerich, A., A.P. Carter, S.L. Reck-Peterson, and R.D. Vale. 2007. Force-induced bidirectional stepping of cytoplasmic dynein. *Cell.* 131:952–965. http://dx.doi.org/10.1016/j.cell.2007.10.016

Gittes, F., B. Mickey, J. Nettleton, and J. Howard. 1993. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J. Cell Biol.* 120:923–934. http://dx.doi.org/10.1083/jcb.120.4.923

Grill, S.W., J. Howard, E. Schäffer, E.H.K. Stelzer, and A.A. Hyman. 2003. The distribution of active force generators controls mitotic spindle position. *Science.* 301:518–521. http://dx.doi.org/10.1126/science.1086560

Hayden, J.H., S.S. Bowser, and C.L. Rieder. 1990. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: Direct visualization in live newt lung cells. *J. Cell Biol.* 111:1039–1045. http://dx.doi.org/10.1083/jcb.111.3.1039

Hentrich, C., and T. Surrey. 2010. Microtubule organization by the antagonistic mitotic motors kinesin-5 and kinesin-14. *J. Cell Biol.* 189:465–480. http://dx.doi.org/10.1083/jcb.200910125

Hill, T.L., and M.W. Kirschner. 1982. Bioenergetics and kinetics of microtubule and actin filament assembly-disassembly. *Int. Rev. Cytol.* 78:1–125. http://dx.doi.org/10.1016/S0074-7696(08)60105-9

Itabashi, T., J. Takagi, Y. Shimamoto, H. Onoe, K. Kuwana, I. Shimoyama, J. Gaetz, T.M. Kapoor, and S. Ishiwata. 2009. Probing the mechanical architecture of the vertebrate meiotic spindle. *Nat. Methods.* 6:167–172. http://dx.doi.org/10.1038/nmeth.1297

Jamison, D.K., J.W. Driver, A.R. Rogers, P.E. Constantinou, and M.R. Diehl. 2010. Two kinesins transport cargo primarily via the action of one motor: implications for intracellular transport. *Biophys. J.* 99:2967–2977. http://dx.doi.org/10.1016/j.bpj.2010.08.025

Jamison, D.K., J.W. Driver, and M.R. Diehl. 2012. Cooperative responses of multiple kinesins to variable and constant loads. *J. Biol. Chem.* 287:3357–3365. http://dx.doi.org/10.1074/jbc.M111.296582

Jannasch, A., V. Bormuth, M. Storch, J. Howard, and E. Schäffer. 2013. Kinesin-8 is a low-force motor protein with a weakly bound slip state. *Biophys. J.* 104:2456–2464. http://dx.doi.org/10.1016/j.bpj.2013.02.040

Kapitein, L.C., E.J.G. Peterman, B.H. Kwok, J.H. Kim, T.M. Kapoor, and C.F. Schmidt. 2005. The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. *Nature.* 435:114–118. http://dx.doi.org/10.1038/nature03503

Kapoor, T.M. 2017. Metaphase spindle assembly. *Biology (Basel).* 6:1–36.

Kashina, A.S., R.J. Baskin, D.G. Cole, K.P. Wedaman, W.M. Saxton, and J.M. Scholey. 1996. A bipolar kinesin. *Nature.* 379:270–272. http://dx.doi.org/10.1038/379270a0

Korneev, M.J., S. Lakämper, and C.F. Schmidt. 2007. Load-dependent release limits the processive stepping of the tetrameric Eg5 motor. *Eur. Biophys. J.* 36:675–681. http://dx.doi.org/10.1007/s00249-007-0134-6

Laan, L., J. Husson, E.L. Munteanu, J.W.J. Kerssemakers, and M. Dogterom. 2008. Force-generation and dynamic instability of microtubule bundles. *Proc. Natl. Acad. Sci. USA.* 105:8920–8925. http://dx.doi.org/10.1073/pnas.0710311105

Lansky, Z., M. Braun, A. Lüdecke, M. Schlierf, P.R. ten Wolde, M.E. Janson, and S. Diez. 2015. Diffusible crosslinkers generate directed forces in microtubule networks. *Cell.* 160:1159–1168. http://dx.doi.org/10.1016/j.cell.2015.01.051

Mastronarde, D.N., K.L. McDonald, R. Ding, and J.R. McIntosh. 1993. Interpolar spindle microtubules in PTK cells. *J. Cell Biol.* 123:1475–1489. http://dx.doi.org/10.1083/jcb.123.6.1475

McEwen, B.F., A.B. Heagle, G.O. Cassels, K.F. Buttle, and C.L. Rieder. 1997. Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* 137:1567–1580. http://dx.doi.org/10.1083/jcb.137.7.1567

Nicklas, R.B. 1983. Measurements of the force produced by the mitotic spindle in anaphase. *J. Cell Biol.* 97:542–548. http://dx.doi.org/10.1083/jcb.97.2.542

Nicklas, R.B. 1988. The forces that move chromosomes in mitosis. *Annu. Rev. Biophys. Biophys. Chem.* 17:431–449. http://dx.doi.org/10.1146/annurev.bb.17.060188.002243

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. http://dx.doi.org/10.1083/jcb.95.1.91

Rubinstein, B., K. Larripa, P. Sommi, and A. Mogilner. 2009. The elasticity of motor-microtubule bundles and shape of the mitotic spindle. *Phys. Biol.* 6:016005. http://dx.doi.org/10.1088/1478-3975/6/1/016005

Rusan, N.M., C.J. Fagerstrom, A.M.C. Yvon, and P. Wadsworth. 2001. Cell cycle-dependent changes in microtubule dynamics in living cells expressing green fluorescent protein-alpha tubulin. *Mol. Biol. Cell.* 12:971–980. http://dx.doi.org/10.1091/mbc.12.4.971

Saxton, W.M., D.L. Stemple, R.J. Leslie, E.D. Salmon, M. Zavortink, and J.R. McIntosh. 1984. Tubulin dynamics in cultured mammalian cells. *J. Cell Biol.* 99:2175–2186. http://dx.doi.org/10.1083/jcb.99.6.2175

Scholey, J.E., S. Nithianantham, J.M. Scholey, and J. Al-Bassam. 2014. Structural basis for the assembly of the mitotic motor Kinesin-5 into bipolar tetramers. *eLife.* 3:e02217. http://dx.doi.org/10.7554/eLife.02217

Sharp, D.J., G.C. Rogers, and J.M. Scholey. 2000. Microtubule motors in mitosis. *Nature.* 407:41–47. http://dx.doi.org/10.1038/35024000

Shimamoto, Y., Y.T. Maeda, S. Ishiwata, A.J. Libchaber, and T.M. Kapoor. 2011. Insights into the micromechanical properties of the metaphase spindle. *Cell.* 145:1062–1074. http://dx.doi.org/10.1016/j.cell.2011.05.038

Shimamoto, Y., S. Forth, and T.M. Kapoor. 2015. Measuring pushing and braking forces generated by ensembles of kinesin-5 crosslinking two microtubules. *Dev. Cell.* 34:669–681. http://dx.doi.org/10.1016/j.devcel.2015.08.017

Takagi, J., T. Itabashi, K. Suzuki, Y. Shimamoto, T.M. Kapoor, and S. Ishiwata. 2014. Micromechanics of the vertebrate meiotic spindle examined by stretching along the pole-to-pole axis. *Biophys. J.* 106:735–740. http://dx.doi.org/10.1016/j.bpj.2013.12.033

Tanenbaum, M.E., R.D. Vale, and R.J. McKenney. 2013. Cytoplasmic dynein crosslinks and slides anti-parallel microtubules using its two motor domains. *eLife.* 2:e00943. http://dx.doi.org/10.7554/eLife.00943

Valentine, M.T., P.M. Fordyce, T.C. Krzysiak, S.P. Gilbert, and S.M. Block. 2006. Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads in vitro. *Nat. Cell Biol.* 8:470–476. http://dx.doi.org/10.1038/ncb1394

Weinger, J.S., M. Qiu, G. Yang, and T.M. Kapoor. 2011. A nonmotor microtubule binding site in kinesin-5 is required for filament crosslinking and sliding. *Curr. Biol.* 21:154–160. http://dx.doi.org/10.1016/j.cub.2010.12.038