

Force and Length in the Mitotic Spindle

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Abstract

The mitotic spindle assembles to a steady-state length at metaphase through the integrated action of molecular mechanisms that generate and respond to mechanical forces. While molecular mechanisms that produce force have been described, our understanding of how they integrate with each other, and with the assembly-disassembly mechanisms that regulate length, is poor. We review current understanding of the basic architecture and dynamics of the metaphase spindle, and some of the elementary force producing mechanisms. We then discuss models for force integration, and spindle length determination. We also emphasize key missing data that notably includes absolute values of forces, and how they vary as a function of position, within the spindle.

Introduction

Cell division, the process by which a parent cell divides into two daughters, is fundamental to life. An important aspect of cell division is to ensure that genomic information is conserved; chromosome segregation errors in man can cause birth defects and contribute to cancer. In all eukaryotes, chromosome segregation is accomplished by the mitotic spindle, a bipolar assembly of dynamic microtubules. Work over the last 20 years has identified and characterized many of the molecules needed for mitosis, and we may be close to a complete list in some systems. Despite this progress, surprisingly little is known about the underlying mechanical principles that govern the assembly and function of the spindle. Here we review current biophysical understanding, with a focus on force and position in animal spindles; we refer the reader elsewhere for molecules [1].

Metaphase, the state in which paired sister chromosomes balance at the center of the spindle, is a natural starting point for a consideration of spindle biophysics because it is a stable steady-state. The metaphase spindle is highly dynamic, with large fluctuations and directed fluxes in both physical and chemical processes, yet the average amount and position of all spindle components is constant over time. The stability of this steady-state is evident from the remarkable ability of metaphase spindles to correct transient fluctuations in morphology and position (Fig. 1), and to recover from transient physical and chemical perturbations (e.g. [2-9]).

The spindle is made of molecules (mostly proteins, but see [10, 11]) and subject to chemical influences, but here we will view it as an intrinsically mechanical object. Mechanical forces help assemble the spindle [12], move chromosomes within it [13, 14], stabilize [15] and correct [16] the attachment of chromosomes to microtubules, and regulate anaphase entry [17]. Spindle forces are generated by molecular motors, microtubule assembly dynamics, elastic elements and friction (Fig. 2); because the structure is at steady-state, the action of these forces on any mechanically independent spindle component must integrate to zero. A notable aspect of most integrated spindle forces is that they are position-dependent, which is required for them to position objects in specific places. At least three positioning tasks are accomplished to generate the metaphase spindle: the spindle positions within the cell (Fig. 1A), typically near the center of the longest axis [18-20]; the chromosomes align at the center of the spindle (Fig. 1B), generating the arrangement called the “metaphase plate” [21]; the poles position a certain distance from each other (or perhaps from the chromosomes), determining spindle length (Fig. 1C).

The shape of the spindle and its likely filamentous organization was described by Flemming more than 125 years ago [22]. Polarization microscopy in the 1950s proved that spindles are built from filaments that run parallel to the direction of chromosome motion, which we will call the spindle axis [23]. Rapid assembly and disassembly of these filaments in response to physical and chemical perturbations lead Inoué and Sato to propose that their polymerization dynamics produce mechanical force, for example to power chromosome motion [7]. The filaments were identified as microtubules, non-covalent polymers of the protein tubulin, by a combination of biochemistry, pharmacology and electron microscopy [24, 25]. Today, we know that the main structural element of the spindle is a lattice of oppositely oriented microtubules (Fig. 2A) that undergo rapid polymerization and depolymerization powered by GTP hydrolysis. Spindle microtubules are organized in space, and their dynamics are regulated by proteins that include motor proteins [26] and microtubule-binding proteins [27]. We will use the term “motor protein” to refer to molecules in the kinesin and dynein families that use ATP hydrolysis energy to walk along microtubules.

These generate sliding force between microtubules and other objects, and play a major role in force production (Fig. 3).

1. Spindle architecture and dynamics

Most animal spindles can be thought of as a superposition of kinetochore, nonkinetochore and astral microtubules that differ in their architecture, dynamics and function, though they all assemble from the same pool of tubulin subunits (Fig. 2B-C-D).

Kinetochore microtubules (K-MTs) (Fig. 2B) have plus-ends embedded in kinetochores (protein structures where microtubules attach to chromosomes) and minus-ends at or near poles [28]. Their main functions are to exert pulling forces on chromosomes at kinetochores, and to silence the spindle assembly checkpoint signal that is generated by unattached kinetochores. Some types of spindle may lack one of the other microtubule classes, but K-MTs appear to be indispensable. In mammalian cells, each chromosome has one kinetochore that binds to the plus-ends of 10-30 K-MTs [29], and most extend continuously from kinetochore to pole [28]. The K-MTs attached to a single kinetochore tend to bundle with each other and with an approximately equal number of nonkinetochore microtubules [28], to form a 'kinetochore fiber' (k-fiber) that is prominent in light level micrographs. Within a k-fiber, microtubules are evenly spaced, 50-100 nm apart [28], and they behave as one mechanical unit upon physical manipulation [30]. Interactions between k-fibers are weak, except at the poles where they converge [2, 31, 32]. K-MTs probably have two origins, capture of plus-ends of microtubules from the other two classes [33], and direct nucleation at kinetochores followed by integration into the spindle [34]. The blue zones in Fig. 2B illustrate the K-MT nucleation potential. K-MTs turnover much more slowly than the other microtubule classes, presumably because both ends are capped, with a half-life of ~7 min in metaphase spindles [35]. Complete replacement of K-MTs presumably requires that their plus-ends detach from the kinetochore. K-MTs turnover while remaining attached by polymerizing at kinetochores (black arrow), sliding toward the pole at ~0.5 $\mu\text{m}/\text{min}$ (Fig. 2B, green arrow) [36], and depolymerizing at poles (black arrow). In mammalian spindles, the instantaneous polymerization rate at kinetochores is quite variable, because chromosomes oscillate around their mean position on the metaphase plate. Sliding and depolymerization rates appear rather constant from published data [37], though they have yet to be measured with high accuracy. Polymerization, sliding and depolymerization must precisely balance at steady-state. How this occurs is an interesting unsolved problem that is part of the question of spindle length regulation, discussed below.

Nonkinetochore microtubules (nK-MTs) (Fig. 2C) collectively span the region from one spindle pole to the other, and constitute all microtubules that lie between spindle poles other than K-MTs (they have also been called interpolar microtubules [38]). NK-MTs comprise the majority of microtubules in mammalian spindles that have been studied by electron microscopy. During metaphase, they bundle together 30-50 nm apart in groups of 2-6, with anti-parallel interactions apparently preferred [38]. The function of nK-MTs is poorly understood. Since they are the majority class of microtubules, and interact in an anti-parallel fashion, they are thought to help integrate the whole spindle and keep the poles apart, i.e. to ensure its bipolarity. Arguing in favor of this role, bipolar meiotic spindles can assemble from nK-MTs alone in *Xenopus* egg extract [39]. Contrary to many textbook models, the minus-ends of most nK-MTs are not simply located at poles, but rather throughout the spindle [40]; many nK-MTs have minus-ends embedded in k-fibers, where they presumably couple mechanically to K-MTs [38]. Most of our understanding of nK-MT

dynamics comes from *Xenopus* egg extract spindles, where nK-MTs comprise >90% of all microtubules. Nucleation of nK-MTs is thought to occur throughout the spindle [41], as indicated by the blue shaded zone in Figure 2C. NK-MTs turnover very rapidly [42], presumably by dynamic instability of plus-ends. Sliding velocities in *Xenopus* extract spindles have been measured by speckle imaging and single molecule imaging. All nK-MTs slide poleward, at an average velocity of ~2 $\mu\text{m}/\text{min}$, though sliding velocities are quite variable, and can differ greatly even between nearby microtubules [43], indicating that lateral cross-links in the spindle must be weak and/or dynamic. Sliding velocity decreases away from the metaphase plate, which lead to the suggestion that poles may assemble where the sliding velocity reaches zero [44]. Such a velocity gradient is only possible if nK-MTs are short compared to the length of the *Xenopus* meiotic half-spindle, which is probably the case, though we lack quantitative electron microscopy data. Much less is known about nK-MTs dynamics in mammalian spindles, in part because their rapid turnover makes photo-marking difficult. Filling this gap is important to elucidate integrated spindle mechanics.

Astral microtubules (A-MTs) (Fig. 2D) have their minus-ends attached to centrosomes, where they are nucleated. Many of their plus-ends extend toward the cortex, and these are thought to mediate one key function of A-MTs, which is to position the spindle within the cell [45]. A-MTs presumably extend into the spindle as well. These are very obvious in *C. elegans* embryonic spindles that lack nK-MTs [46], but in mammalian cells they are difficult to distinguish from nK-MTs. A-MTs turnover at a rate comparable to nK-MTs, and for the subset of A-MTs that elongate away from the spindle, turnover by dynamic instability of plus-ends has been visualized, with growth and shrinkage rates of ~10-15 $\mu\text{m}/\text{min}$ [47]. A-MT minus-ends are thought to be capped by gamma-tubulin complexes at the centrosomes, and do not appear to slide [48].

2. Molecular forces in the spindle

Even cursory examination of the spindle suggests that mechanical forces are involved in moving chromosomes, and there has been interest in the origin of these forces since the time of Flemming. As colorful theories of fluid flow, electrostatics and the like were gradually discarded, students of the spindle came to focus on two types of active forces (where chemical energy is converted into mechanical work), polymerization dynamics and motor proteins, and two types of passive forces, elasticity and friction (Fig. 3A-D). Elasticity and friction can also be thought of as material properties that reflect responses to applied force. We prefer to call them forces, to draw attention to the fact that all the forces – including the passive ones – that act on the spindle must sum to zero, since the spindle as a whole is at steady-state. This powerful concept is often under-appreciated by biologists who focus on active force production. Furthermore, elastic and frictional forces may derive in part from motor proteins, which is important to consider when interpreting results of genetic and pharmacological inhibition experiments. In Figure 3E we summarize current understanding of how forces are generated at key locations in the spindle. Passive forces are largely not included, reflecting the paucity of current understanding. Our treatment of molecular forces is necessarily brief; for more rigorous descriptions, see [49].

Microtubule polymerization dynamics. The concept that spindle fibers could push by polymerizing and pull by depolymerizing (Fig. 3A) was proposed by Inoué and Sato [7], and the thermodynamics by which microtubules could generate these forces became evident when dynamic instability was described [50]. Assembly of GTP-tubulin and disassembly of GDP-tubulin are both thermodynamically favorable in the cytoplasm, and can thus perform mechanical work [51].

Addition of one GTP-tubulin dimer provides a gain in free-energy of $5-10 k_B T$, such that a microtubule growing by a dimer 8 nm tall (for all 13 protofilaments) could generate up to ~ 50 pN of force; disassembly of one GDP-tubulin dimer can again release $5-10 k_B T$ [52]. Both microtubule assembly [53] and disassembly [54] forces have been shown to perform work using pure tubulin in reconstituted systems. How might they generate force in the spindle? One plausible pushing mechanism is a Brownian ratchet, where thermal fluctuations generate transient gaps between the plus-end and some object that can be filled by an incoming monomer [55]. In cells, this simple mechanism is complicated by the presence of many proteins that interact with growing plus-ends, including plus-end directed motors [56]. Pushing by growing plus-ends at the cortex has been shown to play an important role in nucleus centering in *S. pombe* [57], but pushing forces may be less important for spindle positioning in larger cells, where longer A-MTs would tend to buckle under compression forces. While pushing at kinetochores has also been seen [58] and proposed to play a role in spindle assembly [59, 60], centromeres are rarely compressed [61, 62]; in our view, pushing at the kinetochore is not proven – there is always some other away-from-pole force acting on chromosomes. Pushing by nK-MTs has been discussed [2] but not tested. We suspect that it may be an important source of forces pushing k-fibers towards poles, an idea which we return to below in the context of force integration and spindle length.

For microtubule disassembly to generate pulling force, it must be mechanically coupled to movement of the pulled object, which is conceptually more difficult than pushing. Pulling by depolymerization has been most studied at kinetochores, where it is currently thought to be a major force driving chromosomes poleward [13, 63]. Consistent with this view, deletion of all known minus-end directed motors in yeast has no effect on chromosome movement [64]. A sleeve with multiple microtubule binding sites (now called a “Hill sleeve”) could, in principle couple depolymerization to sliding by a kind of reverse Brownian ratchet mechanism [65]. The propensity of protofilaments to curve outwards at plus-ends allows, in principle, for a more active “curling power stroke” mechanism that could propel sliding rings toward minus-ends [66]. A recent EM tomography study of kinetochores was interpreted using a variant of this mechanism, where curling protofilaments were proposed to make transient elastic connections to kinetochore fibrils [67]. Progress in kinetochore molecular biology is beginning to reveal the molecules responsible for coupling depolymerization to pulling, with the Ndc80 complex emerging as the most conserved and fundamental coupling element [68]. The magnitude of force from depolymerization at kinetochores has not been measured directly. Nicklas measured the stall force acting on anaphase chromosomes in grasshopper spermatocytes at $\sim 10-50$ pN per K-MT [14]. However, this is probably not a direct measure of force from depolymerization, because speckle tracking in a similar cell type showed that K-MTs in fact *polymerize* at anaphase, and chromosomes only move poleward because microtubules slide poleward faster than the polymerization rate [69]. Thus, Nicklas’ famous measurement may actually represent the frictional resistance to K-MTs being dragged through kinetochore attachment sites by forces from elsewhere in the spindle, i.e. the friction associated with the kinetochore “slipping clutch” [70]. Nicklas’s work is notable as an example of direct force measurements in a field that has mostly inferred forces indirectly. New force measurements are now needed on today’s molecularly tractable systems.

Molecular motors. A sliding filament mechanism for spindle forces was proposed in 1969 [71], inspired by previous work on muscle contraction. Motor proteins with roles in mitosis were later revealed by molecular genetics [72, 73], and we now know that as many as 10 different motors are required for normal mitosis in some systems. Individual motors walk either toward the microtubule plus- or minus-end (Fig. 3B), and generate on the order of ~ 5 pN of force [74]. Motors have several

functions in the spindle: they move objects relative to microtubules, orient or move microtubule relative to each other, and regulate polymerization and depolymerization at ends [75]. The last activity was unexpected, yet seems to be very important, and it complicates experiments aimed at disentangling force producing mechanisms by genetic ablation of motors. The functions of motor proteins have been extensively reviewed [1, 26]. In our view, one of the limitations of this literature is that forces from polymerization dynamics are mostly ignored, perhaps because forces from motors are easier to conceptualize and measure.

Elasticity. This is the force that causes materials to return to their original shape after being deformed by external forces (Fig 3C). Materials are typically only elastic over small deformations, and short timescales, before material remodeling occurs. The elastic force generated (F), is proportional to the deformation (x) and the spring constant (k) of an object: $F = k \cdot x$. Currently, the most investigated aspect of elasticity in the spindle is reversible stretching of centromeric chromatin in response to kinetochore forces [76], but elasticity surely has much broader importance. For example, if we knew the precise elasticity and shape of spindle microtubules, we could, in principle, infer the forces acting on them. Bending rigidity has been measured for individual microtubules [77], but the situation in spindles, where microtubules are bundled, is more complicated, since elasticity depends not only on the number of microtubules in a bundle, but on the tightness with which they are bundled [78], and the elasticity of any gel-like material in which they are imbedded [79]. Recent measurements of whole *Xenopus* extract spindles using force-sensitive cantilevers revealed viscoelastic (a combination of elastic and viscous) responses to small compressions, and plastic deformation under larger compression [8]. The Young's modulus (k for a material as defined above) was at least ten-fold larger along the spindle axis than normal to this axis, presumably due to the orientation of most microtubules along the spindle axis; ~4 nN along the spindle axis was required to shorten a spindle by 1 μm . Combining these measurements with molecular perturbations should help dissect contributors to spindle mechanics.

Molecular friction. Resistive forces (Fig. 3D) act to oppose movement, and the extent of this opposition typically increases with velocity. The simplest form of friction in biological systems is Stokes' drag, which is exerted on moving objects by a viscous liquid. The importance of this force is likely minimal in spindles, because spindle objects move at relatively slow velocities. For example, only 0.1 pN are in principle required to move a chromosome at typical anaphase velocities [80], which is much smaller than the measured forces generated at kinetochores. In spindles, resistive forces are more likely to derive from the need to break non-covalent bonds between proteins during movement. When a microtubule that is held in place by motors or cross-linkers is forced to move, bonds must be stretched or broken for movement to occur, and this will create an effective frictional force $F = \gamma \cdot v$, where γ is the drag coefficient (which depends on molecular interaction parameters) and v the velocity (p. 40 in [49]). A related type of friction occurs when a large object, such as a chromosome, is dragged through a gel made of filaments that can reversibly break or turnover. Bonds that hold the gel together must break transiently to allow movement. When frictional forces arise from bond breaking, the timescale of movement compared to that of thermally-driven bond dissociation becomes important [81]. If movement is slow compared to bond dissociation, cross-links rapidly equilibrate as the object moves: movement is smooth, and the friction force can be approximated as linear with movement velocity. If, however, movement is fast compared to bond breaking, the movement rate is limited by the rupture rate of the weakest bond in the system: under these conditions, the relationship between the friction force and velocity is more complex, and movement can become episodic [82]. Molecular friction probably plays a central role

in spindle dynamics. We suspect it must be responsible for the fact that most movements within spindles are rather slow, typically 0-3 $\mu\text{m}/\text{min}$, despite generation of large forces (e.g. nN forces on chromosomes [14]) from polymerization dynamics and motors. We also note that the relative importance of viscosity and elasticity will depend on the deformation timescale of the material, which has not been measured for the complex, active meshwork that comprises the spindle.

3. Toward a primitive force map of the spindle

How do the microscopic assembly processes and forces discussed above integrate to generate the mesoscopic dynamics of the spindle? Much less is known about this than about the microscopic forces themselves. In part, this is because mesoscopic forces are difficult to measure, and in part it reflects our incomplete understanding of the material properties of the spindle, and therefore of elastic and friction forces. In this section, we review current understanding of integrated spindle forces in an effort to move toward a force (or stress) map of the spindle (Fig. 4A). At the most basic level, such a map entails knowing which parts of the structure are under compression, and which are under tension. Although molecular experiments are commonly interpreted in these terms, we feel that much of the most informative data comes from mechanical perturbations, many of which pre-date the molecular era.

Force map based on tensed k-fibers. The morphology of anaphase chromosome movement has long been interpreted in terms of pulling forces on chromosomes exerted at kinetochores, but it was less obvious that tension is already exerted at metaphase. This was conclusively demonstrated by ablating one kinetochore of a metaphase pair, and observing that its sister moved poleward [83] (Fig. 4B). Tension on metaphase kinetochores was also evident from the effect of depolymerizing k-fibers, which decreased the distance between sister kinetochores [61] (Fig. 4C). Given these findings, two questions arise: how is tension generated on k-fibers, and how is it balanced by compression in other spindle components? Kinetochores themselves are known to generate pulling forces by microtubule depolymerization and perhaps also motors activity (Fig. 3E). More speculatively, poles were proposed to generate tension by similar mechanisms [37, 84, 85], but direct evidence for generation of pulling forces at poles is lacking. Continuous tension on sister chromatids at metaphase must be balanced by compression in some other spindle element [80]. Early force maps were informed by microtubule shape. In some systems, K-MTs are typically straight while nK-MTs are curved, and more splayed at metaphase than anaphase [86]. These observations suggested that nK-MTs bear the compressive load needed to balance tension at kinetochores, leading to a force map based on tensed k-fibers (Fig. 4A, left) that is widely assumed to hold for all spindles. Pulling forces between the poles and the cortex may also play a role in balancing tension at kinetochores, but in many systems the spindle makes only weak interactions with the cortex, and it appears that forces are mostly balanced within the spindles itself.

Limitations of the tensed k-fibers force map. One prediction of the tensed k-fibers map is that ablating some k-fibers will result in a longer spindle and straighter remaining fibers. Removal of all k-fibers by genetic ablation of kinetochores indeed caused lengthening of the remaining spindle [68]. However, other experiments produced results that are less consistent. UV microbeam severing of a few k-fibers (and likely other microtubules) in one-half of a vertebrate spindle resulted in spindle shortening, with the remaining k-fibers bowing outwards (Fig. 4D) [87]. Photorelease of a caged-microtubule depolymerizing drug in *Xenopus* extract spindles caused rapid loss of nK-MTs, spindle shortening, and buckling of all visible k-fibers (Fig. 4E) [88]. These observations suggest that some

element other than k-fibers is under tension from pole-to-pole, and that removing either K-MTs or nK-MTs results in compressive forces being exerted on remaining k-fibers, causing bending and even buckling. These data create an apparent paradox: how can a solid rod (k-fiber microtubules) be under tension in one place (kinetochores) and compression in another (nearer poles)? For this to be possible, the rod would have to make mechanical interactions with other structures along its length that could oppose forces generated at kinetochores. One simple experiment reveals that such interactions must exist: laser cutting a k-fiber 1-2 μm away from its kinetochore had no apparent effect on tension at that kinetochore, nor on the microtubule sliding rate of the cut fiber (Fig. 4F) [34] (similarly observed in [87]). These observations suggest that tension at the kinetochore is opposed by forces directed toward the pole acting on the first few microns of the k-fiber.

Revised force map. Integrating the classic view with the results discussed above, we propose a revised force map (Fig. 4A, right), where k-fibers are tensed near kinetochores, and compressed near poles. This requires that poleward force is exerted all along their lengths, as proposed by Östergren in his “traction fiber” model [89]. Consistent with this view, unbalancing of the number of K-MTs on each side of a metaphase chromosome leads to movement of the chromosome to a new position, and quantitative analysis suggested that the poleward force generated by a k-fiber is proportional to its length [90], although this result did not hold in a different system [91]. Because all microtubules are under compression near poles in this map, some as yet unidentified element under tension is required to balance the forces. A hypothetical tensile element within the spindle has been termed the “spindle matrix” in the literature. Molecular candidates for such a matrix include NuMA [92], Skeletor [93], poly(ADP-ribose) [10], though none are known to comprise an elastic system that stretches from pole to pole. An interesting candidate outside the spindle (as defined by its microtubules) is the cage of ER membranes that surrounds spindles [94], which may include remnants of the interphase nuclear envelope [95] and nuclear lamins in some systems [96]. More work is required to probe whether a tensile element exists, either inside or outside spindles.

Origin of poleward force in the revised force map. The nature of the putative poleward force that can hold a k-fiber stub in place (Fig. 4E), and presumably also drive poleward sliding of the k-fiber, is mysterious in mammalian systems. It must be active, i.e. generated by motors and/or polymerization dynamics since the poleward sliding of K-MTs would dissipate elastic forces directed toward the pole, and generate frictional forces in the other direction. In the classic tensed k-fibers force map (Fig. 4A, left), k-fibers are viewed largely disconnected from the rest of the spindle. In the revised force map (Fig. 4A, right) they must connect strongly to nearby spindle elements through the force-generating connections. However, any cross-links must be quite weak and/or structurally dynamic, since lateral forces in the spindle, as assayed by microneedle perturbation (Fig. 4G), tend to be weak everywhere except the poles [31, 32]. Transient interactions mediated by motor proteins might meet the criteria of being structurally dynamic yet strong. The only well-characterized force that pushes microtubules toward poles is anti-parallel sliding driven by the tetrameric, plus-end directed motor Kinesin-5: this motor seems to play a central role in keeping the poles apart, and driving poleward sliding of microtubules, during metaphase in *Xenopus* extract [44] and *Drosophila* embryo spindles [97]. Although K-MTs do not participate directly in anti-parallel interactions, they might couple laterally to nK-MTs that do. However, Kinesin-5 is not required for maintenance of spindle length, or microtubule sliding, in mammalian metaphase spindles [37]; Kinesin-5 is apparently only required for initial separation of poles during spindle assembly. Other plus-end directed motors might push microtubules poleward. For example, Kid attached to chromosome arms pushes chromosomes outwards [98], which implies that it exerts

poleward forces on nK-MTs, which probably couple mechanically to k-fibers. The role of forces from chromosomes is discussed below in the context of monopolar spindles. Alternatively, we speculate that polymerization pressure from nK-MTs whose minus-ends are anchored in K-MTs may generate poleward force on k-fibers [2]. In our view, elucidating the nature of poleward force on k-fibers at metaphase is one of the most interesting unsolved problems in spindle physiology.

4. Position-dependence of force — the case of spindle length

Nature of the problem

Metaphase spindles are characterized as much by positions as by forces (Fig. 1). Positioning the spindle in the cell and the chromosomes in the spindle are both centering problems, which require position-dependent forces giving rise to a stable equilibrium when an object centers. Positioning the poles is different, since it requires the establishment of a spatial scale [99]. How elementary forces (Fig. 3) are made position-dependent in the spindle is largely unsolved. Spindle centering (Fig. 1A), and the related problem of aster centering, were the subject of recent reviews [18-20], and we will not discuss them further. For the remainder of this review, we will focus on the question of how spindle poles are positioned, or equivalently, how the $\sim 5\text{-}50\ \mu\text{m}$ length scale of bipolar spindles arises from building blocks (tubulin subunits) that are only few nanometers in length; in doing so, we briefly discuss the problem of chromosome positioning. We first discuss three key observations that set the stage for thinking about this problem (Fig. 5), and then move to specific models (Fig. 6).

Scaling with cell size (Fig. 5A). To achieve its function of physically separating chromosomes, spindle length must, to some extent, scale with cell size. This problem was recently investigated in early *Xenopus* embryos, where early cleavage divisions cause cell size to decrease from $\sim 1200\ \mu\text{m}$ to $\sim 12\ \mu\text{m}$ over a few hours. In blastomeres smaller than $\sim 300\ \mu\text{m}$, spindle length scaled approximately linearly with cell length, so in this regime the cell length scale somehow sets the spindle length scale; in larger blastomeres, spindle length plateaued at $\sim 60\ \mu\text{m}$, implying an upper limit to length that must be set by factors intrinsic to the spindle [100]. Egg extract meiotic spindles [101], that are $\sim 30\ \mu\text{m}$ long independent of the container size or spindle density [88], must also use intrinsic mechanisms to set length. Interestingly, this set point is different in two related *Xenopus* species [102], but exactly how spindle dynamics differ between species so as to change the set length is not yet clear. These data suggest that spindle length determination is not one problem but two, extrinsic and intrinsic, and we discuss them separately below (Fig. 6).

Perturbation experiments (Fig. 5B). A broad range of physical and chemical perturbations revealed the dynamic nature of spindle length [7]. More recently these were complemented by genetic perturbations (Fig. 5B legend). These perturbation experiments suggest that spindle length (and mass) are determined as an emergent property of a dynamical system, rather than being specified by some tape-measure-like molecule, as in the case of muscle sarcomeres [103]. They also reveal a relationship between assembly processes and mechanical forces, with assembly pushing and disassembly pulling. At a coarse-grained level it is perhaps obvious that more polymerization leads to longer microtubules which make longer spindles, but at a microscopic level it is far from clear how this would work.

The monopole question (Fig. 5C). An important question for spindle length determination, and also for metaphase chromosome positioning, is the extent to which pole- and chromosome-positioning forces are the same in monopolar and bipolar spindles. Monopoles arise spontaneously in some systems [104], and can be generated experimentally by preventing centrosome duplication [105] or inhibiting Kinesin-5 [106]. Structurally and conceptually they are simpler than bipoles, because all microtubules have the same polarity (presumably - this has not been proven), so forces from anti-parallel interactions can be neglected. Chromosome-to-pole distances are typically similar in monopoles and bipoles (Fig. 5C), which lead Salmon and Rieder to propose that the forces positioning these objects relative to each other are the same in both cases [104]. This would imply that the spindle length problem is one of positioning both poles relative to chromosomes, not to each other. Chromosomes in monopoles are positioned by polar ejection forces that act on chromosome arms [104] (Fig. 3E), and perhaps also on kinetochores [107]. Polar ejection forces were proposed to decrease with distance from the pole [104], as A-MT density decreases (Fig. 5C, purple arrows). If pulling forces from kinetochores were constant (or increased with k-fiber length [108]), this would lead to a steady-state in chromosome position. Consistent with this view, the distribution of ejection force along the spindle axis was recently estimated in bipoles and found to decrease with distance from the pole [109]. The idea that bipolar spindles can be thought of as two monopoles joined at chromosomes [110] is appealing, but new data do not completely support this view. The polar ejection force was proposed to be generated by the chromokinesin Kid, based on the observation that inhibition of this motor strongly reduces chromosome-to-pole distance in monopoles [98]. However, inhibition of Kid only reduces bipolar spindle length by 20% in mammalian cells [111], and does not affect spindle length in *Xenopus* extract spindles, despite leading to scattered chromosomes along the spindle axis [112]. Bipolar spindles may thus have additional forces that keep the poles apart, at least in some systems. In *C. elegans* spindles, where there is little or no anti-parallel overlap, the forces in monopoles and bipoles may be more similar. In our view, it is still unclear to what extent bipolar mammalian spindles can be viewed simply as two half-spindles connected by chromosomes.

Models and key experimental data

We cartoon potential spindle length-determining mechanisms in Figure 6, starting with extrinsic mechanisms. We note that these mechanisms are not necessarily exclusive, and different systems might use different mechanisms. Also, we can imagine one mechanism being used to set an approximate length scale, and a second to tune length around that scale

A. Extrinsic mechanisms (Fig. 6A)

A1. Physical translation of cell length to spindle length. Here, cell length specifies spindle length by a direct force between the spindle and the cell cortex, or another object within the cell that has cell-like dimensions. Consistent with this model, compressing a cell results in spindle elongation [2, 4]; however, these perturbations may also affect intrinsic physical and chemical mechanisms.

A2. Component limitation. Here, cell volume specifies spindle mass by fixing the amount of one or more spindle components that are present at fixed concentration in the cytoplasm. The most obvious limiting factor is tubulin itself, and we know that ~50% of available tubulin assembles into the spindle in mammalian cells [113]. However, cells tend to synthesize proteins in the ratios required to build assemblies, so other spindle proteins are probably just as limiting. Tubulin concentration obviously sets an upper bound on microtubule mass. It is a less obvious how it might set a length scale, but this is possible in theory. In the presence of nucleating sites, tubulin will polymerize into microtubules until it is sufficiently depleted from the cytoplasm that dynamic

instability enters the bounded regime [114]. In this regime, the length distribution is exponential, which means that microtubules have a well-defined mean length. The larger the number of nucleating sites, the shorter this mean length will be, all other factors being equal. In principle, kinesins which can both walk to plus-ends and trigger depolymerization can also generate a microtubules length scale [75]. Whether a natural length scale individual microtubules contributes to setting spindle length is not clear. In our view, it is very likely that component limitation is one factor in scaling spindle length with cell length in the small cell regime (Fig. 5A), but how this limitation plays into the intrinsic models discussed below is far from clear.

B. Intrinsic physical mechanisms (Fig. 6B)

In general, these models work by creating position-dependent forces on spindle poles, as illustrated conceptually in the grey panel. One important model we do not discuss below is the polar ejection force model (Fig. 5C), where bipole length is set by the same polar ejection forces that set the chromosome-to-pole length in monopoles [104].

B1. Opposed motors. Here, one set of motors (e.g. Kinesin-5, plus-end directed) acts to elongate the spindle, while another (e.g. kinesin-14 family motor, minus-end directed) acts to shorten it [115, 116]. A problem with this class of models, which has been widely discussed in the literature, is that neither motor is known to generate a force that is naturally length- or position-dependent, so it is unclear how a steady-state length emerges. This problem can be solved by having length change continually in the context of a short cell cycle where motor activity is temporally regulated; in this case no steady-state in length emerges or is needed [116]. It can also be solved by adding an elastic element with a specified rest length [115]. In that case the motors serve to modulate the natural length of the elastic element, so the model reverts to model B3 below. Alternatively, mechanisms that make motor forces length- or position-dependent may in fact exist (e.g. through a traction fiber model [108]). However, until such mechanisms are experimentally demonstrated, we feel that opposed motor models on their own are unsatisfactory, because of this lack of a natural steady-state in length. Developing techniques to measure forces in a position-dependent manner (in reconstituted systems and within the spindle) will be essential to testing this class of models.

B2. Slide-and-cluster. This model also uses two motors, but they do not directly oppose each other. One (presumably Kinesin-5) slides microtubule outwards, while the other (presumably Kinesin-13 or Dynein) clusters minus-ends in the spindle axis [44]. Microtubules are nucleated near chromosomes, and lost by turnover, so a length scale emerges primarily as the outwards sliding velocity multiplied by the microtubule lifetime. Adding the pole-clustering motor generates distinct poles by causing the sliding velocity to decrease with distance from the chromosomes, which has been experimentally observed for nK-MTs in extract spindles [44]. Sharp poles form where outwards velocity decreases to zero, or decreases to the average depolymerization rate at poles. This model is appealing in that it robustly generates bipolar spindles with a natural intrinsic length scale. Its main deficiencies are *i*) a lack of realism (the model was only analyzed in a one-dimensional approximation); *ii*) a lack of consistency with k-fiber data (if K-MT sliding is blocked by Ndc80 antibody injection [68] or depolymerase RNAi treatments [117], a non-zero spindle length is still achieved), though k-fibers could simply conform to an nK-MT-specified length; *iii*) its requirement for long microtubule lifetimes to work. More work is required to test whether the slide-and-cluster concept operates in real spindles.

B3. Elastic structural scaffold. An elastic structural scaffold with a fixed rest length could determine spindle length. Whatever this tensile element is, it must be dynamic as the spindle appears structurally plastic in fusion experiments [118]. Although we find this model unappealing in the sense that it seems to simply pass the length-scale problem to another set of (unknown)

molecules, the force map experiments discussed above (Fig. 4) do point to the possible existence of a tensile element in some spindles.

C. Intrinsic chemical mechanisms (Fig. 6C)

C1. Morphogen gradient. Here, one or more morphogens diffuse from a source at the chromatin to a sink in the cytoplasm [119]. The resulting spatial gradient provides a length scale via a threshold concentration that controls biochemical activities. Spatial gradients of Ran-GTP [120] and Aurora-B kinase activity [121] with spindle-like length scales have been demonstrated, and these molecules are known to regulate many key spindle proteins. However, a direct role for morphogen gradients in setting spindle length has not yet been shown; moreover, doubling DNA content (and presumably chromatin-generated morphogen) in the spindle only changes spindle length by 10% in *Xenopus* egg extract [100, 118]. Although intrinsic length-determining models involving chemical and physical influences seem very different, the two can be related. For example, if the morphogen controlled the activity of microtubule depolymerases at the poles, which is plausible (e.g. [122]), it would indirectly regulate forces on poles. Chemical gradients might also interact with the component limitation model (A2). In a very large cell, the morphogen gradient effectively defines a volume of cytoplasm that is much smaller than the cell. In this volume, some limiting spindle component(s) may set spindle length, as per the component limitation model (Fig 6A, A2). While chemical gradients emanating from chromatin are likely to play some role in spindle assembly, more experiments are required to test if they directly set the length, or mass, scales of the spindle.

5. Conclusion

A central theme in this review is the interplay between forces and assembly dynamics that lies at the heart of spindle mechanics, and makes the spindle very different from a muscle, or an automobile engine. Microtubule dynamics generate force and are also affected by force, making the interplay challenging to study. As Nicklas remarked, "this raises the intriguing possibility that spindle function, i.e. force production, regulates spindle structure by directly affecting assembly thermodynamics, altering the length and stability of microtubules" [80]. We agree wholeheartedly with Nicklas' suggestion. At kinetochores, we may be close to revealing the molecular basis of this interplay. Elsewhere in the spindle, forces, assembly dynamics, and the position-dependencies of both that allow for the metaphase steady-state are still quite mysterious, and worthy of study by a new generation. Developing a system in which physical, chemical and genetic perturbations can be done in combination will be essential to addressing these questions.

Figure 1. Three steady-states in position are reached during metaphase. Position-dependent forces (black arrows) must help reach the steady-state positions and correct any deviations (fainter colors) from them. A) During symmetrical cell division, the spindle (green) must be positioned at the center of the cell (blue). B) The chromosomes (blue) must be placed in the middle of the two poles. C) The spindle poles (blue) must be positioned a certain distance away from each other (and the chromosomes) to dictate spindle length. The three steady-state positions are critical in determining where the sister chromosomes will travel after anaphase, and thus essential to accurate chromosome segregation.

Figure 2. Microtubule architecture and dynamics in the mitotic metaphase spindle of mammalian cells. A) Architecture of the mammalian mitotic spindle: microtubules (green), sister chromosomes (blue) and kinetochores (red) for attachment of chromosomes to microtubules. B-D) Three classes of microtubules within the spindle, with different minus-end locations (black circles), dynamics (black arrows) and nucleation zones (blue). B) Kinetochore-microtubules continuously slide toward the pole (green arrow), polymerize at the kinetochore and depolymerize at the pole. Kinetochore-microtubules form large bundles (thicker green line) and have much longer lifetimes than the other two classes of microtubules. C) Nonkinetochore-microtubules are nucleated throughout the spindle, and continuously slide poleward (green arrow) with dynamic plus-ends and unprobed minus-ends. D) Astral microtubules are nucleated at centrosomes, don't slide, have dynamic plus-ends and fixed minus-ends. Astral microtubules may also overlap with other microtubules (question mark).

Figure 3. Molecular force generators and their sites of action in the mammalian metaphase spindle. Arrows depict object (square) direction of movement (small arrows) and experienced force (large arrows). A) Microtubules (green) assembling (top) and disassembling (bottom) can push or pull an object, respectively. To couple disassembly to object movement, a connecting element (red ellipse) is required. B) A molecular motor can power object movement toward microtubule plus-ends (purple) or minus-ends (blue). C) An elastic element (spring) can pull objects inward when stretched, or push objects outward when compressed. D) Friction forces oppose movement. They can be generated by bond breakage (top, blue bonds moving up and down) and mesh reorganization (bottom) required for object movement. E) Spindle locations where the above forces operate. Only dominant forces are cartooned. Friction and elasticity likely operate everywhere, but are only drawn at the kinetochore. (1) Anywhere anti-parallel microtubules overlap, microtubule cross-linking motors operate. This site is depicted both near and far from the metaphase plate. Kinesin-5 family members push microtubules apart: this is the best characterized outward force, and is required for bipolarity establishment in most spindles [44, 97]. *C. elegans* embryonic spindles largely lack nK-MTs and do not require this force [123]. (2) At kinetochores, where microtubules disassemble and pull (and assemble and may push), and where Ndc80 (red arms) is thought to provide microtubule attachment. The elastic centromere (spring) is deformed [76] and friction (double arrow) occurs. Plus- and minus-end motors (e.g. Cenp-e and Dynein) can also operate here, as can microtubule depolymerases (e.g. MCAK and Kif18) and other end binding proteins [13, 26, 75, 124, 125]. (3) At the poles, dynein and/or minus-end kinesins organize and focus minus-ends, presumably by holding on to one microtubule while moving on another [126]. K-MTs depolymerize at poles (depolymerases may be involved [85]), and whether this generates pulling forces has been suggested [37, 84] but not directly measured. (4) On chromosome arms, plus-end-directed chromokinesins (e.g. Kid [98]) push microtubules, exerting away-from-the-pole force (polar ejection force [104]). (5) At the cortex, dynein pulls on A-MTs, and may be the main spindle-

centering force in mammalian cells [127]. How motor activity is coupled to depolymerization (and polymerization) at the cortex is unclear.

Figure 4. Toward a primitive force map of the mammalian metaphase spindle. Experiments informing on mechanical properties of the spindle. A) Classic tensed k-fibers force map (left), and revised force map proposed herein (right). Red bar represents a possible non-microtubule element under tension. B) Laser ablating one kinetochore results in poleward movement of the sister kinetochore [83]. C) Addition of nocodazole results in reduced tension on the kinetochores [61]. D) Cutting several k-fibers results in bending of the few remaining fibers and shortening of that half-spindle [87]. E) Release of a microtubule depolymerizer drug results in bending of the stable k-fibers and loss of kinetochore tension as the spindle shortens [88]. F) Laser cutting a k-fiber near the kinetochore does not prevent tension generation on that kinetochore, or microtubule sliding (green arrow) [34, 87]. G) A microneedle can move a chromosome across the metaphase plate while the k-fiber stays connected at the pole [31, 32].

Figure 5. Nature of the metaphase spindle length problem. A) For small cells, spindle length scales with cell size, but for larger cells [100] and in extract [101], spindle length reaches an upper limit. B) The spindle is a dynamic structure. Physical perturbations reversibly change the spindle length steady-state: the spindle lengthens upon egg [4] or spindle [2, 8] compression, and shortens when subject to high hydrostatic pressure [9], low temperature [5, 6], or pole-to-pole microneedle compression [3]. Similarly, addition of hexylene glycol [88] or D₂O [128] increases spindle length, while colchicine reversibly decreases spindle length [4]. While these physical and chemical perturbations affect total spindle tubulin polymer mass, we do not know whether they affect spindle length by changing microtubule length, growth parameters or numbers in the spindle. Genetic perturbations that affect both spindle assembly and maintenance (e.g. RNAi, depletions) revealed that microtubule destabilizers contribute to spindle shortening [88, 115], and microtubule stabilizers [115] and nucleators [129] contribute to lengthening; the location of destabilizers may be important, and their activities may oppose each other [130]. Chemical and genetic perturbations of motors [37, 44, 115], and kinetochore-microtubule attachment [68], can also affect spindle length and are not included here; their role may be system-dependent. C) Bipolar and monopolar spindles have the same chromosome-to-pole distance [104]. Purple arrows represent the position-dependence of polar ejection forces [109] (powered in part by chromokinesins, see Fig. 3E).

Figure 6. Three classes of models able to provide a stable metaphase spindle length scale. A) Spindle-extrinsic mechanisms. For example, cell size (A1) or availability of a spindle component (e.g. tubulin monomer) (A2) could determine spindle length. B) Spindle-intrinsic physical mechanisms: inward forces could increase with spindle length (left), or outward forces could decrease with spindle length (right). Proposed mechanisms include opposed motors (B1), a slide-and-cluster model (B2, where half-spindle length is proportional to the product of microtubule sliding velocity v and lifetime Δt), and an elastic structural scaffold (B3). C) Spindle-intrinsic chemical mechanisms. For example, a morphogen (grey molecule) gradient (C1) could determine spindle length. Right cartoon represents the morphogen concentration decay away from chromosomes; the dotted lines represent the concentration threshold determining pole position.

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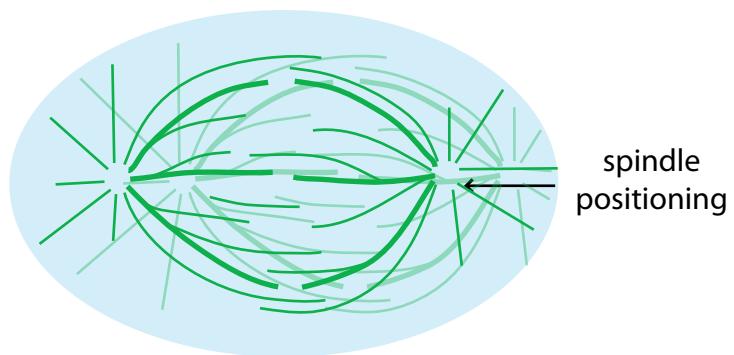
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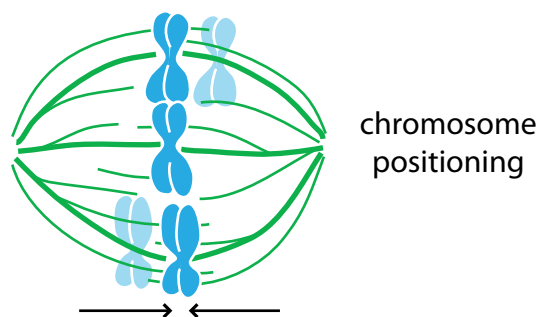
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Figure 1

A



B



C

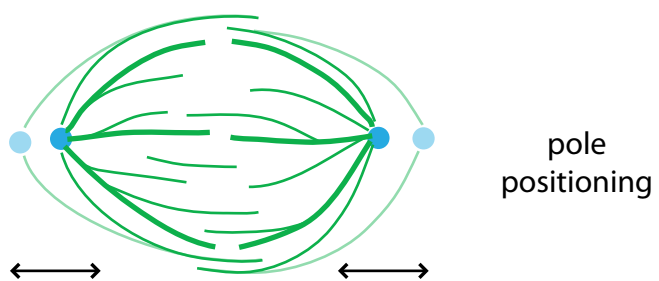


Figure 2

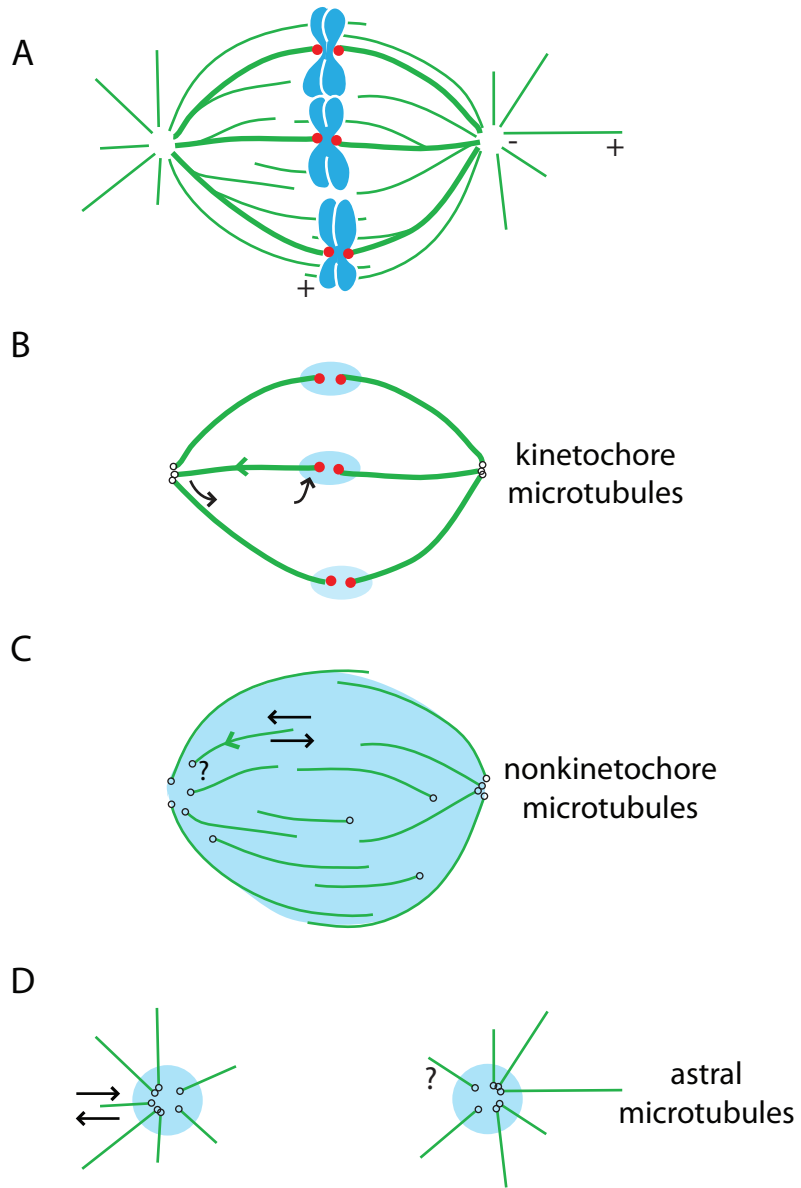


Figure 3

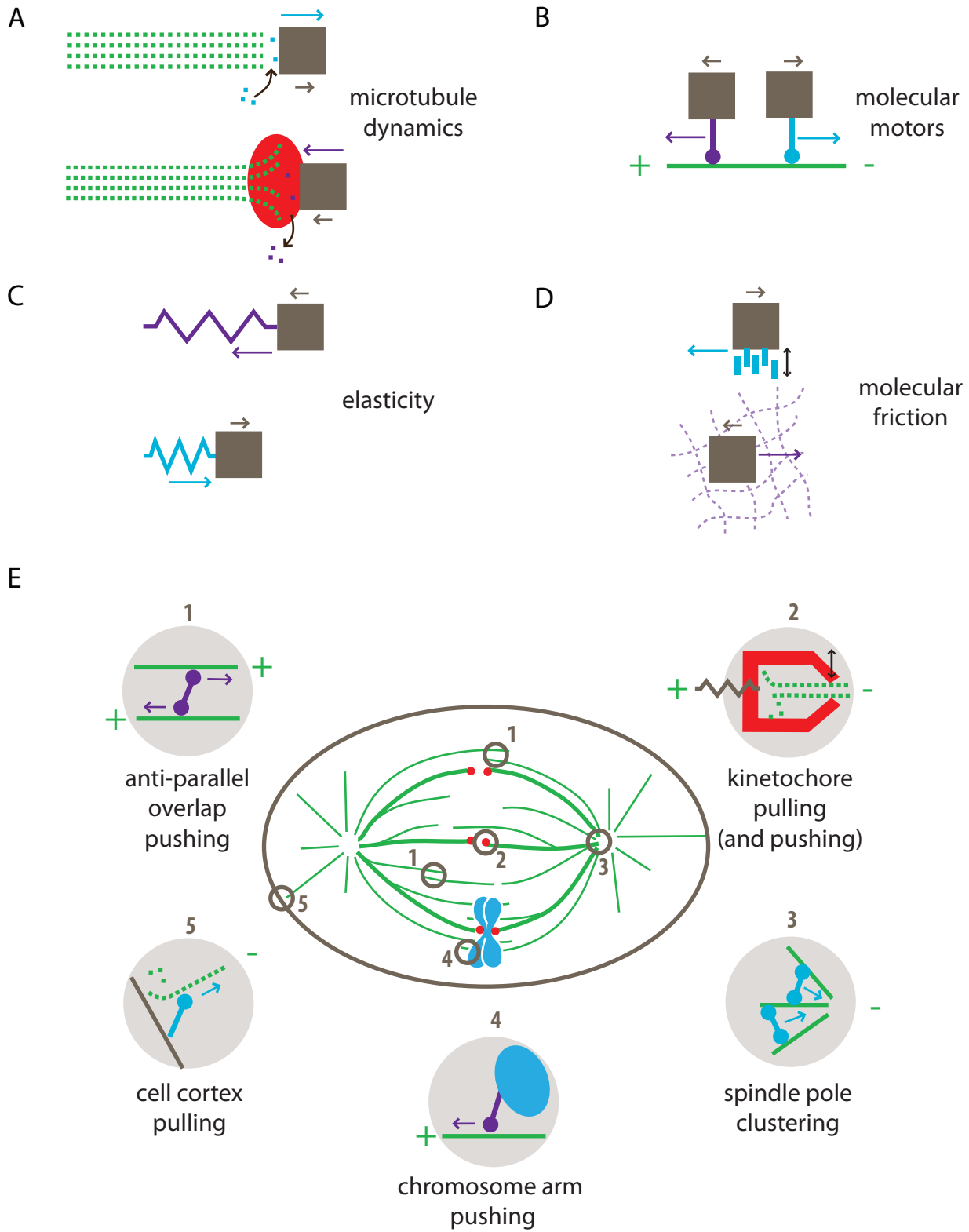


Figure 4

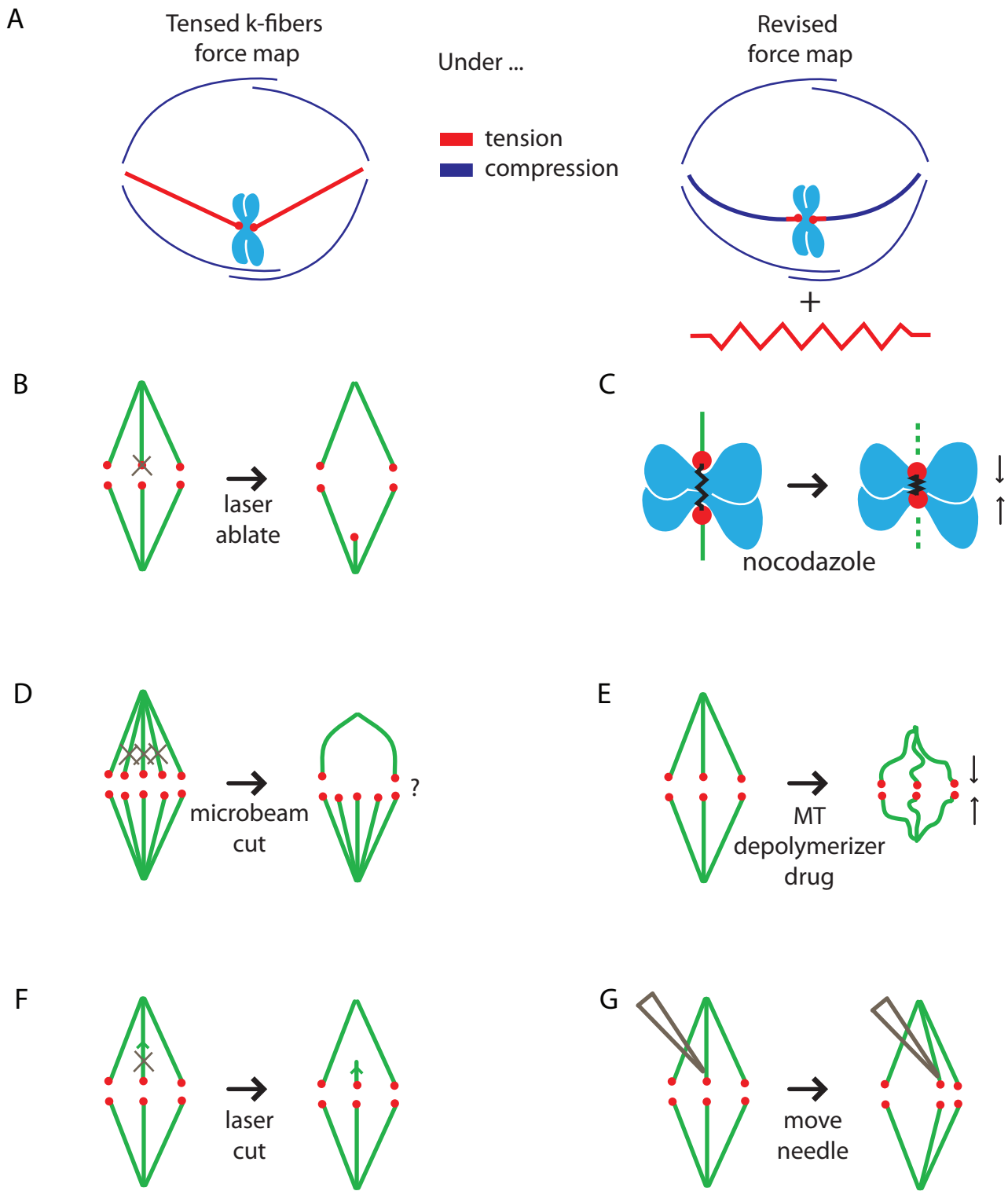
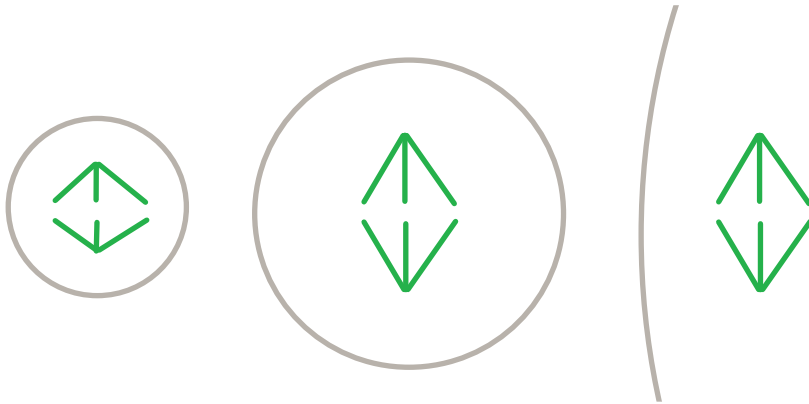
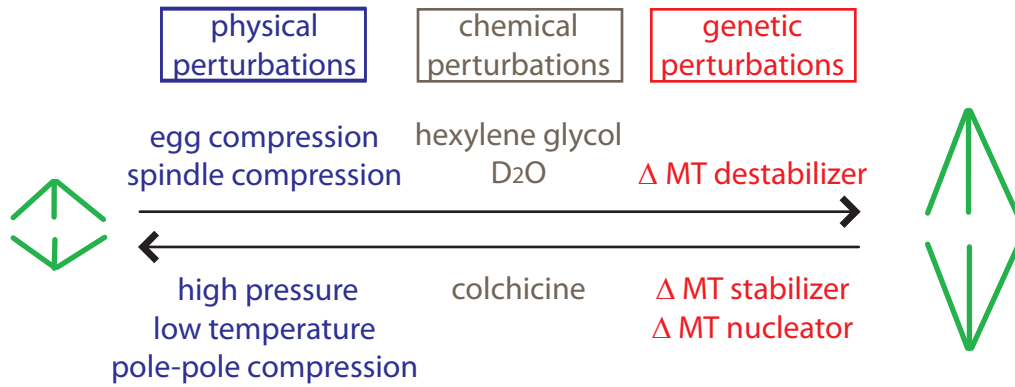


Figure 5

A



B



C

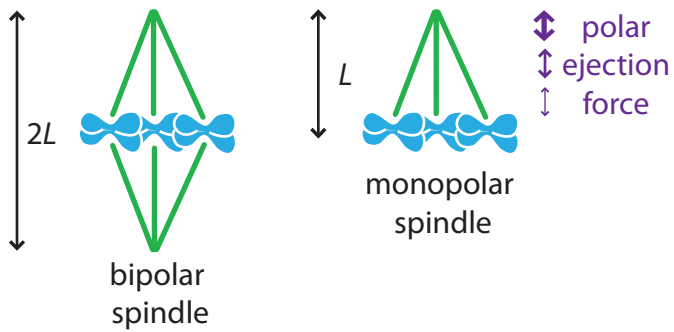


Figure 6

