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The spindle: Integrating architecture and mechanics across scales

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Abstract

The spindle segregates chromosomes at cell division, and its task is a mechanical one. While we have a nearly complete list of spindle components, how their molecular-scale mechanics give rise to cellular-scale spindle architecture, mechanics and function is not yet clear. Recent *in vitro* and *in vivo* measurements bring new levels of molecular and physical control and shed light on this question. Highlighting recent findings and open questions, we introduce the spindle's molecular force generators, and we discuss how they organize microtubules into diverse architectural modules and give rise to the mammalian spindle's emergent mechanics. Throughout, we emphasize the breadth of space and time scales at play, and the feedback between spindle architecture, dynamics and mechanics that drives robust function.

Keywords

Spindle; self-organization; mechanics; material properties; architecture; dynamics

The spindle: mechanical integrity with dynamic parts

When all eukaryotic cells divide, they build a microtubule-based machine called the **spindle** (see Glossary) to segregate their chromosomes into two daughter cells. This machine's job must be accomplished robustly and accurately. For example, billions of cells divide every day in our bodies, yet chromosome segregation errors are rare [1]. Errors can lead to cell

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death and disease in somatic cells, and developmental disorders in the germline [2]. Key biochemical and physical processes drive spindle function across scales. Here we focus on the latter, specifically on how molecular-scale forces integrate to give rise to the mammalian mitotic spindle's robust cellular-scale **architecture, mechanics** and function. In building itself, the spindle faces a formidable task: to build an intricate micron-sized structure that lasts an hour, from thousands of nanometer-sized molecules that turn over every few seconds or minutes [3, 4]. As an analogy, imagine if a skyscraper built itself, and every month replaced its beams and bolts. The spindle is dynamic and **self-organizes**, and understanding how such active biological structures and their mechanics emerge from their parts remains a challenge [5]. New measurements both *in vitro* and *in vivo* are becoming possible due to technical advances, shedding new light on this question.

In this review we begin by highlighting the molecular-scale **active** and **passive forces** that build the spindle. Second, we discuss new insights into how these force generators self-organize to build key architectural modules of the spindle. Third, we describe how the above forces and modules give rise to the spindle's **emergent** mechanics and **dynamics**, and ultimately to its robust function. Combining improved molecular and architectural control with new mechanical measurements both *in vitro* and *in vivo* is helping to link concepts emerging from 'bottom up' approaches (reconstituting systems from their parts) and 'top down' approaches (breaking systems apart). We highlight recent advances, focusing on mitotic spindles in mammalian systems, and important questions that remain unanswered.

Molecular-scale force generators build, maintain and power the spindle

Mechanical force is central to the spindle's function: it builds the spindle [6], moves and segregates chromosomes [7], and stabilizes attachment of chromosomes to the spindle [8]. While we have now identified most of the proteins that compose the mammalian spindle [9, 10], and are beginning to uncover some of their molecular-scale mechanics, we still have a poor understanding of the mechanical principles underlying spindle self-organization and function. At the molecular level, active force generators (motors) transduce chemical energy into mechanical work to drive and maintain specialized organization, such as a microtubule **aster** (Fig. 1A-B). In turn, passive forces (**elasticity** and **friction**) store or dissipate, but do not consume chemical energy (Fig. 1C-D): they resist deformation, limiting changes in spindle structure from active forces, thereby giving the spindle structural robustness in space and time.

Active forces drive spindle self-organization

Two molecular-scale processes convert chemical energy from ATP or GTP into mechanical work to build and maintain the spindle: molecular motors that walk on microtubules (Fig. 1A), and microtubule dynamics as an assembly/disassembly motor (Fig. 1B).

Walking motors: diverse parts for diverse functions—Motors walking on microtubules fall into two categories: kinesins, most of which walk towards microtubule plus-ends, and dynein, which walks towards minus-ends. These generate 1-10 pN of force per motor, taking nm-sized steps with ms timescales. Kinesins have diverse structures and biophysical properties (Fig. 1A, top), and they preferentially localize to different

microtubule architectures and move different cargos. This molecular-scale diversity leads to diverse functions in the spindle [11]: for example, the tetrameric kinesin-5 Eg5 crosslinks and slides apart antiparallel microtubules to help establish spindle bipolarity; the processive and strong kinesin-7 CENP-E pulls chromosomes to the spindle center; the non-processive chromokinesins push chromosomes to the spindle center while avoiding ripping them apart.

In contrast to the wide diversity of kinesins, cytoplasmic dynein 1 is the only dynein active at mitosis. Yet, dynein plays diverse roles at different locations and times during mitosis [12]: for example, it focuses **spindle poles**, positions the spindle, and helps ensure the correct attachment of chromosomes to microtubules. One way dynein can accomplish such diverse roles is through its different adaptor proteins (Fig. 1A, top). NuMA can target dynein to spindle microtubule minus-ends [13] and to the cell **cortex** [14], and Spindly can target it to **kinetochores** [15], the structure linking chromosomes to microtubules. In addition to its targeting role, Spindly also regulates dynein's motility [16]. Mapping dynein's adaptors [17] at mitosis and whether and how they control dynein motility [18] will be an important next step. Determining how specific biophysical properties (e.g. processivity, force-velocity relationship) of kinesins and dynein complexes link to their spindle function is an area of active interest.

How do molecular motors, acting at a local scale, collectively give rise to a globally complex structure like the spindle? Such action requires coordination and regulation of motor activities across spatial and time scales. We now discuss examples of how local features can regulate global force generation outputs in the spindle by telling motors where, when and how to act. First, motors can be post-translationally modified according to their location, for instance, and this can regulate their function [19]. Second, microtubule posttranslational modifications can regulate motor activity [20], creating diverse "roads" with similar building blocks (Fig. 1A, middle). For example, recent work showed that CenpE-based chromosome motility is biased toward the spindle center because microtubules inside the spindle body are more detyrosinated (a post-translational modification) than astral microtubules pointing to the cell cortex, and CenpE motility is enhanced on detyrosinated microtubules [21]. Mapping microtubule post-translational modifications across the spindle [22] and how this "tubulin code" [23] affects motor function will be an important future direction. Third, the spatial arrangement of spindle microtubules can impact force generation. For example, a recent study *in vitro* demonstrated that the number of Eg5 motors scaled with the overlap length between antiparallel microtubules, and thus generated force proportional to overlap length [24] (Fig. 1A, bottom). Thus, force generated by motors not only builds microtubule modules in the spindle, but can be shaped by local microtubule organization. More broadly, mapping how the identity and number of motors working together regulate output force production (e.g., [25]) will help us understand how diverse motor forces integrate inside the spindle (e.g., [26]). Towards this goal, the ability to control motor position and numbers *in vitro* [27] will be an important tool.

Microtubule dynamics generate force—Microtubule polymerization and depolymerization, powered by GTP hydrolysis, are essential to spindle remodeling, mechanics and function. This dynamic instability [28], in which microtubules can exist in a steady-state of growth or shrinkage, facilitates search and capture of chromosomes and

allows the spindle to constantly rebuild itself. Both microtubule polymerization and depolymerization are thermodynamically favorable in the cell, and as such can perform mechanical work [29]. *In vitro*, microtubule polymerization can generate pushing forces [30] while depolymerization of a single microtubule can, through a coupler that holds onto the depolymerizing microtubule, generate pulling forces of up to ~30 pN [31, 32] (Fig. 1B, top). *In vivo*, microtubule plus-end dynamics (both polymerization and depolymerization) are regulated by proteins such as +TIPs that track plus ends, and which include polymerases and depolymerases that enhance dynamics in an ATP-dependent manner [33] (Fig. 1B, middle). Microtubule depolymerization plays a major role in moving chromosomes to poles, and indeed, removing all minus-end directed motors in yeast does not impact the ability of the spindle to move chromosomes poleward [34]. Tubulin depolymerization may be an ancient biological motor [35] akin to similar filament polymerization motors in bacteria. How depolymerization force is transduced to the kinetochore, and how the kinetochore as a coupler regulates force transmission (Fig. 1B, bottom), remain questions of active interest [36]. Finally, just as studying mutants of kinesins and dynein is helping reveal how their engines work, using recombinant tubulin mutants will be key to uncovering tubulin mechanochemistry [37].

Passive forces make the spindle robust

Passive forces limit spindle dynamics by storing or dissipating energy from active force generators, helping to create a stable structure. Two types of passive forces provide stability to the spindle by opposing active forces: elasticity (Fig. 1C) and friction (Fig. 1D). Elastic force limits the *magnitude* of a structure's deformation, and drives its ability to return to its initial state after storing energy [38]. Most simply, elastic force (F) scales with the **stiffness** (k) and deformation (Δx) of the structure, and, as a restoring force, is directed opposite to the direction of the displacement: $F_{elasticity} = -k \cdot \Delta x$. Cellular structures are typically only elastic over deformations that are small and short-lived, beyond which they remodel [39]. At the molecular level, elasticity can result from stretching or bending a molecule or assembly of molecules, such as a crosslinker stretching or a microtubule bending (Fig. 1C, top). In turn, molecular friction limits the *timescale* of deformations by dissipating energy and slowing objects down, and is associated with structural rearrangements that lead to shape changes that remain [38]. Most simply, frictional force (F) scales with the **viscous drag coefficient** (γ) and object velocity (v), and opposes the direction of motion: $F_{friction} = -\gamma \cdot v$. The simplest form of friction is **viscous** drag, generated on a moving object by the surrounding fluid. Because of viscous drag, small objects (e.g. a few μms, like chromosomes) moving at low typical velocities (e.g. ~1 μm/min, like chromosomes) in the cytoplasm immediately stop moving when forces are removed, a low **Reynolds number** scenario [40, 41]. Yet, drag force in cells is small compared to forces cellular machines can generate: for example, in principle only ~0.1 pN of drag occurs when a chromosome moves through the cytoplasm at anaphase velocities, while the spindle may be able to generate 10,000 times more force [42]. Instead, dominant frictional forces in the spindle come from breaking interactions in the spindle's microtubule network (Fig. 1D, top). This molecular friction may give rise to the slow movements observed inside the spindle despite machinery that can generate much larger forces.

Microtubule networks give rise to both elasticity and friction in the spindle. An important source of elasticity is microtubule rigidity itself. Microtubules are stiff and will extend straight for several millimeters without external force on them [43]. Forces in the spindle are sufficient to bend microtubules, but how much elastic force this requires [44] remains unclear since we do not know the architecture or mechanics of microtubule bundles [45], or of the material around them [46]: both the number of microtubules in a bundle (Fig. 1C, middle) and how they are crosslinked (Fig. 1C, bottom) will impact, and can thus regulate, bundle stiffness. An important source of friction in the spindle comes from motor and non-motor proteins that crosslink microtubules [47], though which molecular interactions contribute most to friction remains an open question. As two microtubules move relative to each other, or as a large object moves in the spindle network, friction originates from breaking non-covalent bonds between spindle components (Fig. 1D, middle) or network reorganization and turnover (Fig. 1D, bottom); both of these factors can thus regulate friction in the spindle. Simple versions of frictional interactions can be probed *in vitro*. Crosslinkers, proteins that can link two microtubules together, resist microtubule sliding in a velocity dependent-manner, which penalizes fast movements and helps stabilize spindle architecture. For example, the motor Eg5 generates friction against microtubule sliding that scales with the number of Eg5 molecules, length of microtubule overlap, and sliding velocity [24]. Forming stable microtubule arrangements may require passive crosslinkers [48], and compaction of crosslinkers has been proposed to help increase friction at shortening overlaps [49]. Notably, friction along microtubules can be direction-dependent and lead to directed forces in active, energy-consuming networks: for example, NuMA, which clusters microtubule minus-ends at poles, is easier to pull toward the minus-end than the plus-end, and as such can move towards minus-ends in certain active networks [50]. The structural origins and implications of this asymmetric friction for protein localization and spindle function are still unclear. Relatedly, the confinement of diffusible, non-motor crosslinkers in a network may also generate directed forces [51]. These two recent examples of non-motor proteins giving rise to directed forces broaden our understanding of the role of passive forces in the spindle.

Finally, we note that passive forces generated by and at chromosomes and kinetochores are likely also essential for robust chromosome segregation. Centromere [52] and kinetochore [53] stiffness may promote chromosome biorientation, and impact signaling at the kinetochore [54]; in turn, friction at the kinetochore-microtubule interface [55] may regulate chromosome attachment stability and spindle mechanics [56]. However, the basis and role of these passive forces remain poorly understood. For example, we do not even know over which magnitude, axis and timescale the centromere is elastic and **plastic** [57]. Understanding the passive forces at centromeres, kinetochores, and their interface with the spindle will be an important goal as approaches become available to probe them.

From molecular-scale forces to cellular-scale architecture

Flemming described the spindle's characteristic architecture more than a century ago [58]. Our understanding of how it arises is however still incomplete, though rapidly progressing [59, 60]. Because spindle microtubules are so densely packed, they cannot be resolved by conventional light microscopy. Thus, most information at this scale comes from electron

microscopy [61, 62]. The spindle's diverse functional needs require diverse microtubule structural modules. These modules are structurally and functionally defined by the number, density, length, and relative overlap and orientation of the microtubules that make them (Fig. 2A), and are built from active and passive force generators. Below, we discuss three key modules, among others, of the mammalian spindle's microtubule architecture, and how they are built from similar components (Fig. 2B): its bipolar array of antiparallel microtubules, its asters making focused poles, and its parallel microtubule bundles binding kinetochores and making '**kinetochore-fibers**'. The fact that microtubules are polar is essential to these modules' architecture and function: microtubule plus- and minus-ends have different biochemical activities [63] and dynamics, with plus-ends being shorter-lived than minus-ends, and microtubule associated proteins can also "read" the orientation of the intervening microtubule polymer. For simplicity, here we focus on the metaphase spindle: it is a stable steady-state structure that is highly dynamic, consuming energy to maintain its shape and dynamics, such as constant poleward movement of microtubules (termed 'flux') [64].

Building the spindle from purified parts and reconstituting cell division *in vitro* remain a dream. Although we can now reconstitute some of these spindle structural modules [65, 66] *in vitro*, begin patterning their geometry [67], and dissect their size regulation [68], positioning [69], mechanics [70] reconstitution cannot as yet approach the mammalian spindle's complex architecture and mechanics. Even how the above three architectural modules (antiparallel, aster and parallel structures) come together to make a spindle is not fully understood. In part, this is because we do not understand the underlying basis of emergent spindle properties: for example, how nm-scale pulls and pushes give rise to the spindle's steady-state μm-size and its **material properties** are poorly understood. Finally, as big steps towards reconstitution of cell division *in vitro* we note that we can now encapsulate spindle-like structures [71] and *Xenopus* spindles from cytoplasmic extract in droplets [72, 73].

Establishing spindle bipolarity

A critical feature of spindle architecture is its bipolarity: the two spindle extremities ('poles') ultimately guide the segregation of chromosomes to two different positions. The mammalian spindle is composed of two oppositely (though not uniformly) oriented arrays of microtubules, with minus-ends enriched around poles and plus-ends enriched in the spindle center, together with microtubule antiparallel overlaps [74]. In mammals, active force from the kinesin-5 Eg5 is required to build a bipolar spindle [75], sliding antiparallel microtubules apart (Fig. 2C). The longer the antiparallel overlaps, the more Eg5 molecules bind and the more outward force is generated [24], presumably generating shorter overlaps, and thus creating the possibility of a stable feedback module between architecture and force generation. Passive force from microtubule crosslinkers may also help form stable microtubule overlaps [48]. We note that Eg5 teams, like teams of other crosslinking motors, do not push parallel overlaps apart as multiple motors would simply fight each other, with some walking on one microtubule and some on the other. Instead, Eg5 provides friction between parallel microtubules [24], potentially helping stabilize their organization. Most though not all [76] mammalian cells can maintain spindle bipolarity without Eg5. They do

so using the plus-end-directed kinesin-12 Kif15 [77, 78], though how they do so remains debated [79–81].

Building and maintaining focused spindle poles

Spindle poles are the two spindle extremities, and in mammals microtubule minus-ends focus together at poles. How are poles built? While centrosomes are structures that nucleate and organize microtubules, and are typically located near spindle poles, they are not needed to build or maintain spindle poles in mammalian cells: spindle microtubules can be nucleated and their minus-ends focused in the absence of centrosomes [82]. For more than 20 years, we have known that dynein can build microtubule asters in cytoplasmic extract [83], and focus microtubules into spindle poles [84]. How dynein could do this while binding indiscriminately all along parallel microtubules is not immediately clear – since such motors will generate gridlock, with different dynein motors pulling a given microtubule in different directions. However, gridlock would be lifted if dynein were preferentially recruited at the end of the microtubule [85] – which it is [86, 87]. Indeed, when using laser ablation to create synchronized minus-ends in the spindle, dynein is rapidly recruited to these minus-ends and carries them as cargos [86, 87]. Dynein walks its minus-end as cargo to the minus-end of a neighbor track that points to the pole, clustering minus-ends there and crosslinking them (Fig. 2D). Thus, an early pole geometry self-reinforces by providing tracks for new minus-ends to follow. NuMA first recognizes minus-ends and recruits dynein there, and this targeting of dynein to minus-ends, rather than all along microtubules, is essential for pole formation [13]. This is a simple example of how spatial regulation of molecular-scale activities can give rise to complex cellular-scale architectures.

Dynein's ability, in cooperation with NuMA, to continually find and transport new minus-ends can not only drive pole formation, but maintain pole – and whole spindle – robustness and function. For example, dynein-based active force is thought to carry newly nucleated microtubule minus-ends to poles [88] and to continually refocus the pole despite competing forces and spindle dynamics [89]. In turn, passive forces from compliant linkages between microtubules [90] help keep pole integrity while accommodating microtubule movements. In addition, dynein force at minus-ends may ensure segregation without a direct connection to the pole, by anchoring one kinetochore-fiber's minus-ends along another fiber that directly connects to the pole [87]. Further, i*n vitro* work suggests that dynein acting at minus-ends of a microtubule network provides a contractile force keeping the spindle together [91, 92], and whether this is true in the spindle remains an open question. The observation that dynein can cluster minus-ends *in vitro* without NuMA [92], but requires NuMA to do so *in vivo*, raises the possibility that NuMA may help dynein generate more force or better hold on to microtubule cargos. Whether NuMA as an adapter could also regulate dynein's motility during minus-end transport remains an interesting, untested possibility. Finally, we note that while perturbing pole focusing in mammals correlates with chromosome segregation defects, whether pole focusing itself is important – and whether it is directly or indirectly important – is not fully clear. Indeed, other systems such as plants lack focused spindle poles. Focused mammalian spindle poles may provide structural stability to the spindle and help it connect to the centrosome; a spindle-to-centrosome connection drives spindle positioning through astral microtubules, and is well suited to ensure centrosome segregation.

Building kinetochore-fibers

In mammalian cells, kinetochore-fibers are bundles of about 10-30 parallel [93] microtubules whose plus-ends attach to a kinetochore. Many – but not all – their microtubules span all the way to the spindle pole, and their microtubules are 50-100 nm apart [62]. By binding to kinetochore-microtubule plus-ends, the kinetochore promotes the formation of this parallel microtubule bundle and stabilizes its microtubules [4]. Kinetochore-fibers can form when kinetochores capture centrosomal microtubule plus-ends, or when non-centrosomal microtubules attach to kinetochores and get transported to poles with their plus-ends staying at kinetochores [59, 94] (Fig. 2E); in meiosis, actin bundles have also been proposed to help kinetochore-fiber formation [95]. How kinetochore-fibers rapidly [96] acquire all their microtubules, where microtubules are bundled within the fiber and by what active and passive force generators, and whether and how fiber microtubules are bound by different proteins than other microtubules (e.g., [97, 98]) are not well understood. Answering these questions will help us better understand kinetochore-fibers and their function. As an example of how kinetochore-microtubules could be different, their lifetime is much longer than that of non-kinetochore-microtubules in the spindle [4], and this may lead to enrichment on kinetochore-fibers of microtubule-binding proteins with slower binding rates [74]. Finally, we note that while having many microtubules in a kinetochore-fiber may help ensure robust attachment and generate more force, how individual microtubules coordinate their plus- and minus-end dynamics within a kinetochore-fiber remains an open and important question. Reconstitution of kinetochore-fiber-like bundles *in vitro*, when conceptual and technical knowledge becomes available to do so, should help shed light on this question.

The spindle: cellular-scale mechanics and function

How do molecular-scale active and passive forces, and key spindle structural modules, integrate in space and time to give rise to the mammalian spindle's cellular-scale forces? Self-organization connects a wide range of spatial scales (Fig. 3A) and time scales (Fig. 3B) in the mammalian spindle: from ~10 nm motor step sizes to 10-20 μm long spindles, and from ~10-100 ms motor steps to a ~1 hr spindle lifetime. Below, we discuss recent progress in understanding the spindle's material properties (Fig. 3C), how force propagates through the spindle (Fig. 3C), and the interplay between architecture, mechanics and dynamics in the spindle (Fig. 3D). We highlight how these are well suited to support robust spindle function.

Spindle material properties and local environment

The spindle's material properties will determine how it responds to forces in space and time – both forces it generates itself and forces from outside the spindle. Thus, establishing a basic material framework is essential to understanding how it performs its mechanical functions. However, the material properties of mammalian spindles remain largely unknown: these spindles cannot yet be reconstituted *in vitro*, and accessing them deep inside cells with force probes remains challenging. The spindle's material properties are likely complex due to its anisotropy (structure that is not uniform in all directions) and the dynamics of the microtubules that comprise it. Nicklas used microneedles in insect spermatocyte cells [42], whose cortex appears to easily deform, to provide us with a foundation for understanding

spindle material properties – before modern molecular tools were available. Unfortunately, using microneedles has proven much more challenging in vertebrate cells [99].

Much of what we know about spindle material properties and their underlying basis comes from work on Xe*nopus* extract meiotic spindles. These spindles can be assembled outside cells and directly contacted by force probes. Pushing on the spindle's sides reveals that it is stiffer along its long than short axis [39], likely due to microtubules' primarily orienting along the long axis of the spindle; this makes sense given that the spindle's main task is to generate force along its long axis. Further, the spindle has a **viscoelastic** response to small deformations and a plastic response to larger ones [39].

This viscoelasticity stems from the solid-like elasticity of the spindle microtubule network and the liquid-like friction this network imparts at the spindle scale (**viscosity**); plastic responses occur over minutes, and may correspond to the time needed for motors to move microtubules and reorganize the spindle under force [39]. In turn, skewering the spindle body with two microneedles moving them relative to each other, reveals the material properties of the local environment inside the spindle [47], as chromosomes and microtubules experience it (Fig. 3C, orange and red). Moving a needle along the spindle's long axis leads to a local viscous response, without altering global spindle size or shape; moving a needle along the spindle's short axis reveals material properties that change with the timescale of force application [47], reflecting complex coupling between elasticity and viscosity [100]. In simplified terms, over the short axis the spindle behaves as a solid (elastic) over short and long force application timescales, and as a liquid (viscous) over intermediates ones. Over short timescales (<1-10 s), the stiffness is dominated by the most rigid spring in this system, proposed to be non-kinetochore-microtubules [47]. Over long timescales (>100 s), the stiffness is dominated by the most compliant spring, proposed to be kinetochore-microtubules [47]. Over intermediate timescales (~10-100 s), the viscous response dominates as dynamic crosslinks locally reorganize: this is the timescale of metaphase chromosome movement, and suggests that short-axis spindle deformations as chromosomes move would dissipate locally, without a global effect on spindle structure. Thus, the force the spindle exerts on its components varies dramatically depending on how fast components move, and in which direction they go. The spindle pole has been proposed to couple mechanical elements along the spindle's long and short axes [47]. What specific active and passive molecular force generators give rise to spindle's material properties is a question of active interest. Further, whether mammalian spindles, with their different architecture, have analogous material properties and underpinnings remains to be discovered. There will likely be basic similarities, and for example cell compression reveals that mammalian spindles are also stiffer along their long axis than short axis [101].

Finally, we note that viscoelastic models are unlikely to be sufficient to understand the spindle as a material. The spindle's metaphase steady-state is a non-equilibrium one by nature: it constantly consumes energy at the molecular level, and thus it is **'active matter'** [102]. This active nature gives the spindle special abilities, e.g. that of self-repairing [103] and fusing with another spindle [104]. Concepts from active matter theory, which describes such materials, can thus be useful for understanding the spindle's physical properties [105, 106].

Force propagation and load-bearing through the spindle

Determining how the spindle generates and responds to mechanical force is key to understanding the spindle's physical and signaling [107] functions. This requires a basic map of how forces propagate through the spindle, starting with which components are tensed and compressed – and where. For chromosomes rather than microtubules to move when kinetochore-microtubules depolymerize at kinetochores, these microtubules must be anchored in the spindle. Laser ablation of kinetochore-fibers can abruptly change available **load-bearing** paths within the spindle and can thus reveal which anchor points are necessary and which are not [108]. In mammalian cells, such work revealed that despite the spindle being often drawn with kinetochore-fibers connecting to other microtubules at poles, kinetochore-fibers need not be anchored at the pole to exert force on kinetochores; further, the ability of a severed kinetochore-fiber, cut away from its pole, to bear **load** appears to scale with its length [19, 109–112]. While neighbor kinetochore-fibers appear poorly coupled in the spindle center [109, 113] and more strongly coupled around poles [114], the spindle can locally bear the load of chromosome movement within a few μm of kinetochores along both spindle axes [109] (Fig. 3C, yellow). Distributed, redundant load-bearing may help mechanically isolate chromosomes in the spindle, and thereby make their movement robust to spindle structure remodeling. In different systems, both NuMA and PRC1 have been proposed help locally bear load, and presumably do so by crosslinking the kinetochore-fiber to neighbor spindle microtubules [109, 110]. Whether these molecules play this role by exerting passive force, or acting in concert with a motor generating active force, is not known.

How exactly load is dynamically borne at the molecular scale – while non-kinetochore-microtubules turnover with seconds timescales [3] – and which structures are compressed to bear load remains unclear. The spindle is a closed-loop force network where total forces must sum to zero [40], and thus tension on kinetochore-fibers near kinetochores (which generate inward force) implies that another structure must be compressed (generating outward force). A mechanical role for a non-microtubule 'spindle matrix' has been proposed, but not established, and is not easily consistent with direct observations [115]. Instead, such a matrix may lead to cytoplasmic compartmentalization at mitosis [116]. What mechanisms generate outward force in the metaphase spindle to counter the inward pulling forces generated at kinetochores remains poorly understood. At anaphase, work in diverse systems suggests that outward pushing forces by microtubules between chromosomes can power chromosome segregation [117–121]. Antiparallel microtubule bundles connecting sister kinetochore-fibers on most chromosomes [122], termed bridge-fibers, have been proposed to generate these pushing forces in mammalian mitotic cells [120]. Whether these bridge microtubules or broader groups of antiparallel microtubules primarily drive segregation, and what active force generators power segregation, are areas of active interest. In any case, mammalian segregation can happen without kinetochore-fibers being connected to the pole [86, 87]. Finally, it is clear that spindles of different species sometimes rely on different molecular mechanisms for function; an exciting direction will be comparative mechanics, i.e. mapping how spindles from different species distribute load in similar and different ways and how this impacts their mechanical function. For example, microtubule

poleward flux has been proposed to help mechanical coordination across kinetochore-fibers in insect cell spindles [123].

Another exciting future direction will be to measure absolute, rather than relative, forces in the mammalian spindle and kinetochore – currently a major missing puzzle piece. Microneedle experiments revealed that the anaphase stall force needed to stop chromosome movement in insect cells is around 1 nN [7]. Whether this stall force reflects a limit in active or passive (e.g. frictional [124]) force generation at kinetochores is unclear. More recently, molecular force sensors revealed that kinetochore protein CenpC is on average under 1-2 pN of force at metaphase in insect cells, suggesting that chromosome biorientation produces hundreds of pN of force at these kinetochores [125]. Finally, we note that a force of 4 nN is required to shorten *Xenopus* extract meiotic spindles by 1 μm in length using cantilevers [39]. Looking forward, absolute force measurements in mammalian cells would allow us to probe models for generating and responding to force in the spindle and at kinetochores – and their molecular basis.

The spindle: mechanical feedback and redundancy

The spindle has a remarkably dynamic and robust structure and function. It is flexible enough to allow its own remodeling and self-repair, and to allow chromosomes to move within it. Yet its metaphase structure is stable, responding to physical cues [101, 126, 127] and chemical cues [128] in a reversible manner. Ultimately, this stability relies on energy consumption at the molecular scale, together with self-organization principles that translate molecular-scale rules to cellular-scale architecture. We illustrate this statement with two key self-organization concepts: *mechanical feedback* and *redundancy* that are key to physical robustness, just as biochemical feedback and redundancy are key to signaling robustness.

First, we emphasize the importance of feedback between architecture, mechanics and dynamics in the spindle (Fig. 3D). As an example of mechanics-architecture feedback, local organization of microtubules in a module will help select which proteins will bind to them, which will determine the mechanics of this module [66]. In turn, active and passive mechanics of the module will impact its architecture. Load-bearing by the spindle could further rewire its architecture – like load-bearing in actin networks alters their architecture [129, 130]. As an example of dynamics-mechanics feedback, microtubule dynamics generate force and are also regulated by force [131] in the spindle and at the kinetochore [132]. Spindle stability, including kinetochore-microtubule attachment stability [133], must be constantly tuned, with too stable or too unstable structures leading to dramatic consequences. As an example of architecture-dynamics feedback, spindle architectural modules (e.g. kinetochore-fibers) can regulate microtubule dynamics, and microtubule dynamics can both allow and drive spindle architectural remodeling. Notably, while energy input and feedback systems are key to integrated spindle architecture and function, they are also at the heart of why understanding the spindle's emergent mechanics and function is so challenging [5].

Second, mechanical redundancy or 'back up plans' in the spindle support stable spindle structure and function, but also make it challenging to dissect key contributors. For example active forces constantly remodel the spindle, while passive forces ensure small and slow

deformations under force; redundant mechanisms for maintaining spindle bipolarity (motors Eg5 and Kif15) [77, 78]; direct and indirect chromosome segregation, whereby chromosomes can segregate with or without their kinetochore-fibers directly contacting the pole, respectively [87]; local, distributed and redundant load-bearing in the spindle [19, 109, 110, 112]. Looking forward, methods to externally control architecture, mechanics and dynamics will be essential to ultimately understand how the spindle can at once so robustly maintain its mechanical integrity and function, and yet be so dynamic in space and time.

Concluding Remarks and Future Directions

How active and passive molecular-scale forces give rise to the mammalian spindle's robust architecture and function remains poorly understood. Still, progress suggests that key insights may be within reach. Answers may not only impact our understanding of cell division, but of other biological processes driven by active networks, such as cell migration. We can now assemble complex microtubule modules *in vitro*, with ever increasing control of both architecture and force generators, and can now better characterize their force output. In parallel, we now have better molecular control of spindle components inside cells [134, 135], simple ways of perturbing mechanics and architecture *in vivo* – and inspiration from other systems to bring mechanical measurements to mammalian cells [7, 39, 47]. As information at both molecular and cellular scales becomes better defined, we believe that theory and modeling will have a key role in helping connect them. Further, to critically test models of both self-organization and emergent mechanics of the spindle, new abilities will be needed. These include the ability to reconstitute more complex spindle-like structures *in vitro*, to control and rewire molecular-scale forces *in vitro* and *in vivo*, for example through optogenetics [136–139] to more easily map spindle architecture at the level of individual microtubules [140], to quantify spindle forces in space and time, and to image the action of individual molecules [141] in the spindle. Combining these abilities will be especially powerful for future investigations (see Outstanding Questions), and as such we highlight the importance of working in systems where genetic, chemical and physical tools can be used together.

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Glossary

Active force
force that consumes energy and that can drive self-organization by transducing chemical energy into mechanical work

Active matter
system in which the individual units are internally driven, energy consuming

Antiparallel microtubules
pair of microtubules oriented such that their plus- and minus-ends point in opposite directions

Architecture
design and construction of a physical structure

Aster
assembly of microtubules where ends are gathered together to form a star shape

Cortex
specialized protein layer attached to the inner surface of the cell's plasma membrane that helps give the cell its overall shape

Dynamics
change in parts over time, such as movement, turnover, growth, or shrinkage

Elasticity (elastic)
ability of a structure, by storing energy, to return to its original shape after being deformed

Emergent
characteristic of a phenomenon whereby interactions among smaller components give rise to larger entities that exhibit properties that the smaller components do not exhibit

Friction (frictional)
molecular-scale resistance that opposes motion and dissipates energy

Kinetochore
protein structure linking chromosomes to spindle microtubules

Kinetochore-fiber
parallel bundle of microtubules and associated proteins binding kinetochores at their plus-ends and anchoring chromosomes in the spindle

Load
force to which a given object is subjected

Load-bearing
supporting force transmission

Material properties
properties that define a material's response to force

Mechanics
generation of, response to or transmission of mechanical force

Passive force
force that does not consume energy but resists object deformation in space and time

Plasticity (plastic)
property of an object to permanently retain its deformed shape after transient force on it is removed

Reynolds number
dimensionless value that measures the ratio of inertial forces (tendency of an object to continue its same motion under no force) to viscous forces (tendency of a fluid to resist motion of an object moving through it)

Self-organization (self-organizes)
order arising from self-driven parts that consume energy

Spindle
bipolar array of microtubules that mediates chromosome segregation at cell division

Spindle pole
spindle extremity, where many microtubule minus-ends focus together in mammalian cells

Stiffness
rigidity of a structure i.e. the extent to which it resists deformation in response to force

Viscoelasticity (viscoelastic)
property of materials that exhibit both viscous (i.e. frictional) and elastic characteristics when deformed

Viscosity (viscous)
measure of resistance that opposes motion and dissipates energy, associated with structural rearrangements that lead to shape changes that remain

Viscous drag coefficient
shape-dependent value that reflects the viscous resistance of an object moving through a fluid

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Trends Box

- To perform its function, the spindle must be dynamic and flexible and yet able to generate force and maintain its integrity.
- Active and passive molecular force generators build and maintain the spindle. New approaches are revealing new properties of each, for example how total force output scales with the number of force generators.
- Molecular motors can build diverse microtubule organization modules, for example by selectively pulling on microtubule ends or by selectively acting on antiparallel microtubule arrangements.
- Physical perturbations reveal how spindle material properties vary with the axis and timescale of force application, and that the spindle locally bears the load of chromosome movement.
- Spindle self-organization connects scales from nanometers to tens of microns, and from tens of milliseconds to an hour. The spindle’s robust function emerges from the feedback between its architecture, dynamics and mechanics across scales.

Outstanding Questions Box

- Active forces: How do the specific biophysical properties of kinesins and dynein complexes link to their diverse functions in the spindle? How do the identity and numbers of molecular motors working together determine total force output? How do kinetochores harness the force of microtubule depolymerization to move chromosomes?
- Passive forces: How stiff are different microtubule bundles in the spindle, and how does crosslinking architecture impact their stiffness? What molecular processes contribute most to molecular friction in the spindle?
- How do active and passive molecular-scale forces integrate across space and time to give rise to cellular-scale spindle forces?
- Microtubule architectural modules of the mammalian spindle: How do spindles maintain bipolarity without the activity of the Eg5 motor? What's the role of a focused spindle pole? What's the architecture of a kinetochore-fiber and how is it built?
- How do diverse spindle architectural modules organize together to give rise to the mammalian spindle? Where does the steady-state size of the spindle emerge from of a series of molecular-scale pulls and pushes?
- Mechanics of the mammalian spindle: What are the material properties of the spindle, and what are their molecular and physical bases? How is the load of chromosome movement locally borne in the spindle, and which structures are compressed to support this load? What's a basic force map of the spindle, i.e. which components are tensed and compressed where, and what are absolute forces at play?
- How do spindle architecture, dynamics and mechanics affect and integrate with each other, and together ensure robust function of the mammalian spindle?

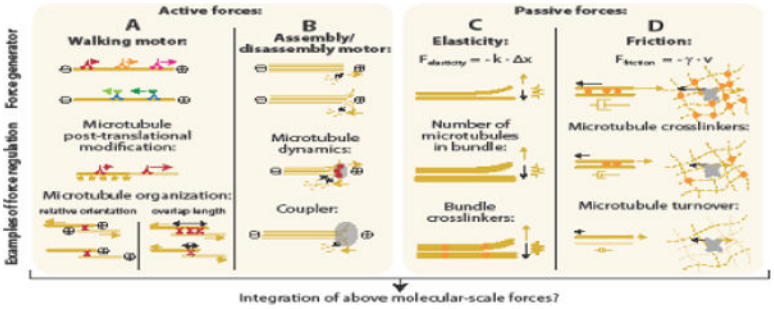

Figure 1. Molecular-scale force generators and their regulation in the spindle

(A-B) Cartoon of active force generators in the spindle. **(A)** Walking motors consume ATP to transport cargos. Kinesins (red, orange, pink) largely walk to plus-ends, and are diverse. Dynein (blue) walks to minus-ends, only one is active in the spindle and adaptors (green) can regulate it. Microtubule post-translational modifications (stars) and microtubule organization (relative orientation, and overlap length (black arrow)) can regulate the total force output (yellow and red arrows) of motors (red). **(B)** Microtubule dynamics, an assembly/disassembly motor consuming GTP, can generate pushing (polymerization, top) and pulling (depolymerization, bottom) forces (yellow arrows). Proteins (red) regulating microtubule dynamics can regulate force generation, and the nature of coupler (grey oval e.g. kinetochore) can impact exerted force. **(C-D)** Cartoon of passive force generators in the spindle, which do not consume energy but respond (black arrows) to applied active forces (yellow arrows). **(C)** Elastic force (spring) limits deformation and stores energy, scaling with object stiffness (k) and deformation magnitude (Δx). The number of microtubules and crosslinkers (orange) between them will regulate elasticity of a given microtubule ensemble, and of the spindle. **(D)** Frictional force (dashpot) slows deformations down and dissipates energy, scaling with effective viscous drag coefficient (γ) and deformation velocity (v). Frictional force occurs when non-covalent bonds must break for a deformation to occur, e.g. when crosslinked microtubules slide past each other (left) or chromosomes move within a microtubule network (right). Crosslinker(orange) detachment and microtubule turnover (shaded microtubule) are two ways to disrupt bonds and regulate friction in the spindle.

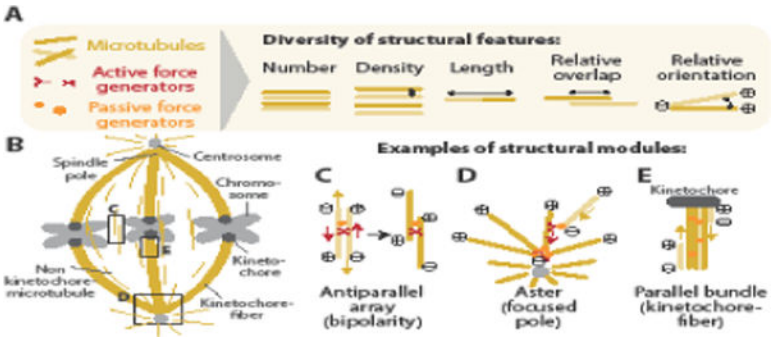

Figure 2. Building diverse spindle structural modules from the same microtubule building blocks

(A) Cartoon of different nm-scale active (red) and passive (orange) force generators organizing microtubules into diverse μm-scale structures that drive diverse spindle functions: these are defined by the number, density, length, relative overlap and orientation of the microtubules that make them. **(B)** Cartoon depicting architectural features of the mammalian spindle and highlighting three key structural modules shown in detail in (C-E).

(C) An antiparallel array of microtubules that establishes spindle bipolarity. To make a bipolar spindle, the tetrameric kinesin-5 Eg5 (red) crosslinks microtubules and generates an active force that slides antiparallel microtubule overlaps apart.

(D) A focused pole that provides spindle structural support and a spindle-to-centrosome (grey) connection point. To make a spindle pole, dynein (red) and NuMA (orange) transport microtubule minus-end cargos to other minus-ends and crosslink them together, forming the aster center.

(E) Kinetochore-fibers that robustly connect chromosomes to the spindle. To make a kinetochore-fiber, kinetochores can capture the plus-ends of centrosome-nucleated microtubules (left), and microtubules nucleated near kinetochores can grow out towards poles with their plus-ends staying at kinetochores (right). By binding to kinetochore-microtubule plus-ends, the kinetochore promotes the formation of this parallel microtubule bundle and stabilizes its microtubules to make them longer-lived than others in the spindle. Microtubule crosslinkers (orange) exert passive forces to stabilize these three and other structural modules in the spindle.

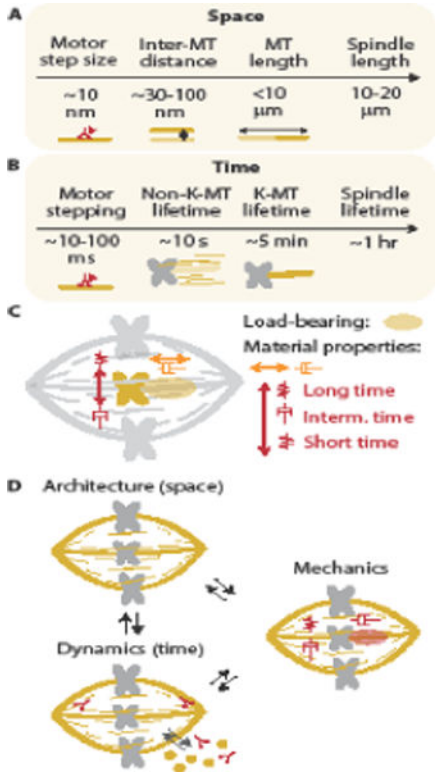

Figure 3. Integrating spindle architecture, dynamics and mechanics across scales

(A) Spatial scales in the mammalian spindle vary across a wide (10^3) range: motor step sizes, nearest neighbor distance between spindle microtubules (inter-MT distance), microtubule (MT) length, and spindle length.

(B) Time scales in the mammalian spindle vary across a wide (10^4-10^5) range: motor stepping, non-kinetochore-microtubule (non-K-MT) and kinetochore-microtubule (K-MT) lifetimes, and the spindle's lifetime at each mitosis.

(C) Spindle architecture and dynamics help sculpt the spindle's mechanics. The network of crosslinked spindle microtubules bears the load of chromosome movement locally (yellow oval) in the mammalian spindle. This network determines the spindle's material properties: moving a needle inside a *Xenopus* extract spindle along its long axis (orange) leads to a viscous response (orange dashpot likely reflecting microtubule crosslinks and dynamics), while moving it along the short axis leads (red) to a response that depends on the perturbation timescale. The response to a slow and fast perturbation is more elastic (red springs likely reflecting microtubule network stiffness), and that to an intermediate timescale perturbation that matches chromosome movement is more viscous (red dashpot also likely reflecting microtubule crosslinks and dynamics).

(D) The feedback triangle between the spindle's architecture, dynamics and mechanics ensures robust spindle function.