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Perspective

Mechanobiology of the Mitotic Spindle

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SUMMARY

The mitotic spindle is a microtubule-based assembly that separates the chromosomes during cell division. As the spindle is basically a mechanical micro machine, the understanding of its functioning is constantly motivating the development of experimental approaches based on mechanical perturbations, which are complementary to and work together with the classical genetics and biochemistry methods. Recent data emerging from these approaches in combination with theoretical modeling led to novel ideas and significant revisions of the basic concepts in the field. In this Perspective, we discuss the advances in the understanding of spindle mechanics, focusing on microtubule forces that control chromosome movements.

Segregation of the genome from a mother cell into two daughter cells during cell division is one of the fundamental processes of life. Physical separation of the chromosomes to opposite poles of the cells is carried out by the spindle (Figure 1), a fascinating and complex micro machine made of microtubules and numerous other proteins (McIntosh et al., 2012; Pavin and Tolić, 2016; Prosser and Pelletier, 2017). The principal job of the spindle is to segregate the chromosomes without mistakes. However, sometimes errors in chromosome segregation occur and they can cause aneuploidy, a state characterized by a wrong number of chromosomes in the cell. This type of aberration is found in almost all human cancers and is a common cause of miscarriages and genetic disorders, such as Down syndrome (Santaguida and Amon, 2015; Knouse et al., 2017; Webster and Schuh, 2017). Thus, studying the spindle is not only important as a basic research to understand how the spindle functions but also to better understand the causes of these serious diseases.

Spindles have been studied for more than 150 years (McIntosh and Hays, 2016). However, it is still not clear how the spindle orchestrates the forces that segregate the chromosomes. Why is this biologically crucial micro machine still not understood? Several thousands of studies on the spindle used various approaches from molecular and cell biology and genetics and have provided a list of hundreds of involved proteins, including tens of motor proteins that walk along microtubules, as well as a massive amount of experimental data on the spindle. But it is not always easy to make a coherent picture out of these data because the main players have complex interactions. Some proteins perform different functions at different locations. For example, the motor proteins Eg5 from the kinesin-5 family not only slide the microtubules apart in the spindle center but also bundle microtubules together at the spindle poles (Mann and Wadsworth, 2018b). An additional complication arises when different proteins perform the same function, for example, Eg5 and Kif15 from the kinesin-12 family both slide microtubules apart (Tanenbaum et al., 2009). These examples show why it is not easy to identify the role of each protein and how they interact together to make a functioning spindle. In this perspective article, we discuss the mechanobiology of the mitotic spindle mainly in mammalian cells, putting into context the methods to experimentally dissect the spindle and the advantages of combining them with theoretical approaches, with focus on microtubule-based forces that control chromosome movements and positioning.

STUDYING SPINDLE MECHANOBIOLOGY BY MECHANICAL PERTURBATIONS

The complexity of the mitotic spindle is motivating the development of a variety of approaches complementary to genetics and biochemistry. As the spindle is essentially a mechanical machine, the understanding of its functioning requires approaches based on mechanical perturbations. One of the most fruitful mechanical tools has been laser ablation, which allows for the cutting of a microtubule bundle and the direct identification of the direction of forces based on the movement of the microtubule fragments (Figure 2A). The rationale is that if the fragments moved toward each other, the microtubule was under compression before the cut (Figure 2A, left), whereas movement of the fragments apart was a signature of tension (Figure 2A, middle). If the microtubule fragments rotate, this implies that rotational forces were present before the cut (Figure 2A, right). The movement of the fragments can also be more complex, including a combination of linear and rotational movements. This reasoning holds for any material and was used to study forces acting on the kinetochore, a protein complex linking the chromosome with microtubules. In a pioneering study, one kinetochore was ablated by a laser, which resulted in the movement of the sister kinetochore toward the associated pole, demonstrating inter-kinetochore tension (McNeill and Berns, 1981). Since then, laser ablation has been used in numerous works that, for example, explored the mechanics of spindle microtubules, kinetochores, and centrosomes in mammalian cells (Aist et al., 1993; Skibbens

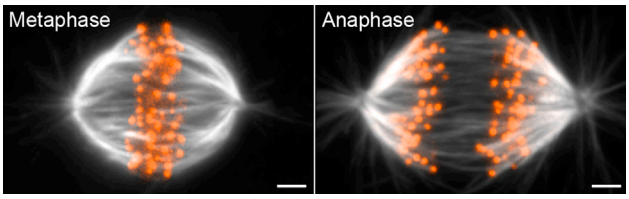

Figure 1. Mitotic Spindle in Metaphase (Left) and Anaphase (Right)
Spindles in human cells (U2OS) with a stable expression of CENP-A-GFP marking kinetochores (orange) and immunostained for tubulin with Alexa Fluor-594 (white). Scale bar represents 2 μm.

et al., 1995; Khodjakov and Rieder, 1996; Khodjakov et al., 2000; Maiato et al., 2004; Dick and Gerlich, 2013; Elting et al., 2014; Sikirzhytski et al., 2014; Cojoc et al., 2016; Kajtez et al., 2016; Vukušić et al., 2017).

Whereas laser ablation perturbs the existing forces in the spindle, other techniques allow for applying external forces to the spindle. In groundbreaking studies, Bruce Nicklas used a microneedle to stretch, move, or halt chromosomes to measure the forces acting on them (Figure 2B) (Nicklas and Staehly, 1967; Nicklas, 1983). External forces applied by a microneedle on the spindle (Takagi et al., 2019) or by squeezing the whole cell (Dumont and Mitchison, 2009a; Itabashi et al., 2012) were used to change the forces throughout the spindle to study its mechanical properties (Figure 2C). Optical and magnetic tweezers were used to displace the spindle, providing insight into the forces coming from the cell cortex to position the spindle (Figure 2D) (Tolić-Nørrelykke et al., 2005; Garzon-Coral et al., 2016).

These mechanobiology techniques are combined with light microscopy to see the structure that will be perturbed and the outcome of the experiment. The use of fluorescently labeled proteins helps to see the spindle architecture, including the localization of different proteins over time before and after the perturbation. Recent developments of super-resolution techniques open possibilities to explore a more detailed picture (Sahl et al., 2017). The gold standard for the most detailed spindle architecture, where single microtubules can be identified, is still electron microscopy, which unfortunately cannot be used on live cells. All these techniques based on microscopy and mechanical perturbations reveal different aspects of the spindle, working together toward a complete picture of spindle mechanobiology.

THEORETICAL MODELING HELPS TO IDENTIFY PRINCIPLES OF SPINDLE FUNCTIONING

Experiments based on mechanical perturbations typically provide movies that contain ample information about different aspects of the spindle dynamics. To identify the key concepts in spindle functioning, it is important to find patterns and propose hypotheses. In the first step, the hypotheses can be expressed by cartoon models. However, cartoon models cannot be tested because they are not quantitative. In such situations, theory is a great tool and is becoming increasingly used in the studies of the spindle and cell biology, in general. Designing a model allows us to formulate the hypotheses, whereas solving the model and verifying it experimentally allows us to identify the key players and concepts.

In general, a model is a simplified view of the real system, and thus, cannot describe all of its features, but it becomes a powerful tool when it describes the main aspects (Mogilner et al., 2006). A successful model should not only reproduce the behavior of the system and pass the experimental tests but also provide exciting predictions for what would happen in new situations, motivating new experiments that would otherwise not be an obvious choice. An exciting example of theoretical work in the context of spindle mechanics are studies that explored how kinetochores are captured by microtubules, which is an important part of the spindle assembly (Wollman et al., 2005; Paul et al., 2009; Magidson et al., 2015). The studies found that the prevailing search-and-capture mechanism (Kirschner and Mitchison, 1986) should be revised, as it requires a significantly longer time to capture all kinetochores than what are observed in cells. For example, the computational model predicted that enlarging the kinetochore can speed up the capture process substantially, which inspired new experiments and led to new concepts (Magidson et al., 2015). Similarly, a theoretical study that explored the formation of interpolar microtubule bundles (Nédélec, 2002), which is another important aspect of the spindle assembly, initiated a body of work on the spindle assembly and functioning based on simulations combined with experiments (Dinarina et al., 2009; Loughlin et al., 2010).

Things become even more interesting when theory is not used only within the limited scope of the scientific question that motivated the design of the theory, but rather when the same theory is used to see the bigger picture. One of the most fascinating examples of the power of theory comes from 19th century physics when James Clerk Maxwell developed the theory of electromagnetism, which he used to predict the existence of electromagnetic waves and their velocity. Surprisingly, this velocity was the same as the velocity of light, which led him to propose that light is an electromagnetic wave. We expect in the future important and surprising advances led by theory also in the studies of the spindle, especially because of the large complexity of the spindle.

FORCE- AND LENGTH-DEPENDENT MECHANISMS WITHIN THE METAPHASE SPINDLE

The spindle relies on self-regulating mechanisms for forces and length. Mechanical forces can speed up or slow down chemical reactions. Force affects microtubule polymerization, the speed of which depends on the difference in the rates of addition and removal of subunits. Compression force that arises when a growing microtubule tip encounters an obstacle decreases the rate of addition because the gap needed for the addition of a subunit, which appears as the tip position fluctuates and occurs less frequently, resulting in the slowdown of microtubule polymerization (Dogterom and Yurke, 1997). Similarly, microtubule depolymerization can generate force to move the cargo attached to the plus end (Lombillo et al., 1995; Grishchuk et al., 2005). Force acting on the microtubule plus end changes microtubule dynamics and the detachment rate of the cargo, such as the kinetochore (Akiyoshi et al., 2010). In contrast to most chemical bonds, which show increased dissociation under force (Kramers, 1940; Howard,

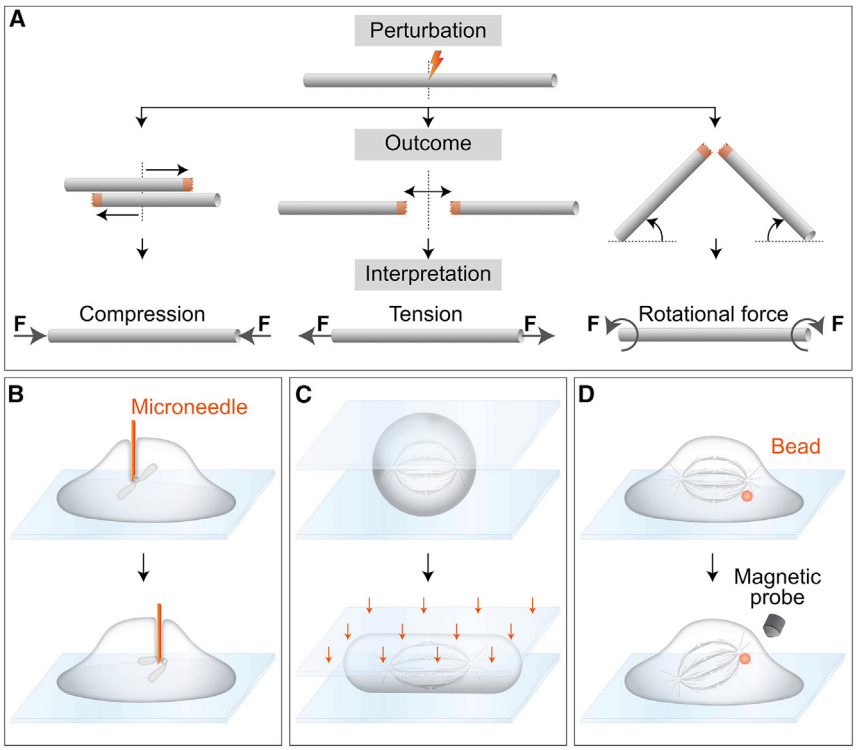

Figure 2. Experimental Tools Based on Mechanical Perturbations to Study the Mechanobiology of the Spindle

(A) Laser cutting (bolt sign) of a microtubule (gray rod) is used as a perturbation (top row), which can result in three different outcomes (middle row) with corresponding interpretations of forces acting on the microtubule (bottom row). Microtubule ends that are created by laser cutting are marked in orange.

(B) Microneedle tip (orange) contacts a chromosome and moves it rightwards.

(C) A cell underneath the coverslip flattens when compression force (orange arrows) is applied.

(D) Magnetic bead (orange) associated with the spindle moves the spindle when magnetic force is exerted on it.

In all figures, please see text for details and references.

2009), tension stabilizes kinetochore attachment to depolymerizing microtubules (Figure 3A) (Akiyoshi et al., 2010), resembling a catch-bond mechanism (Sarangapani and Asbury, 2014).

Length of the microtubules in the spindle also needs to be regulated. One of the key mechanisms is based on the length-dependent accumulation of motor proteins at the microtubule end, where they regulate microtubule dynamics (Figure 3B). Motors, kinesin-8 in particular, bind along the entire microtubule, and thus, a longer microtubule collects more motors. As these motors walk processively and reach the plus end without detachment, they accumulate at this end, which is known as the antenna model (Varga et al., 2006, 2009). Once at the end, these motors induce microtubule catastrophe (Tischer et al., 2009), limiting the microtubule length. This mechanism promotes the positioning of kinetochores at the spindle center (Stumpff et al., 2008; Klemm et al., 2018).

Similar to the length of individual microtubules, the overlap length of antiparallel microtubules in the spindle midzone is regulated by kinesin-4, a microtubule depolymerase recruited by the crosslinker PRC1 in an overlap length-dependent manner, as suggested by *in vitro* experiments (Bieling et al., 2010). Within the overlap, Ase1/PRC1 prevents the microtubules from sliding apart completely and thereby stabilizes the overlap presumably by entropic forces generated by Ase1/PRC1 molecules diffusing within the overlap (Braun et al., 2011; Lansky et al., 2015). In addition to diffusing crosslinkers, antiparallel overlaps contain motor proteins that slide the microtubules with respect to each other. The forces generated by these motor proteins typically depend on the overlap length. For example, *in vitro* work has shown that kinesin-5 within an antiparallel overlap generates a higher force when the overlap is longer (Shimamoto et al., 2015). In summary, regulation of length together with forces determines how the spindle self-organizes and ultimately segregates chromosomes (Dumont and Mitchison, 2009b; Pavin and Tolić, 2016; Oriola et al., 2018).

FORCES ACTING ON CHROMOSOMES IN METAPHASE

The life of a spindle starts with its formation by interactions between microtubules and chromosomes mediated by the microtubule-associated proteins and the kinetochore proteins during prometaphase and ends with chromosome segregation in anaphase, followed by spindle disassembly in telophase. To gain a complete insight into spindle functioning, it is important to understand all the dynamic phases of its life. As mitosis belongs to the most complex processes in the cell, studying only one phase is already a big challenge. The most studied phase is metaphase because the spindle is in a steady state, which makes responses to perturbations simpler to interpret than in the dynamic prometaphase and anaphase.

Metaphase is defined by the eye-catching chromosome alignment near the equatorial plane of the spindle. One of the key questions about the metaphase spindle is what forces act on kinetochores to keep them close to the spindle equator. Kinetochores are attached to the plus ends of the microtubules forming a kinetochore fiber, which pulls the kinetochore toward the pole, as demonstrated by laser ablation of the kinetochore (Figure 4, point 1) (McNeill and Berns, 1981). At the kinetochore, microtubules polymerize, which was discovered by microinjecting biotinylated tubulin into mitotic cells to identify the sites of microtubule growth in the spindle (Figure 4, point 2) (Mitchison et al., 1986). Kinetochore microtubules continuously translocate toward the pole in a process known as poleward flux, visible as the poleward movement of the photoactivated microtubule segments (Figure 4, point 3) (Mitchison, 1989). This movement together with a constant spindle length during metaphase implies that the kinetochore microtubules simultaneously polymerize at the kinetochore and depolymerize at the spindle pole (Figure 4, main drawing).

The pulling forces on kinetochores are opposed by the elastic forces of the stretched chromatin (Pickett-Heaps et al., 1982), a

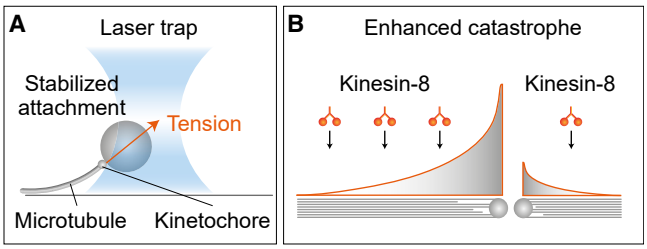

Figure 3. Force- and Length-Dependent Microtubule Dynamics
(A) The effect of force on microtubule dynamics can be studied by optical tweezers. Tensile force exerted by the laser trap is applied to the kinetochore at the microtubule tip, resulting in stabilized kinetochore-microtubule attachment.
(B) Kinesin-8 motors (orange) bind along the microtubules and accumulate at their tip, where they enhance microtubule catastrophe. Longer microtubules collect more motors at the tip (compare profiles highlighted in orange) and thus more likely undergo catastrophe.

finding supported by measurements showing that bioriented kinetochores are further apart than nonattached ones (Figure 4, point 4) (Waters et al., 1996). At the same time, chromatin is under polar ejection forces, which are exerted by microtubules growing from the pole and pushing chromosome arms away from the pole, which is transmitted to the kinetochore (Figure 4, point 5) (Rieder et al., 1986).

These mechanobiology experiments led to a picture where the chromosome movements on the spindle are driven by pulling forces exerted by kinetochore microtubules that pull the kinetochore poleward and polar ejection forces exerted by non-kinetochore microtubules that push the chromosome arms away from the pole (Rieder and Salmon, 1994). To explore the consequences of these concepts, computational models have been introduced. An early work based on simulations suggested that a combination of these forces can explain chromosome congression and their behavior during metaphase (Khodjakov et al., 1999), which was later explored in elegant computational models that describe the force balance on the chromosome (Joglekar and Hunt, 2002; Civelekoglu-Scholey et al., 2006). The models show that these forces can explain the general behavior of chromosomes during metaphase. In particular, the models predict that kinetochores move back and forth around the equatorial plane. The predicted kinetochore oscillations are similar to those observed in cells (Civelekoglu-Scholey et al., 2013), supporting the model.

FORCES ACTING ON KINETOCHORE FIBER MINUS END

To understand the forces acting on the kinetochore fiber and thus on the chromosome, it is important to know the molecular and biophysical events that occur not only on the plus end of the kinetochore fiber but also on its minus end. The minus ends of kinetochore fibers are mainly localized at the spindle pole (McDonald et al., 1992). They are focused at the pole by the minus-end-directed motors dynein and HSET/kinesin-14 (Endow et al., 1994; Merdes et al., 1996, 2000; Goshima et al., 2005; Kleylein-Sohn et al., 2012) and are continuously depolymerized by Kif2a/kinesin-13 (Rogers et al., 2004; Ganem et al., 2005). Based on these findings obtained by the classical methods of protein depletion or inactivation in combination with microscopy, it was suggested that these motors pull the kinetochore fiber poleward.

Kinetochore fibers do not interact only with the kinetochore and the spindle pole but are embedded in a dense network of spindle microtubules. Understanding the interactions that the kinetochore fiber makes with other spindle structures is thus crucial to gain a complete picture of spindle mechanics, and is a new direction in the field that is currently being intensely explored (Vladimirou et al., 2013; Kajtez et al., 2016; Elting et al., 2017).

To study the forces arising through various interactions that a kinetochore fiber makes with neighboring structures, laser cutting has been the most informative approach because it uncouples the kinetochore fiber from the rest of the spindle. When a kinetochore fiber is cut, the stub that remains attached to the kinetochore has a newly created minus end, which is stable and does not depolymerize, thus allowing for studies of the stub movement to assess the forces acting on it (Figure 5A). The minus end of the stub remains free for some time but is eventually pulled toward the pole and reincorporated into the spindle (Maiato et al., 2004). These pulling forces are generated by dynein that accumulates together with nuclear mitotic apparatus protein (NuMa) at the minus end of the stub and walks along the neighboring spindle microtubules toward the pole (Figure 5A, long time) (Elting et al., 2014; Sikirzhytski et al., 2014).

FORCES ARISING FROM THE INTERACTION BETWEEN THE BRIDGING AND KINETOCHORE FIBER

Laser cutting experiments of the kinetochore fiber led to the discovery of the mechanism of reincorporation, but the same experiments were used to study the forces in the spindle (Kajtez et al., 2016). In the short time between the cut of the kinetochore fiber and its reincorporation in the spindle, the stub shows an interesting behavior, where it rotates with the minus end moving away from the spindle (Figure 5A, short time). The rotation stops when the stub becomes aligned with the sister kinetochore fiber. This rotational movement of the stub led to a new hypothesis that rotational forces are present in the kinetochore fiber before the cut.

What is the origin of these rotational forces? A possible answer could be that the sister kinetochore fibers are connected not only by the soft chromatin but also more firmly by a flexible material that is bent due to rotational forces before the cut and straightens as these forces vanish after the cut. This flexible material could be a microtubule bundle, which was indeed observed in the region between sister kinetochores, and its movement together with the kinetochore fibers after the cut demonstrated their mechanical connection (Kajtez et al., 2016). This bundle is called as a bridging fiber because it acts as a bridge between sister kinetochore fibers. This finding is in agreement with the electron microscopy images showing that in the region between the pole and the vicinity of the kinetochore, numerous microtubules intermingle and form a single thick bundle, which separates into two bundles—the kinetochore fiber that ends at the kinetochore and the bridging fiber. The bridging fiber passes the region of the sister kinetochores and contacts not only the sister kinetochore fiber but also the neighboring fibers (McDonald et al., 1992; Mastronarde et al., 1993; O'Toole et al., 2020). Based on the findings of the bridging fiber, a new picture of spindle mechanics

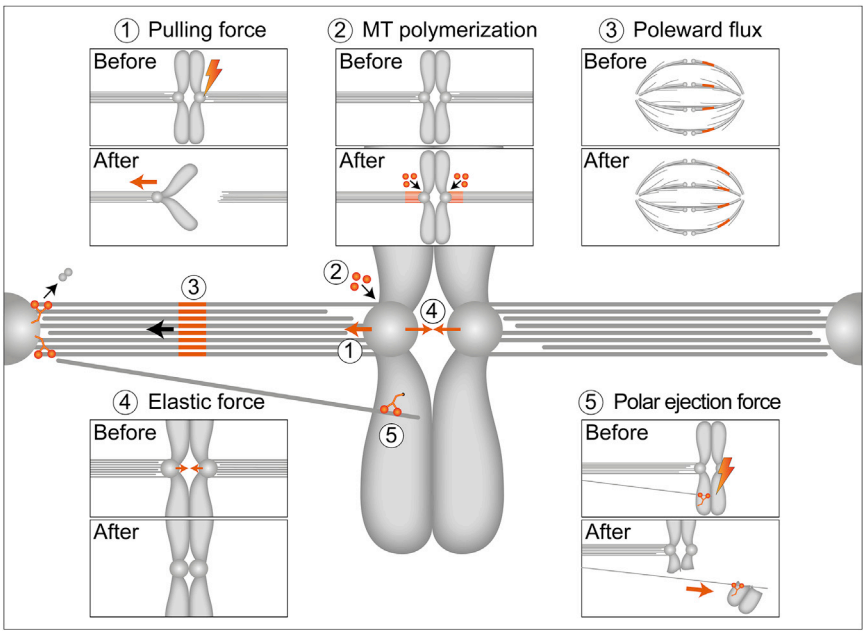

Figure 4. A Segment of a Metaphase Spindle with Associated Forces

Microtubules (gray lines) extend from the spindle poles (gray hemispheres) toward kinetochores (gray spheres) and chromosomes. Motor proteins (orange pictograms) bound to spindle poles and chromosomes interact with microtubules.

Inset 1, laser cut (bolt sign) of the right kinetochore results in the movement of the left kinetochore leftwards.

Inset 2, integration of labeled tubulin (orange spheres) at the microtubule tips reveals microtubule polymerization at the kinetochores.

Inset 3, labeled microtubule segments (orange lines) move toward the spindle pole, which is known as poleward flux.

Inset 4, elastic force in the chromatin (orange arrows) brings the kinetochores toward each other when pulling forces by microtubules are eliminated.

Inset 5, upon laser cut (bolt sign), a chromosome fragment moves away from the pole due to forces exerted by chromokinesins (orange pictogram), motor proteins attached to chromosome arms.

emerged, where the interaction between the bridging and kinetochore fibers implies new forces on the kinetochore fiber that are transmitted to the chromosome (Simunić and Tolić, 2016; Tolić and Pavin, 2016; Tolić, 2018).

Together with the prevailing picture of the system, theoretical models need to be revised to include the bridging fiber. In a revised model, sister kinetochore fibers interact with the bridging fiber (Kajtez et al., 2016), which is modeled as an interpolar bundle whose curved shape results from bending forces (Rubinstein et al., 2009). A model with a bridging fiber elucidates the intricate force balance in the kinetochore and bridging fiber (Kajtez et al., 2016). The bridging fiber is under compression, which balances the tension acting on kinetochores and within the neighboring region of the kinetochore fiber (Figure 5B). Interestingly, the pole-proximal part of the kinetochore fiber is also under compression, in contrast to the kinetochore-proximal part of the same fiber, which is under tension. This apparent paradox that tension and compression coexist along a single kinetochore fiber was identified and discussed previously (Dumont and Mitchison, 2009b), and the compression in the bridging fiber offers a simple solution.

The bridging fiber model was strengthened by testing the prediction that cutting of the kinetochore fiber at different locations will have a different effect on inter-kinetochore tension (Figure 5C). Indeed, experiments showed that the distance between sister kinetochores decreased to a larger extent when the cut was closer to the kinetochore (Kajtez et al., 2016; Milas and Tolić, 2016; Elting et al., 2017; Maiato et al., 2017). This result could not be explained by a classical view of spindle mechanics but supports the bridging fiber model.

Sophisticated experiments in which a kinetochore fiber was pulled by a microneedle showed that sister kinetochore fibers are strongly linked and do not pivot around the kinetochore region but rather around the pole (Figure 5D) (Suresh et al., 2020). These findings suggest reinforcements near kinetochores, consistent with a bridging fiber. After extensive pulling, kinetochore fiber grows by polymerization at the kinetochore (Long et al., 2020).

ROTATIONAL FORCES AND THE TWISTED SPINDLE

In the bridging fiber model, surprising findings appear when the model is used to describe a three-dimensional architecture of the whole spindle. In this case, rotational forces can have an arbitrary direction (Figure 6A, bending + twisting) rather than acting within a plane (Figure 6A, bending). This model predicts that the microtubule bundles in the spindle extend in three dimensions, having a twisted shape rather than lying in a plane, like meridians on the Earth (Figure 6B) (Novak et al., 2018).

Motivated by this unusual prediction, experiments showed that the bridging fibers indeed display a twisted shape (Novak et al., 2018). The twisted shape is visible as the rotation of bridging fibers around the spindle axis when the spindle is observed along the axis. The fibers show a left-handed twist, making the whole spindle a chiral structure (Figure 6B). Twist was observed also in rod-shaped spindles in yeasts, where individual microtubules within the bundle twist around each other (Ding et al., 1993; Winey et al., 1995). Recent 3D reconstructions of the microtubule organization in spindles of human cells show an occasional twist of microtubules within a bundle (O'Toole et al., 2020). It will be interesting to explore the twist of microtubules within bundles and of entire bundles in spindles of different species.

The twisted shapes of microtubule bundles are most likely generated by motor proteins, given that motors exert rotational forces on the microtubule in addition to linear forces. *In vitro* studies have shown that the mitotic motors kinesin-14 (Ncd) (Walker et al., 1990; Nitzsche et al., 2016; Mitra et al., 2020), kinesin-5 (Eg5) (Yajima et al., 2008), kinesin-8 (Kip3) (Bormuth et al., 2012; Bugiel et al., 2015; Mitra et al., 2018), and

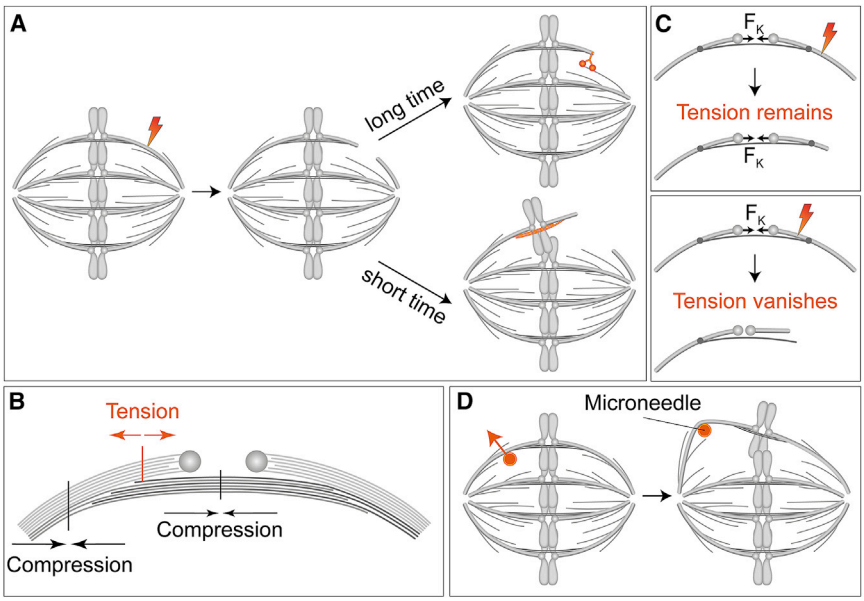

Figure 5. Forces Acting on Kinetochore and Bridging Fiber

(A) Movement of the microtubule stub created by a laser cut (orange bolt), was studied on long and short timescales. On a long timescale, motor proteins (orange pictogram) accumulate at the newly created minus end of the microtubule stub and pull on it. On a short timescale, elasticity within the bridging fiber (orange line) straightens it and drives the rotational movement of the microtubule stub.

(B) Tension in the kinetochore fiber (orange arrows) is balanced by compression in the bridging fiber (gray arrows, middle). Kinetochore microtubules (light gray lines) are bound to kinetochores (gray spheres) and interact laterally with bridging microtubules (dark gray lines).

(C) Upon laser cutting far away from the kinetochore, the long stub of the kinetochore fiber that remains after the cut stays connected to the bridging fiber, which withstands the inter-kinetochore tension, thus the kinetochores keep their distance (top). Upon laser cutting close to the kinetochore, the kinetochore fiber becomes disconnected from the bridge and the tension is released, which leads to a decrease of the distance between the kinetochores (bottom).

(D) Pulling of a kinetochore fiber by a microneedle (orange circle) rotates the kinetochore fiber around the spindle pole, but sister kinetochore fibers remain aligned in the region close to the kinetochores.

cytoplasmic dynein (Can et al., 2014), can generate rotational forces on the microtubule by stepping sideways while moving along the microtubule. In the spindle, the twisted shape of the bundles depends on kinesin-5 (Novak et al., 2018) and likely also on other motors, which may generate rotational forces in the overlap zone of antiparallel microtubules and at the spindle pole. In the overlap zone, motors may twist the antiparallel microtubules around each other, while the motors attached to the pole may rotate the microtubules as they walk along them (Tolić et al., 2019). To explore these hypotheses, new experiments and theoretical models are needed with the aim to understand how rotational forces are generated and balanced in the spindle. Moreover, the biological function of the spindle chirality is currently unknown. The findings of rotational forces on the scale of individual motor proteins and the entire spindle open an exciting new area of research on the mechanisms and the biological roles of rotational forces in mitosis.

THE ROLE OF SLIDING FORCES IN THE BRIDGING FIBER

The mechanical role of the bridging fiber is not only restricted to the metaphase but also prominent in the anaphase (Figure 7). The anaphase appears to be very dynamic and the relative movements of the main constituents of the spindle occur: sister kinetochores move with respect to kinetochore fibers, kinetochores fibers move with respect to the poles, and microtubules in the overlap region move with respect to each other (Gorbsky et al., 1987; Saxton and McIntosh, 1987; Zhai et al., 1995). These coherent movements drive separation of sister kinetochores and spindle elongation, processes that are crucial for chromosome segregation (Scholey et al., 2016; Asbury, 2017; Vukušić et al., 2019b).

The role of bridging fibers in kinetochore separation was shown by laser cutting experiments, which revealed that a single unit consisting of two sister kinetochore fibers and their bridge is able to separate the kinetochores, even when they are not connected to the spindle pole (Vukušić et al., 2017). The antiparallel microtubules in the bridging fiber slide apart, and thus, the bridging fiber is under compression as in metaphase. These sliding forces push the attached kinetochore fibers apart due to strong crosslinks between the bridging and kinetochore fibers, separating sister kinetochores and pushing the spindle poles apart (Vukušić et al., 2017). While the kinetochores move apart, the overlap region of the bridging microtubules shrinks (Figure 7) (Pamula et al., 2019). The kinetochores keep moving with the same velocity after exiting the overlap region, suggesting that even a short overlap is sufficient to properly separate the kinetochores. An alternative model based on the inside-out pushing of central spindle microtubules against the chromosomes has been proposed for chromosome segregation in *Caenorhabditis elegans* (Dumont et al., 2010; Nahaboo et al., 2015; Laband et al., 2017), which has also been suggested to be relevant for human cells (Yu et al., 2019).

What roles sliding forces play within the bridging fiber in the metaphase remains an open question. Sliding in the bridge may drive poleward flux of the bridging microtubules and also of kinetochore microtubules due to lateral attachments between the bridging and kinetochore microtubules of the same orientation, as in the anaphase. Interestingly, the same motors that elongate the spindle in the anaphase, kinesin-4 and kinesin-5 (Vukusic et al., 2019a), are involved in driving poleward flux in the metaphase (Steblyanko et al., 2020). Kinesin-4 is localized on chromosome arms, where it exerts polar ejection forces, which contribute to the flux (Steblyanko et al., 2020). However,

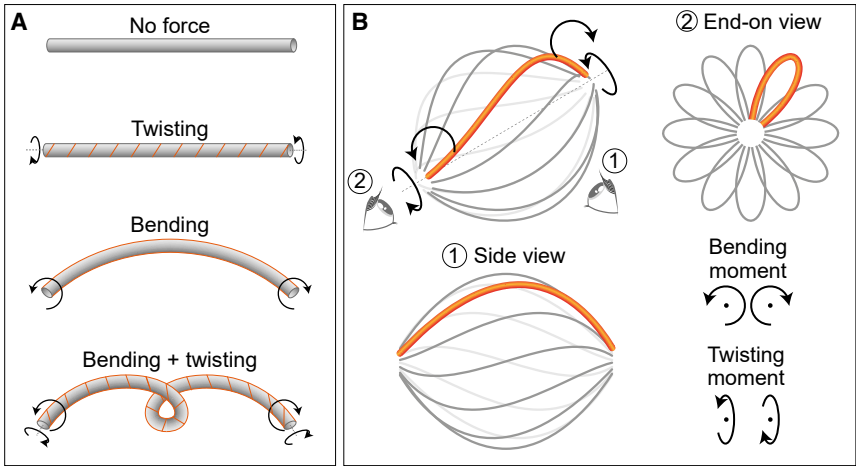

Figure 6. Bending and Twisting by Rotational Forces and Spindle Chirality
(A) Elastic rod (gray) is straight in a relaxed state. It becomes twisted (orange line) when the twisting moment is applied, bent when bending is applied, and attains a complex shape when both moments, bending and twisting, are applied.
(B) Three projections of a simplified spindle showing its chiral structure. A view of a spindle from an arbitrary angle (top, left), together with eye signs marking the view angle for the side view (1) and the end-on view (2). Interpolar microtubule bundles are depicted by gray lines and one bundle is highlighted in orange. Bending and twisting moments (curved arrows) acting on the bundle generate the observed chiral shapes.

kinesin-4 is also localized in the bridging fiber during the metaphase (Jagrić et al., 2019), as well as kinesin-5 (Kajtez et al., 2016; Mann and Wadsworth, 2018a); thus, it will be interesting to explore the role of these motors in the generation of sliding forces within the bridging fiber.

Forces arising from the interaction between the kinetochore fibers and the bridging fibers may be important for kinetochore positioning at the spindle equator. Optogenetic experiments showed that the bridging fiber improves kinetochore alignment (Jagrić et al., 2019). We speculate that if a kinetochore pair is displaced toward one pole, the overlap between the kinetochore fiber on the other side and the bridging fiber is longer and thus contains more motors, which generate larger force that pulls the kinetochores toward the spindle center. Thus, the bridge may help to keep the kinetochores in the center by sliding and length-dependent forces on kinetochore fibers.

Sliding forces are generated in the central part of the spindle. However, to understand the spindle mechanobiology, it is also important to understand the forces in the polar regions. Electron microscopy and tomography have shown that minus ends of microtubules are not only found at the pole but also along the microtubules throughout the spindle (Mastronarde et al., 1993; O'Toole et al., 2020). It will be interesting to explore how the dynamics of these minus ends are regulated, which together with the sliding determines the spindle length and keeps it constant during the metaphase.

CONCLUSION AND OUTLOOK

The largest steps in the understanding of spindle mechanobiology have been driven by applying new tools based on mechanical perturbations, in pioneering as well as most recent works. This trend will surely continue, and combinations of the existing tools will also be important. For example, performing laser ablation or microneedle experiments on a super-resolution microscope will reveal the dynamics and interactions between separate microtubule bundles or even individual microtubules, which cannot be distinguished by confocal microscopy.

Spindle mechanobiology uses force and length as the language to tell its story because the regulation of these two players defines how the spindle self-organizes and performs its function. Thus, the cell needs to sense them in order to control them. Moreover, the cell uses force and length sensing to modulate different biological functions, such as progression through mitosis. Many force and length regulation mechanisms are known, but due to their complexity, a lot remains to be learned. Here, theory is a great tool to synthesize diverse ideas into a bigger coherent picture.

The tools and concepts discussed here are relevant not only for the understanding of the mechanobiology of a well-functioning spindle but also for situations where the spindle makes errors in chromosome segregation. As such errors are characteristics of several serious diseases, revealing their mechanical origins and the molecular players involved is of general interest because of potential medical applications.

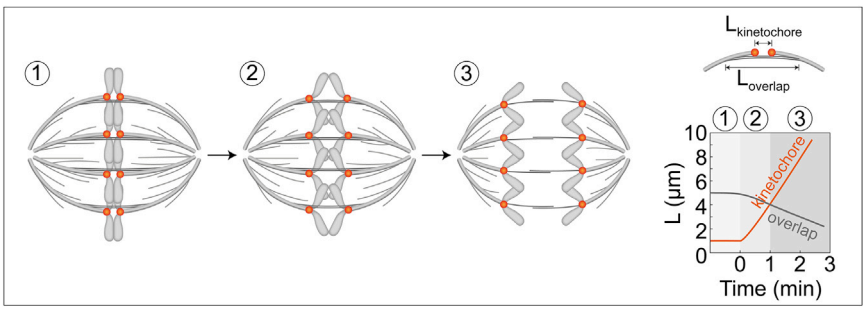

Figure 7. Chromosome Movements and Bridging Microtubule Overlap during Anaphase
Mitotic spindle during late metaphase (1), early anaphase (2), and mid-anaphase (3). The distance between kinetochores (orange circles) increases in time, whereas the antiparallel overlap region of bridging microtubules shrinks. Typical time course for kinetochore distance and length of the overlap is plotted in the graph.

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