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Review

Biomechanics of chromosome alignment at the spindle midplane

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SUMMARY

During metaphase, chromosomes are aligned in a lineup at the equatorial plane of the spindle to ensure synchronous poleward movement of chromatids in anaphase and proper nuclear reformation at the end of mitosis. Chromosome alignment relies on microtubules, several types of motor protein and numerous other microtubule-associated and regulatory proteins. Because of the multitude of players involved, the mechanisms of chromosome alignment are still under debate. Here, we discuss the current models of alignment based on poleward pulling forces exerted onto sister kinetochores by kinetochore microtubules, which show length-dependent dynamics and undergo poleward flux, and polar ejection forces that push the chromosome arms away from the pole. We link these models with the recent ideas based on mechanical coupling between bridging and kinetochore microtubules, where sliding of bridging microtubules promotes overlap length-dependent sliding of kinetochore fibers and thus the alignment of sister kinetochores at the spindle equator. Finally, we discuss theoretical models of forces acting on chromosomes during metaphase.

Introduction

Essential to the reliable inheritance of the genome is the mitotic spindle, which drives physical separation of a complete set of chromosomes into two equal parts destined to the two daughter cells. This self-assembled mechanical micro-machine generates forces that are precisely regulated in space and time to move the chromosomes[1]. The spindle is made of microtubules and numerous microtubule-associated proteins (MAPs)[2,3]. Microtubules of the spindle can be divided into three main groups based on their position and function: kinetochore microtubules bind the kinetochore, a protein complex at the centromere of each chromosome, and form a kinetochore fiber (k-fiber). Overlap microtubules grow from the opposite spindle halves and overlap in the middle. In numerous cell types and organisms, they link sister k-fibers like a bridge, which is why they are called bridging fibers[4,5]. Astral microtubules extend from the spindle pole towards the cell periphery and contact the cell cortex.

Before chromosome segregation in anaphase, chromosomes are neatly aligned at the spindle equator (Figure 1A,B), undertaking different paths to get there[6–10]. This process, termed chromosome congression, is coupled closely in time with chromosome biorientation, the formation of stable attachments of sister kinetochores to microtubules that emanate from the opposite spindle poles (Figure 1C). Proper kinetochore–microtubule attachments are monitored by the spindle assembly checkpoint and required for correct chromosome segregation[11,12]. Similarly, the alignment of chromosomes at the spindle equator is important for mitotic fidelity because it promotes synchronous anaphase poleward movement of chromatids and proper telophase nuclear reformation[13,14] (Figure 1D).

Central to chromosome positioning within the spindle are microtubules, polar polymeric structures whose plus ends are more dynamic and undergo dynamic instability characterized by persistent periods of growth and shrinkage[15,16]. *In vitro* experiments have demonstrated that growing or shrinking microtubules can generate pushing or pulling forces, respectively[17,18], suggesting that these forces drive movements within cells including chromosome positioning on the spindle[19].

More than 100 proteins are involved in chromosome alignment and for many of them the mechanisms are not known[6]. Thus, chromosome alignment is a complex process that is still not fully understood. In this review, we present the prevailing view and recently introduced concepts of how the forces that align the chromosomes are generated and regulated. We discuss how the alignment is achieved through the regulation of the dynamics of microtubule plus ends at kinetochores and minus ends at the spindle pole, through polar ejection forces arising through interactions between spindle microtubules and chromosome arms and through mechanical coupling between kinetochore and bridging microtubules.

Physical mechanisms that can center chromosomes

In order to position something in the center of an object, such as the spindle, there needs to be a mechanism that measures length. How can the spindle measure length to position the chromosomes in its midplane? There are three classes of mechanisms that sense spindle length, based on microtubule length-dependent pushing forces, pulling forces and microtubule dynamics.

Microtubule length-dependent pushing forces

The idea that chromosomes experience pushing forces within the spindle has a long history. More than 80 years ago, Darlington hypothesized that chromosomes move towards the spindle equator because they are repelled by the poles due to electric charges[20]. Darlington's initial idea about the existence of

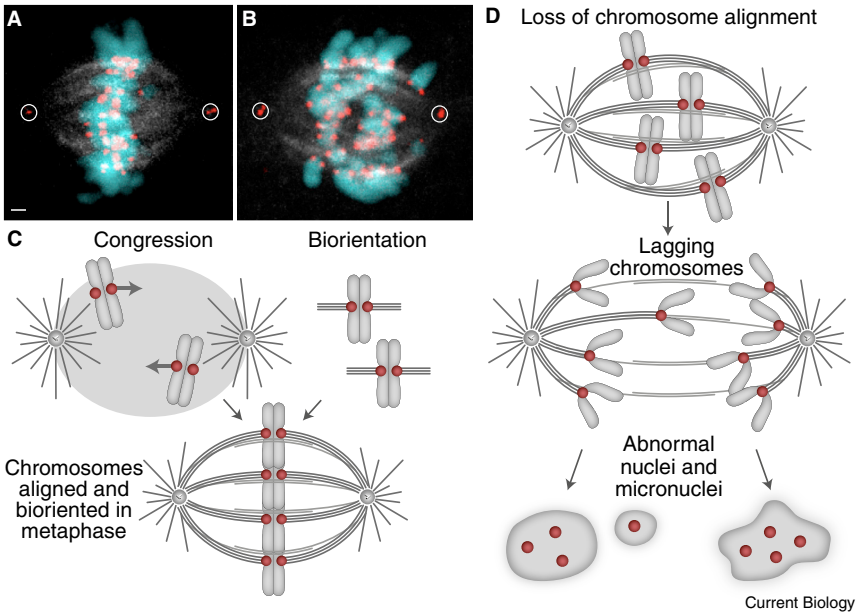

Figure 1. Chromosome alignment and its biological relevance.
(A) Spindle in a human retinal pigment epithelial 1 (RPE1) cell with aligned chromosomes (cyan) at the equatorial plane during metaphase. Kinetochores and centrin are shown in red and PRC1-labeled bridging fibers in grey. White circles indicate centrosomes. Scale bar, 1 μm. (B) Example of a spindle with misaligned chromosomes due to co-depletion of Kif18A/kinesin-8 and Kif4A/kinesin-4; legend as in (A). (C) During spindle assembly chromosomes congress to the spindle equator and become bioriented, meaning that their kinetochores (red) are attached to microtubules (grey lines) extending from the opposite spindle poles (grey spheres). (D) Loss of chromosome alignment leads to asynchronous poleward movements of chromatids in anaphase and impaired nuclear formation in telophase. For all figures, please find a more detailed discussion and references in the text.

repulsive forces and their ability to help center the chromosomes on the spindle was right, though the origin of forces was later shown to be mechanical and microtubule-dependent[21].

Microtubule pushing forces depend on the distance from the centrosome for three reasons: first, the density of microtubules that are nucleated at the centrosome is high close to the centrosome, and many of them reach a unit area situated close to the centrosome, producing a high pushing force (Figure 2A). Far away from the pole, microtubules are rare and few of them reach large distances, generating a smaller pushing force per unit area. In mathematical terms, microtubule density decreases with the distance from the centrosome, d, as $1/d^2$ for an ideal isotropic aster[22] (Figure 2A). Note that an isotropic distribution is a mathematical idealization of a microtubule aster, whereas in spindles of human cells microtubules are nucleated not only at the centrosome but also along existing microtubules by the augmin complex, leading to a weaker reduction of microtubule density with distance than for centrosomal nucleation alone[23].

Second, the length distribution of microtubules is roughly exponential, with many short microtubules and few long ones (Figure 2A). Such a distribution is a consequence of microtubule dynamic instability[24,25] and has been observed in electron tomography images of spindles in *Caenorhabditis elegans* embryos[26]. The exponential length distribution amplifies the microtubule density effect described above, resulting in an even larger difference in the number of microtubules reaching shorter and longer distances.

The third effect is based on microtubule buckling (Figure 2A). If the pushing force exerted by a growing microtubule exceeds a critical force, the microtubule buckles under its own compression. The critical force for buckling, also called the 'Euler force', depends on microtubule length, L, as $1/L^2$ (Figure 2A)[27]. Therefore, a shorter microtubule has a larger Euler force and thus produces a stronger push than a long one.

When all three effects are put together, a chromosome that is displaced towards one spindle pole has more microtubules extending from the nearer than the farther pole, pushing it away. Moreover, the Euler force of the microtubules extending from the nearer pole is higher. Due to the higher number of microtubules and their higher force, the chromosome will be pushed away from the nearer pole towards the spindle center (Figure 2A).

Microtubule length-dependent pulling forces

In contrast to the microtubule pushing forces within the spindle, which are widely accepted to depend on microtubule length and the distance from the centrosome, the length-dependence of the pulling forces is controversial. In pioneering work on spindle forces, Ostergren proposed that a longer traction fiber of a displaced chromosome generates a stronger pulling force toward the more remote pole, causing the stabilization of chromosome positioning at the equatorial plate[28]. However, at that time microtubules had not yet been discovered, and these concepts were not developed further.

Ostergren's hypothesis was tested three decades later by elegant experiments in which multivalent chromosomes with three or four kinetochore fibers were created by γ-irradiation in grasshopper spermatocytes[29]. These chromosomes shifted closer to the pole to which the greater number of kinetochore fibers were attached, and the analysis of the stable asymmetric positions of the chromosomes supported Ostergren's hypothesis that the magnitude of poleward force along a kinetochore fiber is proportional to the length of the fiber. Similarly, analysis of chromosome positions following partial reduction of kinetochore microtubule number confirmed the length-dependence of the poleward force[30].

The discovery of the dynamic instability of microtubules led to the idea that the events on the plus and minus ends of kinetochore microtubules regulate the pulling forces on the kinetochore[31,32]. Thus, the field shifted its focus towards the forces generated at microtubule ends, whereas pulling forces exerted along the length of the microtubule were largely neglected.

Kinetochore microtubules are not isolated within the spindle, but are laterally attached to non-kinetochore microtubules[33,34]. Motor proteins may bind within the overlaps of kinetochore and non-kinetochore microtubules and longer overlaps

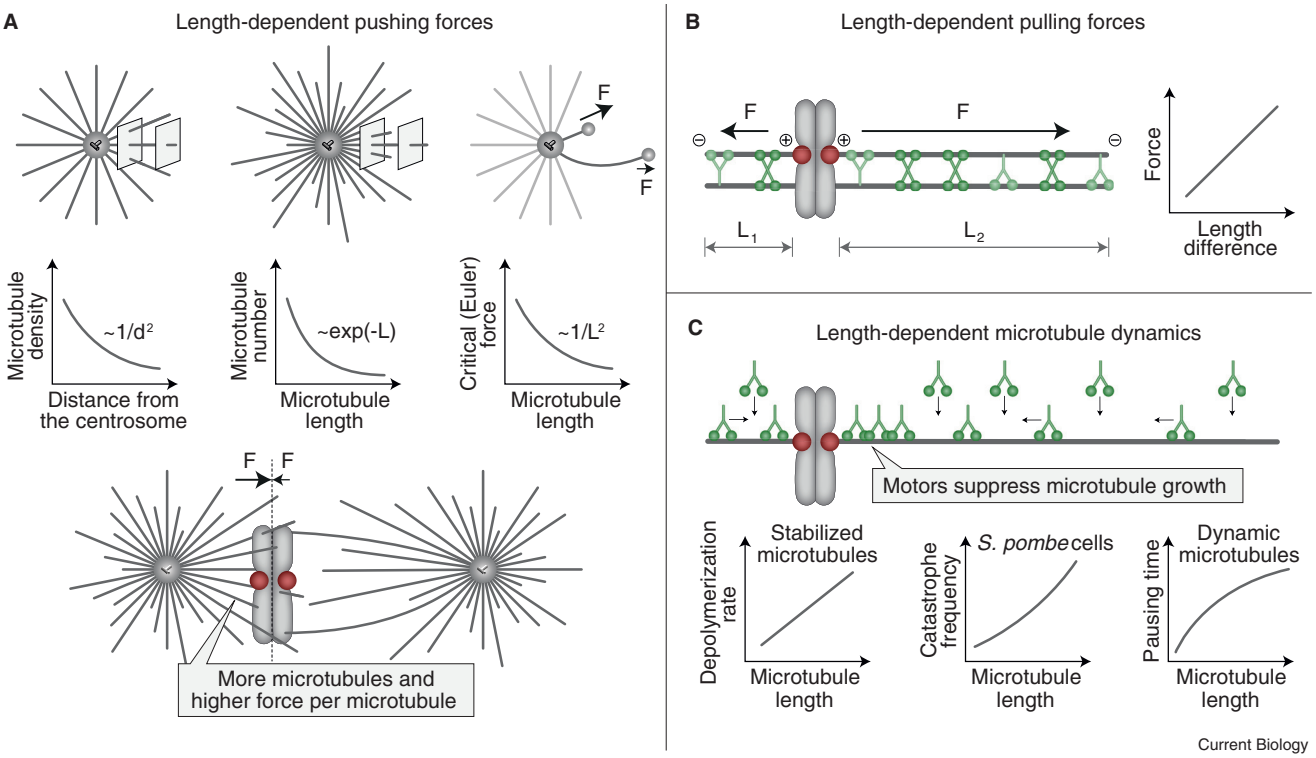

Figure 2. Principles of length measurements within the spindle.
(A) Pushing forces exerted by growing microtubules decrease with an increasing distance from the centrosome because microtubule density decreases due to aster geometry (left aster and graph), a roughly exponential distribution of microtubule lengths due to microtubule dynamics (middle aster and graph), and a decreasing critical (Euler) force, *F*, at which the microtubule buckles (right aster and graph). A displaced chromosome is contacted by more microtubules from the nearer pole, and they can exert a higher force per microtubule than the long ones extending from the other spindle half, resulting in a net force towards the spindle center (bottom). (B) Pulling forces, *F*, exerted by motor proteins (tetrameric, dark green and/or dimeric, light green) attached along the k-fibers depend on the length of the overlap between the k-fiber and non-kinetochore microtubules, *L*. The net force is proportional to the difference in the overlap length on either side (graph at the right). (C) Motor proteins (green) that walk towards the microtubule plus end with a low detachment rate accumulate there in a microtubule length-dependent manner. If these motors are modulators of microtubule dynamics, then microtubule depolymerization rate, catastrophe frequency, or the fraction of time that the microtubule spends in a pausing state depend on the microtubule length (graphs).

accumulate more motors, consequently exerting larger forces (Figure 2B). The total force on the chromosome is then directed towards the spindle center and proportional to the difference in the length of the overlap on either side (Figure 2B). Such a centering mechanism was recently proposed for spindles in human cells[35].

Microtubule length-dependent regulation of microtubule dynamics

The pulling force generated by the depolymerizing plus end of a microtubule does not depend on microtubule length and thus cannot center the chromosome, but some motor proteins can 'measure' microtubule length and make microtubule dynamics length-dependent (Figure 2C). Such length-dependent mechanisms are achieved by the motors that bind along the microtubule lattice and walk all the way to the microtubule plus end. Thus, the longer the microtubule, the more motors accumulate at its plus end. This effect, known as the 'antenna model', has been shown for kinesin-8[36–38] and kinesin-4[39]. For the antenna model to be functional, the motors must be highly processive, i.e. walk for a large distance along the microtubule without detachment, and must walk faster than the microtubule grows in order to reach the plus end. If the motors showing this behavior are regulators of microtubule dynamics, then the dynamics will be regulated in a length-dependent manner. For example, due to the kinesin-8 Kip3 from budding yeast, long, stabilized microtubules *in vitro* depolymerize faster than short microtubules (Figure 2C)[36]. Similarly, in the fission yeast *Schizosaccharomyces pombe*, the dynamics of the longest microtubule in an interphase bundle is regulated in a length-dependent manner, although a different feature is affected, namely the catastrophe rate of longer microtubules is higher than that of shorter ones (Figure 2C)[40]. Finally, *in vitro*, dynamic microtubules become less dynamic and spend more time in a pausing state when the human kinesin-8 Kif18A accumulates at their plus end[41,42] (Figure 2C).

Molecular mechanisms that generate and regulate pulling forces on kinetochores

Kinetochore microtubules pull on kinetochores

Back in the 1980s, laser ablation experiments on prometaphase or metaphase mitotic spindles revealed the existence of poleward pulling force on chromosomes exerted by k-fibers. Ablation of one of the two sister kinetochores led to the movement of the whole chromosome towards the spindle pole to which the

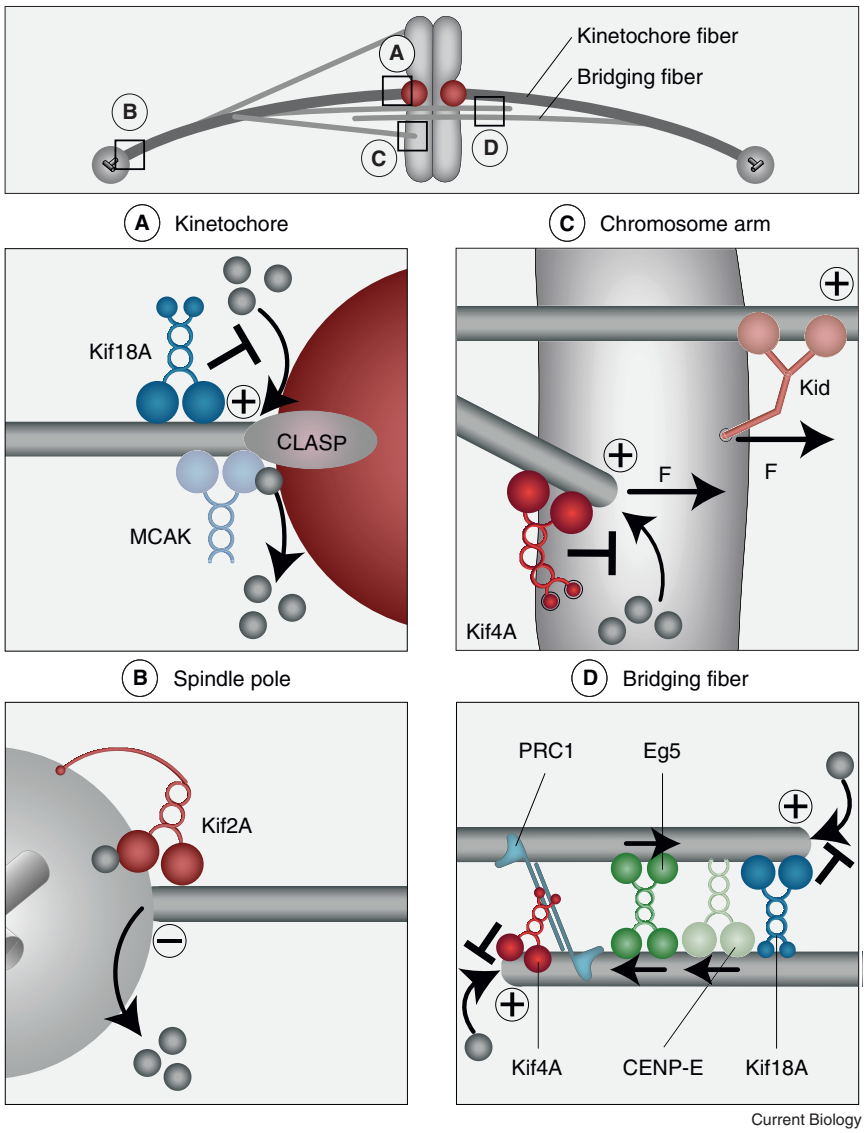

Figure 3. Molecular players involved in kinetochore alignment.
A chromosome with k-fibers (dark grey) and non-kinetochore microtubules (bridging microtubules overlapping in the middle and other microtubules interacting with chromosome arms, all in light grey) is sketched at the top with boxes marking the enlarged areas shown below. (A) At the kinetochore, Kif18A suppresses microtubule dynamics, CLASP promotes microtubule polymerization and MCAK promotes depolymerization. (B) At the spindle pole, Kif2A promotes microtubule depolymerization. (C) At the chromosome arm, chromokinesins generate polar ejection forces. Kid moves the chromosome along the microtubule and Kif4A suppresses microtubule dynamics. Growing microtubules also generate polar ejection forces as they push into the chromosome. (D) Within the bridging fiber, Eg5 and CENP-E slide the antiparallel microtubules apart. Kif18A and Kif4A, which interacts with the crosslinker PRC1, suppress the dynamics of microtubule plus ends, thereby controlling the length of antiparallel overlaps.

non-ablated kinetochore was oriented[43]. Similarly, ablation of chromosome arms of mono-oriented chromosomes, in which only one sister kinetochore is attached to microtubules emanating from the spindle pole, caused the kinetochores to move towards the attached spindle pole[21]. Ablation of the region between sister kinetochores resulted in the movement of each kinetochore towards the pole it was attached to[44]. These experiments have demonstrated that k-fibers exert pulling forces on kinetochores.

Mitotic chromosomes are elastic and upon biorientation their centromeres become stretched by kinetochore microtubules pulling on both sister kinetochores[45,46]. This pulling generates opposing forces that tend to return the centromere to its non-stretched configuration. In line with this, the inter-kinetochore distance between bi-oriented sister kinetochores is larger than that of mono-oriented kinetochores, suggesting increased tension when both sister kinetochores are attached to opposing spindle poles[47].

Even though attached to kinetochores, kinetochore microtubule plus-ends remain dynamic, yet with slower tubulin turnover compared to non-kinetochore microtubules[48]. Due to the mechanical coupling between kinetochore microtubules and kinetochores, dynamic instability of kinetochore microtubules contributes to the oscillatory motion of the kinetochores along the spindle axis[49,50]. These abrupt changes between poleward and anti-poleward movement of kinetochores, termed 'directional instability', are less prominent during chromosome congression in prometaphase when chromosomes have directional persistence towards the spindle equator due to differences in the durations but not in the velocities of movements towards and away from the equator[49]. Once aligned close to the equator, sister kinetochores move in a coordinated manner within a narrow region in the central part of the spindle.

Molecular mechanisms that regulate the dynamics and length of kinetochore microtubules

How are the length-measurement mechanisms and the forces that control chromosome alignment implemented in cells at the molecular level? Motor proteins can make microtubule dynamics length dependent, which has a centering effect on chromosomes, the principles of which are described above (Figure 2C). This centering mechanism works well due to the precise regulation of microtubule dynamics achieved by a large number of motor proteins and other microtubule-associated proteins.

Microtubule plus ends, responsible for pulling forces on kinetochores, are a hub for multiple microtubule regulators (Figure 3A). One of the most important regulators is kinesin-8, which promotes microtubule catastrophe in budding yeast[51],

increases catastrophe frequency in fission yeast[40,52] and promotes microtubule destabilization in *Drosophila*[53]. Similar activity was observed for the human homolog Kif18A[38], although more recent studies indicate that it suppresses microtubule dynamics rather than induces microtubule depolymerization[41,42]. Accordingly, depletion of Kif18A results in increased spindle length and loss of inter-kinetochore tension[38,54,55]. With its role at kinetochore microtubule plus ends, kinesin-8 is required for proper mitotic chromosome movement and alignment[38,41,53,54,56–61]. Quantitative tracking of kinetochore positioning upon Kif18A depletion indicated that Kif18A limits kinetochore movements around the spindle equator by affecting the frequency of kinetochore directional switches and by decreasing the velocity of kinetochore movements[54], although another study reported the opposite effect on kinetochore velocity, possibly due to a lower time resolution of imaging[38].

Several plus end-tracking proteins have been implicated in kinetochore alignment. MCAK/Kif2C/Kinesin-13 is a microtubule depolymerase that localizes on centromeres and kinetochores and is thus a strong candidate for force generation involved in chromosome movements and positioning[62,63] (Figure 3A). MCAK is a motor which diffuses along the microtubule lattice without directional bias, i.e. it targets and destabilizes both microtubule ends *in vitro*[64]. Depletion of MCAK leads to chromosome alignment defects, decrease of chromosome oscillation speed and directional coordination between sister kinetochores, without affecting the period of oscillations[65–67]. Based on these results and the observation that MCAK preferentially accumulates on the leading sister kinetochore — the one moving towards its associated pole — it was suggested that MCAK sets the velocity of chromosome oscillations together with Kif18A. In this model, MCAK depolymerizes microtubules within the k-fiber of the leading kinetochore, whereas Kif18A suppresses dynamics at the trailing kinetochore — the one moving away from its associated pole — thereby providing resistance to sister pair movement[65,67].

Upon microtubule attachment, cytoplasmic linker-associated proteins (CLASPs) remain localized at the kinetochore–microtubule interface[68–70] (Figure 3A). With their redundant roles in promoting microtubule rescue and suppressing microtubule catastrophe, without affecting the overall microtubule polymerization rate, CLASPs act as microtubule stabilizers[71,72]. By stabilizing kinetochore microtubules, CLASPs increase tension on kinetochores, and decrease both oscillations and microtubule growth[69]. Thus, CLASPs help keep the kinetochores in tight alignment at the spindle equator.

Contrary to the highly dynamic plus ends that interact with kinetochores, minus ends are mostly anchored at the microtubule nucleation sites, i.e. centrosomes or microtubule lattice in the case of augmin-dependent microtubule nucleation[73]. The key player involved in the minus end dynamics is Kif2A/kinesin-13, which depolymerizes microtubules[74,75] (Figure 3B), though other proteins are likely also involved as Kif2A antibody injection does not completely eliminate depolymerization[76].

Dynamics at the microtubule ends underlie a process termed 'poleward flux', defined as a continuous translocation of tubulin subunits in the direction of the minus end[77]. Even though the molecular mechanisms responsible for this process are not yet fully elucidated, two main models have been suggested. One model proposes that flux is driven by kinesin-13-mediated depolymerization at spindle poles with simultaneous CLASP-mediated polymerization at kinetochore microtubule plus-ends[75,78,79]. A different model explains the origin of poleward flux as a response to sliding of antiparallel interpolar microtubules, which is transmitted to kinetochore microtubules due to their coupling mediated by different crosslinking molecules[80,81]. Recently, it was proposed that poleward flux is driven by Kif4A/kinesin-4 on chromosome arms, and that the distribution of poleward flux across the spindle is achieved by coupling of non-kinetochore and kinetochore microtubules[82]. Microtubule flux has been implicated in regulation of spindle length, correction of erroneous kinetochore–microtubule attachments and equalization of forces at kinetochores prior to segregation[13,74,75].

Polar ejection forces act on chromosome arms

The existence of polar ejection forces, generated by microtubules that push the chromosomes away from the pole, was first demonstrated by laser ablation of chromosome arms on chromosomes in monopolar and bipolar spindles, which resulted in transport of the created acentric chromosome fragments away from the pole[21,50]. Polar ejection forces originate from interactions between non-kinetochore microtubules and chromosome arms, with anti-poleward forces being generated by microtubule polymerization against chromosome arms, or by activity of chromokinesins, proteins that bind to both microtubules and chromosomes[21,83–86] (Figure 3C). It was shown that polar ejection forces exerted by individual microtubules on metaphase chromosomes are consistent with forces generated by polymerizing microtubules pushing against chromosomes or by individual kinesin motors[87]. However, chromokinesins contribute to polar ejection forces to a larger extent than the pushing forces of polymerizing microtubules, given that a larger fraction of acentric chromosome fragments is able to congress to the spindle equator when chromokinesins are present[88].

Among chromokinesins, generation of polar ejection forces primarily depends on Kid/kinesin-10 activity to move chromosomes toward the microtubule plus ends, a conclusion based on the experiments showing that Kid is involved in chromosome alignment[89,90], chromosome oscillations, and chromosome arm orientation[91,92]. By directly suppressing dynamics of microtubule plus ends, chromokinesin Kif4A/kinesin-4 independently contributes to polar ejection force modulation[41,91,92] (Figure 3C).

Within the spindle, polar ejection forces depend on the surface area of chromosome arms available for interaction with microtubules, as laser ablation of a larger portion of a chromosome arm allows the kinetochore-containing chromosome fragment to move further away from the equator, evident in the increase of its oscillation amplitude[93]. Similarly, stronger polar ejection forces acting on peripheral chromosomes due to their large size in comparison with central chromosomes were proposed to cause the more extensive oscillations of central versus peripheral chromosomes[76].

Furthermore, polar ejection forces were hypothesized to depend on microtubule density, meaning that polar ejection forces should increase towards the spindle pole due to an increase in microtubule density (Figure 2A). The precise spatial distribution of polar ejection forces across the spindle was determined experimentally based on the relationship between

reduction in chromosome size after laser ablation and increased oscillation amplitude, yielding a force map in which polar ejection forces increase most rapidly near the equator and flatten towards the poles[93]. These experiments led to a model in which polar ejection forces limit the extent of oscillations by exerting tension on the leading kinetochore during its movement away from the equator, thus inducing microtubule rescue and chromosome reversal[93]. Accordingly, elevated polar ejection forces achieved by overexpression of Kid stabilized synthetic kinetochore–microtubule attachments, whereby both sister kinetochores are attached to microtubules from the same spindle pole, through higher tension exerted on kinetochores and by preventing chromosomes from moving closer to the poles where error correction takes place[94]. Altogether, by operating in concert with the mechanisms of length-dependent modulation of microtubule dynamics, polar ejection forces contribute to the positioning of chromosomes at the spindle equator by promoting reversal in their movement as the chromosomes approach the pole.

Forces originating from mechanical coupling of k-fibers and bridging fibers

Initial electron microscopy studies of the mitotic spindle in PtK1 cells and grasshopper cells proposed that interpolar microtubules contribute to the structural integrity of the spindle and provide mechanical support for the forces exerted on chromosomes[95–97]. Indeed, recent findings show that sister k-fibers are physically linked with an antiparallel interpolar microtubule bundle, termed the bridging fiber[5,33]. These fibers have been observed also in electron microscopy images of human cells[34,98,99]. Electron tomography reconstructions of spindles in human RPE1 cells revealed that the minus ends of bridging microtubules are typically found near the wall of a kinetochore microtubule[34]. In the vicinity of the kinetochore, the bridging fiber consists of 10–15 microtubules and lies next to the k-fiber, which also consists of 10–15 microtubules. As the bridging microtubules pass the kinetochores, roughly half of them interact with the sister k-fiber while others fan out and comingle with nearby k-fibers. Most bridging microtubules from one side of the spindle also interact with those extending from the other side[34].

Bridging microtubules are mainly nucleated in an augmin-dependent manner[100], in agreement with the localization of their minus ends along kinetochore microtubules. Crosslinking of parallel overlap regions between bridging and kinetochore microtubules is mediated by NuMa[35,101], whereas anti-parallel overlaps within bridging fibers are linked together by the protein regulator of cytokinesis 1 (PRC1). Several motor proteins that slide microtubules or regulate microtubule dynamics are also found within the bridging fiber (Figure 3D), including Eg5[33,102], CENP-E[82], Kif4A, Kif18A, and MKLP1[103]. Eg5 is likely the main microtubule slider as its inactivation during metaphase results in spindle shortening and collapse[104]. The reduced poleward flux velocity of the bridging microtubules observed after CENP-E depletion suggests that this motor also has a role in sliding of bridging microtubules[35]. The overlap length of the antiparallel overlaps within the bridging fiber is regulated by Kif4A and Kif18A[103].

By spanning the gap and acting as a bridge between sister k-fibers, bridging fibers balance the tensile forces at kinetochores[33] and restrict extensive stretching of the centromere[105]. This mechanical support for k-fibers extends up to ~2 μm laterally from each sister kinetochore[33,105] and is defined as an overlap region selectively marked by the microtubule crosslinker PRC1[33,106].

As PRC1-labeled bridging fibers show one-to-one association with a pair of sister k-fibers[106], this could give rise to flux-dependent equalization of tension at kinetochores[13] and a closed-loop force network independent of centrosomes[107]. Indeed, it was shown that bridging microtubules slide apart and serve as a platform for force generation that underlies microtubule poleward flux[35]. Upon loss of k-fibers in the spindle, bridging fibers undergo similar rates of poleward flux, suggesting that bridging fiber flux is independent of k-fibers. Interestingly, poleward flux of k-fibers is slower than that of bridging fibers, indicating that the coupling between bridging and k-fibers is not rigid but allows for sliding. This sliding opens a new perspective on the physical mechanisms of chromosome positioning, where forces are generated within the overlaps between bridging and k-fibers. Such forces belong to the class of length-dependent pulling forces, which have a centering effect as described above (Figure 2B).

The typical amplitude of chromosome oscillations in human cells is about 1.2 μm[54], which lies within the PRC1-labeled overlap region. Interestingly, upon acute PRC1 removal by an optogenetic approach, kinetochores are found to extrude out of the narrow region in the central part of the spindle, suggesting that bridging fibers have a role in buffering chromosome movements within this region. As PRC1 removal results in elongated overlaps of antiparallel microtubules, this suggests that chromosome centering is achieved by overlap length-dependent forces transmitted to the associated k-fibers[103].

To explain this, imagine a bioriented chromosome positioned away from the spindle equator (Figure 4A). The kinetochore facing the nearer pole has a shorter k-fiber than its sister that faces the farther pole, implying a shorter and longer overlap with the bridging fiber, respectively. As the length of the overlap determines the strength of the coupling, the friction force due to sliding of bridging microtubules is higher for longer overlaps. This leads to a higher poleward flux velocity of the longer k-fiber and directs the net force towards the spindle equator. This was corroborated by speckle microscopy experiments that revealed a difference in the poleward flux of longer and shorter k-fibers in human spindles[35].

Following the same rationale, shorter and longer k-fibers have shorter and longer antiparallel overlap with the bridging fiber, respectively (Figure 4B). Here, more motor proteins that slide the microtubules apart accumulate in the longer overlap, which leads to a higher force sliding the k-fiber along the bridging fiber. Thus, the net force on both k-fibers is directed towards the spindle equator. Experiments in which the bridging fiber overlap regions were elongated, thus also creating longer overlap regions with k-fibers and resulting in faster k-fiber flux, support this idea[35].

The centering efficiency depends on the relative asymmetry of the chromosome position within the overlap. This means that the same displacement of the chromosome implies a larger relative asymmetry and thus better centering when the overlap is short in comparison with longer overlaps. Accordingly, spindles in treatments which result in longer overlap regions of bridging fiber,

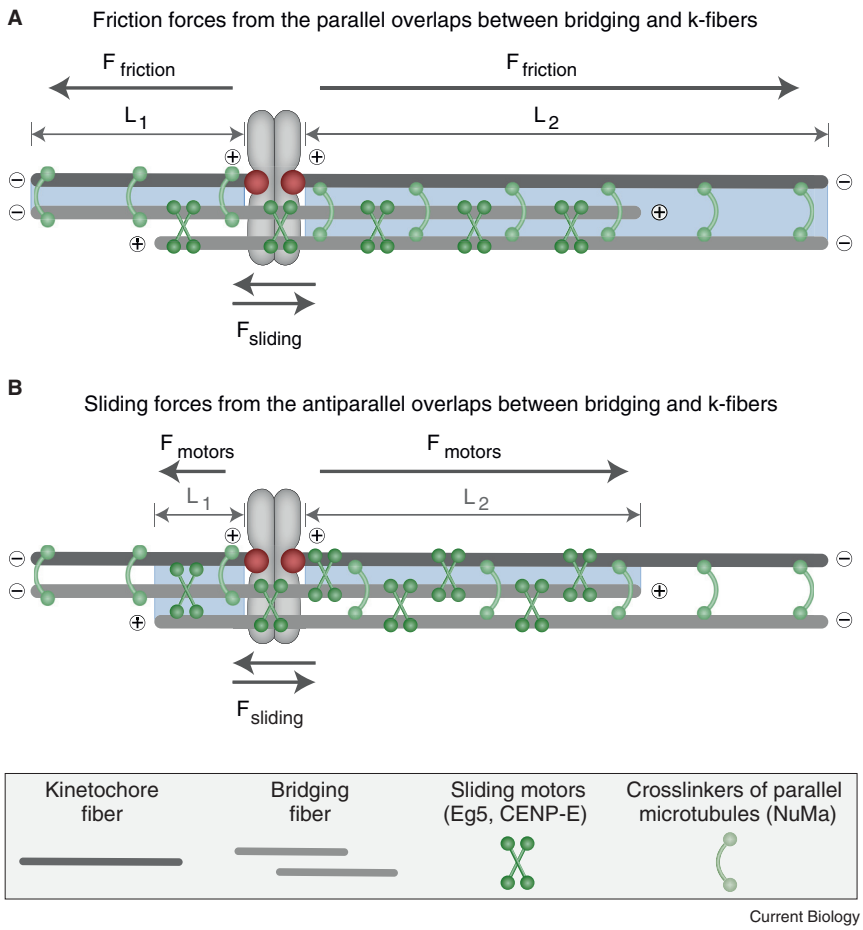

Figure 4. Forces arising from mechanical coupling of k-fibers and bridging fibers center the chromosomes.
(A) A displaced chromosome has a longer overlap between the k-fiber extending towards the distal pole and the parallel bridging microtubules coming from the same pole, L_2, than the k-fiber extending towards the proximal pole and the bridging microtubules on that side, L_1. The friction force, $F_{friction}$, is generated by the sliding force of bridging microtubules, $F_{sliding}$, that is transmitted to the k-fibers in the overlaps between parallel bridging and kinetochore microtubules (areas shaded in blue) crosslinked by NuMa (green C-shaped pictograms). These friction forces are larger for longer overlaps, leading to a net force towards the spindle center. (B) A displaced chromosome also has a longer antiparallel overlap between the k-fiber extending towards the distal pole and the bridging microtubules coming from the opposite pole, L_2, than the k-fiber extending towards the proximal pole and the bridging microtubules from the opposite side, L_1. The sliding force generated by motors (green X-shaped pictograms) within these overlaps (areas shaded in blue), F_{motors}, is larger for longer overlaps, leading to a net force towards the spindle center.

such as depletion of Kif18A, exhibit chromosome misalignment[35,103].

Comparison of mechanisms for chromosome alignment in yeast and mammalian spindles

With their plus ends, 10–20 microtubules attach to kinetochores and make up k-fibers in human cells[34,108]. Unlike human spindles, in which there are only few direct connections between spindle poles and kinetochores[34], spindles in budding yeast contain a single and in fission yeast around three kinetochore microtubules which originate at the spindle pole body and directly attach to kinetochores[109–111]. Furthermore, microtubules that overlap in the central part of the spindle also emanate from the spindle pole body in lower eukaryotes[109,110]. This is not the case in human spindles, where the majority of overlapping microtubule minus ends are incorporated in the k-fiber lattice, mediated by augmin-dependent nucleation[34,112], which is not observed in yeasts.

Yeast spindles do not show poleward flux[113] (Figure 5A), which may be related to the fact that fission yeast lacks kinesin-13[114], though this kinesin family is present in budding yeast[115]. Yeasts also lack chromokinesins responsible for polar ejection forces[114]. Thus, chromosome positioning in yeasts is exclusively dependent on microtubule polymerization and depolymerization at kinetochores[113,116]. When a pair of kinetochores is displaced towards one spindle pole, it is crucial that the longer kinetochore microtubules undergo catastrophe and start to shrink, to bring the kinetochores back to the spindle center (Figure 5A). Catastrophe in yeasts is mainly regulated by kinesin-8 that accumulates on longer microtubules, as described above, thereby preventing excessive growth of trailing microtubules and excessive movements of kinetochores away from the spindle center.

In higher eukaryotes, beside microtubule dynamics at kinetochores, microtubules undergo poleward flux and depolymerization at the minus end close to the spindle pole[31,32] (Figure 5B). As spindle length is constant during metaphase, the plus ends at the kinetochores undergo net polymerization. However, analysis of speckles on k-fibers in PtK1 cells demonstrated that as the kinetochores oscillate around the spindle midplane, k-fibers go through periods of polymerization and depolymerization at the plus end[117]. In contrast, speckle measurements in RPE1 cells, which show ~40% faster flux than in PtK1 cells[118], revealed that kinetochore microtubule plus ends predominantly grow or pause, suggesting that microtubule depolymerization at the plus ends plays a minor role in kinetochore movements[35] (Figure 5B). It is, therefore, possible that depolymerization at the plus ends is more important for spindles with slow than fast flux. Furthermore, the difference in microtubule dynamics in yeast and human cells is in agreement with the observation that yeast kinesin-8 promotes depolymerization or catastrophe[36,40,51], whereas human kinesin-8 suppresses microtubule dynamics[41,42].

Theoretical models of chromosome alignment

Experiments on the metaphase spindle revealed which forces are most relevant for chromosome positioning at the metaphase

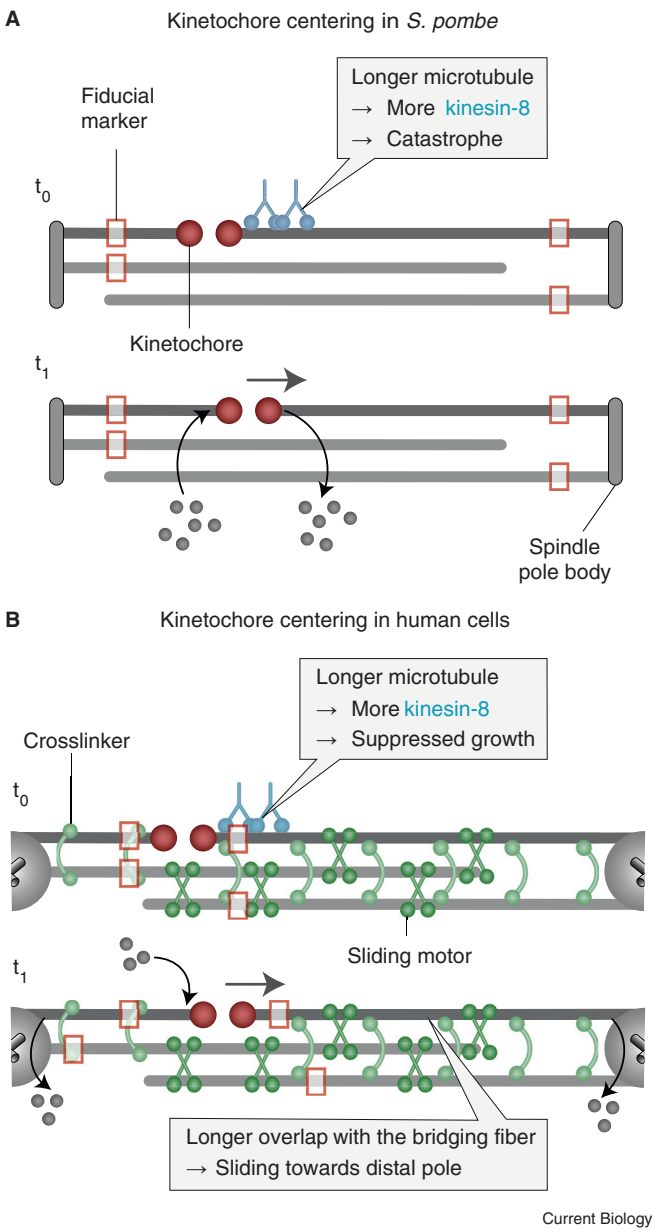

Figure 5. Mechanisms of kinetochore centering in yeast and human spindles.
(A) In fission yeast, spindle microtubules do not undergo poleward flux (red squares denote fiduciary marks on the microtubules). When kinetochores (red circles) move towards one pole, the longer kinetochore microtubules on the trailing side accumulate more kinesin-8 motors (blue), which induce their catastrophe and subsequent shrinkage, thereby bringing the kinetochores back towards the spindle center. (B) In human spindles, microtubules undergo poleward flux (note the movement of red squares between times t_0 and t_1). Motors that slide bridging microtubules (green X-shaped pictograms) together with microtubule depolymerization at the minus end drive the flux, which is transmitted to k-fibers via crosslinkers (NuMa, green C-shaped pictograms). As the crosslinkers allow for sliding between microtubules, k-fiber flux is generally slower than the bridging microtubule flux (note that the red squares on the k-fiber move less from time t_0 to t_1 than those on the bridging fiber). Because the longer k-fiber has a longer overlap with the bridging fiber, the force on the longer k-fiber is higher (Figure 4), resulting in a higher flux velocity (note that the red square on the right k-fiber moves more from time t_0 to t_1 than the one on the left k-fiber). Thus, the net movement of the k-fibers and kinetochores is towards the spindle center. In addition, kinesin-8 motors (blue) accumulate on the longer k-fiber and suppress its growth, promoting kinetochore centering.

plate. However, experiments alone are often not sufficient to explore different hypotheses and understand their consequences. Here, theoretical approaches are a useful tool[119].

To answer how a combination of the known forces can drive chromosome movement in higher eukaryotic cells, several theoretical models have been proposed. In an elegant computational model of the force balance on chromosomes, the major centering force is described by a phenomenological function that represents the polar ejection force[120]. This centering force, working together with microtubule dynamic instability, can explain the experimentally observed chromosome movement. In a computational model for chromosome movement in *Drosophila* embryos, the main forces are collective motor forces, microtubule polymerization and depolymerization, and a polar ejection force as the major centering force[121]. To explain a difference in the movement between central and peripheral chromosomes in PtK1 cells, polar ejection forces are described by introducing two phenomenological functions: a shallow function for polar ejection forces on central chromosomes and a steep function for peripheral chromosomes[76].

A novel centering mechanism that relies on the interaction between bridging microtubules and k-fibers was recently formulated by a 'flux-driven centering' model[35]. A key feature of this model is that motor proteins accumulate in the overlaps between k-fibers and bridging microtubules. As the number of motors is proportional to the overlap length, off-centered kinetochores have a different number of motors on the two k-fibers, which generates a centering force. In this model, kinetochores become centered only if the poleward flux of the bridging microtubules is faster than the flux of k-fiber, which was measured experimentally[35].

In lower eukaryotic cells, the major centering mechanism differs from those in higher eukaryotes. In early theoretical studies the major centering mechanism relies on a chemical gradient with a maximum in the middle of the spindle, which regulates microtubule catastrophe frequency[122,123]. Such spatially regulated microtubule catastrophe, together with force-dependent rescue, reproduces the observed chromosome movement. For chromosome congression, a mechanism which relies on length-dependent suppression of microtubule polymerization governed by kinesin-5 motors was proposed[124]. A centering mechanism that relies on length-dependent forces can also explain chromosome congression and chromosome movement. This was shown in a model with a phenomenological parameter in the force–velocity relationship, which depends on microtubule length[125]. A centering mechanism based on length-dependent microtubule catastrophe regulated by kinesin-8 motors can also explain the chromosome movements[126]. Finally, a model that describes the dynamics of kinesin-8 motors shows a length-dependent accumulation of these motors at growing microtubules, which promote microtubule catastrophe of longer microtubules, keeping kinetochores predominantly under tension and supporting their centering[60]. Taken together, the

studies describing yeast spindles have shown that length-dependent regulation of kinetochore microtubule dynamics by kinesin-8 motors is crucial for kinetochore centering. Experiments have shown that kinesin-8 plays an important role also in human spindles[35,38,41,54], and we expect that future theoretical studies will show how the activity of this motor works together with other mechanisms to position the kinetochores at the spindle midplane.

Conclusions and outlook

Chromosome alignment at the metaphase plate is the most eye-catching image of mitosis, with biological relevance for proper chromosome segregation and nuclear reformation. Yet, the mechanisms driving alignment are still under debate due to the large number of players and processes involved. We have discussed the generally accepted mechanisms based on the regulation of microtubule plus end dynamics by motors, notably kinesin-8, which can 'measure' microtubule length and suppress excessive microtubule growth and thus also the excessive kinetochore movements away from the spindle midplane. Polar ejection forces arising through interactions between spindle microtubules and the chromosome arms also promote chromosome alignment because they are highest close to the pole and decay towards the equator.

A recently introduced concept based on mechanical coupling between kinetochore and bridging microtubules provides a new perspective on chromosome alignment. In this model, bridging microtubules slide apart and this sliding is transmitted to kinetochore microtubules. The longer the overlaps between kinetochore and bridging microtubules, the larger the forces, resulting in a net force towards the spindle center. We propose that this mechanism based on length-dependent relative sliding of kinetochore along bridging microtubules works together with the length-dependent regulation of microtubule dynamics and polar ejection forces to ensure alignment of kinetochores at the equatorial plane of the spindle in metaphase. The centering mechanism based on sliding opens an attractive new avenue of research on the molecular players involved in the sliding of bridging fibers, regulation of their plus and minus ends, and their coupling with k-fibers. It is tempting to imagine that this mechanism also works in prometaphase during chromosome congression to promote chromosome movement from polar regions of the spindle towards the equator, which will be an exciting topic for future studies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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