Thirty years of search and capture: The complex simplicity of mitotic spindle assembly

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Cell division is enacted by a microtubule-based, self-assembling macromolecular machine known as the mitotic spindle. In 1986, Kirschner and Mitchison proposed that by undergoing dynamic cycles of growth and disassembly, microtubules search for chromosomes. Capture of microtubules by the kinetochores progressively connects chromosomes to the bipolar spindle. 30 years later, "search and capture" remains the cornerstone of spindle assembly. However, a variety of facilitating mechanisms such as regulation of microtubule dynamics by diffusible gradients, spatially selective motor activities, and adaptive changes in chromosome architecture have been discovered. We discuss how these mechanisms ensure that the spindle assembles rapidly and with a minimal number of errors.

The origin of "search and capture"

In his seminal review of the mitotic cycle, Daniel Mazia boldly stated: "Nothing we have learned about mitosis since it was discovered a century ago is as dazzling as the discovery itself" (Mazia, 1987). This statement reflected Mazia's (and the whole field's) frustration with the lack of mechanistic understanding of cell division. Although voluminous phenomenological data had been gathered on the mitotic apparatus (spindle), the principles governing its assembly remained elusive. Interestingly, Mazia's opus major was published merely a year after the original formulation of the "search and capture" (S&C) hypothesis (Kirschner and Mitchison, 1986). If Mazia had read this paper (it was not cited in his 1987 review), he might have changed his stance. Indeed, S&C offered the first plausible mechanism to drive spindle assembly in animal cells and signified the transition from descriptions to molecular investigations of the process.

The S&C hypothesis stems from the discovery of microtubule dynamic instability (Mitchison and Kirschner, 1984). In sharp contrast to other cytoskeletal filaments, the plus ends of a typical microtubule oscillate between periods of growth and shrinkage caused by the addition and loss of αβ-tubulin subunits. The frequency of shrinkage events increases dramatically when a cell enters mitosis, transforming the longer, more stable interphase microtubule cytoskeleton into two dynamic radial arrays nucleated by the duplicated centrosomes. During the growth phase, a microtubule tip moves over several micrometers, and its trajectory is likely to vary from one period of growth to another. This unique behavior inspired the discoverers of dynamic instability to propose that microtubules could "search" for a target positioned within their reach, which would eventually be hit by a growing microtubule (Fig. 1 A). If the target were capable of "capturing" and "capping" this microtubule, thereby suppressing its dynamics, such a hit would result in the formation of a stable connection between the target and the centrosome. In the context of spindle assembly, the proposed targets were kinetochores, the paired macromolecular assemblies residing at the centromere of each mitotic chromosome. The S&C mechanism would therefore progressively incorporate multiple individual chromosomes into a common bipolar microtubule array with microtubule minus ends converging on the centrosomes and plus ends directed toward the equator (Fig. 1 A).

Less than four years after the formulation of the hypothesis, microtubule capture by chromosomes was directly visualized in live cells (Hayden et al., 1990; Rieder and Alexander, 1990). These elegant studies established that the first step of a chromosome's incorporation into the spindle involves a direct contact between the kinetochore and a single microtubule (Fig. 1 B). This and similar observations were consistent with the concept of S&C. However, as is common in cell biology, S&C was born as an intuitive cartoon rather than a testable model. The question of whether dynamic instability constituted a searching behavior efficient enough to incorporate all of the chromosomes into a common spindle was not addressed until almost a decade after the original formulation of the hypothesis. The first theoretical evaluation suggested that dynamically unstable microtubules would in fact find a target much faster than conventional steadily growing filaments (Holy and Leibler, 1994). However, the absolute time required to find all 46 chromosomes in a human cell via completely unbiased stochastic S&C was calculated to be several times longer than the typical duration of mitosis (Wollman et al., 2005). Furthermore, the establishment of proper connections to microtubules would depend on how a chromosome is positioned within the nascent spindle (Fig. 1 C). These considerations imply the existence of

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Abbreviations used in this paper: CPC, chromosome passenger complex; S&C, search and capture.

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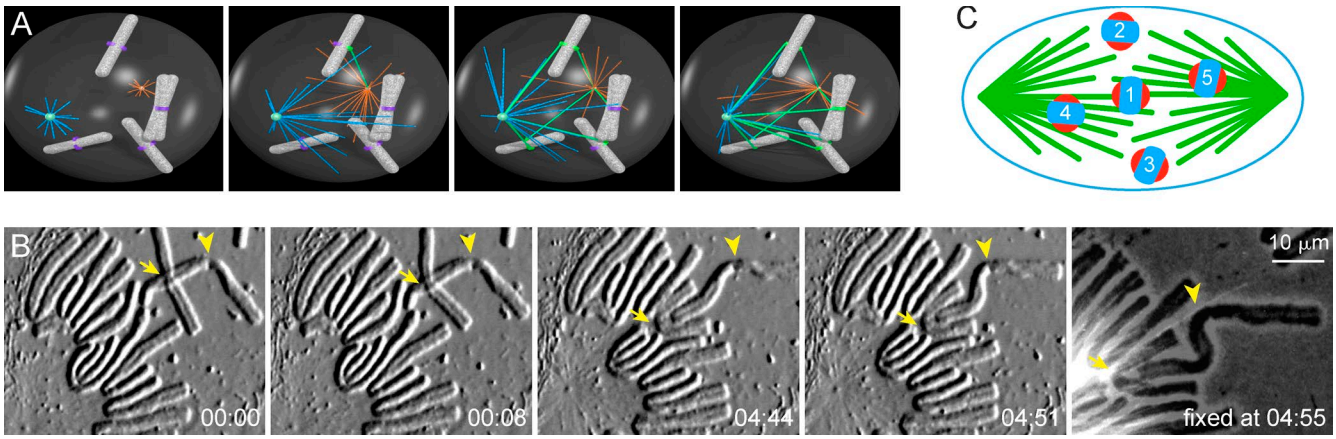

Figure 1. **Assembly of the mitotic spindle by microtubule S&C.** (A) Sequence of events envisioned in the classic formulation of the S&C hypothesis. Microtubules nucleated at the duplicated centrosomes form two radial arrays (blue and orange). Dynamically unstable microtubules explore space until a growing plus end encounters a kinetochore (magenta). This encounter results in the capture and partial stabilization of the microtubule (denoted by a color change of both the microtubule and kinetochore to green). Captured microtubules connect individual kinetochores to the centrosomes (spindle poles). In this scenario, each kinetochore ultimately becomes attached to microtubules, although the duration of spindle assembly varies significantly as a result of the stochastic nature of the process. (B) Direct visualization of microtubule capture by kinetochores in a newt lung cell (adapted with modifications from Rieder and Alexander, 1990). Because of the large cell size, individual chromosomes are often positioned in areas with a low density of microtubules and remain motionless for extended periods before suddenly moving poleward (arrows) at a velocity that often exceeds 30 μm/min. Immunofluorescence of the assembling spindle fixed immediately after initiation of the poleward movement reveals a kinetochore interacting with a single microtubule (arrowheads). Note that the initial contact is not to the plus end but to the wall of the microtubule (i.e., lateral interaction). Time is given in minutes:seconds. (C) The efficiency and fidelity of S&C depends on kinetochore orientation and the distance from centrosomes. Chromosomes positioned near the intersection of the spindle equator and spindle axis would have a reasonable chance of forming proper amphitelic attachments (1). Chromosomes located at the periphery of the spindle have reduced chances of capturing microtubules (2 and 3). In contrast, chromosomes positioned near a centrosome are exposed to a high density of unipolar microtubules (4 and 5), which promotes either erroneous attachment of both sister kinetochores to the same spindle pole (syntelic attachment; 4) or attachment of only one kinetochore (monotelic attachment; 5).

additional factors that facilitate spindle assembly, and a series of such mechanisms has been identified in recent years. A common theme emerging from these studies is that S&C depends on spatially selective biochemical pathways that promote the formation of microtubules in the vicinity of kinetochores and also differentially engage molecular motors to position chromosomes in the areas with maximal exposure to spindle microtubules. Furthermore, proper attachment of chromosomes to the spindle is assisted by orderly changes in the shape of the cell and the adaptive architecture of kinetochores. Together, these facilitating mechanisms ensure that stochastic encounters between microtubules and kinetochores result in a rapid yet low error incorporation of all chromosomes into the mitotic apparatus.

Guidance by gradients

In the original formulation of S&C, radial arrays of microtubules nucleated by the duplicated centrosomes were expected to be the sole source of spindle microtubules. However, the density of microtubule ends and therefore the probability of capture decreases rapidly as the distance between the centrosome and chromosomes increases, and a 200-nm small kinetochore has only a slight chance of being contacted by a microtubule originating from a centrosome positioned 15–20 μm away within the 10–15-min period typical of spindle assembly (Wollman et al., 2005). This problem can be overcome if the density of microtubules near kinetochores is increased, and multiple mechanisms have been identified that selectively promote microtubule nucleation and stabilize microtubule plus ends near the chromosomes, leading to the formation of kinetochore-attached microtubule bundles termed kinetochore fibers (K-fibers).

The role of chromosome arms in mitosis was once compared with that of a "corpse at a funeral"; the DNA comprising the bulk of the genome provides the reason for the proceedings, but does not actively participate in the event (Mazia, 1961). However, this view was challenged by the demonstration that viral DNA injected into a frog egg, or plasmid DNA-coated beads incubated in metaphase egg extract, induce the formation of spindle-like structures in the absence of centrosomes or kinetochores (Karsenti et al., 1984; Heald et al., 1996). It is now understood that mitotic chromatin exudes "perfume" in the form of biochemical gradients that promote spindle microtubule assembly.

The most prominent gradient is of RanGTP (discussed extensively in Forbes et al., 2015). RanGTP is produced by the guanine nucleotide exchange factor for Ran, regulator of chromosome condensation 1 (RCC1), which ubiquitously decorates chromatin. In contrast, Ran's GTPase-activating factor (RanGAP) is cytoplasmic. The opposing activities of RCC1 and RanGAP result in a steep gradient of RanGTP centered on chromosomes (Kalab et al., 2002, 2006). Similar to its function in nucleocytoplasmic transport, RanGTP releases cargoes from nuclear transport receptors called importins (Gruss et al., 2001; Nachury et al., 2001; Wilde et al., 2001). Among these cargoes are many proteins that function in microtubule polymerization and organization, such as TPX2 (Gruss and Vernos, 2004), the spindle microtubule cross-linking kinesin XCTK2, which requires a RanGTP gradient for proper localization and motility (Weaver et al., 2015), and components of the nonspecific lethal (NSL) complex, which binds to and stabilizes K-fiber minus ends (Meunier and Vernos, 2011; Meunier et al., 2015). An important source of RanGTP-induced spindle microtubules that serves to increase the density of microtubule plus ends in the vicinity of chromosomes is microtubule-templated nucleation mediated by the Augmin complex, which requires the micro-

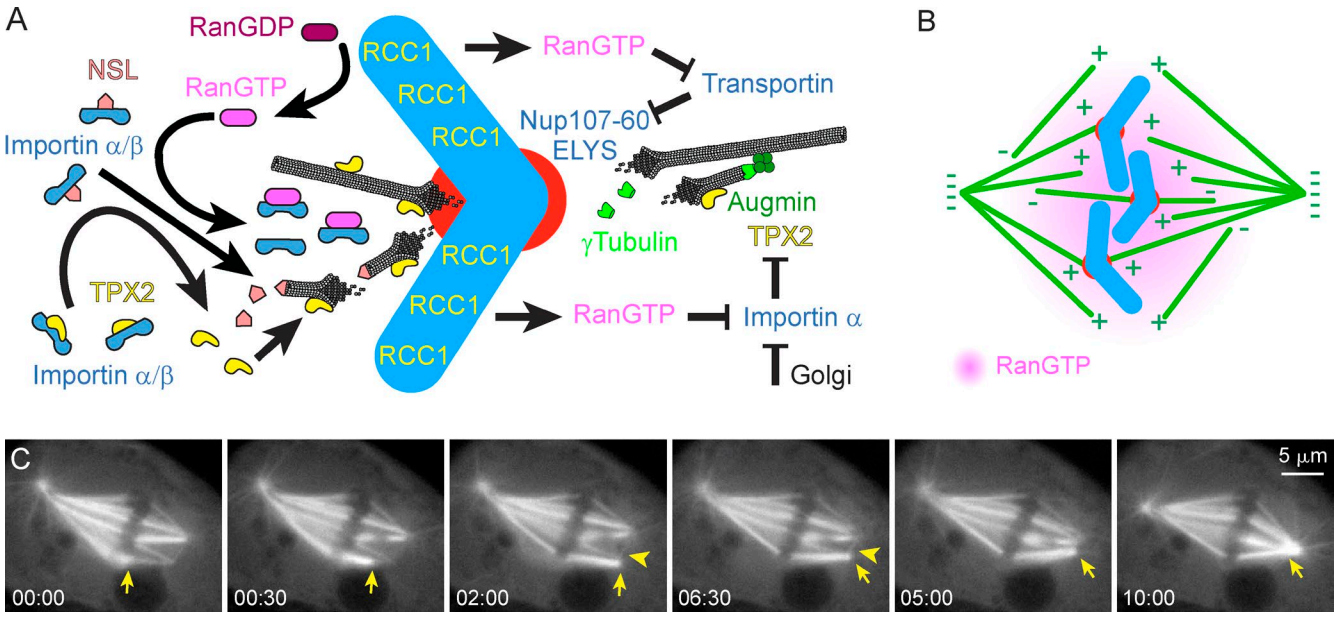

Figure 2. **Chromatin gradients guide spindle assembly.** (A) RCC1 localized to chromosomes increases the local concentration of RanGTP (pink) and promotes microtubule polymerization by liberating cargoes such as TPX2 (yellow) and the nonspecific lethal (NSL) complex (beige) from inhibitory interaction with importins (blue). Importin-α is also sequestered at the Golgi apparatus. Microtubules positioned near the kinetochore are likely to be rapidly captured, which initiates formation of a nascent K-fiber with a capped minus end (left side). At the kinetochore (red), microtubule nucleation is stimulated when RanGTP relieves inhibition of nucleoporins ELYS and Nup107–160 by transportin, allowing recruitment of γ-tubulin ring complexes (light green; right side). Within the spindle, templated microtubule nucleation is mediated by Augmin (dark green) and stimulated by TPX2. (B) Because of its rapid diffusion, RanGTP forms a gradient resulting in a differential regulation of microtubule nucleation/dynamics near versus away from the chromosomes. This, in turn, facilitates integration of chromosomes and their associated kinetochore microtubules into a common spindle. (C) A K-fiber formed via the kinetochore-mediated mechanism (arrows) grows via polymerization at plus ends, generating a larger target for astral microtubules. Direct contact (capture) between the elongating K-fiber and an astral microtubule (arrowheads) is followed by poleward transport and incorporation of the fiber's free end into the spindle. Time is given in minutes:seconds.

tubule nucleator γ-tubulin (Petry and Vale, 2015) and is stimulated by TPX2 (Petry et al., 2013). In addition to being liberated from importins by RanGTP, the inhibition of TPX2 is also relieved by the Golgi protein GM130, which sequesters importin-α to Golgi membranes (Wei et al., 2015). Thus, the RanGTP gradient promotes both de novo nucleation of microtubules near kinetochores and amplification of microtubule growth toward chromosomes (Fig. 2 A). Under normal conditions, these mechanisms increase the probability of kinetochore capture. However, if the chromatin and kinetochores become spatially separated, the gradient can erroneously guide microtubules away from the kinetochores. This was directly observed in mitotic cells with unreplicated genomes, where the bulk of chromatin along with its associated RanGTP gradient resides in the cell periphery and astral microtubules extend from the centrosomes toward the chromatin, which becomes particularly prominent when K-fiber formation is inhibited (O'Connell et al., 2009).

RanGTP is thought to contribute to spindle assembly in all metazoan cells, but it is most crucial in the second meiotic division of vertebrate eggs (Kalab et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999; Dumont et al., 2007), when centrosomes are absent and the chromosomes and spindle are tiny relative to the size of the cell, making spindle assembly via unbiased S&C virtually impossible. However, eggs contain stockpiles of cellular material including Ran pathway components, and the RanGTP generator RCC1 is not significantly enriched or activated on chromosomes, begging the question of why a large unbound pool of RCC1 does not obscure a chromatin-centered RanGTP gradient. Using *Xenopus laevis* egg extracts, Zhang et al. (2014) found that the cytoplasmic pool of RCC1 is inactive because of its association in a complex together with Ran and Ran binding protein 1 (RanBP1). This mechanism is critical for spatial control of the RanGTP gradient and spindle assembly.

Importantly, chromosomes still regulate microtubule nucleation and stability in the absence of a RanGTP gradient (Maresca et al., 2009) as a result of a second chromatin-induced pathway mediated by the chromosome passenger complex (CPC; Zierhut and Funabiki, 2015). The kinase subunit of the CPC, Aurora B, locally phosphorylates and inactivates microtubule-destabilizing proteins including MCAK (Andrews et al., 2004; Lan et al., 2004; Sampath et al., 2004) and Stathmin/Op18 (Kelly et al., 2007). Spatial activation of Aurora B in mitosis occurs through a kinase cascade initiated by the kinase Haspin, which phosphorylates histone H3. Phospho-H3 is bound directly by another CPC component, Survivin, enriching Aurora B and promoting its trans-autophosphorylation (Zierhut and Funabiki, 2015). A powerful nucleosome depletion and add-back approach in *Xenopus* egg extracts demonstrated that histone H3 phosphorylation is the only target of Haspin important for the spatial regulation of Aurora B (Zierhut et al., 2014).

By concentrating at the centromere that underlies each kinetochore, the CPC is known to control and correct erroneous microtubule attachments to kinetochores, thereby promoting biorientation of chromosomes so that chromatids are attached to opposite spindle poles and poised for segregation (Lampson and Cheeseman, 2011). Active Aurora B may diffuse away and phosphorylate substrates at a distance (Wang et al., 2011), thus also regulating microtubule depolymerization within the spindle. Interestingly, another kinase of the Aurora family, Aurora A, is situated at the spindle poles, where erroneously attached

chromosomes frequently accumulate, and generates a pole-centered phosphorylation gradient that also contributes to error correction (Chmátal et al., 2015; Ye et al., 2015).

In the context of S&C, microtubule growth induced in the vicinity of chromatin significantly accelerates spindle assembly (Fig. 2 B). The proximity of the freshly formed microtubules to the kinetochores facilitates their rapid capture. In addition, RanGTP appears to act directly at kinetochores, relieving inhibition of a complex containing nuclear pore proteins and γ-tubulin (Bernis et al., 2014; Yokoyama et al., 2014) and activating TPX2 to generate kinetochore-associated microtubules (Fig. 2 A; Tulu et al., 2006). Once captured, the plus ends of microtubules that reside at the kinetochore tend to grow continuously, resulting in a steady elongation of the nascent K-fiber (Maiato et al., 2004). As these fibers extend outwards, they provide large "antennae" that are rapidly discovered by the astral microtubules and are incorporated into the common spindle (Fig. 2, B and C). The minus end capture of preformed K-fibers is particularly evident when spindles transition from a monopolar to a bipolar configuration (Khodjakov et al., 2003).

Motor-mediated mechanisms

In the S&C hypothesis of 1986, the molecular nature of a capture event was not defined, but was assumed to dampen dynamics at the tip of the kinetochore-associated microtubule (Kirschner and Mitchison, 1986). However, observations of microtubule capture in cells revealed that kinetochores initially come in contact with the wall of a microtubule (Fig. 1 B) and that these lateral interactions are subsequently replaced by attachment to the microtubule plus end (Rieder and Alexander, 1990; Tanaka et al., 2005; Magidson et al., 2011; Kalinina et al., 2013). Indeed, direct single-step capture of the tip is significantly less probable than an encounter at a random point along the length of a microtubule, particularly because microtubules are known to pivot in space, which significantly enhances their ability to search for kinetochores (Kalinina et al., 2013). Molecular mechanisms that govern conversion from lateral to end-on attachments remain poorly understood and may occur as a direct transition of the same microtubule (Gandhi et al., 2011). Alternatively, lateral interactions may facilitate capture of other plus ends by orienting kinetochores favorably within the spindle (Magidson et al., 2011, 2015). The transition from lateral interaction to end-on attachment involves the coordinated activities of several molecular motors and microtubule depolymerases (Shrestha and Draviam, 2013). In fact, a second fundamentally important property of capture revealed by live-cell observations was the activity of molecular motors residing at the kinetochore. Kinetochores were seen to initiate rapid movement toward the minus end of a captured microtubule immediately after lateral contact (Rieder and Alexander, 1990).

The S&C hypothesis predated the discovery of motor proteins in the spindle, and in its primitive form, the duplicated centrosomes were thought to dictate the bipolar configuration of the spindle. It is now recognized that cytoplasmic dynein and a large family of kinesin motor proteins normally act to drive microtubule self-organization and spindle bipolarity (Gatlin and Bloom, 2010). The fundamental role of molecular motors is best illustrated in egg extract systems that lack centrosomes or kinetochores (Heald et al., 1996), and upon elimination of the centrosome in vertebrate cells (Khodjakov et al., 2000; Basto et al., 2006). Spindle motors support S&C in a couple of ways. First, they generate the bipolar microtubule array, which provides tracks for polarized chromosome movements that facilitate their biorientation. Second, plus end–directed motors that function to cross-link microtubules and sort them into an antiparallel array, including kinesin-5 (Eg5/Kif11) and kinesin-12 (Xklp2/Kif15), establish a spindle axis, whereas minus end–directed motors, including kinesin-14 (XCTK2/HSET) and dynein, provide balancing forces and act to focus spindle poles (Fig. 3, boxes 1 and 2; Walczak et al., 1998).

Although some motors act on spindle microtubules to organize them, others are present on kinetochores and chromosome arms and position them near the spindle equator, where conditions favor the attachment of sister kinetochores to microtubules from opposite spindle poles. The hierarchy of these chromosomal motors has recently been described (Fig. 3, boxes 3–5; Barisic et al., 2014). Dynein, which also exists in a kinetochore-bound pool, participates in the initial capture of astral microtubules, promoting lateral attachment and the movement of chromosomes toward the minus ends at spindle poles (Yang et al., 2007). This force is counteracted by the chromosome arm–associated chromokinesins kinesin-10 (Kid/NOD) and kinesin-4 (Kif4/Xklp1) that push the arms away from the centrosome (Wandke et al., 2012). As a result of this tug of war, scattered chromosomes are drawn from the cell periphery to the vicinity of spindle poles (Barisic et al., 2014). From there, chromosomes are subsequently delivered to the spindle equator by the kinetochore-associated kinesin-7 CENP-E (Kapoor et al., 2006; Cai et al., 2009). Interestingly, CENP-E prefers the less dynamic microtubules directed toward the spindle equator that contain posttranslationally modified α-tubulin lacking the C-terminal tyrosine. In vitro reconstitution experiments revealed that CENP-E–dependent transport is enhanced on detyrosinated microtubules, and treatment causing ubiquitous tubulin detyrosination in cells caused chromosome transport in random directions away from spindle poles (Barisic et al., 2015). Thus, motors organize the bipolar antiparallel microtubule array and drive chromosome movements that promote their congression and biorientation and are guided by biochemical cues, including RanGTP-induced gradients and αβ-tubulin posttranslational modifications.

Dynamic features of cellular geometry

S&C-driven spindle assembly is profoundly affected by geometric constraints such as the size and shape of the cell and the spatial organization of spindle components at the onset of mitosis. Because the length of dynamic microtubules is limited, accessory mechanisms must exist to prevent the excessive scattering of chromosomes or actively gather them within the searchable volume. In extremely large cells, such as animal oocytes, the chromosomes are driven into a compact group by actin filaments (Lénárt et al., 2005). In somatic cells, a cage of intermediate filaments that surrounds the nucleus during interphase averts the dispersion of chromosomes after nuclear envelope breakdown (Mandeville and Rieder, 1990). Similarly, chromosomes can be confined by the remnants of the nuclear envelope that usually surround the spindle (Tsai et al., 2006; Ma et al., 2009). Recent work suggests that the compartmentalization of space by the residual nuclear envelope operates as a matrix that not only confines larger mitotic apparatus components like chromosomes but also creates a diffusion barrier that concentrates soluble tubulin, as well as proteins involved in the regulation of mitosis within the spindle region. Conversely, this barrier prevents the invasion of cytoplasmic organelles into

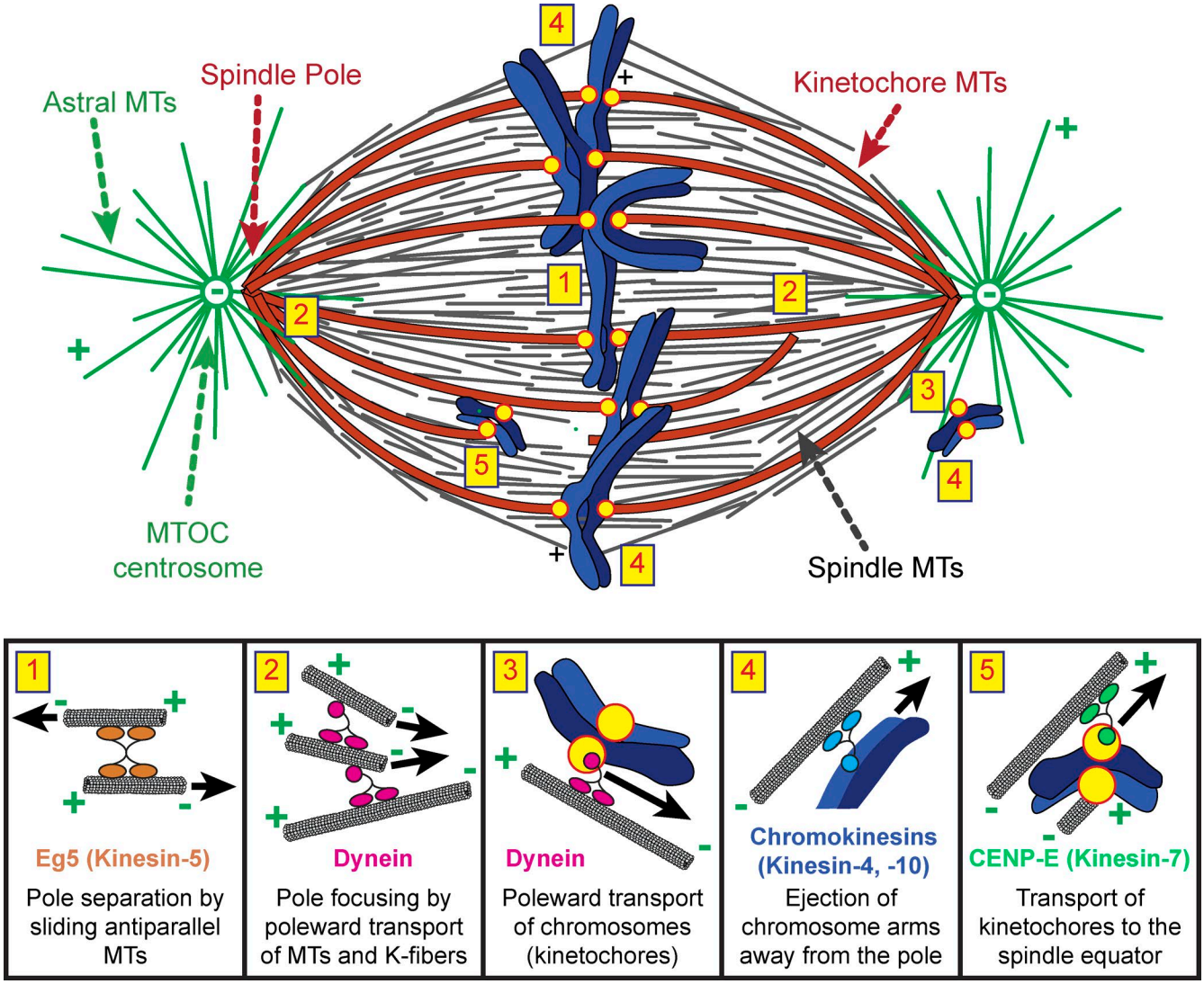

Figure 3. **Motor activities in spindle assembly.** Microtubule (MT)-bound motors promote bipolar spindle formation, whereas chromosome-associated motors drive proper kinetochore orientation and chromosome movement to the equator. Box 1: Motor-dependent mechanisms establish bipolarity as Eg5 (kinesin-5) motors slide antiparallel microtubules apart with their minus ends leading and their plus ends directed toward the spindle equator. Box 2: Minus end–directed motors such as dynein move microtubules poleward with their minus ends leading, thereby incorporating K-fibers into the spindle and focusing spindle poles. Box 3: Kinetochore-associated dynein transports chromosomes along astral microtubules toward the spindle poles from the periphery. Box 4: Plus end–directed chromokinesins (kinesin-4 and -10) eject chromosome arms outward. Box 5: CENP-E (kinesin-7) transports unattached kinetochores toward the equator along spindle microtubules. MTOC, microtubule organizing center.

the spindle compartment to avoid their steric interference that would impede interactions between kinetochores and microtubules (Schweizer et al., 2015). Intriguingly, the spindle matrix contains proteins such as BuGZ that harbor low-complexity hydrophobic sequences and undergo a temperature-dependent phase transition that promotes microtubule polymerization (Jiang et al., 2014). Therefore, the search for chromosomes normally takes place not throughout the cytoplasm but within a compact and biochemically distinct subcellular environment that promotes spindle assembly. Regulation that affects the size, shape, and composition of this compartment may indirectly yet profoundly affect the efficiency and fidelity of spindle assembly. For example, a common feature of animal cells is that they round up during mitosis. Preventing this morphological change either by perturbing cortical actin or by pure mechanical means impedes the gathering of chromosomes into a compact group near the geometric center of the cell. This in turn limits the efficiency of S&C and leads to a higher probability of chromosome loss (Lancaster et al., 2013; Cattin et al., 2015).

Although gathering the chromosomes within the reach of spindle microtubules is necessary, it also poses a problem for S&C-driven spindle assembly. In a crowded environment, many kinetochores become inaccessible to microtubules because of occlusion by chromosome arms (Fig. 4 A). The number of kinetochores that are invisible to microtubules increases rapidly as the number of chromosomes grows. Computational analyses suggest that only ∼3% of kinetochores would be accessible to microtubules if 46 average-size chromosomes were randomly distributed within a typical-size spherical nuclear volume (Paul et al., 2009). To overcome this problem, cells have developed mechanisms that shape, orient, and distribute chromosomes into spatial patterns that actively present kinetochores to the searching microtubules during prometaphase (Kitajima et al., 2011; Magidson et al., 2011). These mechanisms involve the

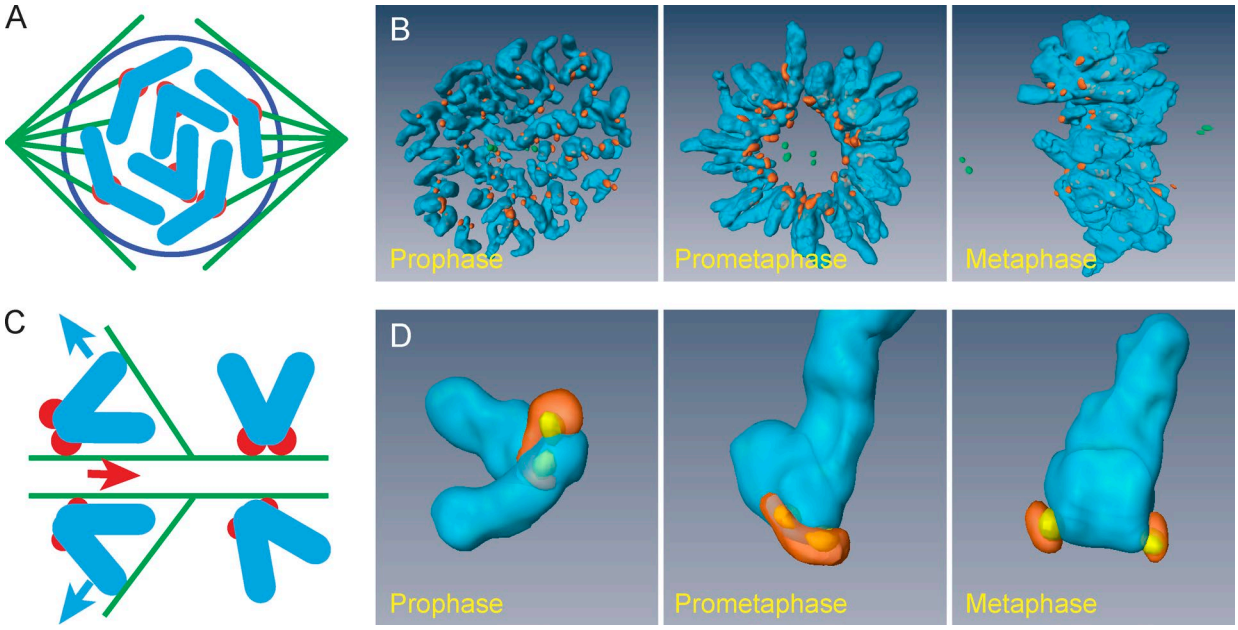

Figure 4. **Cellular geometry and dynamic kinetochores.** (A) The efficiency of S&C is affected by the spatial organization of chromosomes. The arms of peripheral chromosomes (blue) shield kinetochores (red) positioned deeper inside the nucleus from astral microtubules (green). (B) Typical spatial patterns observed in mammalian cells at progressive stages of spindle assembly. At prophase, duplicated centrosomes (green) separate to opposite sides of the nucleus, and the distribution of kinetochores (orange) appears to be random. During early prometaphase, chromosomes form a toroid with most kinetochores residing on the surface of the nascent spindle and chromosome arms pointing outwards. Upon formation of stable end-on kinetochore attachments, chromosomes repopulate the central part of the spindle, becoming uniformly distributed at the spindle equator at metaphase. (C) The ejection force of the arms (blue arrows) opposed by the inward forces generated at the kinetochore (red arrow) rotates the centromere so that sister kinetochores become preferentially oriented toward opposite spindle poles. Notice that larger kinetochores support a more significant rotation (top), whereas smaller kinetochores lose contact with microtubules, dampening the inward force (bottom). (D) Typical examples of chromosome architecture during various stages of mitosis. Note that chromosome arms (blue) are bent inside the prophase nucleus but become straightened by the ejection force (prometaphase and metaphase). Inner kinetochores (CENP-A; yellow) remain compact throughout mitosis, whereas the outer kinetochore (CENP-F; orange) encircles a large part of the centromere during prometaphase and compacts after the formation of end-on attachments (metaphase).

interplay between a chromokinesin-mediated ejection force on the arms (Rieder and Salmon, 1994; Vanneste et al., 2011) and inward-directed forces produced by the kinetochore-associated microtubule motors, which arrange the chromosomes into a toroid around the nascent spindle. In this belt-like configuration (Fig. 4 B), kinetochores become exposed to a high density of microtubules, which promotes efficient capture. Subsequently, stronger and more stable end-on attachments allow chromosomes to gradually repopulate the central part of the spindle. Conditions that prevent the formation of the chromosomal belt (e.g., the inactivation of chromokinesins) prolong spindle assembly and markedly increase the number of lagging chromosomes that segregate improperly during the ensuing anaphase (Magidson et al., 2011, 2015).

Another set of geometric constraints that inevitably affect the efficiency and fidelity of S&C-based spindle assembly are the size and relative positions of sister kinetochores assembled at the centromere (Östergren, 1951). Intuitively, small sister kinetochores positioned on opposite sides would ensure error-free spindle assembly, as they would be sterically shielded by the centromere from capturing microtubules that emanate from the same spindle pole. Indeed, when sister kinetochores become juxtaposed, the number of syntelic attachments increases dramatically (Lončarek et al., 2007; Sakuno et al., 2009). However, small kinetochores cannot capture microtubules very efficiently and would increase the time required for spindle assembly. Interestingly, recent computational analyses suggest that the intuitive reciprocal relationship between efficiency and fidelity in S&C-driven spindle assembly is incorrect. A model that considers the formation of end-on attachments in a spindle environment dominated by lateral microtubule interactions predicts that the enlargement of kinetochores during prometaphase would both accelerate spindle assembly and suppress the number of errors (Magidson et al., 2015). This unexpected synergy is the result of rotational alignment of centromeres with respect to the spindle axis driven by opposing forces acting at the kinetochores versus chromosome arms. The extent of angular prealignment is less for smaller kinetochores, which are not capable of remaining in direct contact with microtubules during extensive rotations (Fig. 4 C). Indeed, enlargement of kinetochores during earlier stages of spindle assembly followed by their compaction upon the formation of end-on attachment (Fig. 4 D) has been directly observed in cells (Thrower et al., 1996; Hoffman et al., 2001; Magidson et al., 2015) as well as in egg extracts (Wynne and Funabiki, 2015). Interestingly, computational modeling suggests that once angular chromosome alignment is attained, efficient correction of erroneous attachments will be achieved simply because of the rapid turnover of microtubules at kinetochores (Zaytsev and Grishchuk, 2015). Thus, dynamic changes in chromosome architecture in the context of spatial cues and constraints, together with the high turnover rate of microtubules within the spindle, promote the proper attachment of sister kinetochores to opposite spindle poles.

Conclusion

Cell biology is a rapidly advancing field, and new observations frequently disprove mechanistic hypotheses after just a few short years. Yet, nothing that we have learned about mito-

sis in the last 30 years, which includes the discovery of scores of factors involved in spindle assembly, has been inconsistent with the basic principles of S&C. Instead, the many facilitating mechanisms elucidated over the years have been organically incorporated into the model. It is important to emphasize that these mechanisms are often not essential: spindles form in the absence of mitotic gradients (Maresca et al., 2009), or when the function of key motors is blocked (Ganem et al., 2005; Gayek and Ohi, 2014) or spindle geometry is perturbed (Ganem et al., 2009; Silkworth et al., 2009; Lancaster et al., 2013). As long as the minimal requirements for S&C (i.e., dynamic microtubules and capture by kinetochores) are in place, a functional spindle can assemble. However, the duration of spindle assembly and the number of erroneous chromosome attachments increase dramatically in the absence of facilitating S&C mechanisms. Importantly, both of these side effects compromise the fate of daughter cells: the prolongation of mitosis has been shown to halt progression through the ensuing cell cycle (Uetake and Sluder, 2010), and erroneous segregation of a chromosome can trigger perpetuating chromosomal instability (Thompson and Compton, 2008). Thus, the complexity of numerous nonessential mechanisms sustains the wonderfully simple principle of S&C.

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