Annual Review of Plant Biology

Spindle Assembly and Mitosis in Plants

Bo Liu and Yuh-Ru Julie Lee

Department of Plant Biology, University of California, Davis, California, USA;
email: bliu@ucdavis.edu, yjlee@ucdavis.edu

Annu. Rev. Plant Biol. 2022. 73:227–54

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-070721-084258

Keywords

Aurora kinase, central spindle, γ-tubulin, kinesins, mitosis, microtubule nucleation, prophase spindle, spindle assembly checkpoint

Abstract

In contrast to well-studied fungal and animal cells, plant cells assemble bipolar spindles that exhibit a great deal of plasticity in the absence of structurally defined microtubule-organizing centers like the centrosome. While plants employ some evolutionarily conserved proteins to regulate spindle morphogenesis and remodeling, many essential spindle assembly factors found in vertebrates are either missing or not required for producing the plant bipolar microtubule array. Plants also produce proteins distantly related to their fungal and animal counterparts to regulate critical events such as the spindle assembly checkpoint. Plant spindle assembly initiates with microtubule nucleation on the nuclear envelope followed by bipolarization into the prophase spindle. After nuclear envelope breakdown, kinetochore fibers are assembled and unified into the spindle apparatus with convergent poles. Of note, compared to fungal and animal systems, relatively little is known about how plant cells remodel the spindle microtubule array during anaphase. Uncovering mitotic functions of novel proteins for spindle assembly in plants will illuminate both common and divergent mechanisms employed by different eukaryotic organisms to segregate genetic materials.

Contents

1. INTRODUCTION

The bipolar spindle apparatus, constructed by polar polymers of microtubules made of α- and β-tubulin heterodimers, is one of the most sophisticated biological machineries produced by eukaryotic cells to faithfully separate the duplicated nuclear genome. While *Annual Reviews* journals frequently update readers about advances in our understanding of this machinery, the knowledge emphasized primarily comes from fungal and animal model systems. Since the *Annual Review of Plant Physiology and Plant Molecular Biology* presented the knowledge of dynamic plant spindles in the fluorescence microscopic view of the cytoskeleton in the context of their structure and function over 30 years ago (15, 82), this important subject has not been reviewed again here. Historically, meticulous observations of the plant spindle apparatus using innovative microscopic techniques have provided novel insights into dynamic events taking place during spindle assembly, and related mechanisms have gradually been understood via the discovery of responsible proteins associated with their underlying mechanisms. First, we highlight several inspiring views gained from the examination of informative plant models that offer great advantages for microscopic observations. In the literature, the difference between animal and plant spindles is often depicted as being centrosomal versus acentrosomal, respectively. In fact, Bajer (10) learned many years ago that microtubules established converging centers representing focused spindle poles without the presence of the centrosome in the African blood lily *Haemanthus* (now known as *Scadoxus*). Researchers also learned that microtubules were nucleated from sites such as kinetochores in addition to spindle poles during spindle assembly in *Lilium* (51). While textbooks often depict kinetochore fibers as microtubule filaments running from the spindle pole to kinetochores, studies detected that they are more like microtubular fir trees, with fine microtubules nucleated from the trunk of

Kinetochore fibers: microtubules assembled at and/or linked to kinetochores; they are responsible for poleward movement of sister chromatids

the fiber (10). Unusual mitotic spindles, such as those formed during generative cell division in the pollen tube of the spiderwort *Tradescantia*, have led to appreciation of the great morphological plasticity of the spindle apparatus in different cell types (109). Many of these beautiful plant cells have provided insights into common events taking place during spindle assembly, and these historic findings continue to inspire those who examine the molecular mechanisms that regulate microtubule organization in the spindle apparatus.

Mitotic spindle: a bipolar microtubule array assembled by kinetochore fibers and interpolar microtubules that are engaged in the midzone

The general description of acentrosomal spindle formation in plant cells remains unchanged and is briefly summarized here in the context of microtubule organization. At prophase, microtubules are nucleated on the nuclear envelope and ultimately organized into the prophase spindle, which often contains two perfectly focused poles (**Figure 1*a***). By contrast, fungal cells produce an intranuclear spindle array with microtubules that initiate from duplicated spindle pole bodies prior to having chromosomes attached, and animal cells have microtubules nucleated from

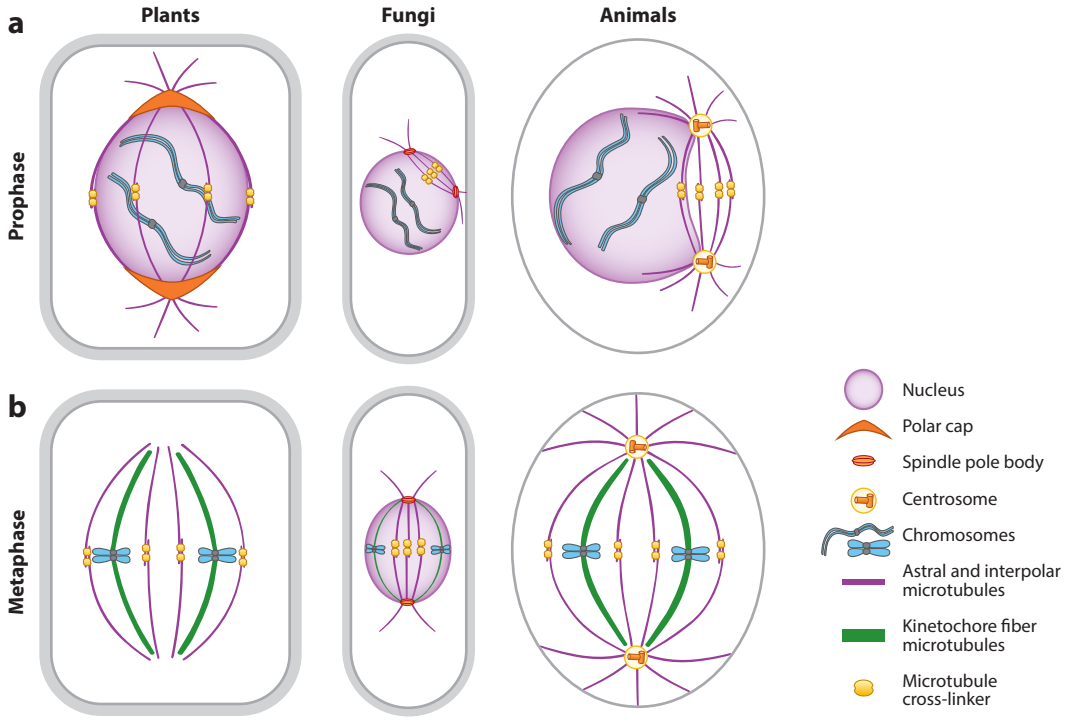

Figure 1

Comparison of the prophase and mitotic spindles in plants, fungi, and animals. (*a*) Toward the end of prophase, microtubules on the nuclear envelope are bipolarized into a spindle array, with oppositely positioned converging centers of the polar caps acting as the spindle poles in plant cells. Microtubules generated from the two poles are engaged toward plus ends by cross-linkers of microtubule motors, or microtubule-associated proteins (MAPs). In addition, astral microtubules also are nucleated from the polar caps. In fungi, duplicated spindle pole bodies nucleate intranuclear microtubules that are engaged in the middle, while astral microtubules are generated on the cytoplasmic side of the spindle pole bodies. In centrosome-bearing animal cells, duplicated centrosomes nucleate microtubules of outward astral microtubules and inward interpolar microtubules that are cross-linked in the midzone. (*b*) At metaphase, the mitotic spindle array is assembled by kinetochore fiber microtubules flanked by microtubules that may be inherited from the prophase spindle in plant cells. These microtubules converge toward the two poles but are not tightly focused. In fungi, the intranuclear spindle elongates concomitant with the further separation of the spindle pole bodies from which additional microtubules are nucleated to attach chromosomes. The metaphase spindle in animal cells has chromosome-attached kinetochore fibers placed between interpolar microtubules, and additional astral microtubules generated from the centrosomes connect spindle poles to the cell cortex.

duplicated centrosomes that are engaged in the bipolar array next to the prophase nucleus (**Figure 1*a***). After nuclear envelope breakdown, chromosomes are attached to rapidly polymerizing microtubules, which leads to the formation of oppositely oriented kinetochore fibers linked to each chromosome. These individual kinetochore fibers, or so-called microtubular fir tree complexes, join to form a spindle apparatus with an undefined degree of convergence (**Figure 1*b***). Compared to plants, fungi form the intranuclear spindle microtubule array with conspicuous microtubules originating from the spindle pole bodies that also nucleate microtubules to attach chromosomes (**Figure 1*b***). In animal cells, centrosomal spindles have astral microtubules, which often reach the cell periphery, and cross-linked interpolar microtubules intercalating the kinetochore microtubule fibers, which bring the chromosomes into the microtubule array (**Figure 1*b***). Successful chromosome congression at the metaphase plate triggers anaphase onset, marked by the shortening of kinetochore fibers in a process described as anaphase A, leaving behind growing microtubules in what is often called the central spindle. In most plant cells, anaphase B, or the elongation of the central spindle, is not obvious, perhaps because of the geometric restriction imposed by the cell wall. Microtubules in the central spindle serve as the precursors of the cytokinetic phragmoplast array.

Prophase spindle: a bipolar microtubule array formed on the nuclear envelope at prophase before the formation of kinetochore microtubule fibers

Central spindle: the middle section of a spindle, made of nonkinetochore fiber microtubules, that becomes more pronounced between segregated sister chromatids

Kinesin: an ATPase enzyme that mostly functions as a motor for transport along microtubule tracks

Microtubule-associated proteins (MAPs): regulate various aspects of microtubule dynamics by direct interaction

While earlier historic observations were interpreted without the emphasis on specific proteins, functional characterizations of regulators of spindle assembly have continuously shed light on molecular mechanisms regulating each specific step that plant cells take to assemble the dynamic microtubule array. In this review, we capture some key aspects of spindle assembly, albeit not conclusive, based on many advances made in the past three decades. While many of the proteins associated with spindle microtubules also function in assembling the dynamic phragmoplast microtubule array, their cytokinetic roles are not included in this review. Furthermore, we apologize to authors whose works are not cited here because of unintentional omissions. This article is intended to stimulate further discussion and investigation of spindle apparatus assembly through the use of innovative technologies, including genetic manipulations of gene expression and microscopic observations of proteins. We are in an excellent position to advance our knowledge of plant spindles when observations made in classical model systems converge with discoveries of new proteins characterized in modern models ranging from bryophytes to angiosperms.

2. MICROTUBULE-NUCLEATING FACTORS, MICROTUBULE-ASSOCIATED PROTEINS (MAPS), AND KINESIN MOTORS FOR SPINDLE ASSEMBLY

Cycling eukaryotic cells tear down interphase microtubule arrays and assemble the bipolar spindle microtubule array typically within approximately 30 min. Structures such as the nuclear envelope and chromosomes create spatial territories that allow proteins to be locally activated or inactivated in order to regulate microtubule dynamics and organization. Some evolutionarily conserved proteins are responsible for different aspects of producing the spindle apparatus: Microtubule-nucleating factors generate new microtubules, microtubule-associated proteins (MAPs) regulate their dynamic properties and selectively bundle parallel or antiparallel microtubules inside the spindle, and kinesin motors directionally slide microtubules. Genes encoding spindle-associated factors are likely expressed in a cell cycle–dependent manner, and their functions often are posttranslationally regulated via phosphorylation by cyclin-dependent or other mitotic kinases. Discoveries of spindle assembly factors (SAFs) have benefited from the employment of approaches such as protein purification from synchronized animal cells in culture, generation of monoclonal antibodies against proteins enriched in centrosomes and spindles, and molecular genetics in model fungal species. More recently, genome-wide RNA interference–based screening has also

uncovered additional factors for spindle assembly. A comprehensive list of proteins important for spindle assembly and mitosis has been generated, and many are conserved among eukaryotes, including some in flowering plants that have been summarized recently (152). Proteins discussed here are categorized as microtubule-nucleating factors, MAPs, and microtubule-based motor kinesins. Results of the functional analysis of some evolutionarily conserved factors in plants often challenge some widely accepted models established by studies of vertebrate spindles, as discussed later.

2.1. Microtubule-Nucleating Factors

The most prominent microtubule-nucleating factors are γ-tubulin, which is nonpolymerizing, and the proteins complexed with it. There are five γ-tubulin complex proteins (GCPs) that share sequence and structural similarities in the N terminus, which interacts with other GCP proteins, and the C terminus, which interacts with γ-tubulin. A ring-shaped complex of >2 MDa, known as the γ-tubulin ring complex (γ-TuRC), is assembled by γ-tubulin and the GCP2–GCP6 proteins. The γ-TuRC is an asymmetric structure made of seven tetramers of dimerized GCPs, with γ-tubulin associated with each GCP (80). It starts with four tetramers of γ-tubulin-GCP2/γ-tubulin-GCP3 followed by one γ-tubulin-GCP4/γ-tubulin-GCP5 tetramer, one γ-tubulin-GCP4/γ-tubulin-GCP6 tetramer, and, finally, another γ-tubulin-GCP2/γ-tubulin-GCP3 tetramer. The γ-TuRC serves as the template for the nucleation of new microtubules, but its nucleation function is activated by its associated factors (138). Plants produce all proteins in the γ-TuRC, including the associated mitotic-spindle organizing protein 1 (MZT1) of ~8 kDa and the WD40 repeat protein called neural precursor cell expressed, developmentally down-regulated gene 1 (NEDD1) (39). Although γ-tubulin and the core γ-TuRC subunits GCP2 and GCP3, as well as MZT1 and NEDD1, are essential, corresponding mutant gametophytic cells produced by the heterozygous sporophytes are still able to assemble bipolar spindles, as demonstrated in *Arabidopsis thaliana* (52, 102, 112, 154). By contrast, GCP6 is not essential in *A. thaliana* as the null *gcp6* mutant plant can produce bipolar spindles and undergo growth and reproduction, albeit with morphological defects in spindle organization (91). The *gcp6* mutant does not produce the intact γ-TuRC as γ-tubulin only associates with GCP2 and GCP3 but not GCP4 or GCP5 in the absence of GCP6. It would be interesting to learn whether GCP4 and GCP5 are nonessential as well, although downregulation of GCP4 expression leads to severe growth reduction in addition to producing spindles with disorganized poles (59). The findings in plant cells support the notion that there may be other functional forms of the γ-tubulin complex besides the γ-TuRC described above (105).

In plant cells, a dominant form of γ-TuRC-dependent microtubule nucleation takes place on the wall of extant microtubules (99). This microtubule-dependent microtubule nucleation event hinges on targeting the γ-TuRC to the microtubule wall instead of to the minus end. This task is fulfilled by an eight-protein complex called augmin, first uncovered in a screen of factors required for γ-tubulin association with spindle microtubules in cultured insect cells (38). Plants produce the augmin complex, although its individual AUG1–AUG8 subunits exhibit high degrees of amino acid sequence divergence from their animal counterparts, and it plays a critical role in both parallel and branched microtubule nucleation on existing microtubules of interphase and mitotic arrays (48, 49, 81, 103). Augmin's association with existing microtubules is brought about by its AUG8 subunit bearing a microtubule-binding site. In *A. thaliana*, the mitotic function of augmin is specified by one of the AUG8 isoforms, endosperm defective 1 (EDE1) (69). The discovery of this augmin-dependent microtubule nucleation phenomenon provides a molecular mechanism that accounts for the fine-branched microtubules in the microtubular fir trees that had been observed inside the endosperm spindles (10). Although augmin is essential for plant life, cells with

γ-Tubulin: an evolutionarily conserved member of the tubulin family that functions in microtubule nucleation and does not polymerize into filaments

γ-Tubulin ring complex (γ-TuRC): an asymmetric ring-shaped complex of >2 MDa that is formed primarily by the association of γ-tubulin and five related γ-tubulin complex proteins (GCPs)

Augmin: an eight-subunit protein complex that functions by targeting the γ-TuRC to existing microtubules and activating it for parallel and branched microtubule nucleation

compromised or completely lost augmin function (e.g., in mutant gametophytic cells produced by a heterozygous parent) still can produce convergent bipolar spindles, albeit elongated and perhaps less robust than those produced by wild-type *A. thaliana* cells (49, 69). Such phenotypes leave questions about whether augmin spatially regulates microtubule nucleation events but is not essential for the activity per se and how a null *aug* mutation causes lethality.

There are several factors such as centrosomin and pericentrin that function in anchoring the γ-TuRC to the centrosome and activating its nucleation activity in animal cells. To date, no homologs have been identified in plants, so whether there are functional counterparts of these factors on the prophase nuclear envelope and spindle poles that have no obviously discernable sequence homology to proteins such as pericentrin remains to be explored.

2.2. MAPs in the Spindle Apparatus

MAPs interact with microtubules directly, but the consequences of the interaction on microtubules are drastically different among different MAPs. Plants produce both evolutionarily conserved MAPs and unique ones that contribute to spindle assembly.

2.2.1. The targeting protein of XKLP2 (TPX2) family proteins. Among MAPs that are associated with the spindle microtubule array in vertebrates, perhaps the most well-known is targeting protein of XKLP2 (TPX2), which exhibits centrosome- and spindle pole–concentrated localization. Most importantly, TPX2 serves as the essential targeting and activation factor of the mitotic kinase Aurora in addition to playing a critical role in the spindle pole–biased localization of mitotic kinesins upon activation by Ras-related nuclear protein (Ran)-GTP (143). Recently, TPX2 was also found to act as an activating factor for branched microtubule nucleation (1). Although fungi do not have TPX2 homologs, plants produce the canonical TPX2 homologs with all domains found in vertebrate counterparts, and TPX2 in *A. thaliana* exhibits a pole-biased association of spindle microtubules and can induce ectopic microtubule nucleation upon overexpression (113). The TPX2 protein, however, is dispensable as the null *tpx2* mutants grow indistinguishable from the wild type in *A. thaliana*; instead, a related TPX2-like 3 (TPXL3) protein becomes essential (18). Therefore, the TPX2-centric mechanisms that regulate spindle assembly are inapplicable while the mitotic function of this canonical TPX2 protein remains to be characterized in plants.

2.2.2. The microtubule plus-end-tracking proteins (+TIPs). Evolutionarily conserved proteins in the *Xenopus* MAP of 215 kDa (XMAP215) family function as microtubule polymerases and make essential contributions to the generation of dynamic microtubules by antagonizing depolymerizing challenges brought about by depolymerases (36). The proteins also track the polymerizing plus end of microtubules, and they are grouped into +TIPs because of their shared microtubule plus-end-tracking phenomenon. The plant homologs of XMAP215 known as microtubule organization 1 (MOR1) were initially identified in *A. thaliana* and are detected on spindle microtubules as well as on all other microtubule arrays (40, 53). However, bipolar spindles can still be assembled despite some abnormalities, and mitosis progresses, but the phragmoplast microtubule array is more seriously distorted in the loss-of-function mutants (53, 107, 139). Therefore, unlike XMAP215-dependent principles found in vertebrates, mechanisms independent of MOR1 likely drive spindle assembly in plants.

The CLIP-associated protein (CLASP) is structurally related to XMAP215. Both contain tumor overexpressed gene (TOG) domains made of Huntingtin, elongation factor 3; subunit A of protein phosphatase 2A; and PI3 kinase target of rapamycin 1 (HEAT) repeats, and both play critical roles in bipolar spindle assembly and chromosome attachment to spindle microtubules in animals (67). However, such a role is not found in the plant CLASP protein, despite its high

degree of homology to its animal counterparts. Not only is the protein not associated with spindle microtubules, but it is also dispensable for growth and reproduction, although its mutants show reduced axial organ elongation (5, 54). Again, rules established by the animal CLASP do not apply in plant spindles.

One of the most critical +TIPs for animal cell division is the end-binding 1 (EB1) protein as revealed by severe phenotypes in the assembly, dynamics, and orientation of spindles when its expression is repressed in cultured cells (120). Plant EB1 proteins exhibit plus-end-tracking activities that are identical to those found in other organisms, and one is devoted to cell division and is released from the nucleoplasm to the cytoplasm upon nuclear envelope breakdown (47, 56). This mitotic EB1 isoform associates with polymerizing microtubules in mitotic arrays but only becomes indispensable when plant cells are challenged by microtubule depolymerizing agents. Surprisingly, the loss of all EB1 proteins does not affect plant growth and reproduction (16).

2.2.3. Microtubule-bundling factors in the MAP65 family. A key feature of the bipolar spindle is that microtubules from the two half-spindles are engaged by interdigitation in the central spindle. In vertebrate spindles, microtubules initiated from one kinetochore fiber are cross-linked with those from the opposite kinetochore fiber to form so-called bridging microtubules via the action of the microtubule cross-linker protein regulating cytokinesis 1 (PRC1) (126). PRC1 is a homolog of MAP65 that is initially purified from plant cells and primarily depicted as the bundler of antiparallel microtubules in the phragmoplast midzone (128). In *A. thaliana*, there are nine MAP65 isoforms, and to date MAP65-3 and MAP65-4 have been found to be mitotically active, and MAP65-4, but not MAP65-3, decorates the spindle midzone in addition to its activity in the phragmoplast (76). If the MAP65 proteins play a mitotic function similar to that of the vertebrate PRC1, it must be shared among two or more isoforms because the loss of a single *MAP65* gene does not prevent mitosis from taking place successfully.

2.2.4. The microtubule-severing MAP katanin. The remodeling of microtubule arrays from one to the next requires severing activities brought about by the ATPase enzyme katanin, which is made of the catalytic p60 (a MAP) and regulatory/targeting p80 subunits and known for rapidly severing long microtubules in preparation for spindle assembly (90). While the function of plant katanin is extremely important in the remodeling of the cortical microtubule array in rapidly expanding cells, it is not essential for the assembly of mitotic microtubule arrays, as demonstrated in mutants lacking either catalytic or regulatory subunits (24, 146). In *A. thaliana*, katanin is associated with mitotic microtubule arrays, but defects in organizing these arrays caused by its loss are not severe enough to prevent the mutant cells from correcting them and completing the cell division cycle (110, 121). Therefore, there are other factors that act synergistically with katanin, provided that microtubule turnovers are essential for microtubule remodeling in plant cell division.

2.2.5. Other novel plant MAPs that decorate spindle microtubules. It is intriguing that none of the abovementioned MAPs are required for spindle assembly in plants, while their animal counterparts are considered essential SAFs. One plausible explanation is that plants produce novel MAPs that function redundantly with homologs of these animal MAPs. In fact, multiple approaches, including a holistic MAP purification attempt, have successfully detected novel MAPs such as spiral 1 (SPR1) with ~110 amino acid polypeptides, tortifolia 1 (TOR1)/SPR2 with Armadillo (ARM) repeats, and basic proline-rich proteins (BPPs). These proteins all decorate spindle microtubules upon expression under their native promoters, and thus they likely represent the localizations of the native proteins (25, 41, 101, 125). However, whether they functionally participate in microtubule assembly and/or reorganization during mitosis is largely unexplored.

Spindle assembly checkpoint (SAC): a cell cycle checkpoint mechanism that monitors the congression of chromosomes at the metaphase plate

In metazoans, known essential SAFs also include MAPs such as abnormal spindle protein (ASP), CAMSAP/Nezha/Patronin, microspherule protein 1 (MCRS1), and nuclear mitotic apparatus (NuMA). While plants do not make CAMSAP and NuMA, they produce obvious homologs of ASP and MCRS1 (152). In *A. thaliana*, ASP is associated with all mitotic microtubule arrays, but its loss does not cause a noticeable phenotype in cell division and plant growth (Y. Lee & B. Liu, unpublished data). In the meantime, MCRS1 is essential but most likely not involved in mitosis because it is neither associated with microtubules nor required for mitosis during gametogenesis (H. Huo, M. Luo, Y. Lee & B. Liu, unpublished data).

2.3. Kinesins in Spindle Assembly

Microtubule-based motors play pivotal roles in microtubule assembly/disassembly, organization, and chromosome motility during karyokinesis and include both kinesins and cytoplasmic dynein in organisms such as vertebrates (135). Plants lack cytoplasmic dynein, and angiosperms produce an expanded family of kinesins that belong to some of the 14 known subfamilies (119). In mitosis, the vertebrate cytoplasmic dynein plays versatile and prominent roles in organizing spindle poles, anchoring spindles to the cell cortex, and silencing the spindle assembly checkpoint (SAC) (118). If these functions are universally important for mitosis, they must have been performed by kinesins for plant cell division. As analyzed in *A. thaliana*, kinesins involved in cell division often exhibit cell cycle–dependent expression patterns (141). A survey of 72 kinesins that are produced by the moss *Physcomitrium patens* detected 43 motors associated with mitotic microtubule arrays or chromosomal structures (92).

Kinesins can sometimes be classified by the location of the conserved catalytic motor domain at the N terminus, middle, or C terminus of polypeptides. This rule is not applicable to plant kinesins because members of a single subfamily, such as Kinesin-14, may have the motor domain at different positions. Alternatively, they may be described according to the directionality of their motilities for being plus-end- or minus-end-directed motors, while Kinesin-13 is nonmotile and often acts as a microtubule depolymerase. Functions of diversified kinesins are governed by respective domains outside of the catalytic motor domain that often are characteristic of each subfamily or even of each member within a subfamily, as reviewed previously (73, 104). To date, only Kinesin-14 motors exhibit minus-end-directed motility, and other examined plant kinesins all travel in the opposite direction. Unfortunately, we have obtained only a small fraction of the big picture depicting how motors in the expanded plant kinesin family may drive the assembly of the dynamic spindle array. The summary below includes both limited experimental evidence and some wild speculation.

2.3.1. Kinesins associated with chromosomes and kinetochores. The most urgent task of karyokinesis after nuclear envelope breakdown is to engage chromosomes with microtubules to promote an end-on attachment of microtubules at kinetochores during prometaphase. There is no question that the final alignment of chromosomes at the metaphase plate is brought about by kinesins as they are the only microtubule motors in plants. To date, one Kinesin-4 member is detected on chromosomes and one Kinesin-7 motor on kinetochores in *P. patens* (92). Although such localizations certainly provide informative evidence for their participation in mitosis, proof of chromosome congression function has not yet been obtained. Instead, it was found that the loss of all three Kinesin-4 motors severely inhibits cell elongation and, consequently, axial organ growth but does not prevent mitosis from taking place successfully in *A. thaliana* (60). Out of 15 Kinesin-7 motors, 1 is associated with the spindle as well as other microtubule arrays and surprisingly interacts with the cohesin-breaking separase to promote microtubule polymerization for cell polarization in *A. thaliana* (96). Therefore, we have yet to learn how kinesins act on plant

kinetochores/chromosomes in the context of their functions in spindle assembly and chromosome alignment.

2.3.2. The role of Kinesin-5 in establishing the bipolarity of the spindle apparatus. Motors in the Kinesin-5 subfamily are often considered the most important factor for producing the bipolar spindle, as demonstrated by the inactivation of the founding member block-in-mitosis C (BIMC), which caused the failure of the separation of spindle poles in the fungus *Aspergillus nidulans* (34). A single plant often makes multiple isoforms of Kinesin-5 that show associations with all spindle microtubules, as well as other mitotic arrays, without an enrichment in the central region that has interdigitated microtubules, and the functions of Kinesin-5 isoforms are said to be associated with the phragmoplast for cytokinesis (7, 14, 92). Among the four Kinesin-5 motors in *A. thaliana*, however, a point mutation in the *RSW7* gene encoding Kinesin-5C caused a temperature-sensitive phenotype of collapsed spindle poles, resembling the phenotype seen in the fungal *bimC* mutant (12, 13). However, the loss of Kinesin-5C does not cause any noticeable phenotype in mitosis (37), perhaps due to the functional redundancy among different isoforms. Similarly, suppression of Kinesin-5 expression causes defects in chromosome segregation in anaphase but not bipolarization of spindle microtubules prior to anaphase onset in *P. patens* (92). Therefore, Kinesin-5 is possibly joined by other motors, such as Kinesin-12, as suggested in vertebrate spindles (137), to assist in the construction of the bipolar array.

2.3.3. Kinesin-12 and poleward tubulin flux. Kinesin-12 was first discovered as a motor required for centrosome separation in vertebrates (17). In *A. thaliana*, however, the motors in this subfamily have been mostly found in the spindle midzone for establishing the minimal microtubule overlapping zone or at the cortical division site for maintaining the division plane established by the microtubule preprophase band (PPB) (70, 71, 98). Of the six Kinesin-12s in *A. thaliana*, however, one has been implicated in spindle assembly: It acts in the spindle midzone and contributes to microtubule sliding and bundling, resulting in the birth of a robust spindle apparatus (45). Such a function, if essential, must have been shared with other motors because its loss leads to few, if any, changes in overall plant growth and reproduction.

2.3.4. Kinesin-14 and the establishment of spindle poles. Among the six classes of the plant Kinesin-14 motors, motors similar to those found in other kingdoms, e.g., the yeast karyogamy 3 protein (Kar3p), fruit fly nonclaret disjunctional (Ncd), and human SET (HSET), have been tied to the formation of convergent spindle poles in plant cells. These particular Kinesin-14 motors have a nucleotide-independent microtubule-binding domain at the N terminus and catalytic motor domain at the C terminus that are often separated by coiled coils responsible for dimerization (124). They move toward microtubule minus ends while carrying parallel microtubules as the cargo so that the filaments converge toward their minus ends; this action is often enhanced by achieving a processive motility through motor oligomerization (150). The loss of the *A. thaliana* Kinesin-14 motor kinesin-like protein in *Arabidopsis thaliana* A (KatA)/*Arabidopsis thaliana* kinesin 1 (ATK1) leads to the segmentation of spindle poles and consequently missegregation of chromosomes during male meiosis but does not seem to jeopardize mitosis (28, 86). Because losing a highly homologous ATK5 kinesin causes very similar defects in spindle pole organization without harming the outcome of mitosis and the simultaneous loss of both ATK1 and ATK5 leads to lethality (2, 4, 117), these motors may oligomerize in vivo in order to boost the robustness of the assembly of the spindle apparatus with convergent poles. The dependence of spindle pole organization on ATK1 and ATK5 has been recapitulated in maize in the context of both meiosis and endosperm development (46, 50). Plants produce other Kinesin-14 motors with elevated

expressions during mitosis (141). Because these motors possess structural novelties not found in other known motors in this subfamily, they likely exercise yet-unknown functions.

Aurora kinases (AURs): evolutionarily conserved M-phase-specific kinases, including α-Aurora, which exhibits a spindle pole–biased association with microtubules, and β-Aurora, which forms a chromosomal passenger complex in the centromere in plants

2.3.5. Do Kinesin-13 and Kinesin-8 function as microtubule depolymerases in plant mitosis? In vertebrates, Kinesin-13 does not walk on microtubules. Instead, it functions as an essential enzyme that depolymerizes microtubules at their ends so that long cytoplasmic filaments are depolymerized and replaced by shorter ones assembled into the spindle array at prometaphase and kinetochore microtubules can be shortened at their ends during anaphase (145). Such activity was also associated with the Kinesin-8 motor that walks to the plus end to depolymerize microtubules. Plants produce both Kinesin-8 and Kinesin-13. However, several lines of evidence show that they may function differently from their counterparts in fungi and animals. For example, Kinesin-13A decorates Golgi stacks and regulates cell morphogenesis in highly differentiated leaf trichome cells and also plays a role in secondary cell wall thickening in the xylem tissue in *A. thaliana* (84, 106). Furthermore, in *P. patens*, neither Kinesin-8 nor Kinesin-13 exhibits microtubule depolymerase activities in vitro, and both are dispensable as their null mutants did not prevent mitotic progression (74). Therefore, the microtubule depolymerization activity characteristic of Kinesin-13, if essential, must have landed on other proteins that may or may not be kinesins.

3. REGULATION OF CYTOSKELETAL PROTEINS BY PHOSPHORYLATION

Cell cycle–dependent functions of microtubule-associated factors are often turned on and off by reversable phosphorylation events. Cyclin-dependent kinases (CDKs) have been detected on spindle microtubules (148). Coincidentally, the M-phase-specific cyclin B3;1, like CDKA;1, shows a spindle association in both mitotic and meiotic cells (19, 23). Collectively, proteins associated with spindle microtubules likely are targeted by CDKA/cyclin B (CYCB) for M-phase-specific phosphorylation. While proteins such as Kinesin-5 and others have been speculated to be possible substrates, one MAP65 isoform is a confirmed substrate, and abolishing its CDK-dependent phosphorylation leads to excessive accumulation of spindle microtubules so that cell cycle progression is inhibited (127). Although there is little if any doubt that such phosphorylation is essential for mitosis, it is unknown what other spindle- and chromosome-associated factors may be regulated by CDKA/CYCB.

Eukaryotes also produce kinases other than CDKs that act more specifically on the spindle apparatus and chromosomes at M phase. These so-called mitotic kinases primarily comprise Aurora kinases (AURs) and Polo-like kinases in fungi and animals. However, plants have only AURs (152). Like vertebrates, plant AURs are classified into spindle-microtubule-associated α-Aurora and centromere-localized β-Aurora (147). AURs are probably master regulators of plant spindle assembly and mitosis because either α- or β-Aurora is essential in *A. thaliana* (58, 140). In contrast to vertebrates in which spindle-microtubule-associated AURA (a.k.a. AURK or AUR-A) is dependent on TPX2 for localization and activation, the TPXL3, but not the canonical TPX2, complexes with α-Aurora and becomes its essential activator in *A. thaliana* (18). One of the key targets of α-Aurora/TPXL3 is the γ-TuRC because its spindle association is dependent on the kinase complex (33). New substrates of α-Aurora/TPXL3 will likely be detected by in-depth studies of plant mitosis.

Besides CDK and Aurora, one of the seven never-in-mitosis A (NIMA)-related kinases in *A. thaliana*, NEK6, also is associated with all microtubule arrays in dividing cells but is mostly implicated in polarized cell expansion (97). In addition, the RUNKEL (RUK) kinase, possessing a

microtubule-binding site, decorates all mitotic microtubule arrays as well, but its function is mostly linked to phragmoplast expansion during cytokinesis (65). The chromosome-associated Haspin-like kinase demonstrates cell cycle–dependent phosphorylation of histone H3, an essential event in mitosis (8, 66). Whether these kinases function in concert with CDK and/or Aurora to regulate the remodeling of spindle microtubule arrays and chromosomes, as well as their interaction for mitotic progression in plant cells, has yet to be tested.

Microtubule-organizing centers (MTOCs): cytoplasmic sites enriched with microtubule-nucleating factors to generate new microtubules; often they are not structure based

Polar caps: acentrosomal microtubule-organizing centers established on the nuclear envelope during prophase in plant cells

4. THE BIRTH OF THE BIPOLAR SPINDLE MICROTUBULE ARRAY

To carry out karyokinesis, the spindle microtubule array must establish two poles toward which sister chromatids will be segregated. Centrosomal spindles establish this feature by placing two duplicated centrosomes at the opposite poles. Acentrosomal spindles do so in the absence of structurally defined microtubule-organizing centers (MTOCs). It is worth noting that the term MTOC was created over half a century ago to describe acentrosomal spindle poles based on where spindle microtubules were believed to be generated in dividing plant cells (115). However, the term MTOC is often used as a synonym for the centrosome in the literature now. In fact, cells of plants and other eukaryotes can have locally organized microtubule-nucleating factors to produce unstructured MTOCs. During female meiosis in vertebrates, for example, spindle poles are established by many SAFs, found in the pericentriolar region in centrosomal spindles, assembling into acentriolar microtubule-organizing centers (aMTOCs) while a suite of proteins join together by phase separation into a liquid-like meiotic spindle domain (132). Because plants lack many factors described in the mouse meiotic spindle poles, such as NuMA and proteins associated with cytoplasmic dynein, it is unclear how they establish aMTOCs that could serve as spindle poles. Prior to producing a bipolar array, microtubules are nucleated and organized on the nuclear envelope. When plant cells enter prometaphase, microtubules are nucleated and assembled around chromosomes and individual kinetochore fiber–chromosome complexes are unified in the spindle apparatus. Here, we discuss spindle assembly by emphasizing stage-dependent microtubule organization and potential novel regulators in plants.

4.1. Assembling the Prophase Spindle

In contrast to the separation of duplicated centrosomes at prophase in cultured animal cells, in plant cells a bipolar spindle microtubule array is produced with focused poles on the nuclear envelope without the assistance of the centrosome. In somatic plant cells that form the PPB for division plane orientation, this cortical array likely contributes to the bipolarity of the prophase spindle and its position as well as its orientation via the physical connection of bridging microtubules (3). In fact, spindle bipolarization is seriously hindered when formation of the PPB is compromised by mutations in *A. thaliana* (122). When mitosis takes place in the absence of the PPB, such as that in the endosperm, microtubules on the nuclear envelope may be organized with more than two poles, followed by the dissolution of excess poles prior to or at prometaphase onset (131). The bipolar character of the prophase spindle can be best represented by increasing the concentration of γ-tubulin and perhaps the γ-TuRC toward the poles in what are often referred to as polar caps (72) (**Figure 1*a***). This phenomenon is obvious in cells of some bryophytes that have γ-tubulin concentrated in a centriole-lacking, centrosome-like appearance, which are described as polar organizers (22). In *A. thaliana*, the establishment of the polar cap is dependent on the γ-TuRC, as demonstrated by the inability of the *gcp6* mutant to organize perinuclear microtubules into a bipolar array (91). Such a function likely is regulated by the mitotic kinase α-Aurora, which regulates

the activity of the γ-TuRC on mitotic microtubule arrays (33), and perhaps poleward transport of the γ-TuRC by kinesins in order to generate spindle pole–biased microtubule nucleation activities.

Whether there is a structural basis for the prophase spindle poles remains an intriguing and unresolved question. In bryophytes such as the liverwort *Marchantia*, electron-dense, endoplasmic reticulum–connected membranous elements are organized near the nuclear envelope and serve as polar MTOCs of the prophase spindle (6, 35). However, this structural feature has not been identified in angiosperms, although its existence is often speculated. One possibility is that proteins required for spindle pole assembly may undergo liquid phase separation to form membraneless assemblies that serve as aMTOCs during prophase spindle assembly.

After nuclear envelope breakdown, the focused spindle poles typically dissolve and are replaced by broader ones after kinetochore fibers are produced that account for most of the microtubules of the new spindle apparatus (**Figure 1*b***). On the other hand, kinetochore fibers can be assembled into a bipolar spindle array even if there are defects in the prophase spindle array, as shown in a mutant with defects in PPB assembly (122). Therefore, one may question the biological significance of having the prophase spindle if it is not required for karyokinesis. When the prophase spindle is defective, cells devote a significantly longer time to establishing the bipolar mitotic spindles with chromosomes aligned on the metaphase plate (91, 122). A recent study also demonstrated that cytoplasmic MTOCs such as the prophase spindle poles play a role in spindle orientation, and this function is particularly pronounced during asymmetric cell division (63). Therefore, the bipolar prophase spindle contributes to the robustness of mitosis and perhaps plays a cytokinetic role, as exemplified by the physical connection between the PPB and spindle poles for cell division plane determination. This connection is also echoed by a recent finding that the loss of the PPB- and cortical division site–associated MAP TANGLED 1 (TAN1) leads to a temporary collapse of spindle microtubule arrays followed by a recovery that allows mitosis to progress (87).

4.2. Formation of the Spindle Poles

Compared to prophase spindles with highly focused poles, the mitotic spindle apparatus has poles exhibiting great plasticity in terms of width so that the spindles are often described as anastral or, for those with wide poles, barrel-shaped. No matter how wide the poles are, however, spindle microtubules always demonstrate the nature of convergence, behaving as if they were bending toward the magnetic poles of so-called microtubule-converging centers or polar cones (88, 129). Although we are still missing the full understanding of the mechanisms that regulate the formation of the spindle poles, some key proteins have been detected in *A. thaliana* and other plants.

α-Aurora plays a pivotal role in the activation of spindle pole organization, and perhaps this is part of the reason why the kinase is indispensable. In a mutant with α-Aurora with partially compromised function, spindle poles become diffuse and disorganized, and the phenotype can be recapitulated by repressing the expression of TPXL3, the protein that targets and activates α-Aurora (33). The α-Aurora/TPXL3 complex, required for γ-tubulin association with spindle microtubules, is biased toward the two poles. Because the γ-TuRC is essential for spindle pole organization (91), it is plausible that the α-Aurora/TPXL3 complex activates the microtubule nucleation function of the γ-TuRC, and such pole-biased activity perhaps critically contributes to the formation of spindle poles (**Figure 2**).

One well-accepted model for producing acentrosomal spindle poles involves the function of microtubule minus-end-directed motors, such as cytoplasmic dynein and Kinesin-14 in animal cells. In a variety of different plant mutants lacking the conserved Kar3/NCD-type Kinesin-14 motors, the spindle poles become greatly widened (4, 46, 86). It will be interesting to test whether

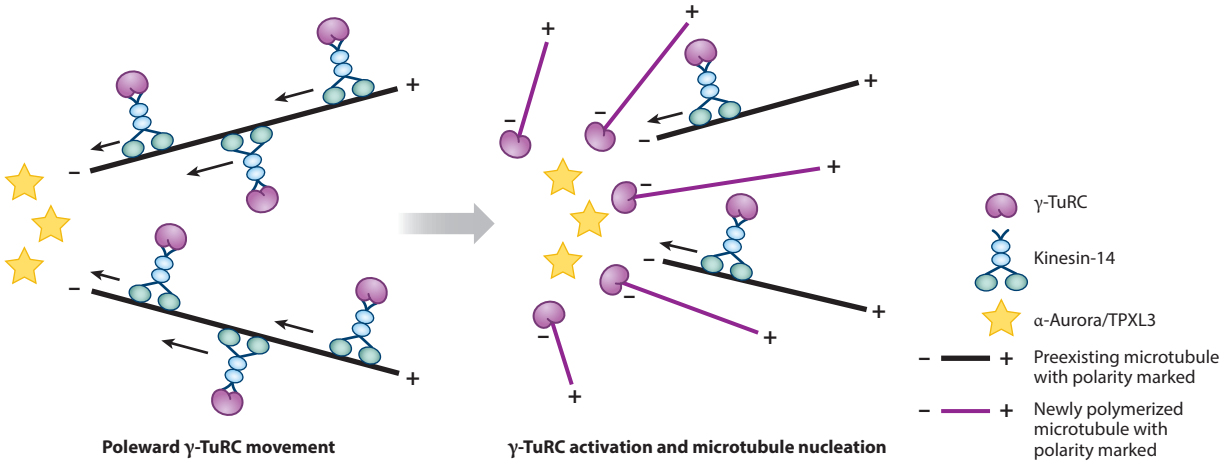

Figure 2

Spindle pole organization. Microtubule-nucleating factors such as the γ-TuRC and its associated proteins are concentrated toward the spindle pole by Kinesin-14, which generates microtubule minus-end-directed motility. Toward the spindle pole, these factors are phosphorylated by the pole-biased α-Aurora/TPXL3 complex and activated to nucleate new microtubules. New microtubules polymerized toward the spindle midzone are stabilized and join others in the array. Abbreviations: γ-TuRC, γ-tubulin ring complex; TPXL3, TPX2-like 3.

these Kinesin-14 motors function in the poleward motility of the γ-TuRC, as demonstrated in cultured human cells (68), or act in an independent pathway to regulate spindle pole organization.

4.3. Kinetochore Fibers in the Mitotic Spindle

A well-received model for kinetochore fiber formation in vertebrate mitosis involves a Ran-GTP gradient generated in the vicinity of chromosomes that induces microtubule nucleation in a TPX2-dependent manner (27). In plants, however, the dispensability of the canonical TPX2 for spindle assembly raises the question of whether a similar rule applies. While chromosomes are capable of generating new microtubules, other microtubules that grow out from sites such as spindle poles also may encounter chromosomes. The end-on kinetochore fibers are probably formed following the initial lateral interaction between microtubules and kinetochores, when microtubules from spindle poles search for the chromosomes and/or kinetochores capture microtubules. This notion is supported by the observation of spindle assembly during mitosis in generative cells of the spiderwort *Tradescantia virginiana* (78). One or more plus-end-directed kinesin motors may be responsible for the transition from the lateral kinetochore–microtubule association to the end-on attachment, and such motors are yet to be identified in plants. Each kinetochore fiber, described as a microtubular fir tree in *Haemanthus* endosperm, has many fine microtubules nucleated and branched away from the trunk that links to the kinetochore (10). A chromosome-kinetochore fiber complex perhaps may be an autonomous structure and may be considered as a minispindle module (21) (**Figure 3**). Many such complexes, flanked by fine microtubules, give rise to the mitotic apparatus as "a forest of microtubular fir trees" (11, p. 175). In the context of bipolarization, we envision the minispindle module as oppositely oriented, kinetochore-attached trunks of microtubules that generate branched microtubules in an augmin-γ-TuRC-dependent manner. Additional antiparallel microtubules, such as those in the prophase spindle or interpolar microtubules, perhaps are independently nucleated to augmin continuously (**Figure 3**). Together, they form a dynamic spindle array.

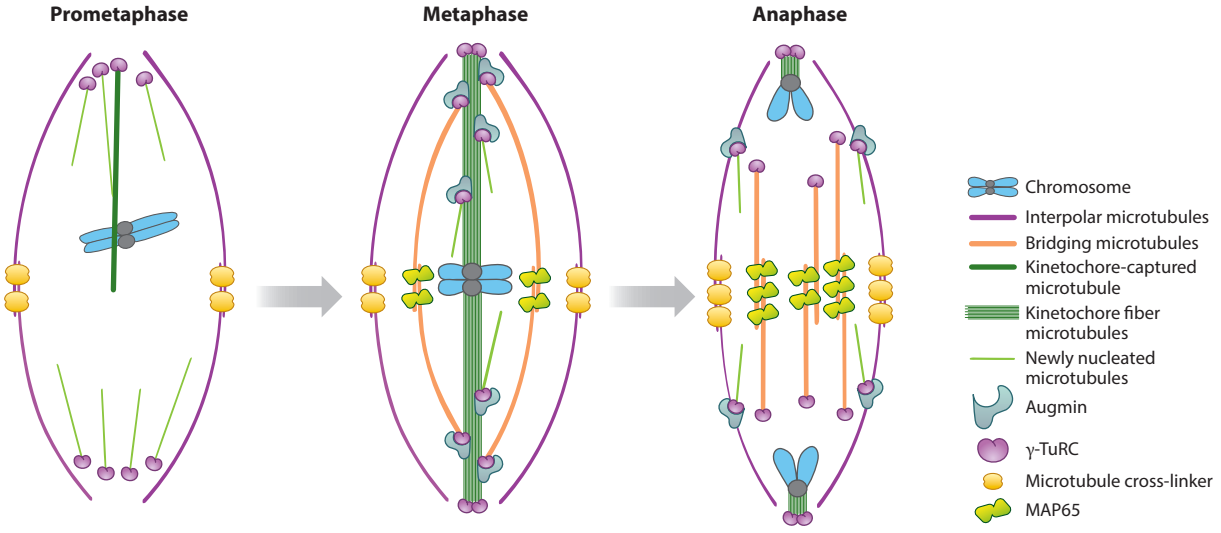

Figure 3

Organization of the minispindle module and development of the central spindle. At prometaphase following nuclear envelope breakdown, chromosomes encounter microtubules that are nucleated from spindle poles by the γ-TuRC, and kinetochores capture nearby microtubules. The lateral kinetochore–microtubule interaction is eventually replaced by end-on attachment of microtubules when the kinetochore is brought to their plus ends. Microtubules attached to the kinetochore are stabilized and have additional microtubules nucleated by the γ-TuRC join them, giving rise to the kinetochore fiber. At metaphase, each pair of kinetochore fibers has opposite microtubule trunks linked to the paired kinetochores. Branched microtubules are nucleated from the trunk by augmin and the γ-TuRC and cross-linked with microtubules generated from the opposite side by proteins such as MAP65 to form bridging microtubules. With the chromosome in the amphitelic attachment, the kinetochore fibers and bridging microtubules form an autonomous minispindle. Surrounding minispindles are microtubules inherited from the prophase spindle or the interpolar microtubules that remain connected in the spindle midzone by other proteins, such as the kinesin motors that engage them by minus-end-directed motility and maintain the minimal overlapping region by plus-end-directed motility. Following the shortening of kinetochore fibers at anaphase, microtubules are continuously generated by augmin/γ-TuRC- and microtubule-dependent microtubule nucleation and stabilized by cross-linking factors. Together with the cross-linked microtubules inherited from earlier interpolar microtubules, they form the central spindle. Abbreviations: γ-TuRC, γ-tubulin ring complex; MAP65, microtubule-associated protein 65.

4.4. Organization of Antiparallel Microtubules in the Spindle Midzone

To construct a bipolar spindle array, microtubules generated from two half-spindles are engaged with each other toward their plus ends. Three proteins are often associated with the central spindle to generate overlapping regions of these antiparallel microtubules: the microtubule cross-linkers in the MAP65/anaphase spindle elongation 1 (Ase1)/protein regulating cytokinesis 1 (PRC1) family, Kinesin-5, and Kinesin-14. A plausible model established in vertebrates has PRC1 cross-link antiparallel microtubules so that Kinesin-5 can generate outward force by sliding antiparallel microtubules apart, while Kinesin-14 antagonizes the action so that the microtubules remain engaged (144). Because all three players are highly conserved among eukaryotes, such a model is often applied universally. To date, most experimental findings in plants such as tobacco and *A. thaliana* suggest that MAP65 functions to organize the phragmoplast microtubule array, while Kinesin-14 functions in spindle pole organization, as discussed in Section 4.2. Although antiparallel microtubules in the central spindle are often discussed in the context of spindle elongation during anaphase B, there may be more than one population, e.g., interpolar microtubules nucleated from poles and those nucleated from the paired kinetochore fibers (**Figure 3**). These two populations are believed to synergistically contribute to forming the spindle midzone.

The prophase spindle often is considered the precursor of the mitotic apparatus following the encounter of microtubules and chromosomes. This may be true in some specialized cells, such as the generative cell of male gametophytes (78). By contrast, discrete lagging chromosomes are able to generate kinetochore microtubule fibers (21). This finding is consistent with the observed microtubule nucleation activities at the kinetochores in lily meiotic cells and the kinetochore-based, γ-TuRC-dependent microtubule polymerization activity in mitotic vertebrate cells (51, 93). We suggest that the prophase spindle microtubules, independent of those associated with kinetochores, persist in contributing to the bipolarization of the spindle apparatus at later stages of mitosis. These microtubules inherited from the prophase spindle may become unnoticeable in fluorescent images when taken to reveal dense kinetochore fibers but are clearly visible in *Haemanthus* endosperm cells when microtubules are stained by immunogold labeling and visualized by video-enhanced microscopy (130). These microtubules are not as stable as kinetochore fibers and perhaps are not targeted by MAP65 but may be targeted by other proteins such as kinesin motors.

By contrast, microtubules that originate from kinetochore fibers probably are targeted by MAP65/Ase1/PRC1 proteins. In *A. thaliana*, MAP65-4 highlights microtubules in the spindle midzone that emanate from kinetochore fibers at metaphase where MAP65-3 is undetectable (76). This phenomenon is like the association of the human MAP65 homolog PRC1 with so-called bridging microtubules in the spindle midzone (116). It will be important to test whether one or more other MAP65 isoforms function redundantly with MAP65-4 in the organization of the spindle midzone independent of MAP65-3.

4.5. Missing Players in the Anaphase Spindle

In most studied cases, the poleward segregation of sister chromatids is accomplished by microtubule depolymerization at microtubule plus ends facing the kinetochore and minus ends facing the spindle pole. In animal cells, this segregation is accomplished by the microtubule depolymerase Kinesin-13, coupled with the action of kinetochore-attached, microtubule minus-end-directed motors, such as cytoplasmic dynein, that push the kinetochore against microtubule plus ends to induce depolymerization (89). Such an action is often described in the Pac-Man model, depicting kinetochore-driven microtubule depolymerization while sister chromatids remain engaged with the shortening kinetochore fibers. To date, there is no evidence showing the action of Kinesin-13 at the kinetochore in examined plant species (74, 84). Perhaps one or more of the yet-to-be-examined Kinesin-14 motors, owing to their minus-end-directed motility, may have taken the mission to drive anaphase A. Serendipitous investigations have also generated evidence suggesting that alternative mechanisms, other than microtubule depolymerization at the ends, may contribute to poleward chromosome movement, e.g., the contribution of the putative spindle matrix made of nonmicrotubule factors such as actin and myosin (114).

Anaphase B can be observed during plant mitosis, albeit in a much smaller magnitude than that in animals, and this spindle elongation process aids in the further separation of chromosomes (43). Aside from the action of cytoplasmic dynein to pull astral microtubules at the cell cortex, bipolar, tetrameric Kinesin-5 motors are known to drive antiparallel microtubule sliding in the central spindle, which is coupled with poleward microtubule flux during anaphase B in animal models (123). Although Kinesin-5 is well conserved in plants and is associated with the bipolarization of the spindle microtubule array (13), whether one or more isoforms play an anaphase role is unknown. However, Kinesin-5 has been suggested to generate microtubule-sliding force in the phragmoplast (7), which may be a continuation of similar activity at anaphase. In summary, we know very little, if anything, about the molecular mechanisms that regulate anaphase progression in plant cells as most models established in animals are not applicable.

5. KINETOCHORES AND THE SPINDLE ASSEMBLY CHECKPOINT

5.1. Centromere Proteins

The centromere represents the constriction point on a eukaryotic chromosome. It is distinguished from the rest of the chromatin by the recruitment of a histone H3 variant, called centromeric histone 3 (CENH3), to the nucleosomes of centromeric chromatin. CENH3, highly variable among eukaryotes, is known to the cell biology community as centromere protein A (CENP-A) and was once named histone three–related 12 (HTR12) in flowering plants (136). Built on the centromeric chromatin is the proteinaceous kinetochore that serves as the primary attachment point of spindle microtubules to a chromosome during mitosis and meiosis. Kinetochores in mitotic animal cells exhibit a laminar structure of inner kinetochore and outer kinetochore domains docked on the centromere (chromatin) domain, and they have distinct proteins associated with them (29). Prior to microtubule attachment, the outer kinetochore is decorated with a fibrous corona with proteins that promote interaction with microtubules and regulate the SAC (61). However, such a laminar structure has not been confirmed in plant kinetochores when processed similarly to animal cells for ultrastructural studies. Furthermore, samples processed by rapid freezing and freeze substitution also did not render structural features found in animal kinetochores (F. Guo & B. Liu, unpublished observation). Instead, plant kinetochores exhibit a ball-like appearance and often are described as a ball and socket (9). The structural difference between plant and animal kinetochores implies that they may have different assemblies of proteins during mitosis. Such a structural difference may be linked to differences in molecular mechanisms that regulate spindle assembly in plants versus animals.

Centromere/kinetochore biology is indebted to autoimmune patients with calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (CREST) syndrome who have produced anticentromere antibodies (ACAs). The ACAs led to the discoveries of many proteins collectively called centromere proteins (CENPs) (20). Although initially discovered in vertebrates, some CENPs are conserved across kingdoms (142). Due to the lack of molecular tools in early examinations of plant centromeres/kinetochores by light microscopy, a few ACAs were adopted to probe mitotic cells of plants such as *Haemanthus* and *Tradescantia* that produce sizable chromosomes (95, 108). Although what the ACAs recognized was unknown at the time, the detection of plant and animal kinetochores by common antibodies suggested that certain kinetochore proteins likely are conserved across the two kingdoms. Decades of heroic efforts to identify CENPs were supplemented by discoveries of more proteins through molecular genetic and proteomic studies of the kinetochore so that there is now a clear roadmap depicting how proteins are organized from the centromeres to kinetochore microtubules in vertebrates (61). A similar roadmap is yet to be constructed in plant cells because many vertebrate centromere and kinetochore proteins do not have obvious homologs when compared to polypeptide sequences deduced from sequenced plant genomes.

Linking the centromeric chromatin and the kinetochore is the constitutive centromere-associated network (CCAN), which is made of four protein complexes (42). Among the dozens of proteins in the vertebrate CCAN, most are not found in land plant proteomes (152), suggesting that plants may have a suite of novel CENPs to construct the CCAN. However, among the few that are conserved, CENP-C is detected in the inner kinetochore domain throughout the cell division cycle in maize (31). Three other CCAN proteins were hypothesized to be CENP homologs in land plants but lack the kinetochore localization when examined in the moss *P. patens* (64). CENP-C directly interacts with CENP-A, bringing the network of the KNL1, MIS12, and NDC80 complexes (KMN) to the centromere (42). Unlike those in the CCAN, proteins in the KMN network are mostly conserved in plants with significant homologies with their animal counterparts and are associated with the kinetochores (64, 152). The KMN network governs

the association of kinetochores with the plus end of kinetochore fiber microtubules through the NDC80 protein, which bears a microtubule-binding calponin homology (CH) domain (149). The NDC80 complex recruits the Ska complex, which also directly binds to microtubules and strengthens the kinetochore–microtubule attachment (44). In *P. patens*, depletion of representative kinetochore proteins led to not only a lagging chromosome phenotype but also, surprisingly, defects in cytokinesis, implying that perhaps the kinetochore harbors signaling events that are critical for later stages of the cell division cycle (64).

5.2. β-Aurora and the Chromosomal Passenger Complex

Besides attaching chromosomes to microtubules, the kinetochore also serves as a signaling hub to monitor the bivalent attachment of replicated chromosomes, marking the successful assembly of the mitotic spindle, and modulates the functions of proteins associated with the mitotic apparatus through phosphorylation and dephosphorylation. One of the most noticeable controllers at the centromere/kinetochore is a form of the AUR known as Aurora B in animals and β-Aurora in plants (32). In animals and fungi, Aurora B forms the chromosomal passenger complex (CPC) with scaffolding proteins inner centromere protein (INCENP), Borealin, and Survivin (26). The CPC has earned its name for first appearing at the centromere but not traveling with the segregating chromosomes at anaphase. Thus, it not only targets proteins associated with the centromeric chromatin but also phosphorylates microtubule-associated factors in the spindle midzone and proteins in the actomyosin contractile ring for cytokinesis in fungi and animals. Following the identification of β-Aurora, it was speculated that one of the scaffolding factors of the CPC existed in *A. thaliana* because of very limited sequence homology with animal INCENP (55). This INCENP-related Wyrd (WYR) protein plays a role in mitotic division and is critical for cell fate determination in the female gametophyte. The BOREALIN-RELATED (BORR) protein has also been discovered among proteins encoded by genes that exhibit cell cycle–dependent expression patterns even though the homology is also very limited (58). BORR plays a role in the centromeric localization of β-Aurora, and together they localize to the midzone of the central spindle during late anaphase and telophase but do not accumulate in the expanding phragmoplast. Hence, the plant CPC likely shares the mitotic function at the centromere with the animal counterpart but not during cytokinesis.

Phosphorylation of residual proteins and microtubule-associated factors at the centromere and kinetochore are catalyzed by β-Aurora in plants (152). Although it is unclear what proteins at the centromere and kinetochore, other than CENH3, are phosphorylated by β-Aurora, perhaps it is safe to hypothesize that plants solely rely on this kinase for error correction when it regulates functions of yet-to-be-identified microtubule-associated factors.

5.3. Activation of the Spindle Assembly Checkpoint

To establish and monitor chromosome biorientation or the amphitelic kinetochore–microtubule attachment when sister kinetochores are attached to microtubules from opposite poles in animal cells, proteins are recruited to the kinetochore and assembled into the fibrous corona. Among them, a critical factor is the Rod-ZW10-Zwilch (RZZ) complex, which is responsible for recruiting additional proteins such as Spindly and cytoplasmic dynein with its regulatory protein dynactin. Once the kinetochore is attached to microtubules, proteins in the fibrous corona are removed by cytoplasmic dynein–dependent, microtubule minus-end-directed transport (61). However, other than a protein distantly related to ZW10 (133), plants do not have obvious homologs of most of the proteins in these complexes (152), implying different proteins may be employed to fulfill this essential task during mitosis. Additionally, plants likely do not assemble a kinetochore-associated RZZ-like complex because the ZW10 homolog, known as MAG2-interacting protein 1 (MIP1) in

A. thaliana, is detected in an endoplasmic reticulum–localized protein complex for storage proteins leaving the endoplasmic reticulum (77). Thus, an unidentified novel machinery presumably fulfills the tasks assigned to the animal RZZ complex.

To monitor the kinetochore–microtubule attachment, the monopolar spindle 1 (MPS1) kinase and proteins collectively known as budding uninhibited by benzimidazole (BUB) and mitotic arrest deficient (MAD) function as the machinery of the SAC prior to anaphase onset (100). They depend on the KMN network to arrive at the kinetochore in order to release the mitotic arrest signal by the assembly of the MCC, which prevents the activation of the anaphase-promoting complex/cyclosome (APC/C) (62). In vertebrates, activated SAC, either at prometaphase or when spindle microtubules are challenged, is reported by the association of MPS1 to the NDC80 complex, where it phosphorylates kinetochore scaffold 1 (KNL1) (also known as Blinkin) at the Met-Glu-Leu-Thr (MELT) motifs in order to recruit the BUB1 and BUB1-related 1 (BUBR1) kinases (62). The localization and function of BUB1 and BUBR1 are thought to be dependent on their binding partner, the WD40 repeat protein BUB3, as demonstrated in vertebrates. When the SAC is triggered at the kinetochore in animal cells, the MAD2 protein is activated by MAD1 while BUBR1 is activated by BUB1 so that a functional MCC can be assembled by BUBR1, BUB3, MAD2, and cell division cycle 20 (CDC20) (83). Consequently, CDC20 cannot activate APC/C, so cells do not enter anaphase.

SAC activation, as described in the previous paragraph, cannot simply be applied in plant cells due to both the sequence divergence and the localizations and functions of SAC proteins (57). While MPS1, BUB3, MAD1, and MAD2 are conserved in plants, gene family expansion has complicated the situation. For example, in *A. thaliana*, three BUB1/MAD3 family (BMF) proteins are related to animal BUB1 and BUBR1/MAD3 by possessing discrete domains. BMF1, not BMF2 or BMF3, is the only one carrying a kinase domain. Furthermore, none of the BMF proteins contain a BUB3-interacting Gle2-binding-sequence (GLEBS), which is essential for the kinetochore localization and function of BUB1 and BUBR1/MAD3 in animal cells. Conversely, BMF1 and BMF2, but not BMF3, are recruited to kinetochores directly by the KNL1 homolog that lacks the MELT motif and functions in preventing chromosome lagging during mitosis, as tested in maize (134). Unlike their animal counterparts, plant MPS1 and BMF1 decorate the kinetochore throughout the mitotic cell cycle, while BMF3 and MAD1 exhibit localization at unattached kinetochores only at late prophase and prometaphase (57). MAD2, however, exhibits a predominant cytoplasmic localization, and its kinetochore localization does not stand out in *A. thaliana*, in contrast to the predominant localization at kinetochores lacking microtubule attachment in maize (153). Furthermore, MPS1, BMF1, BMF2, and MAD2 are not required for the kinetochore localization of other SAC proteins, and only BMF3 is required for MAD1 localization. Such activated SAC-dependent kinetochore localization recapitulates the BUB1-dependent recruitment of MAD1 in vertebrates in order for it to induce the conformational change of MAD2 (85). Therefore, BMF3 associates with unattached kinetochores and recruits MAD1 so that they are phosphorylated by MPS1 before being released to interact with MAD2, which is available in the cytosol following its association with CDC20, the APC/C activator.

Furthermore, none of the SAC proteins are required for plant growth and reproduction, while the animal counterparts often are essential. However, failed SAC often manifests in compromised cell division and, consequently, aborted root growth when low doses of microtubule-depolymerizing agents like oryzalin are applied, as demonstrated by most SAC mutants in *A. thaliana*. For wild-type plants, such doses would only slow down but not stop seedling growth. Sensitivity to microtubule-depolymerizing agents distinguishes BMF1 from other SAC proteins because its absence does not seriously affect cell division and seedling growth upon oryzalin challenges, suggesting that BMF1-dependent phosphorylation is not essential for SAC activation.

BUB1 becomes essential for the SAC in fungi, which, like plants, lack the RZZ complex, but becomes unessential for SAC response in vertebrates (30). Surprisingly the well-conserved BUB3 isoforms in *A. thaliana*, BUB3.1 and BUB3.2, do not appear to function in the SAC but instead function in the phragmoplast midzone where they interact with MAP65-3 to regulate cytokinesis (155). By contrast, the more divergent form of BUB3, BUB3.3, is most likely critical for the SAC because of the oryzalin-hypersensitive phenotype in the knockout mutant. However, a functional BUB3.3–green fluorescent protein (GFP) fusion has not been detected at the kinetochore (57, 75).

These findings of structural and functional features of SAC proteins raise the question of what constitutes the equivalent of the MCC in plants and how it works. We hypothesize that kinetochores lacking the amphitelic microtubule attachment have BUB3.3 recruit BMF3 and MAD1, which are probably phosphorylated by MPS1 and primed to assemble the MCC of BMF3, MAD2, and CDC20, inhibiting anaphase onset in plants (**Figure 4**). When the SAC is turned off,

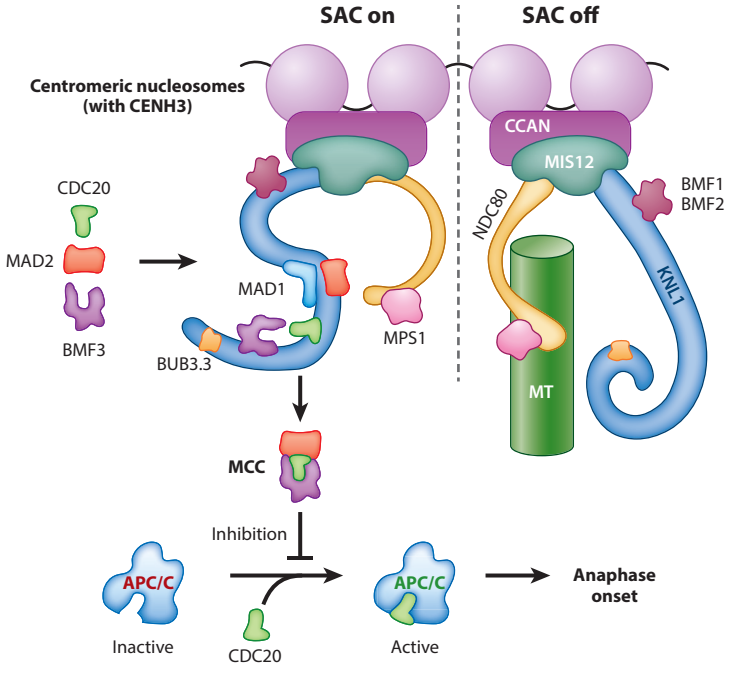

Figure 4

Activation of the SAC in plants. The centromere is defined by nucleosomes containing the CENH3, with which the CCAN, including CENP-C, is associated. The MIS12 complex interacts with CENP-C in order to dock on the CCAN. Both the KNL1 and NDC80 complexes can then associate with the kinetochore via MIS12. The NDC80 complex brings in the SAC kinase MPS1 and interacts with kinetochore fiber microtubules. The KNL1 protein recruits most SAC proteins. While the SAC proteins such as BMF1 localize to the kinetochores throughout mitosis, others such as MAD1 and BMF3 only appear at the kinetochore when the SAC is activated. SAC activation induces the formation of the MCC of, perhaps, MAD2, BMF3, and CDC20 to prevent the activation of the APC/C. The SAC is turned off when microtubules make end-on attachments to the kinetochore via NDC80 so that CDC20 is freed to activate the APC/C in order to trigger anaphase onset. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; BMF, BUB1/MAD3 family; BUB3.3, budding uninhibited by benzimidazole 3.3; CCAN, constitutive centromere-associated network; CDC20, cell division cycle 20; CENH3, centromeric histone 3; CENP-C, centromere protein C; KNL1, kinetochore scaffold 1; MAD, mitotic arrest deficient; MCC, mitotic checkpoint complex; MIS12, minichromosome instability 12; MPS1, monopolar spindle 1; MT, microtubule; NDC80, nuclear division cycle 80; SAC, spindle assembly checkpoint.

yet-to-be-discovered microtubule minus-end-directed motors, likely one or more Kinesin-14s, may be responsible for silencing the SAC by removing proteins such as BMF3 and perhaps MAD1 from the microtubule-attached kinetochore. APC/C can then be activated to trigger anaphase onset.

6. NOVEL FACTORS IN SPINDLE ASSEMBLY AND REMODELING

Serendipitous discoveries have pointed at several auspicious factors that may be involved in spindle assembly in plants. Early electron microscopic observations have revealed coincident distribution of calcium-stored endoplasmic reticulum and kinetochore fibers (151). Whether the membrane system provides structural support for the spindle array and whether stored calcium is a critical regulator of microtubule dynamics inside the spindle are unknown. The nuclear pore complex protein MOS7/NUP88 is also considered an essential spindle assembly factor (111). Inside animal spindles, certain NUP proteins play a moonlighting role recruiting the γ-TuRC for microtubule nucleation (93). Whether a similar connection is established in plant cells is unclear. Furthermore, fine actin microfilaments are detected inside plant spindles and often are reorganized according to microtubule arrays (79, 94). How the two cytoskeletal elements work in concert for the progression of mitosis through coordinated remodeling of their three-dimensional reorganization has yet to be explored.

Taken together, spindle assembly is probably a natural consequence when chromosomes find microtubules. Perhaps it should not be surprising to learn that some of the well-studied proteins such as cytoplasmic dynein and TPX2 are either missing or dispensable in the production of the bipolar microtubule array in plants. It reminds us that the centrosome should not be the driver of spindle assembly as vascular plants and red algae together with many cells of centriole-producing organisms can accomplish the task without it. Instead, regulatory proteins form functional units to fine-tune specific features associated with either microtubule dynamics or chromosome motility. While a single unit is likely dispensable, consolidated actions of multiple units become essential for assembling the bipolar spindle array.

SUMMARY POINTS

1. Bipolarization of microtubules takes place independently of chromosomes on the nuclear envelope at prophase.
2. Plant spindles are assembled with the contributions of microtubule-nucleating and microtubule-polymerizing factors but not structurally defined organizing centers.
3. Multiple factors act synergistically for bipolar spindle assembly, and individual factors could be expendable.
4. Formation of the convergent acentrosomal spindle pole is dependent on both microtubule nucleators and Kinesin-14 motors.
5. Microtubule bundles in the spindle midzone are organized by microtubule-bundling microtubule-associated proteins and kinesin motors that regulate spindle bipolarization.
6. Plant cells assemble both conserved and novel proteins into a unique spindle assembly checkpoint network to monitor the alignment of chromosomes at the metaphase plate.
7. Factors that regulate spindle remodeling in anaphase are largely unknown in plants.

FUTURE ISSUES

1. What is the mechanism that regulates the recruitment of proteins such as the γ-tubulin ring complex (γ-TuRC) to trigger microtubule nucleation on the nuclear envelope during prophase?
2. What are the key substrates of α-Aurora kinase that are activated for spindle assembly?
3. How do kinesin motors function in concert to regulate microtubule organization in the spindle pole and central spindle?
4. How does chromosome congression take place at prometaphase?
5. What is the mechanism that regulates the shortening of kinetochore fiber microtubules during anaphase?
6. How do plant cells assemble a functional mitotic checkpoint complex to prevent anaphase onset from taking place precociously?
7. How do plant cells silence the spindle assembly checkpoint when all chromosomes are aligned at the metaphase plate?
8. Are components other than microtubules, such as the actin cytoskeleton, involved in spindle remodeling?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Our investigation of plant mitosis was supported by the National Science Foundation grant MCB-1920358 and the US Department of Agriculture hatch grant CA-D-PLB-2536-H. We thank Xiaojiang Guo and Amanda Liu for preparation of the diagrams.

LITERATURE CITED

1. Alfaro-Aco R, Thawani A, Petry S. 2017. Structural analysis of the role of TPX2 in branching microtubule nucleation. *J. Cell Biol.* 216:983–97
2. Ambrose JC, Cyr R. 2007. The kinesin ATK5 functions in early spindle assembly in *Arabidopsis*. *Plant Cell* 19:226–36
3. Ambrose JC, Cyr R. 2008. Mitotic spindle organization by the preprophase band. *Mol. Plant* 1:950–60
4. Ambrose JC, Li W, Marcus A, Ma H, Cyr R. 2005. A minus-end-directed kinesin with plus-end tracking protein activity is involved in spindle morphogenesis. *Mol. Biol. Cell* 16:1584–92
5. Ambrose JC, Shoji T, Kotzer AM, Pighin JA, Wasteneys GO. 2007. The *Arabidopsis CLASP* gene encodes a microtubule-associated protein involved in cell expansion and division. *Plant Cell* 19:2763–75
6. Apostolakos P, Galatis B. 1985. Studies on the development of the air pores and air chambers of *Marchantia paleacea*. III. Microtubule organization in preprophase-prophase initial aperture cells—formation of incomplete preprophase microtubule bands. *Protoplasma* 128:120–35
7. Asada T, Kuriyama R, Shibaoka H. 1997. TKRP125, a kinesin-related protein involved in the centrosome-independent organization of the cytokinetic apparatus in tobacco BY-2 cells. *J. Cell Sci.* 110:179–89

8. Ashtiyani RK, Moghaddam AMB, Schubert V, Rutten T, Fuchs J, et al. 2011. AtHaspin phosphorylates histone H3 at threonine 3 during mitosis and contributes to embryonic patterning in Arabidopsis. *Plant J.* 68:443–54
9. Bajer A. 1968. Behavior and fine structure of spindle fibers during mitosis in endosperm. *Chromosoma* 25:249–81
10. Bajer A. 1990. The elusive organization of the spindle and kinetochore fiber: a conceptual retrospect. *Adv. Cell Biol.* 3:65–93
11. Bajer AS, Mole-Bajer J. 1989. Microtubules modify kinetochore fiber organization and function: a new aspect of mitosis. *Prog. Clin. Biol. Res.* 318:171–84
12. Bannigan A, Lizotte-Waniewski M, Riley M, Baskin TI. 2008. Emerging molecular mechanisms that power and regulate the anastral mitotic spindle of flowering plants. *Cell Motil. Cytoskelet.* 65:1–11
13. Bannigan A, Scheible W-R, Lukowitz W, Fagerstrom C, Wadsworth P, et al. 2007. A conserved role for kinesin-5 in plant mitosis. *J. Cell Sci.* 120:2819–27
14. Barroso C, Chan J, Allan V, Doonan J, Hussey P, Lloyd C. 2000. Two kinesin-related proteins associated with the cold-stable cytoskeleton of carrot cells: characterization of a novel kinesin, DcKRP120-2. *Plant J.* 24:859–68
15. Baskin TI, Cande WZ. 1990. The structure and function of the mitotic spindle in flowering plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:277–315
16. Bisgrove SR, Lee Y-RJ, Liu B, Peters NT, Kropf DL. 2008. The microtubule plus-end binding protein EB1 functions in root responses to touch and gravity signals in *Arabidopsis*. *Plant Cell* 20:396–410
17. Boleti H, Karsenti E, Vernos I. 1996. Xklp2, a novel Xenopus centrosomal kinesin-like protein required for centrosome separation during mitosis. *Cell* 84:49–59
18. Boruc J, Deng X, Mylle E, Besbrugge N, Van Durme M, et al. 2019. TPX2-LIKE PROTEIN 3 is the primary activator of α-Aurora kinases and is essential for embryogenesis. *Plant Physiol.* 180:1389–405
19. Boruc J, Mylle E, Duda M, De Clercq R, Rombauts S, et al. 2010. Systematic localization of the Arabidopsis core cell cycle proteins reveals novel cell division complexes. *Plant Physiol.* 152:553–65
20. Brenner S, Pepper D, Berns MW, Tan E, Brinkley BR. 1981. Kinetochore structure, duplication, and distribution in mammalian cells: analysis by human autoantibodies from scleroderma patients. *J. Cell Biol.* 91:95–102
21. Brown RC, Lemmon BE. 1989. Minispindles and cytoplasmic domains in microsporogenesis of orchids. *Protoplasma* 148:26–32
22. Brown RC, Lemmon BE. 2007. The pleiomorphic plant MTOC: an evolutionary perspective. *J. Integr. Plant Biol.* 49:1142–53
23. Bulankova P, Akimcheva S, Fellner N, Riha K. 2013. Identification of *Arabidopsis* meiotic cyclins reveals functional diversification among plant cyclin genes. *PLOS Genet.* 9:e1003508
24. Burk DH, Liu B, Zhong R, Morrison WH, Ye ZH. 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13:807–27
25. Buschmann H, Fabri CO, Hauptmann M, Hutzler P, Laux T, et al. 2004. Helical growth of the *Arabidopsis* mutant *tortifolia1* reveals a plant-specific microtubule-associated protein. *Curr. Biol.* 14:1515–21
26. Carmena M, Wheelock M, Funabiki H, Earnshaw WC. 2012. The chromosomal passenger complex (CPC): from easy rider to the godfather of mitosis. *Nat. Rev. Mol. Cell Biol.* 13:789–803
27. Cavazza T, Vernos I. 2015. The RanGTP pathway: from nucleo-cytoplasmic transport to spindle assembly and beyond. *Front. Cell Dev. Biol.* 3:82
28. Chen CB, Marcus A, Li WX, Hu Y, Calzada J-PV, et al. 2002. The *Arabidopsis ATK1* gene is required for spindle morphogenesis in male meiosis. *Development* 129:2401–9
29. Cleveland DW, Mao Y, Sullivan KF. 2003. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell* 112:407–21
30. Currie CE, Mora-Santos M, Smith CA, McAinsh AD, Millar JBA. 2018. Bub1 is not essential for the checkpoint response to unattached kinetochores in diploid human cells. *Curr. Biol.* 28:R909–30

10. Summarizes the kinetochore fiber as an autonomous fir tree–like complex with skewed microtubules growing out of those fibers attached to the kinetochore.

18. The evolutionarily conserved TPX2 does not play a critical role in plant mitosis; the related but divergent TPXL3 protein activates Aurora kinase's function in spindles.

24. Concludes that plants employ proteins other than the conserved microtubule-severing enzyme katanin to induce rapid microtubule turnovers.

31. Dawe RK, Reed LM, Yu HG, Muszynski MG, Hiatt EN. 1999. A maize homolog of mammalian CENPC is a constitutive component of the inner kinetochore. *Plant Cell* 11:1227–38
32. Demidov D, Van Damme D, Geelen D, Blattner FR, Houben A. 2005. Identification and dynamics of two classes of Aurora-like kinases in Arabidopsis and other plants. *Plant Cell* 17:836–48
33. Deng X, Higaki T, Lin H-H, Lee Y-RJ, Liu B. 2021. Acentrosomal spindle morphogenesis is dependent on the unconventional TPX2 family protein TPXL3 and α Aurora kinase in *Arabidopsis thaliana*. *Res. Sq.* **https://doi.org/10.21203/rs.3.rs-519922/v1**
34. Enos AP, Morris NR. 1990. Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. *Cell* 60:1019–27
35. Fowke LC, Pickett-Heaps JD. 1978. Electron microscope study of vegetative cell division in two species of *Marchantia*. *Can. J. Bot.* 56:467–75
36. Gard DL, Becker BE, Romney SJ. 2004. MAPping the eukaryotic tree of life: structure, function, and evolution of the MAP215/Dis1 family of microtubule-associated proteins. *Int. Rev. Cytol.* 239:179–272
37. Gillmor CS, Roeder AH, Sieber P, Somerville C, Lukowitz W. 2016. A genetic screen for mutations affecting cell division in the *Arabidopsis thaliana* embryo identifies seven loci required for cytokinesis. *PLOS ONE* 11:e0146492
38. Goshima G, Mayer M, Zhang N, Stuurman N, Vale RD. 2008. Augmin: a protein complex required for centrosome-independent microtubule generation within the spindle. *J. Cell Biol.* 181:421–29
39. Guo L, Ho CM, Kong Z, Lee Y-RJ, Qian Q, Liu B. 2009. Evaluating the microtubule cytoskeleton and its interacting proteins in monocots by mining the rice genome. *Ann. Bot.* 103:387–402
40. Hamada T, Igarashi H, Itoh TJ, Shimmen T, Sonobe S. 2004. Characterization of a 200 kDa microtubule-associated protein of tobacco BY-2 cells, a member of the XMAP215/MOR1 family. *Plant Cell Physiol.* 45:1233–42
41. Hamada T, Nagasaki-Takeuchi N, Kato T, Fujiwara M, Sonobe S, et al. 2013. Purification and characterization of novel microtubule-associated proteins from Arabidopsis cell suspension cultures. *Plant Physiol.* 163:1804–16
42. Hara M, Fukagawa T. 2020. Dynamics of kinetochore structure and its regulations during mitotic progression. *Cell. Mol. Life Sci.* 77:2981–95
43. Hayashi T, Sano T, Kutsuna N, Kumagai-Sano F, Hasezawa S. 2007. Contribution of anaphase B to chromosome separation in higher plant cells estimated by image processing. *Plant Cell Physiol.* 48:1509–13
44. Helgeson LA, Zelter A, Riffle M, MacCoss MJ, Asbury CL, Davis TN. 2018. Human Ska complex and Ndc80 complex interact to form a load-bearing assembly that strengthens kinetochore–microtubule attachments. *PNAS* 115:2740–45
45. Herrmann A, Livanos P, Zimmermann S, Berendzen K, Rohr L, et al. 2021. KINESIN-12E regulates metaphase spindle flux and helps control spindle size in Arabidopsis. *Plant Cell* 33:27–43
46. Higgins DM, Nannas NJ, Dawe RK. 2016. The maize *Divergent spindle-1* (*dv1*) gene encodes a kinesin-14A motor protein required for meiotic spindle pole organization. *Front. Plant Sci.* 7:1277
47. Ho CMK, Hotta T, Guo F, Roberson RW, Lee Y-RJ, Liu B. 2011. Interaction of antiparallel microtubules in the phragmoplast is mediated by the microtubule-associated protein MAP65-3 in *Arabidopsis*. *Plant Cell* 23:2909–23
48. Ho CMK, Hotta T, Kong Z, Zeng CJ, Sun J, et al. 2011. Augmin plays a critical role in organizing the spindle and phragmoplast microtubule arrays in *Arabidopsis*. *Plant Cell* 23:2606–18
49. Hotta T, Kong Z, Ho C-MK, Zeng CJT, Horio T, et al. 2012. Characterization of the *Arabidopsis* augmin complex uncovers its critical function in the assembly of the acentrosomal spindle and phragmoplast microtubule arrays. *Plant Cell* 24:1494–509
50. Huang Y, Wang H, Huang X, Wang Q, Wang J, et al. 2019. Maize VKS1 regulates mitosis and cytokinesis during early endosperm development. *Plant Cell* 31:1238–56
51. Inoue S. 1981. Cell division and the mitotic spindle. *J. Cell Biol.* 91:131s–47s

49. Functional characterization of the plant augmin complex concludes that there is an evolutionarily conserved mechanism of microtubule-dependent microtubule generation in plant spindle assembly.

52. Janski N, Masoud K, Batzenschlager M, Herzog E, Evrard JL, et al. 2012. The GCP3-interacting proteins GIP1 and GIP2 are required for γ-tubulin complex protein localization, spindle integrity, and chromosomal stability. *Plant Cell* 24:1171–87
53. Kawamura E, Himmelspach R, Rashbrooke MC, Whittington AT, Gale KR, et al. 2006. MICROTUBULE ORGANIZATION 1 regulates structure and function of microtubule arrays during mitosis and cytokinesis in the Arabidopsis root. *Plant Physiol.* 140:102–14
54. Kirik V, Herrmann U, Parupalli C, Sedbrook JC, Ehrhardt DW, Hülskamp M. 2007. CLASP localizes in two discrete patterns on cortical microtubules and is required for cell morphogenesis and cell division in *Arabidopsis*. *J. Cell Sci.* 120:4416–25
55. Kirioukhova O, Johnston AJ, Kleen D, Kagi C, Baskar R, et al. 2011. Female gametophytic cell specification and seed development require the function of the putative *Arabidopsis* INCENP ortholog *WYRD*. *Development* 138:3409–20
56. Komaki S, Abe T, Coutuer S, Inzé D, Russinova E, Hashimoto T. 2010. Nuclear-localized subtype of end-binding 1 protein regulates spindle organization in *Arabidopsis*. *J. Cell Sci.* 123:451–59
57. Komaki S, Schnittger A. 2017. The spindle assembly checkpoint in *Arabidopsis* is rapidly shut off during severe stress. *Dev. Cell* 43:172–85.E5
58. Komaki S, Takeuchi H, Hamamura Y, Heese M, Hashimoto T, Schnittger A. 2020. Functional analysis of the plant chromosomal passenger complex. *Plant Physiol.* 183:1586–99
59. Kong Z, Hotta T, Lee Y-RJ, Horio T, Liu B. 2010. The γ-tubulin complex protein GCP4 is required for organizing functional microtubule arrays in *Arabidopsis thaliana*. *Plant Cell* 22:191–204
60. Kong Z, Ioki M, Braybrook S, Li S, Ye ZH, et al. 2015. Kinesin-4 functions in vesicular transport on cortical microtubules and regulates cell wall mechanics during cell elongation in plants. *Mol. Plant* 8:1011–23
61. Kops GJPL, Gassmann R. 2020. Crowning the kinetochore: the fibrous corona in chromosome segregation. *Trends Cell Biol.* 30:653–67
62. Kops GJPL, Snel B, Tromer EC. 2020. Evolutionary dynamics of the spindle assembly checkpoint in eukaryotes. *Curr. Biol.* 30:R589–602
63. Kosetsu K, Murata T, Yamada M, Nishina M, Boruc J, et al. 2017. Cytoplasmic MTOCs control spindle orientation for asymmetric cell division in plants. *PNAS* 114:E8847–54
64. Kozgunova E, Nishina M, Goshima G. 2019. Kinetochore protein depletion underlies cytokinesis failure and somatic polyploidization in the moss *Physcomitrella patens*. *eLife* 8:e43652
65. Krupnova T, Sasabe M, Ghebreghiorghis L, Gruber CW, Hamada T, et al. 2009. Microtubule-associated kinase-like protein RUNKEL needed for cell plate expansion in *Arabidopsis* cytokinesis. *Curr. Biol.* 19:518–23
66. Kurihara D, Matsunaga S, Omura T, Higashiyama T, Fukui K. 2011. Identification and characterization of plant Haspin kinase as a histone H3 threonine kinase. *BMC Plant Biol.* 11:73
67. Lawrence EJ, Zanic M, Rice LM. 2020. CLASPs at a glance. *J. Cell Sci.* 133:jcs243097
68. Lecland N, Lüders J. 2014. The dynamics of microtubule minus ends in the human mitotic spindle. *Nat. Cell Biol.* 16:770–78
69. Lee Y-RJ, Hiwatashi Y, Hotta T, Xie T, Doonan JH, Liu B. 2017. The mitotic function of augmin is dependent on its microtubule-associated protein subunit EDE1 in *Arabidopsis thaliana*. *Curr. Biol.* 27:3891–97.E4
70. Lee Y-RJ, Li Y, Liu B. 2007. Two *Arabidopsis* phragmoplast-associated kinesins play a critical role in cytokinesis during male gametogenesis. *Plant Cell* 19:2595–605
71. Lee Y-RJ, Liu B. 2013. The rise and fall of the phragmoplast microtubule array. *Curr. Opin. Plant Biol.* 16:757–63
72. Lee Y-RJ, Liu B. 2019. Microtubule nucleation for the assembly of acentrosomal microtubule arrays in plant cells. *New Phytol.* 222:1705–18
73. Lee Y-RJ, Qiu W, Liu B. 2015. Kinesin motors in plants: from subcellular dynamics to motility regulation. *Curr. Opin. Plant Biol.* 28:120–26
74. Leong SY, Edzuka T, Goshima G, Yamada M. 2020. Kinesin-13 and Kinesin-8 function during cell growth and division in the moss *Physcomitrella patens*. *Plant Cell* 32:683–702

57. Dissection of proteins associated with the spindle assembly checkpoint (SAC) in plant mitosis leads to the discovery of novel aspects of SAC regulation.

60. Concludes that Kinesin-4 is dispensable in plant mitosis.

74. Provides evidence demonstrating that neither Kinesin-8 nor Kinesin-13 is an important regulator of spindle assembly in moss cells.

75. Lermontova I, Fuchs J, Schubert I. 2008. The *Arabidopsis* checkpoint protein Bub3.1 is essential for gametophyte development. *Front. Biosci.* 13:5202–11
76. Li H, Sun B, Sasabe M, Deng X, Machida Y, et al. 2017. *Arabidopsis* MAP65-4 plays a role in phragmoplast microtubule organization and marks the cortical cell division site. *New Phytol.* 215:187–201
77. Li L, Shimada T, Takahashi H, Koumoto Y, Shirakawa M, et al. 2013. MAG2 and three MAG2-INTERACTING PROTEINs form an ER-localized complex to facilitate storage protein transport in *Arabidopsis thaliana*. *Plant J.* 76:781–91
78. Liu B, Palevitz BA. 1991. Kinetochore fiber formation in dividing generative cells of *Tradescantia* kinetochore reorientation associated with the transition between lateral microtubule interactions and end-on kinetochore fibers. *J. Cell Sci.* 98:475–82
79. Liu B, Palevitz BA. 1992. Organization of cortical microfilaments in dividing root cells. *Cell Motil. Cytoskelet.* 23:252–64
80. Liu P, Würtz M, Zupa E, Pfeffer S, Schiebel E. 2021. Microtubule nucleation: the waltz between γ-tubulin ring complex and associated proteins. *Curr. Opin. Cell Biol.* 68:124–31
81. Liu T, Tian J, Wang G, Yu Y, Wang C, et al. 2014. Augmin triggers microtubule-dependent microtubule nucleation in interphase plant cells. *Curr. Biol.* 24:2708–13
82. Lloyd CW. 1987. The plant cytoskeleton: the impact of fluorescence microscopy. *Annu. Rev. Plant Physiol.* 38:119–39
83. London N, Biggins S. 2014. Signalling dynamics in the spindle checkpoint response. *Nat. Rev. Mol. Cell Biol.* 15:736–47
84. Lu L, Lee Y-RJ, Pan R, Maloof JN, Liu B. 2005. An internal motor kinesin is associated with the Golgi apparatus and plays a role in trichome morphogenesis in *Arabidopsis*. *Mol. Biol. Cell* 16:811–23
85. Luo Y, Ahmad E, Liu ST. 2018. MAD1: kinetochore receptors and catalytic mechanisms. *Front. Cell Dev. Biol.* 6:51
86. Marcus A, Li W, Ma H, Cyr RJ. 2003. A kinesin mutant with an atypical bipolar spindle undergoes normal mitosis. *Mol. Biol. Cell* 14:1717–26
87. Martinez P, Dixit R, Balkunde RS, Zhang A, O'Leary SE, et al. 2020. TANGLED1 mediates microtubule interactions that may promote division plane positioning in maize. *J. Cell Biol.* 219:e201907184
88. Mazia D. 1984. Centrosomes and mitotic poles. *Exp. Cell Res.* 153:1–15
89. McIntosh JR. 2021. Anaphase A. *Semin. Cell Dev. Biol.* 117:118–26
90. McNally FJ, Roll-Mecak A. 2018. Microtubule-severing enzymes: from cellular functions to molecular mechanism. *J. Cell Biol.* 217:4057–69
91. Miao HY, Guo RF, Chen JL, Wang QM, Lee Y-RJ, Liu B. 2019. The γ-tubulin complex protein GCP6 is crucial for spindle morphogenesis but not essential for microtubule reorganization in *Arabidopsis*. *PNAS* 116:27115–23
92. Miki T, Naito H, Nishina M, Goshima G. 2014. Endogenous localizome identifies 43 mitotic kinesins in a plant cell. *PNAS* 111:E1053–61
93. Mishra RK, Chakraborty P, Arnaoutov A, Fontoura BMA, Dasso M. 2010. The Nup107-160 complex and γ-TuRC regulate microtubule polymerization at kinetochores. *Nat. Cell Biol.* 12:164–69
94. Molé-Bajer J, Bajer AS, Inoué S. 1988. Three-dimensional localization and redistribution of F-actin in higher plant mitosis and cell plate formation. *Cell Motil. Cytoskelet.* 10:217–28
95. Mole-Bajer J, Bajer AS, Zinkowski RP, Balczon RD, Brinkley BR. 1990. Autoantibodies from a patient with scleroderma CREST recognized kinetochores of the higher plant *Haemanthus*. *PNAS* 87:3599–603
96. Moschou PN, Gutierrez-Beltran E, Bozhkov PV, Smertenko A. 2016. Separase promotes microtubule polymerization by activating CENP-E-related kinesin Kin7. *Dev. Cell* 37:350–61
97. Motose H, Hamada T, Yoshimoto K, Murata T, Hasebe M, et al. 2011. NIMA-related kinases 6, 4, and 5 interact with each other to regulate microtubule organization during epidermal cell expansion in *Arabidopsis thaliana*. *Plant J.* 67:993–1005
98. Müller S, Livanos P. 2019. Plant kinesin-12: localization heterogeneity and functional implications. *Int. J. Mol. Sci.* 20:4213
99. Murata T, Sonobe S, Baskin TI, Hyodo S, Hasezawa S, et al. 2005. Microtubule-dependent microtubule nucleation based on recruitment of γ-tubulin in higher plants. *Nat. Cell Biol.* 7:961–68

91. Shows that the γ-tubulin ring complex (γ-TuRC) and other novel forms of the γ-tubulin complex function in microtubule nucleation inside the spindle apparatus.

100. Musacchio A. 2015. The molecular biology of spindle assembly checkpoint signaling dynamics. *Curr. Biol.* 25:R1002–18
101. Nakajima K, Furutani I, Tachimoto H, Matsubara H, Hashimoto T. 2004. *SPIRAL1* encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding Arabidopsis cells. *Plant Cell* 16:1178–90
102. Nakamura M, Yagi N, Kato T, Fujita S, Kawashima N, et al. 2012. Arabidopsis GCP3-interacting protein 1/MOZART 1 is an integral component of the γ-tubulin-containing microtubule nucleating complex. *Plant J.* 71:216–25
103. Nakaoka Y, Miki T, Fujioka R, Uehara R, Tomioka A, et al. 2012. An inducible RNA interference system in *Physcomitrella patens* reveals a dominant role of augmin in phragmoplast microtubule generation. *Plant Cell* 24:1478–93
104. Nebenfuhr A, Dixit R. 2018. Kinesins and myosins: molecular motors that coordinate cellular functions in plants. *Annu. Rev. Plant Biol.* 69:329–61
105. Oakley BR, Paolillo V, Zheng Y. 2015. γ-Tubulin complexes in microtubule nucleation and beyond. *Mol. Biol. Cell* 26:2957–62
106. Oda Y, Fukuda H. 2013. Rho of plant GTPase signaling regulates the behavior of *Arabidopsis* Kinesin-13A to establish secondary cell wall patterns. *Plant Cell* 25:4439–50
107. Oh SA, Pal MD, Park SK, Johnson JA, Twell D. 2010. The tobacco MAP215/Dis1-family protein TMBP200 is required for the functional organization of microtubule arrays during male germline establishment. *J. Exp. Bot.* 61:969–81
108. Palevitz BA. 1990. Kinetochore behavior during generative cell division in *Tradescantia virginiana*. *Protoplasma* 157:120–27
109. Palevitz BA. 1993. Morphological plasticity of the mitotic apparatus in plants and its developmental consequences. *Plant Cell* 5:1001–9
110. Panteris E, Adamakis I-DS. 2012. Aberrant microtubule organization in dividing root cells of p60-katanin mutants. *Plant Signal Behav.* 7:16–18
111. Park GT, Frost JM, Park J-S, Kim TH, Lee JS, et al. 2014. Nucleoporin MOS7/Nup88 is required for mitosis in gametogenesis and seed development in *Arabidopsis*. *PNAS* 111:18393–98
112. Pastuglia M, Azimzadeh J, Goussot M, Camilleri C, Belcram K, et al. 2006. γ-Tubulin is essential for microtubule organization and development in *Arabidopsis*. *Plant Cell* 18:1412–25
113. Petrovská B, Jeřábková H, Kohoutová L, Cenklová V, Pochylová Z, et al. 2013. Overexpressed TPX2 causes ectopic formation of microtubular arrays in the nuclei of acentrosomal plant cells. *J. Exp. Bot.* 64:4575–87
114. Pickett-Heaps J, Forer A. 2009. Mitosis: spindle evolution and the matrix model. *Protoplasma* 235:91–99
115. Pickett-Heaps JD. 1969. The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. *Cytobios* 3:257–80
116. Polak B, Risteski P, Lesjak S, Tolić IM. 2017. PRC1-labeled microtubule bundles and kinetochore pairs show one-to-one association in metaphase. *EMBO Rep.* 18:217–30
117. Quan L, Xiao R, Li W, Oh S-A, Kong H, et al. 2008. Functional divergence of the duplicated *AtKIN14a* and *AtKIN14b* genes: critical roles in Arabidopsis meiosis and gametophyte development. *Plant J.* 53:1013–26
118. Raaijmakers JA, Medema RH. 2014. Function and regulation of dynein in mitotic chromosome segregation. *Chromosoma* 123:407–22
119. Richardson DN, Simmons MP, Reddy ASN. 2006. Comprehensive comparative analysis of kinesins in photosynthetic eukaryotes. *BMC Genom.* 7:18
120. Rogers SL, Rogers GC, Sharp DJ, Vale RD. 2002. *Drosophila* EB1 is important for proper assembly, dynamics, and positioning of the mitotic spindle. *J. Cell Biol.* 158:873–84
121. Sasaki T, Tsutsumi M, Otomo K, Murata T, Yagi N, et al. 2019. A novel katanin-tethering machinery accelerates cytokinesis. *Curr. Biol.* 29:4060–70.e3
122. Schaefer E, Belcram K, Uyttewaal M, Duroc Y, Goussot M, et al. 2017. The preprophase band of microtubules controls the robustness of division orientation in plants. *Science* 356:186–89
123. Scholey JM, Civelekoglu-Scholey G, Brust-Mascher I. 2016. Anaphase B. *Biology* 5:51

124. She ZY, Yang WX. 2017. Molecular mechanisms of kinesin-14 motors in spindle assembly and chromosome segregation. *J. Cell Sci.* 130:2097–110
125. Shoji T, Narita NN, Hayashi K, Asada J, Hamada T, et al. 2004. Plant-specific microtubule-associated protein SPIRAL2 is required for anisotropic growth in Arabidopsis. *Plant Physiol.* 136:3933–44
126. Simunić J, Tolić IM. 2016. Mitotic spindle assembly: building the bridge between sister K-fibers. *Trends Biochem. Sci.* 41:824–33
127. Smertenko AP, Chang H-Y, Sonobe S, Fenyk SI, Weingartner M, et al. 2006. Control of the AtMAP65-1 interaction with microtubules through the cell cycle. *J. Cell Sci.* 119:3227–37
128. Smertenko AP, Kaloriti D, Chang H-Y, Fiserova J, Opatrny Z, Hussey PJ. 2008. The C-terminal variable region specifies the dynamic properties of *Arabidopsis* microtubule-associated protein MAP65 isotypes. *Plant Cell* 20:3346–58
129. Smirnova EA, Bajer AS. 1992. Spindle poles in higher plant mitosis. *Cell Motil. Cytoskelet.* 23:1–7
130. Smirnova EA, Bajer AS. 1994. Microtubule converging centers and reorganization of the interphase cytoskeleton and the mitotic spindle in higher plant *Haemanthus*. *Cell Motil. Cytoskelet.* 27:219–33
131. Smirnova EA, Bajer AS. 1998. Early stages of spindle formation and independence of chromosome and microtubule cycles in *Haemanthus* endosperm. *Cell Motil. Cytoskelet.* 40:22–37
132. So C, Seres KB, Steyer AM, Mönnich E, Clift D, et al. 2019. A liquid-like spindle domain promotes acentrosomal spindle assembly in mammalian oocytes. *Science* 364:eaat9557
133. Starr DA, Williams BC, Li Z, Etemad-Moghadam B, Dawe RK, Goldberg ML. 1997. Conservation of the centromere/kinetochore protein ZW10. *J. Cell Biol.* 138:1289–301
134. Su H, Liu Y, Wang C, Liu Y, Feng C, et al. 2021. Knl1 participates in spindle assembly checkpoint signaling in maize. *PNAS* 118:e2022357118
135. Sweeney HL, Holzbaur ELF. 2018. Motor proteins. *Cold Spring Harbor Perspect. Biol.* 10: a021931
136. Talbert PB, Masuelli R, Tyagi AP, Comai L, Henikoff S. 2002. Centromeric localization and adaptive evolution of an Arabidopsis histone H3 variant. *Plant Cell* 14:1053–66
137. Tanenbaum ME, Macůrek L, Janssen A, Geers EF, Alvarez-Fernández M, Medema RH. 2009. Kif15 cooperates with Eg5 to promote bipolar spindle assembly. *Curr. Biol.* 19:1703–11
138. Tovey CA, Conduit PT. 2018. Microtubule nucleation by γ-tubulin complexes and beyond. *Essays Biochem.* 62:765–80
139. Twell D, Park SK, Hawkins TJ, Schubert D, Schmidt R, et al. 2002. MOR1/GEM1 has an essential role in the plant-specific cytokinetic phragmoplast. *Nat. Cell Biol.* 4:711–14
140. Van Damme D, De Rybel B, Gudesblat G, Demidov D, Grunewald W, et al. 2011. *Arabidopsis* α Aurora kinases function in formative cell division plane orientation. *Plant Cell* 23:4013–24
141. Vanstraelen M, Inzé D, Geelen D. 2006. Mitosis-specific kinesins in *Arabidopsis*. *Trends Plant Sci.* 11:167–75
142. Verdaasdonk JS, Bloom K. 2011. Centromeres: unique chromatin structures that drive chromosome segregation. *Nat. Rev. Mol. Cell Biol.* 12:320–32
143. Wadsworth P. 2015. TPX2. *Curr. Biol.* 25:R1156–58
144. Wadsworth P. 2021. The multifunctional spindle midzone in vertebrate cells at a glance. *J. Cell Sci.* 134:jcs250001
145. Walczak CE, Gayek S, Ohi R. 2013. Microtubule-depolymerizing kinesins. *Annu. Rev. Cell Dev. Biol.* 29:417–41
146. Wang C, Liu W, Wang G, Li J, Dong L, et al. 2017. KTN80 confers precision to microtubule severing by specific targeting of katanin complexes in plant cells. *EMBO J.* 36:3435–47
147. Weimer AK, Demidov D, Lermontova I, Beeckman T, Van Damme D. 2016. Aurora kinases throughout plant development. *Trends Plant Sci.* 21:69–79
148. Weingartner M, Binarova P, Drykova D, Schweighofer A, David JP, et al. 2001. Dynamic recruitment of Cdc2 to specific microtubule structures during mitosis. *Plant Cell* 13:1929–43
149. Wimbish RT, DeLuca JG. 2020. Hec1/Ndc80 tail domain function at the kinetochore-microtubule interface. *Front. Cell Dev. Biol.* 8:43
150. Wittmann T, Hyman A, Desai A. 2001. The spindle: a dynamic assembly of microtubules and motors. *Nat. Cell Biol.* 3:E28–34

132. Microtubule-linked factors of microtubule-associated proteins (MAPs), motors, and regulatory proteins form a liquid-like spindle domain that serves as an acentriolar microtubule-organizing center in female meiosis in frogs.

151. Wolniak SM, Hepler PK, Jackson WT. 1980. Detection of the membrane-calcium distribution during mitosis in *Haemanthus* endosperm with chlorotetracycline. *J. Cell Biol.* 87:23–32
152. Yamada M, Goshima G. 2017. Mitotic spindle assembly in land plants: molecules and mechanisms. *Biology* 6:6
153. Yu H-G, Muszynski MG, Dawe RK. 1999. The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *J. Cell Biol.* 145:425–35
154. Zeng CJT, Lee Y-RJ, Liu B. 2009. The WD40 repeat protein NEDD1 functions in microtubule organization during cell division in *Arabidopsis thaliana*. *Plant Cell* 21:1129–40
155. Zhang H, Deng X, Sun B, Van SL, Kang Z, et al. 2018. Role of the BUB3 protein in phragmoplast microtubule reorganization during cytokinesis. *Nat. Plants* 4:485–94

Annual Review of Plant Biology

Volume 73, 2022

Contents

Errata

An online log of corrections to *Annual Review of Plant Biology* articles may be found at http://www.annualreviews.org/errata/arplant