Cell Motility and the Cytoskeleton 65: 1–11 (2008)

Review Article

Emerging Molecular Mechanisms that Power and Regulate the Anastral Mitotic Spindle of Flowering Plants

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Flowering plants, lacking centrosomes as well as dynein, assemble their mitotic spindle via a pathway that is distinct visually and molecularly from that of animals and yeast. The molecular components underlying mitotic spindle assembly and function in plants are beginning to be discovered. Here, we review recent evidence suggesting the preprophase band in plants functions analogously to the centrosome in animals in establishing spindle bipolarity, and we review recent progress characterizing the roles of specific motor proteins in plant mitosis. Loss of function of certain minus-end-directed KIN-14 motor proteins causes a broadening of the spindle pole; whereas, loss of function of a KIN-5 causes the formation of monopolar spindles, resembling those formed when the homologous motor protein (e.g., Eg5) is knocked out in animal cells. We present a phylogeny of the kinesin-5 motor domain, which shows deep divergence among plant sequences, highlighting possibilities for specialization. Finally, we review information concerning the roles of selected structural proteins at mitosis as well as recent findings concerning regulation of M-phase in plants. Insight into the mitotic spindle will be obtained through continued comparison of mitotic mechanisms in a diversity of cells. Cell Motil. Cytoskeleton 65: 1–11, 2008.

Key words: kinesin-5; kinesin-14; mitosis; monopolar spindle; phylogeny

INTRODUCTION

The mitotic spindle has fascinated biologists for centuries. Before model systems, spindles were observed in anything that could be fixed, sliced, and put under glass. From this diversity, a generality emerged as follows: spindle poles in animal and fungal cells come to a point in a conspicuous star-shaped focus of fibers, termed an aster; whereas spindles in vascular plant cells lack distinct poles, being barrel-shaped and without asters. The aster, it was soon learned, comprises microtubules radiating from a compact organelle, the centrosome, which came to be seen as an essential mitotic structure, manipulating microtubule organization specifically to form the spindle and to support chromosomal movements.

Contract grant sponsor: U.S. Department of Energy; Contract grant number: DE-FG02-03ER15421.

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Received 18 July 2007; Accepted 26 September 2007

Published online 29 October 2007 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/cm.20247

The seed plant spindle posed a challenge to the centrosome-centric view of mitosis. When the morphology of the plant spindle was examined closely, polar microtubules were seen to make multiple foci, perhaps as many foci as chromosome pairs. These foci look somewhat like the tops of fir trees [Bajer and Molè-Bajer, 1986], each of which might function as a pole. Hypothesizing that all these poles contain proteinaceous components of the centrosome, Mazia [1984] invoked a flexible centrosome that functions alike whether gathered together around a centriole or spread over a broad, anastral spindle. Mazia's concept of a diffuse centrosome was plausible for plants and became widely accepted [Baskin and Cande, 1990].

Seed plants are not alone, however, in challenging the idea that spindle assembly requires a centrosome. Animal oocytes form perfectly good spindles without centrosomes. Spindle formation in oocyctes begins with microtubules organizing directly around chromosomes [McKim and Hawley, 1995; Compton, 2000; Karsenti and Vernos, 2001]. In fact, bipolar spindles form in extracts from oocytes around beads coated with chromatin [Heald et al., 1996]. Also, in a variety of cell types, when centrosomes are surgically removed, spindles are able to form in their absence, supporting the idea that a centrosome-free pathway operates in cells other than oocytes [Steffen et al., 1986; Varmark, 2004]. Over the last decade, it has become clear that the two pathways act side-by-side, and that the chromatin-based method is obvious only in the absence of centrosomes [Gadde and Heald, 2004; Wadsworth and Khodjacov, 2004].

With the role of centrosomes diminished for the animal spindle, the concept of a diffuse centrosome in plants loses some of its inevitability. Perhaps the plant spindle, like the animal oocyte, has invented its own centrosome-free pathway? If so, then the "diffuse centrosome" concept may miss the real nature of plant spindle poles.

The uncertain nature of the plant spindle pole illustrates that much remains to be learned from consideration of the plant mitotic spindle. One of us, in 1990, helped review the mitotic spindle in flowering plants [Baskin and Cande, 1990]. The work reviewed was morphological, little molecular information being available. Since then, there has been tremendous progress in identifying the proteins and elucidating the mechanisms powering mitosis, but these advances have concerned animals and fungi almost exclusively. There is surprisingly little information regarding the molecular mechanisms of plant mitosis. This review will present some of what has been learned, a necessarily limited view given the space here, but one we hope will illuminate important progress as well as highlighting dark areas that remain. A comprehensive treatment of the plant spindle has recently appeared [Ambrose and Cyr, in press].

SPINDLE FORMATION

Except for the structure of the poles, metaphase and anaphase spindles in flowering plants resemble those of animals and fungi; however, there are notable differences in prophase, when the spindle forms (Fig. 1). In animal cells, spindle formation begins as the replicated centrosomes separate along the nuclear envelope, with the spindle forming between them. In plant cells, during interphase, microtubules are nucleated from the surface of the nuclear envelope and radiate into the cell, forming an array that resembles an aster with the nucleus itself serving as the center. During prophase, microtubules increase in number at the nuclear envelope and are reoriented to lie tangential to the envelope. Eventually, microtubules are gathered into a pair of cones, one on either side of the nucleus, with the apex of the cones, the presumptive spindle poles, lifted up from the nuclear envelope surface (Fig. 1). This structure has been observed for many years and termed "the prophase spindle" in view of is bipolarity [Baskin and Cande, 1990]. Unlike the broad poles characteristic of the mature plant spindle, the prophase spindle poles are usually focused. Once the nuclear envelope disintegrates, the spindle poles broaden and fragment, taking on their characteristic treetop appearance.

The bipolarity of the spindle is thus established in prophase around the periphery of the nuclear envelope. Once the nuclear envelope breaks down, microtubules permeate the nuclear region, carrying out search-and-capture missions to acquire chromosomes, with the prevailing axis of microtubule growth and bundles being roughly parallel to that of the prophase spindle. Recently, live-cell imaging has confirmed earlier observations on fixed cells that a few microtubules from the prophase spindle penetrate the nucleus even before full nuclear envelope breakdown [Dhonukshe et al., 2006].

In the majority of plant cell types, the axis of the prophase spindle is specified by the cell, and with considerable precision. Starting before prophase, the plane of division is marked by the preprophase band, a ring of microtubules circling the cell cortex (Fig. 1). Despite disappearing by prometaphase, the preprophase band predicts the site where the nascent cell plate meets the parental cell wall at the end of telophase and has traditionally been studied in the context of cytokinesis [Mineyuki, 1999]. The orientation of the nascent cell plate reflects that of the mitotic spindle and experiments where spindle orientation is perturbed show that mechanisms for guiding the expanding cell plate can correct only a small degree of disorientation [Baskin and Cande, 1990]. Therefore, it is cogent to expect the preprophase band to

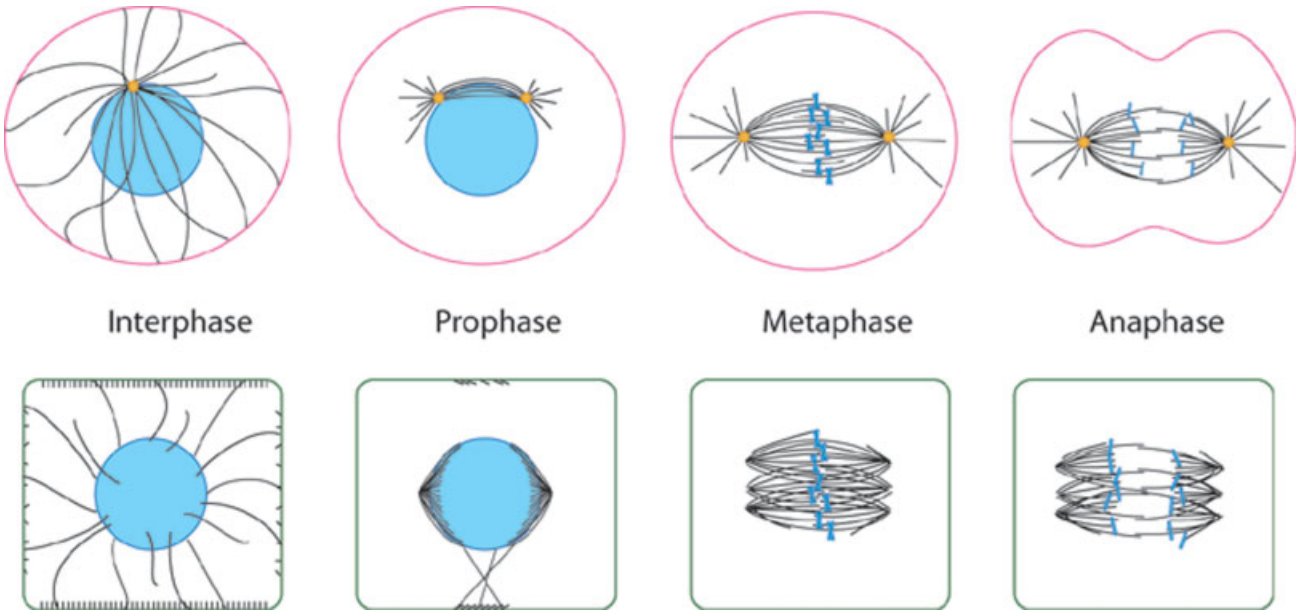

Fig. 1. Cartoon comparing mitotic spindle structures in plants and animals. Top row shows a typical animal somatic cell; bottom row shows typical flowering plant cell. Black lines indicate microtubules, blue indicates nucleoplasm (interphase and prophase) or chromosomes (metaphase and anaphase), and yellow indicates centrosomes. In the plant cell at prophase, microtubules running between nucleus and preprophase band are drawn on one half of the cell only to indicate that their function remains unclear.

help form the prophase spindle; and indeed there is evidence that this is so [Ambrose and Cyr, in press].

Various kinds of links, both direct and indirect, have been observed between the preprophase band and the prophase spindle. The prophase nucleus migrates so as to be bisected by the band, and the prophase spindle along with the underlying nucleus are apparently anchored rather rigidly in place [Mineyuki, 1999]. In subsidiary cells of maize leaves in prophase, the band and spindle (monopolar in this cell type) are so tightly linked that they have been interpreted as forming an integral structure [Panteris et al., 2006], although division in this cell type is quite asymmetric and unusual. Microtubules grow from prophase spindle to preprophase band and also the other way, from band to spindle [Dhonukshe et al., 2005]. Based on observations of prophase in tobacco tissue culture cells (BY-2 cells), Granger and Cyr [2001] hypothesized that the preprophase band locally inhibits microtubule polymerization at the nuclear envelope, thereby depleting microtubules from the equator of the nucleus and encouraging bipolarity. Consistently, the prophase spindle that forms when the BY-2 cell has made two preprophase bands, separated by about one nuclear diameter, is usually multipolar, with the most poles forming at the nuclear equator [Yoneda et al., 2005], being now the nuclear region most distant from the bands. Finally, arabidopsis tissue culture cells that fail to form a preprophase band also fail to form a prophase spindle [Chan et al., 2005]. Taken together, the above observations suggest these prophase structures are linked functionally; however, the form of the linkage remains to be elucidated.

The cells observed by Chan et al. [2005] with neither preprophase bands nor prophase spindles form typical, bipolar mitotic spindles in prometaphase, as do the cells with multipolar prophase spindles studied by Yoneda et al. [2005], and cells in a microtubule motor protein mutant, *atk1* (see later), that have prophase spindles with reduced or absent bipolarity [Marcus et al., 2003]. Some cell types that always lack preprophase bands, such as meiotic cells, also lack a conspicuous prophase spindle [Baskin and Cande, 1990; Otegui and Staehelin, 2000]. In these cells, microtubule association with chromosomes is evidently used for spindle formation. As posited by Lloyd and Chan [2006], plant cells, like animal cells, possess a chromatin-mediated spindle assembly pathway that is always present but only sometimes visible. The preprophase band might be analogous to a centrosome pair: imposing bipolarity on the spindle, from the equator rather than from the poles, but dispensable for mitotic spindle assembly *per se*.

MOTOR PROTEINS

In animals and yeast, the roles of motor proteins have been most clearly elucidated for metaphase and

anaphase. It has become established that spindle function depends on a balance of complementary and antagonistic forces pushing toward the poles (i.e., outward forces) and pushing toward the center (i.e., inward forces) [Saunders and Hoyt, 1992]. The outward forces that separate the spindle poles are mainly delivered by cytoplasmic dynein and plus-end-directed kinesins (kinesin-5); dynein by pulling on astral microtubules from the cell cortex [Sharp et al., 2000b], and kinesin-5 motor proteins by crosslinking antiparallel microtubules at the spindle midzone and walking simultaneously to the plus ends of both, pushing the spindle halves apart [Kapitein et al., 2005]. The inward forces are predominantly delivered by minus-end-directed kinesins (kinesin-14). These motor proteins crosslink antiparallel microtubules in the midzone, as well as parallel microtubules in each spindle half, and walk to their minus ends, pulling the two spindle halves together and focusing the poles [O'Connell et al., 1993; Matthies et al., 1996; Sharp et al., 1999]. Dynein also contributes to pole focusing [Gadde and Heald, 2004]. Importantly, movements in the spindle are not generated by any single motor protein, but by shifts in the balance of forces [Sharp et al., 2000a,b). For example, spindle lengthening at anaphase is likely the result of kinesin-14 being down-regulated, allowing kinesin-5, and therefore the outward force, to dominate.

Illustrating the importance of this force balance, studies have shown that inhibition of a minus-end-directed motor protein can partially suppress the phenotype caused by a defective plus-end-directed motor protein [O'Connell et al., 1993; Sharp et al., 1999]. Many other proteins are undoubtedly involved, and redundancies and alternative pathways exist to vouchsafe spindle function. This has been demonstrated in Drosophila by systematic RNAi of kinesins, which showed that only three of the twenty-five Drosophila kinesins are essential for mitosis [Goshima and Vale, 2003].

The assembly pathway of the plant spindle predicts the involvement of motor proteins, and presumably other structural microtubule-associated proteins at several steps, including the reorganization of microtubules at the surface of the nuclear envelope, the formation of first focused and then "fir-tree" poles, and finally the capture and movement of chromosomes. While cytoplasmic and cilliary dyneins have been lost in flowering plants, kinesins have radiated extensively. Sixty-one kinesins have been identified in the arabidopsis genome [Reddy and Day, 2001; Lee and Liu, 2004], potentially reflecting specialization for new functions [Dagenbach and Endow, 2004] including some of those performed ancestrally by dynein. Twenty-three of these kinesins are up-regulated during mitosis [Vanstraelen et al., 2006]. To date, kinesins shown to have a role in the mitotic spindle belong to the kinesin-14 and kinesin-5 families.

KINESIN-14 MOTOR PROTEINS

Kinesin-14 proteins are C-terminal, minus-end-directed motor proteins that can crosslink two microtubules and slide one relative to the other [Compton, 2000; Sharp et al., 2000b]. They make up the largest family of kinesins in arabidopsis [Reddy and Day, 2001; Richardson et al., 2006; Vanstraelen et al., 2006]. In animals and fungi, these motor proteins are responsible for the inward directed forces on the spindle, balancing the outward forces, focusing the poles, and drawing the spindle halves together. The founder member of the family is nonclaret disjunctional (NCD), discovered on the basis of a Drosophila loss-of-function mutant that caused chromosome nondisjunction and spindles with broad, unfocused poles in oocytes and embryos [Hatsumi and Endow, 1992; Matthies et al., 1996].

In plants, two kinesin-14 family members isolated from Arabidopsis, ATK1 and ATK5, have demonstrated roles in mitotic spindle function. The two proteins share 83% amino acid sequence identity and can crosslink both parallel and antiparallel microtubules in vitro [Ambrose et al., 2005; Ambrose and Cyr, 2007], enabling them in principle to carry out the activities ascribed to this class of kinesin in animal and fungal spindles. ATK5 tracks to the plus end of microtubules in all stages of the cell cycle, independent of the motor domain, but its motor activity is minus-end-directed [Ambrose et al., 2005]. These authors propose that the plus-end tracking targets ATK5 to the spindle midzone, from where it helps to focus the poles by crosslinking parallel microtubules and walking toward the minus ends, as has also been suggested for NCD in Drosophila [Matthies et al., 1996].

In the loss-of-function mutants, *atk1* and *atk5*, mitosis is prolonged and the spindles are somewhat broader than in the wild type [Marcus et al., 2003; Ambrose et al., 2005; Ambrose and Cyr, 2007]. In *atk5,* prophase spindles are longer than those of the wild type [Ambrose and Cyr, 2007], and in *atk1*, prophase spindles have weak or even absent bipolarity, implying that these kinesins act early in mitosis and that forming the prophase spindle involves a force balance. In *atk1,* the metaphase spindle phenotype is more pronounced in meiotic cells [Chen et al., 2002; Marcus et al., 2003], resembling the polar disruption seen in Drosophila *ncd* mutants [Matthies et al., 1996], and is sufficiently severe as to reduce male fertility [Chen et al., 2002]. Given that plant meiocytes have focused spindle poles during metaphase and anaphase [Baskin and Cande, 1990], these spindles may have a stringent need for minus-end directed motor proteins. Importantly, double null mutants for *atk1* and *atk5* are apparently unrecoverable (Richard Cyr, Penn State University, Personal Communication) implying

that, between them, these two motor proteins together provide essential minus-end directed motility for plant mitosis.

KINESIN-5 MOTOR PROTEINS

Key generators of outward force in the spindle are kinesin-5 motor proteins. These proteins anchor each half-spindle together by crosslinking antiparallel microtubules and, at anaphase, separate the spindle halves [Endow, 1999; Goldstein and Philp, 1999; Sharp et al., 2000b]. Because kinesin-5 protein is enriched at the spindle poles in animal cells, this motor protein might also create outward force based on interactions between parallel microtubules [Sawin and Mitchison, 1995]. Kinesin-5 motor proteins form tetramers, built from a pair of dimers joined tail-to-tail. This arrangement allows the tetramer to walk simultaneously on both of the microtubules it crosslinks [Kapitein et al., 2005].

Most animal and fungal genomes contain only one kinesin-5 sequence, and its inhibition by means of mutation [Heck et al., 1993; O'Connell et al., 1993], antibody treatment [Sawin et al., 1992], or exposure to monastrol [Kapoor et al., 2000] invariably leads to spindle collapse and cell cycle arrest. Although the poles separate initially, they later slide back together, chromosomes fail to segregate, and the plus ends of the spindle microtubules radiate outward, with the chromosomes arranged in a ring around the edge [Saunders and Hoyt, 1992; Heck et al., 1993; Endow, 1999; Goshima and Vale, 2003]. Evidence of this kind implies that kinesin-5 motor proteins play an indispensable role in stabilizing the spindle midzone and separating spindle halves at anaphase. An exception to this is the sea urchin mutant for the kinesin-5, *boursin*, which forms multipolar, rather than monopolar, spindles [Touitou et al., 2001]. Similarly, inhibition of kinesin-5 with monastrol in the brown alga *Silvetia compresa* causes both monopolar and multipolar spindles, as well as asters in the cytoplasm [Peters and Kropf, 2006]. These examples suggest that, in some lineages, kinesin-5 proteins have acquired additional roles related to maintaining the integrity of the spindle pole.

Recently, a kinesin-5 in arabidopsis was shown to have a critical role in mitotic spindle function, demonstrating conservation of function between animal and plant kinesin-5 proteins [Bannigan et al., 2007]. The temperature-sensitive mutant *radially swollen 7* (*rsw7*); [Weidemeier et al., 2002] contains a point mutation in the motor domain of the kinesin-5 gene, AtKRP125c, and exhibits massive spindle deformities, reminiscent of those seen in animal and fungal cells with compromised kinesin-5 function (Fig. 2). Spindle collapse in *rsw7* is presumably the result of forces in the spindle becoming unbalanced, such that inward forces dominate and the weakened midline splays as the poles collapse toward each other. Attempts to restore balance by introducing the kinesin-14 mutation, *atk1*, into the *rsw7* background have so far failed because the *atk1rsw7* double mutant appears to be embryo or seedling lethal (A.B. and T.I.B., unpublished data). While the reason for seedling lethality awaits explanation, we suspect that it reflects a specific interaction between these two proteins and that several of the cadre of kinesin-14 proteins provide inward-directed forces for the spindle.

Consistent with a role in crosslinking antiparallel microtubules, kinesin-5 proteins previously have been localized mainly to the midzone of the spindle and phragmoplast in plant cells. The tobacco kinesin-5, TKRP125, is expressed in a cell-cycle dependent manner and localizes to the midzone of both the anaphase spindle and phragmoplast [Asada et al., 1997, Barrosso et al., 2000]. Also, antibodies against the carrot homologue, DcKRP120-2 label the spindle and phragmoplast, accumulating particularly at the phragmoplast midline [Barrosso et al., 2000]. Surprisingly, in arabidopsis, AtKRP125c-GFP, expressed under its native promoter, is distributed along the entire length of microtubules in all arrays throughout the cell cycle and is neither restricted to the spindle nor enriched at the midzone [Bannigan et al., 2007]. The different localizations for plant kinesin-5 motor proteins may reflect the radiation that this clade has undergone in plants (see next section) and the attendant specialization.

PHYLOGENETIC ANALYSIS OF PLANT KINESIN-5 SEQUENCES

Kinesin-5 sequences are well conserved throughout eukaryotes, including plants [Goldstein and Philip, 1999; Lawrence et al., 2002; Richardson et al., 2006]. However, while most organisms have only a single kinesin-5 sequence, plant genomes contain several. In arabidopsis, four sequences have been annotated as related to kinesin-5: AtKRP125a, b, and c, and AtF16L2 [Reddy and Day, 2001]. To understand the relationships of these sequences to each other and to other annotated kinesin-5 sequences, we inferred a phylogenetic tree based on the most highly conserved region of the protein, the motor domain (Fig. 3).

In the resulting phylogeny, plant kinesin-5 motor domain sequences fall into two main groups (Fig. 3). The A group contains two sequences, one from arabidopsis and one from poplar (*Populus tricocarpa*) and is deeply diverged from members of the B group, being almost as distant from the other plant sequences as from the sea urchin sequence (Ot100). The B group contains many sequences, including the other three from arabidopsis, the founding tobacco sequence (TKRP125);

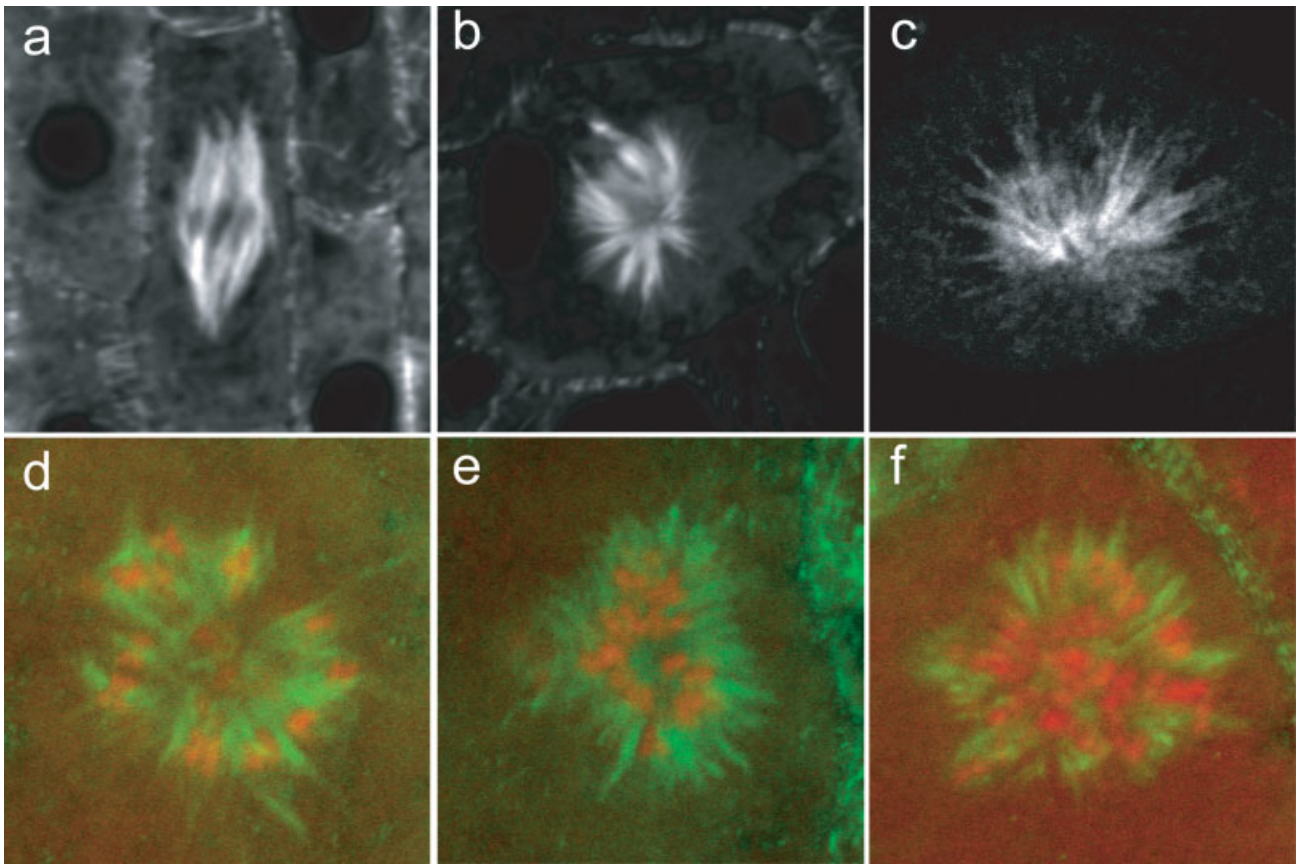

Fig. 2. Confocal micrographs of mitotic spindles. (**a**) Metaphase spindle in wild-type arabidopsis root. (**b, d–f**) Monopolar spindles in *rsw7* exposed to the restrictive temperature (30°C) several hours. (**c**) Monopolar spindle in a pig kidney epithelial cell treated with monastrol. (a–c) spindles labeled with anti-tubulin. (d–f) *rsw7* cells double labeled for microtubules (green) and DNA (red) with chromosomes at the spindle periphery (d), or towards its center (e, f), sometimes numbering more than 10 (f). For methods, see Bannigan et al. [2007].

[Asada et al., 1997], and the biochemically characterized carrot protein (DcKRP120) [Barroso et al., 2000], among others. Within this large assemblage, three subgroups can be distinguished: one that includes the tobacco and an arabidopsis sequence; another that contains the carrot and two arabidopsis sequences, and a third subgroup containing four moss sequences. The three subgroups are robust, as assessed by bootstrap analysis; however, there are not yet sufficient sequences to define the branching patterns within each subgroup.

We propose that AtKRP125a, b, c, and AtF16L2 be renamed as AtKIN5a, b, c, and d, respectively. We make this proposal for several reasons. First, AtF16L2 is an arbitrary name and this sequence is clearly within the kinesin-5 clade (Fig. 3). Second, the original group name, AtKRP125, was based on relatedness to the tobacco protein, TKRP125, the first kinesin-5 studied in plants; whereas, the tree shows that the arabidopsis sequences have various relationships to that sequence. With recent attempts to standardize the nomenclature for all kinesins [Lawrence et al., 2004] it is warranted to name the genes based on their class (KIN5).

The tree supports the idea of functional specialization among plant kinesin-5 motor proteins. Apparently, null alleles of AtKIN5a and AtKIN5b have no detectable phenotype (under standard growth conditions; A.B. and T.I.B., unpublished data) whereas the mutated allele of AtKIN5c (*rsw7*) severely compromises mitosis (Fig. 2) [Bannigan et al., 2007]. Interestingly, for AtKIN5d, there are apparently no available alleles with an insertion in an exon, suggesting that AtKIN5d is essential, conceivably forming a hetero-tetramer with AtKIN5c. On the other hand, in suspension cultured cells, expression of AtKIN5a, b, and c is up-regulated during M-phase whereas expression of KIN5d is not [Vanstraelen et al., 2006], although functional relevance of those expression

levels remains to be demonstrated, given that RSW7-GFP appears to be well expressed during both interphase and mitosis (see above).

STRUCTURAL PROTEINS

While motor proteins may be the *divas* of the spindle, their performance requires a supporting cast of structural proteins—components of the centrosome, kinetochore, and spindle matrix. As with motor proteins, nearly all of the work analyzing the role of structural proteins in spindle function has been done in animals or yeast; however, the roles of a few proteins have been characterized in plants. In general for plant science, a major pathway for identifying and characterizing active proteins has been through mutational inactivation. However, although numerous mutants cause defects in cytokinesis [Lukowitz et al., 1996; Müller et al., 2002; Söllner et al., 2002; Strompen et al., 2002], few have been reported whose phenotypes feature aberrant spindles. Whether this indicates that the plant spindle is swaddled in redundancy or that spindle defects are more often lethal remains to be seen.

A centrosomal component important for spindle formation in animal and fungal cells is γ-tubulin [Varmark, 2004]. It is necessary for microtubule nucleation in both animal and plant cells [Zheng et al., 1995; Murata et al., 2005] and has been localized to the plant spindle, with a preferential localization toward the poles [Liu et al., 1994; Horio et al., 1999; Dryková et al., 2003]. In arabidopsis, there are two functionally redundant γ-tubulin genes; while single mutants have no phenotype, double mutants have severely disrupted spindles [Pastuglia et al., 2006]. These are among the few mutants in plants reported with a defect in the spindle. Similarly, partial RNAi of both γ-tubulin genes together causes mild defects in mitosis, but complete depletion is embryo lethal [Binarová et al., 2006].

Two microtubule-associated proteins that can be tentatively linked to plant spindle structure are MAP65 and MOR1. MAP65 is a microtubule-associated protein named for its weight (65 kDa), represented by gene family in flowering plants, and is homologous to PRC1 in humans, Feo in Drosophila, and Ase1p in fission yeast [Hamada, 2007]. In plants, MAP65 proteins generally crosslink microtubules, and certain members localize to the mitotic spindle at prophase and anaphase, but not at metaphase, in a phosphorylation-dependent manner [Mao et al., 2005; Smertenko et al., 2006]. Those fea-

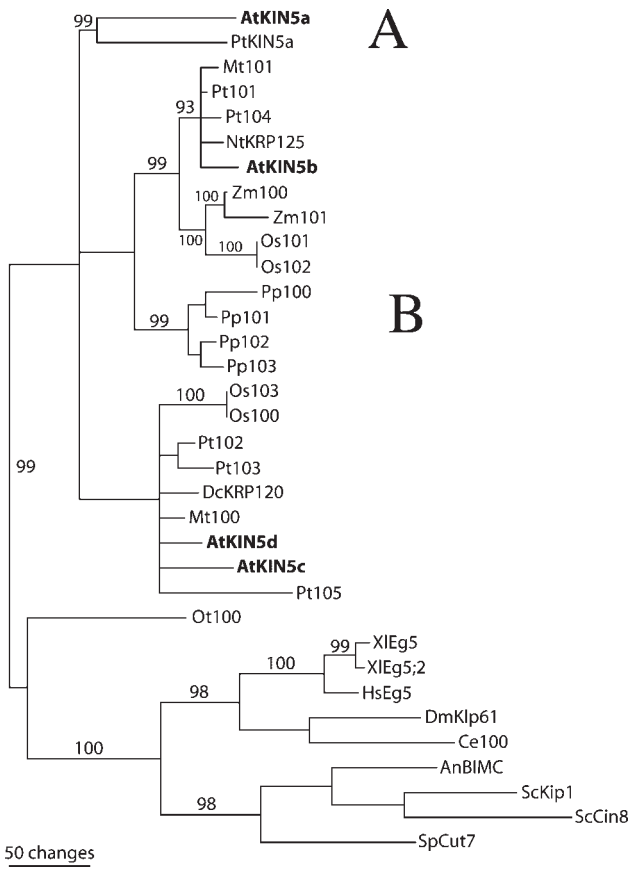

Fig. 3. Kinesin-5 motor domain phylogenetic relationships. Plant and animal kinesin-5 motor domain sequences (as defined by Reddy and Day [2001]) were aligned using CLUSTALW [Thompson, 1994] with gap opening and extension penalties of 5 and 0.05, respectively. Minimal manual editing was performed. Parsimony (shown here) and distance methods were employed to generate phylogenetic trees (PAUP; [Swofford, 2002]) with 10,000 replicate bootstrap analyses. Bootstrap values above 80 are indicated above branches. The original sequences included four *A. thaliana* kinesin-5 motor domain protein sequences (bold): AtKIN5a (AAC98061), AtKIN5b, (AAD21445), AtKIN5c (AAD24373), AtKIN5d (CAB82809), which served as query sequences in BLASTp [Altschul et al., 1990] analyses. Additional kinesin-5 proteins were identified as BLASTp returns with an *E*-value of less than 10^{-48} and included in the alignment, including: *Aspergillus nidulans* (AnBIMC) [P17120], *Daucus carota* (DcKRP120) [AAK91129], *Drosophila melanogaster* (DmKip61) [P46863], *Homo sapiens* (HsEg5) [P52732], *Medicago truncatula* (Mt100) [ABD32308], *M. truncatula* (Mt101) [ABD32688], *Nicotiana tabacum* (NtKRP125) [O23826], *Oryza sativa* (Os100) [AAV44208], *O. sativa* (Os101) [EAZ07994], *O. sativa* (Os102), [EAZ43644], *O. sativa* (Os103) [EAY96319], *Owhathehellii tauri* (Ot100) [CAL21452], *Saccharomyces cerevisae* (ScCin8) [P27895], *S. cerevisae* (ScKip1) [P28742], *Shizosaccharomyces pombe* (SpCut7) [CAA40738], *Xenopus laevis* (XlEg5) [P28025], *X. laevis* (XlEg5-2) [Q91783], *Cenorhabditis elegans* (Ce100) [CAB01170], *Zea mays* (Zm100) [AAK91815], and *Z. mays* (Zm101) [AAK91818]. In addition, the four AtKin 5 protein sequences were also used in BLASTp searches of the newly-completed *Populus trichocarpa* and *Physcomitrella patens* genomes (http://genome.jgi-psf.org) to identify sequences with *E*-values of 0. These sequences are identified by the following protein ID numbers: *Po. trichocarpa*: PtKIN5a (560735), Pt101 (256525), Pt102 (821577), Pt103 (752212), Pt104 (832044), and Pt105 (576621). *Ph. patens*: Pp100 (158752), Pp101 (159492), Pp102 (167794), and Pp103 (96946).

tures of MAP65 suggest that some of them play a role in stabilizing the spindle and phragmoplast during the early stages of formation. Mutation of a MAP65 in arabidopsis can cause division defects, although these appear largely to be associated with the phragmoplast [Müller et al., 2002]. In animal cells, homologues of MAP65 localize to the spindle midzone, forming complexes with members of the kinesin classes 3, 4, 6, or 7 [Hamada, 2007].

The second protein, MOR1, which belongs to the XMAP215/TOG/Dis1 family, localizes to the length of microtubules throughout the cell cycle and its mutation causes multiple cell division defects, including unfocused and multipolar spindles [Kawamura et al., 2006]. In animals and fungi, this class of MAP is well known as a promoter of microtubule growth and regulates microtubule length by antagonizing factors that promote depolymerization [Howard and Hyman, 2007; Niethammet et al., 2007]. Additionally, XMAP215 proteins play a role in supporting microtubule nucleation at the centrosome [Wiese and Zheng, 2006]. In animals and fungi, mutations in XMAP215 proteins disrupt the spindle usually because of the resulting shorter microtubules [Yin et al., 2002; Varmark, 2004], a defect that can be suppressed by concomitant mutation in a depolymerizing kinesin, such as MCAK [Wiese and Zheng, 2006]. When similar kinesins are identified in plants it will be interesting to see whether their knock-down can rescue the *mor1* mitotic phenotype.

CHECK UPS: REGULATION AND CHECKPOINTS

Phosphorylation of spindle-associated proteins is pivotal for regulating mitosis. In mammalian cells, phosphorylation is required to specifically target kinesin-5 (Eg5) to the spindle [Blangy et al., 1995; Sawin and Mitchison, 1995]. Although in plants, AtKIN5c appears to be localized to microtubules throughout the cell cycle [Bannigan et al., 2007], its activity at the spindle midzone could be regulated specifically by phosphorylation. In tobacco, the M-phase cyclin-dependent kinase localizes to the spindle midzone [Weingartner et al., 2001], most likely interacting with its targets, including MAP65 [Mao et al., 2005; Smertenko et al., 2006] and TKRP125 [Barroso et al., 2000]. Moreover in *Vicia faba* root tips, inhibiting cyclin-dependent kinase activity (with bohemine or roscovitine) arrests the cell cycle and also produces radial spindles [Binarová et al., 1998] resembling those of *rsw7* (Fig. 2). The spatial targeting of an activating kinase to the spindle midzone could explain the apparent absence of specific midline localization of the plant outward force-generating kinesin-5 motor proteins.

When kinesin-5 is inhibited in animal cells, chromosomes are not separated; instead they remain attached to the distal ends of microtubules in the monopolar spindles, and the cell cycle stops indefinitely [Endow, 1999]. In contrast, the cell cycle appears to continue in *rsw7*, despite the formation of monopolar spindles resembling those seen in kinesin-5-defective animal cells. This is indicated by the mutant root tips having a constant or decreased mitotic index (cell cycle arrest at metaphase ought to increase mitotic index), and large cells with either one enlarged nucleus or multiple nuclei, typical of nondisjunction followed by nuclear restitution.

The reason for the difference is not clear. In animal cells, the operation of a metaphase checkpoint has been well studied [Amon, 1999; Pinsky and Biggins, 2005]. Briefly, a protein complex at the kinetochore senses tension: even a single kinetochore that is not under tension, which characterizes the state when a kinetochore pair fails to be attached to both spindle poles, is sufficient to delay anaphase onset. Only when all of the kinetochores are subject to productive, (i.e., tension-generating) attachments is the cell allowed to transition into anaphase. Homologues of several metaphase checkpoint proteins have been identified in plants [Yu et al., 1999; Weingartner et al., 2002]. For example, the maize MAD2 protein localizes to kinetochores during prometaphase, as it does in mammalian cells, and disappears from each chromosome as it aligns at the metaphase plate, consistent with tension inactivating the checkpoint signal, chromosome by chromosome [Yu et al., 1999].

If the presence of metaphase checkpoint homologues in plants indicates that they function analogously, then the lack of cell cycle arrest in *rsw7* could be explained by the defective spindle structure manifesting itself late in metaphase or even in anaphase. It is possible that RSW7 is important mostly for spindle elongation (anaphase B) as opposed to chromosome separation (anaphase A). Some evidence indicates anaphase A in plants relies on chromokinesins [Vanstraelen et al., 2006] and microtubule treadmilling, shown in tobacco cells to occur at the same rate as chromosome separation [Dhonukshe et al., 2006]. Consistent with this explanation, in the collapsed spindles of *rsw7*, at least 10 chromosomes (in arabidopsis, $2n = 10$) were often seen attached to the spindle microtubules (Fig. 2d–2f), suggesting that sister chromatids might have separated before the collapse, having satisfied a kinetochore attachment-dependent checkpoint.

On the other hand, mutation of the fission yeast kinesin-5, Cut7, also leads to abnormal spindles and failed mitosis but not to cell cycle arrest [Hagan and Yanagida, 1990], even though the spindle defect is visible at prophase and fission yeast has MAD protein homologues that localize to kinetochores and appear to regulate the metaphase checkpoint [Millband and Hardwick, 2002]. Furthermore, although plants are known to con-

tain homologues of kinetochore attachment checkpoint proteins, direct evidence in plants that anaphase is inhibited when these proteins remain active is lacking. In fact, in the monopolar spindles of *rsw7*, the number of chromosomes visible at the periphery was sometimes *more* than 10, which suggests that spindles form in polyploid cells after one or more failed mitosis and sister chromatids are un-separated. If so, then the kinetochore attachment checkpoint functions differently in plants. In *rsw7*, chromosomes were also sometimes seen amassed at the center of monopolar spindles, although it is not clear whether the chromosomes were translocated to the pole in a pseudo-anaphase or were trapped in the center, never having been properly attached. To resolve these issues, we have tried to image DNA and microtubules simultaneously in living cells by means of dyes such as SYTO82 [Dhonukshe et al., 2006] in a GFP-tubulin line, but have been unsuccessful due to dye toxicity, unreliable chromosome labeling, and interference from labeled mitochondria.

PERSPECTIVES FOR THE FUTURE

During the morphological epoch of mitosis studies, plant spindles were studied along side their animal counterparts, but during the molecular epoch, plants have been left behind. We argue that the plant spindle has interesting things to reveal about the molecular mechanism of mitosis. It has already become clear that the pathway of spindle assembly in anastral oocytes, far from being an oddity, represents an assembly pathway present but hidden in many if not all animal cell types. In plants, we look forward to the elucidation of how a bipolar spindle forms around the surface of the nuclear envelope at prophase, how inward directed forces are provided by the myriad minus-end-directed kinesins at the spindle poles, and how a successful metaphase is announced to the cell. These and other discoveries for plant mitosis will deepen our understanding of this fundamental process for all eukaryotic organisms.

ACKNOWLEDGMENTS

We thank Richard Cyr (Pennsylvania State University) for sharing results prior to publication. *Populus trichocarpa* sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/) and are provided for use in this publication only.

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