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Functions of the *Arabidopsis* kinesin superfamily of microtubule-based motor proteins

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ABSTRACT

Plants possess a large number of microtubule-based kinesin motor proteins. While the Kinesin-2, 3, 9 and 11 families are absent from land plants, the Kinesin-7 and 14 families are greatly expanded. In addition, some kinesins are specifically present only in land plants. The distinctive inventory of plant kinesins suggests that kinesins have evolved to perform specialized functions in plants. Plants assemble unique microtubule arrays during their cell cycle, including the interphase cortical microtubule array, preprophase band, anastral spindle and phragmoplast. In this review, we explore the functions of plant kinesins from a microtubule array viewpoint, focusing mainly on *Arabidopsis* kinesins. We emphasize the conserved and novel functions of plant kinesins in the organization and function of the different microtubule arrays.

INTRODUCTION

The stationary lifestyle of plant cells belies a highly dynamic interior. In contrast to animal cells, the bulk of the directional intracellular movement in plants is thought to be mediated by the actin-myosin cytoskeletal system (Sparkes 2011). Nonetheless, plants possess a large repertoire of microtubule-based kinesin motor proteins (Richardson et al. 2006). In fact, the number of kinesins predicted to be encoded by the *Arabidopsis thaliana* genome (61 kinesins) exceeds the number of kinesins predicted to be encoded by the human genome (45 kinesins) (Reddy and Day 2001; Miki et al. 2005).

Bioinformatic analysis has revealed that the inventory of kinesins in plants is distinctive from that of animals, suggesting that kinesins have evolved to perform specialized functions in plants (Reddy and Day 2001; Richardson et al. 2006). Data from genetic, biochemical and cell biological analyses support the hypothesis that plant kinesins have taken on new functions, perhaps because plants assemble unique microtubule arrays during their cell cycle. In this review, we will explore the functions of plant kinesins from a microtubule array perspective. We focus on *Arabidopsis* kinesins as these are generally the best studied examples.

Kinesins are mechanochemical enzymes that couple the ATP hydrolysis cycle to changes in protein conformation, thus generating force (Vale and Milligan 2000). The kinesin superfamily of proteins is distinguished by the presence of a catalytic core of about 350 amino acids that contains both ATP-binding and microtubule-binding sites. The catalytic domain is typically linked to a short “neck linker” domain that serves to amplify the ATP-dependent conformational changes within the catalytic core and determines the direction of movement along a microtubule track (Vale and Milligan 2000; Endow 1999). The catalytic core and neck linker together make up the motor domain or “head”, which appears as a globular structure when visualized in the electron microscope (Hirokawa et al. 1989). In most kinesins, the head domain is followed by a filamentous “stalk” that consists of a coiled-coil domain and finally a “tail” domain that is thought to mediate binding of kinesin to cargo. The amino acid sequence of the tail domain is typically highly divergent between different types of kinesins (Miki et al. 2005; Reddy and Day 2001).

Based on phylogenetic analyses, kinesins are classified into 14 families (Lawrence et al. 2004). Some of these families appear to have been lost in land plants, while others have expanded and diversified extensively in land plants. The Kinesin-2 family is involved in intraflagellar transport and is missing in land plants, presumably because land plants lack cells with cilia or flagella (Vale 2003). The Kinesin-3 family is involved in long-distance transport of organelles and secretory vesicles (Miki et al. 2005). These kinesins are absent in land plants perhaps because the bulk of the long-distance organelle transport in plants is mediated by the actin-based myosin motor proteins (Sparkes 2011). In addition to the absence of Kinesin-3, land plants may also lack the Kinesin-1 family which also specializes in long-distance organelle transport. Kinesin-1-like sequences have been identified in *Arabidopsis* and other land plants (Richardson et al. 2006), but their identity as bona fide Kinesin-1 has been questioned (Vale 2003). The Kinesin-9 family is also found only in organisms that have ciliated or flagellated cells and is missing in land plants. Kinesin-9 members from the flagellated protozoan *Trypanosoma* were recently shown to play a role in flagellar assembly and motility (Démonchy et al. 2009). The Kinesin-11 family is absent in plants and also in most animal lineages (Richardson et al. 2006) and its functions are poorly understood. Recently, two Kinesin-11 members were shown to participate in kidney and neuronal development by regulating cell-cell adhesion and signaling, respectively (Zhou et al. 2009; Uchiyama et al. 2009).

The Kinesin-7 and Kinesin-14 families have greatly expanded in land plants and together they account for more than half the kinesins encoded by the *Arabidopsis* genome (Richardson et al. 2006). The reasons for the selective expansion of these kinesin families within the plant lineage are not clear. Part of this gene family expansion may simply reflect functional redundancy. On the other hand, some of the plant Kinesin-7 and Kinesin-14 members appear to have taken on new functions in plant cells. Certain members of the plant Kinesin-14 family contain domains that are not found in their non-plant counterparts, supporting the notion that they perform plant-specific functions (Richardson et al. 2006). Similarly, certain members of the plant Kinesin-7 family localize to the cytokinetic apparatus and mitochondria (Nishihama et al. 2002; Strompen et al. 2002; Yang et al. 2003; Itoh et al. 2001), which is distinct from the animal Kinesin-

7 members that work to capture spindle microtubules at kinetochore sites. Therefore, shared membership within a particular kinesin clade does not necessarily mean that the plant and animal kinesins perform similar functions.

Genome analysis indicates that the microtubule-based motor protein dynein is absent in land plants (Wickstead and Gull 2007). Cytoplasmic dynein is responsible for much of the minus-end directed membrane trafficking in animal cells and it also plays a major role in spindle assembly and positioning. One possible explanation for the expansion of the Kinesin-14 family in plants is that Kinesin-14 members substitute for dynein in plant cells (Vale 2003). If so, then some plant Kinesin-14 members would be predicted to be important for minus-end-directed cargo transport and spindle assembly. Of the predicted 21 *Arabidopsis* Kinesin-14 members, only 5 have motor domains at the C-terminus of the protein sequence as expected for minus-end directed motors (Reddy and Day 2001). As discussed later, some of these five Kinesin-14s have been shown to be important for spindle organization, and thus may compensate for the lack of dynein in plants. However, the bulk of the plant Kinesin-14 members have motor domains situated either near the N-terminus or in the middle of the protein sequence and it is unknown if they are capable of minus-end-directed motility. In addition, as noted above, many of these unusual Kinesin-14s are found exclusively in the plant kingdom and thus are probably performing plant-specific functions.

While the mammalian genome contains 45 kinesin-encoding genes, it is thought that there could be twice as many kinesin proteins in mammalian cells since alternative mRNA splicing frequently results in multiple isoforms that perform different functions (Hirokawa et al. 2009; Vale 2003). *Arabidopsis* kinesins have an average of 16 introns (Reddy and Day 2001) and transcriptome analysis has revealed that about 42% of the *Arabidopsis* intron-containing genes are alternatively spliced (Filichkin et al. 2010). Based on the TAIR10 *Arabidopsis* genome annotation, 13 *Arabidopsis* kinesins are alternatively spliced (Table 1). However, whether the different splice forms carry out different functions has not been studied.

Kinesins are essential for many critical cellular processes such as intracellular transport, signaling, cell shape determination and cell division. The way in which kinesins fulfill these diverse functions may be broadly classified into two categories: 1)

microtubule array organization and 2) microtubule-based activities. Kinesins contribute to microtubule array organization through a variety of mechanisms including regulation of microtubule polymer dynamics, crosslinking of microtubules into bundles and translocation of microtubules. Microtubule-based kinesin function typically entails directional transport of cellular components, but can also involve linking of other cellular structures such as actin microfilaments and chromosomes to microtubules. Since kinesins are microtubule-based proteins, this review will take a microtubule-centric view of kinesins and discuss how kinesins participate in both the organization and function of the plant microtubule cytoskeleton.

The major plant microtubule arrays are shown in Figure 1. Each of these arrays reflects the unique cell biology of plants. During interphase, the microtubule cytoskeleton forms a highly dispersed and dynamic cortical array that defines the direction of cell elongation by influencing the deposition of cell wall material (Wasteneys and Ambrose 2009; Lloyd 2011). Upon the onset of mitosis, the interphase cortical microtubule array is disassembled and a highly bundled preprophase band of microtubules is formed, typically encircling the nucleus (Duroc et al. 2011; Wright and Smith 2007). The preprophase band is a transient structure and is destroyed upon nuclear envelope breakdown. Nonetheless, it somehow accurately predicts the future cell division site. The preprophase band also functions in spindle assembly and positioning (Cyr and Ambrose 2008). The mitotic spindle of plant cells is barrel shaped and lacks astral microtubules due to the lack of centrosomes in plants (Wadsworth et al. 2011). Cytokinesis is mediated by a complex structure called the phragmoplast that consists of two opposing sets of microtubules that direct the deposition of material that forms the cell plate (Liu et al. 2011). The phragmoplast expands outwards by adding new microtubules at its periphery while existing microtubules in the phragmoplast center are eliminated. The centrifugally expanding phragmoplast eventually inserts at the cortical site previously occupied by the preprophase band.

Below we discuss the roles of kinesins in the plant microtubule arrays described above. Two recurring themes in this narrative are worth noting at the onset: 1) A particular kinesin may function in multiple stages of the cell cycle and 2) The

organization and function of microtubule arrays typically involves multiple kinesins that may work redundantly, cooperatively or antagonistically with each other (Table1).

INTERPHASE CORTICAL MICROTUBULE ARRAY

The role of kinesins in cortical microtubule (CMT) organization

Plant CMTs bundle extensively in an overlapping manner. Kinesin-based microtubule sliding or translocation contributes heavily to the formation of overlapping microtubule structures in animal cells. However, microtubule sliding is not evident using photobleaching recovery and kymographic analyses of CMTs (Shaw et al. 2003; Shaw and Lucas 2011). Therefore, kinesin-mediated microtubule sliding does not appear to be common in the CMT array. However, these data do not exclude the possibility that microtubule sliding happens only at specific time points or at particular locations in the CMT array.

Several kinesins have been localized to the CMT array where they potentially mediate CMT bundling and/or regulate CMT dynamics. However, it is important to note that localization of a kinesin to the CMT array does not necessarily imply its activity there.

The tetrameric Kinesin-5 motors in animals and fungi are plus-end directed kinesins that cross-link and stabilize anti-parallel microtubules in the spindle (Walczak and Heald 2008). There are four Kinesin-5s in *Arabidopsis* and they show similarity to animal and fungal Kinesin-5s throughout their sequence. One of the Kinesin-5s in *Arabidopsis*, AtKRP125c, decorates CMTs and a temperature-sensitive mutant of AtKRP125c has disorganized CMTs at the restrictive temperature (Bannigan et al. 2007). In addition, this mutant is more sensitive to microtubule depolymerizing drugs at permissive temperature (Bannigan et al. 2007). These results indicate that AtKRP125c plays an important role in CMT organization. In contrast, tobacco TKRP125 and carrot DcKRP120 do not localize to CMTs (Asada et al. 1997; Barroso et al. 2000), indicating that not all plant Kinesin-5s are involved in CMT organization. In addition, Kinesin-5s in animals do not localize to interphase microtubules, indicating that some plant Kinesin-5 motors have acquired a distinct function during interphase. It will be interesting to

determine if AtKRP125c contributes to CMT organization by cross-linking anti-parallel CMTs or through some other activity.

Kinesin-like calmodulin-binding protein (KCBP) is a plant-specific Kinesin-14 member that contributes to CMT organization. The loss-of-function mutant of KCBP has fewer trichome branches than in wild-type plants (Oppenheimer et al. 1997). Trichome branch initiation requires local reorganization and transient stabilization of CMTs (Mathur and Chua 2000; Szymanski et al. 2000). Given that KCBP contains a second ATP-independent MT binding site in its N-terminal tail domain and is capable of bundling microtubules in vitro (Kao et al. 2000), it may regulate trichome formation by directly bundling and stabilizing CMTs.

ATK5 in the Kinesin-14 family is also localized to CMTs and is especially enriched at growing CMT plus-ends (Ambrose et al. 2005). Similar to KCBP, ATK5 also has a second ATP-independent microtubule binding site (Ambrose et al. 2005), which potentially could contribute to bundling of CMTs. However, the *atk5* mutant has normal CMT organization (Ambrose et al. 2005), indicating that it does not play an essential role in CMT organization.

CMTs are dynamic at both ends and their dynamic properties are important for array organization (Shaw et al. 2003). Kinesins may contribute to CMT organization by regulating CMT assembly dynamics. Kinesin-13s in animals are known to act as microtubule depolymerases at both ends of microtubules (Wordeman 2005). Recently, overexpression of Kinesin-13A in *Arabidopsis* was reported to result in partial fragmentation of CMTs (Mucha et al. 2010), indicating that Kinesin-13A might depolymerize CMTs. However, whether Kinesin-13A functions as a microtubule depolymerase remains to be shown.

Armadillo repeat domain-containing kinesins ARK1 and ARK2 in the ungrouped kinesin family are also important in controlling CMT organization perhaps by promoting microtubule depolymerization. Mutations in the ARK1 kinesin lead to abundant microtubules in the endoplasm, disrupted CMT organization and defective root hair growth (Yang et al. 2007; Sakai et al. 2008). In contrast, loss-of-function of ARK2 leads to root cell file twisting and this phenotype can be suppressed by the microtubule depolymerizing drug propyzamide (Sakai et al. 2008). These data are consistent with

the hypothesis that ARK1 and ARK2 destabilize CMTs and it will be interesting to explore the mechanism of this activity.

The tobacco TBK5 Kinesin-14 member is hypothesized to function in relocating and gathering newly formed microtubules and/or microtubule-nucleating units (Goto 2007). TBK5 is highly expressed during interphase in tobacco BY-2 cells (Matsui 2000). Transient overexpression of GFP-TBK5 fusion protein in tobacco cells leads to the loss of CMTs and the formation of a radial microtubule array emanating from a single perinuclear site containing GFP-TBK5 (Goto 2007). However, these results need to be interpreted with caution since overexpression of GFP-TBK5 might cause it to mislocalize and function aberrantly. The subcellular localization and function of endogenous TBK5 needs to be determined. The counterpart of TBK5 in *Arabidopsis* is At5g27950, which shares about 60% amino acid sequence identity with TBK5. It will be interesting to explore the function of At5g27950 in *Arabidopsis* plants.

The role of kinesins in organelle movement during interphase

The correct spatial and temporal localization of organelles and molecules is critical for their function. As mentioned earlier, the long-distance transportation of organelles and molecules in plant cells is largely mediated by the actin-myosin system. Kinesins have been hypothesized to contribute to local positioning of organelles and molecules by mediating short-distance movements along microtubules (Cai and Cresti 2010). However, it remains possible that kinesins drive the long-distance movement of certain organelles/molecules in plants. Evidence is now accumulating for kinesin-based motility of various organelles including the nucleus, mitochondria, chloroplast, Golgi apparatus and Golgi-associated vesicles. Different kinesins are involved in the movement of the various organelles, suggesting specialization of kinesins for cargo transport.

OskCH1 from the Kinesin-14 family in rice is shown to be involved in premitotic nuclear migration (Frey et al. 2010). When OskCH1 is expressed in tobacco BY-2 cells, it localizes along filamentous structures that extend from the nucleus to the cell periphery (Frey et al. 2010). At the onset of mitosis, OskCH1 localization changes and it is present at both poles of the nucleus (Frey et al. 2010). OskCH1 does not label the

PPB, spindle, or phragmoplast (Frey et al. 2010). During late telophase, OsKCH1 is repartitioned to the newly forming nuclei and filaments that extend from these nuclei to the cell periphery (Frey et al. 2010). Overexpression of OsKCH1 delays nuclear migration and mitosis (Frey et al. 2010).

KCH proteins possess a motor domain at the C-terminus and a calponin-homology domain at the N-terminus that allow binding to microtubules and actin filaments respectively (Frey et al. 2009; Xu et al. 2009). Interestingly, the ATPase activity of KCH is dramatically reduced when bound to actin (Umezue et al. 2011), which would predictably impair KCH motor function. Thus, binding to actin might represent a mechanism to regulate KCH motor activity, and this might be important for coordinating the activities of the microtubule and actin cytoskeleton during nuclear migration. It remains to be determined whether the regulation of nuclear migration by KCH kinesin is conserved in plants. In *Arabidopsis*, At2g47500 is most similar to OsKCH1 and shares about 50% amino acid sequence identity with OsKCH1. It will be interesting to test whether At2g47500 is involved in nuclear migration.

Two plant kinesins have been implicated in mitochondria motility and/or function. A tobacco kinesin is associated with mitochondria in pollen tubes and may contribute to the motility and positioning of mitochondria (Romagnoli et al. 2007). AtKP1 in the Kinesin-14 family is localized to mitochondria (Ni et al. 2005). Specifically, it binds to the mitochondrial outer membrane protein voltage-dependent anion channel VDAC3 and regulates aerobic respiration and ATP levels during seed germination at low temperature (Yang et al. 2011), indicating this kinesin is an important regulator of mitochondrial function.

KCA1 and KCA2 in the Kinesin-14 family were recently identified in a genetic screen for chloroplast movement (Suetsugu et al. 2010). KCA1 and KCA2 share 81% protein sequence identity (Vanstraelen et al. 2004). Loss of KCA1 severely impairs chloroplast movement in response to changing light intensities (Suetsugu et al. 2010). The double mutant lacking both KCA1 and KCA2 has no detectable light-induced chloroplast movement and also shows detachment of chloroplasts from the plasma membrane (Suetsugu et al. 2010). Interestingly, these two kinesins have no microtubule-binding activity or detectable ATPase activity (Suetsugu et al. 2010),

consistent with an apparent loss of the nucleotide-sensing switch I domain that is essential for motor function. Instead, the C-terminal domain of KCA1 has been proposed to interact with F-actin and mediate chloroplast movement in an actin-dependent manner (Suetsugu et al. 2010). However, the K_d of KCA1 for actin is about 15 μ M in vitro (Suetsugu et al. 2010), and it is unclear whether this would allow actin binding under physiological conditions. In addition to its role in chloroplast movement, the KCA1 kinesin is likely to be involved in virus infection because it is found to interact with the geminivirus AL1 protein in a yeast 2-hybrid assay (Kong and Hanley-Bowdoin 2002).

Kinesin-13A plays an important role in the dispersion of Golgi stacks and the budding of Golgi-associated vesicles. Kinesin-13A is associated with the Golgi stacks in leaf cells and mutations in Kinesin-13A result in more branches in leaf trichomes and aggregation of Golgi stacks (Lu et al. 2005). This data suggests that the cortical distribution of the Golgi apparatus requires microtubules and Kinesin-13A and that this distribution regulates trichome development. Recently, Kinesin-13A was found to be localized on Golgi-associated vesicles in *Arabidopsis* root-cap peripheral cells (Wei et al. 2009). Peripheral cells of the *kinesin-13a-1* loss-of-function mutants contain fewer and smaller Golgi-associated vesicles (Wei et al. 2009). In addition, the morphology of Golgi cisternae in the *kinesin-13a-1* mutant is significantly different from that of wild-type Golgi cisternae (Wei et al. 2009). Together these results suggest that Kinesin-13A plays an essential role in the structure of Golgi stacks and the formation of Golgi-derived vesicles, however the mechanism for this function remains to be determined.

The role of kinesins in cell wall deposition

CMTs play a critical role in the organization of cellulose microfibrils. CMTs guide the directional movement of cellulose synthase (CESA) complexes within the plasma membrane (Paredez et al. 2006), and are involved in targeting the insertion of CESA complexes into the plasma membrane (Crowell et al. 2009; Gutierrez et al. 2009). Kinesins are ideal candidates for moving cell-wall-related cargo along CMTs, thus ensuring that their deposition is coincident to the CMT orientation. Genetic evidence suggests that FRA1, an *Arabidopsis* Kinesin-4 member, performs such a function. FRA1

is localized to the cell cortex and loss of FRA1 function results in disrupted cellulose microfibril organization without altering CMT organization (Zhong et al. 2002). FRA1 was recently shown to possess motor activity and to move along microtubules in vitro with very high processivity (Zhu and Dixit 2011). FRA1 thus has the potential to regulate cellulose patterning by transporting cell wall-related cargoes over long distances along CMTs.

Mutations in BC12, the homolog of FRA1 in rice, lead to similar defects in cellulose microfibril organization (Zhang et al. 2010; Li et al. 2011), indicating that the function of FRA1 kinesins in cellulose patterning is conserved in both monocots and dicots. Unlike FRA1, BC12 also decorates the PPB and spindle microtubule arrays, indicating a role in mitosis (Zhang et al. 2010). In addition, unlike FRA1, BC12 has a nuclear-localization sequence (Zhang et al. 2010) and it has been shown to act as a transcription factor for the synthesis of the phytohormone gibberellin (Li et al. 2011). The DNA binding ability of BC12 is reminiscent of chromokinesins in the animal Kinesin-4 family (Mazumdar and Misteli 2005).

PREPROPHASE BAND

The role of kinesins in preprophase band (PPB) organization

The available evidence suggests that kinesins potentially contribute to microtubule bundling in the PPB. The PPB is wider in the *atk1* mutant, indicating that the Kinesin-14 member ATK1 plays a role in PPB formation (Marcus et al. 2003). KCBP in the Kinesin-14 family and AtKRP125c in the Kinesin-5 family are also localized to the PPB (Bowser and Reddy 1997; Bannigan et al. 2007). Both of these kinesins can crosslink microtubules into bundles (Kao et al. 2000; Walczak and Heald 2008), but whether they function in this capacity in the PPB remains to be determined.

The role of kinesins in PPB function

How a transient PPB marks the future cell division site is a long-standing open question. The most popular hypothesis for this function posits that the PPB position is

marked by some other factor which persists through mitosis and guides the fusion of the expanding phragmoplast to the PPB site. In this context, several kinesins are found to be important for PPB function by recruiting and/or maintaining components that are required for later phragmoplast insertion at the PPB site. POK1 and POK2 in the Kinesin-12 family are such kinesins and they are important for the localization of two other proteins, TAN and RanGAP, to the PPB site. TAN is a highly basic protein that can directly bind to microtubules and is a key player in determining the cell division plane (Walker et al. 2007). It localizes to the PPB and persists throughout mitosis and cytokinesis, thus qualifying as an excellent candidate for marking the PPB site (Walker et al. 2007). The *tan* mutant resembles the *pok1 pok2* double mutant in that they both have abnormal division planes (Walker et al. 2007). POK1 and POK2 can directly interact with TAN in a yeast 2-hybrid assay (Muller et al. 2006). POK1 and POK2 are required for the recruitment of TAN to the PPB site but the subsequent maintenance of TAN at this site is independent of microtubules (Walker et al. 2007).

RanGAP, the GTPase activating protein of the small GTPase Ran, is another positive marker for the cell division plane. Similar to TAN, RanGAP concentrates at the PPB site and remains associated with it during mitosis and cytokinesis (Xu et al. 2008). In addition, depletion of RanGAP by inducible RNAi leads to misplaced cell walls, similar to *tan* and *pok1pok2* double mutant (Xu et al. 2008). The initial accumulation of RanGAP at the PPB is microtubule dependent but unlike TAN this does not require POK1 or POK2 (Xu et al. 2008). Interestingly, POK1 and POK2 are essential for the retention of RanGAP at the cortical division site after PPB disappearance (Xu et al. 2008), presumably via some other factor that is deposited in a POK1/POK2-dependent manner at the PPB site. TAN is such a candidate that may be important for RanGAP retention at the cortical division site, and it will be interesting to determine if RanGAP and TAN physically interact. The molecular mechanisms for how POK kinesins, TAN and RanGAP work together to mark the division site are unknown. Studying the spatiotemporal localization of POK1 and POK2 with respect to TAN and RanGAP will be key to address this question.

In addition to POK kinesins, ARK3 in the ungrouped kinesin family is also important for PPB function. ARK3 localizes solely to the PPB in a cell-cycle dependent

manner (Malcos and Cyr 2011). The function of ARK3 in the PPB appears to be essential because no null mutant or RNAi lines have been obtained for ARK3 (Malcos and Cyr 2011). Whether ARK3 directs the deposition of other factors that mark the PPB site remains to be determined.

Interestingly, KCA1 in the Kinesin-14 family negatively marks the future division site. When cells enter mitosis, KCA1 accumulates at the plasma membrane except at the PPB site, forming a KCA-depleted zone (KDZ) (Vanstraelen 2006). The PPB microtubules are required for the establishment of the KDZ but not for its preservation. The KDZ spatially overlaps with the actin-depleted zone (Vanstraelen et al. 2006b), providing circumstantial evidence for the idea that KCA1 interacts with actin (Suetsugu et al. 2010). The loss of KDZ (i.e., KCA1 localized to the PPB site) leads to tilted phragmoplasts and misplaced cell plates (Vanstraelen et al. 2006b). It remains to be determined how the KDZ is established and how it determines the cell division plane.

Microtubules bridging the PPB and the nuclear envelope function in early spindle assembly. The absence of these bridge microtubules is associated with a loss of spindle bipolarity, while asymmetric distribution of bridge microtubules with respect to the nucleus correlates with prophase spindle migration, abnormal spindle morphology, and increased bipolarity near the region of highest bridge microtubule density (Cyr and Ambrose 2008). In wild-type cells, the bridge microtubules are cleared when microtubules extend from the spindle poles to the spindle equatorial plane. However, in the *atk1-1* mutant, the bridge microtubules persist and the spindle is disorganized (Marcus et al. 2003). It is hypothesized that ATK1 clears the bridge microtubules by transporting them to the spindle poles (Marcus et al. 2003).

SPINDLE APPARATUS

The role of kinesins in spindle assembly

Members of the Kinesins-14 and Kinesin-5 family are conserved core players that function antagonistically in spindle assembly and function (Vale 2003). Kinesin-14 members contribute to spindle structure by: 1) bundling and sliding microtubules in the spindle midzone to generate inward forces that act to straighten the spindle axis and shorten the spindle length; and 2) focusing spindle poles by bundling parallel

microtubules and/or transporting microtubules towards the poles. ATK1 and ATK5 in the Kinesin-14 family are known to perform these functions. Both ATK1 and ATK5 are present at the spindle poles as well as the midzone (Liu et al. 1996; Ambrose et al. 2005; Ambrose and Cyr 2007). The *atk1-1* mutant has unfocused spindle poles and reduced spindle bipolarity during metaphase (Marcus et al. 2003). These spindles take longer to proceed to anaphase; however, subsequently the spindle abnormalities appear to be corrected by anaphase (Marcus et al. 2003). The spindle defects in the *atk1-1* mutant are more dramatic in male meiotic cells, resulting in abnormal chromosome segregation (Chen et al. 2002). The *atk5-1* mutant also has abnormally elongated, broadened and frequently bent spindles and splayed open spindle poles during mitosis (Ambrose et al. 2005; Ambrose and Cyr 2007), indicating that ATK1 and ATK5 have retained similar functions in mitosis. ATK1 and ATK5 share 83% protein sequence identity (Chen et al. 2002) and either one of these two genes is necessary and sufficient for gametophyte development (Quan et al. 2008). However, the *atk5-1* mutant has nearly normal male meiosis (Quan et al. 2008), indicating that ATK1 plays a dominant role in male meiosis. Both ATK1 and ATK5 are minus-end directed motor proteins (Marcus et al. 2002; Ambrose et al. 2005). Unlike ATK1, ATK5 also has plus-end tracking ability through a second ATP-independent microtubule-binding domain at its N-terminal tail region (Ambrose et al. 2005). In addition, ATK5 is capable of bundling microtubules in vitro (Ambrose and Cyr 2007). These properties are similar to the *Drosophila* NCD kinesin (Furuta and Toyoshima 2008), which also functions in spindle assembly.

KCBP in the Kinesin-14 family is also thought to contribute to the formation of the spindle. In *Arabidopsis*, KCBP localizes to the spindle (Bowser and Reddy 1997), but evidence about the role of KCBP in mitosis is lacking in *Arabidopsis*. Studies in other plants support a role of KCBP in spindle formation. KCBP in *Haemanthus* endosperm is associated with the spindle and is highly concentrated on spindle fibers through metaphase (Smirnova et al. 1998). Injection of antibodies into *Tradescantia virginiana* stamen hair cells to make KCBP constitutively active during prophase results in hastened progression into prometaphase, but the cells later arrest in metaphase (Vos et al. 2000). It has been hypothesized that KCBP promotes the formation of a bipolar

spindle by bundling and sliding microtubules and that this activity needs to be down-regulated subsequently for progression into anaphase (Vos et al. 2000).

Kinesin-5s are important for spindle formation by aligning antiparallel microtubules in the midzone and generating outward forces to balance the inward forces generated by the mitotic Kinesin-14 members. AtKRP125c in the Kinesin-5 family decorates the spindle and mutation of AtKRP125c results in abnormal monopolar or fragmented spindles (Bannigan et al. 2007). These phenotypes are similar to Kinesin-5-defective cells in animals and fungi, indicating that the functions of Kinesin-5 motors in spindle architecture are strongly conserved. Similar to AtKRP125c, the AtKRP125a and AtKRP125b Kinesin-5 members are also upregulated during mitosis (Vanstraelen et al. 2006a). However, single mutants of either AtKRP125a or AtKRP125b do not show defects in mitosis (Bannigan et al. 2007), indicating that AtKRP125c plays a dominant role in mitosis.

Role of kinesins in spindle function

Kinetochores capture is a basic function of spindle microtubules, which is mediated by the Kinesin-7 family (Walczak and Heald 2008). However, it is not clear which kinesins perform this function in plants. *At1g59540* encodes a Kinesin-7 and is upregulated during mitosis (Vanstraelen et al. 2006a). *At1g59540* along with *At5g42790* and *At2g21380* in the Kinesin-7 family are highly expressed in root meristematic cells (Arabidopsis eFP Browser), consistent with a mitotic function. However, whether these kinesins are important for kinetochore capture in plants remains unknown. *At1g21730* and *At4g39050* in the Kinesin-7 family contain a functional mitochondria-targeting signal, which is predicted to translocate these kinesins into the mitochondrial matrix (Itoh et al. 2001). Similar to the *Escherichia coli* motor protein MukB, which is involved in chromosome partitioning, these two kinesins are hypothesized to function in mitochondria nucleoid segregation (Itoh et al. 2001).

Some Kinesin-4 and Kinesin-10 motors in animals act as chromokinesins because they can directly bind to chromosome DNA. They function in chromosome condensation, spindle organization and chromosome alignment (Walczak and Heald

2008; Mazumdar and Misteli 2005). However, chromokinesins in plants are largely unknown. BC12 in the Kinesin-4 family of rice is localized in the nucleus during interphase and is involved in mitosis (Li et al. 2011), so it may act as a chromokinesin. However, the three Kinesin-4 motors of *Arabidopsis* are unlikely to act as chromokinesins since they do not contain a detectable nuclear localization sequence.

The Kinesin-13s in animals are known to regulate spindle microtubule dynamics and generate poleward microtubule flux, which is important for chromosome segregation (Walczak and Heald 2008; Ems-McClung and Walczak 2010). There are two Kinesin-13 motors in *Arabidopsis*, Kinesin-13A and Kinesin-13B. The loss-of-function mutant of Kinesin-13A does not show any noticeable defects in mitosis (Lu et al. 2005), perhaps due to functional redundancy with Kinesin-13B which is upregulated in mitosis (Vanstraelen et al. 2006a). Whether the plant Kinesin-13s function similar to the animals Kinesin-13s remains an open question.

PHRAGMOPLAST

Role of kinesins in phragmoplast assembly

Several kinesins are involved in the establishment and maintenance of the phragmoplast microtubule configuration. PAKRP1 and PAKRP1L in the Kinesin-12 family share about 74% protein sequence identity and both kinesins localize to the midzone of the phragmoplast where the microtubule plus ends of both halves of the phragmoplast face each other (Pan et al. 2004; Lee and Liu 2000). Single mutants of these kinesins do not show any noticeable defects, indicating that they are likely to have redundant functions in the phragmoplast (Lee et al. 2007). However, in the absence of both kinesins, the phragmoplast fails to assemble normally, resulting in defective cell plate formation (Lee et al. 2007). These two kinesins are hypothesized to contribute to phragmoplast formation by preventing the plus ends of the opposing microtubule sets from crossing the midzone. The localization of PAKRP1 and PAKRP1L is dependent on MAP65-3, which specifically bundles the interdigitating antiparallel microtubules in the midzone of the phragmoplast (Ho et al. 2011). In animals, the MAP65 ortholog PRC1 autonomously bundles the overlapping antiparallel microtubules in the central anaphase spindle and recruits Xklp1, a Kinesin-4 motor, selectively to this region (Bieling et al.

2010; Hu et al. 2011). Xklp1 determines the overlap size in the central anaphase spindle by length-dependent microtubule growth inhibition (Bieling et al. 2010; Hu et al. 2011). In plants, PAKRP1 and PAKRP1L may determine the length of the overlapping region in the phragmoplast using a similar mechanism. It will be interesting to determine if PAKRP1 and PAKRP1L regulate microtubule dynamics, and in particular, if they inhibit microtubule plus-end growth as suggested by the double mutant phenotype (Lee et al. 2007).

AtKRP125c in the Kinesin-5 family also functions in phragmoplast assembly. At the restrictive temperature, the conditional mutant of AtKRP125c shows severe phragmoplast defects (Bannigan et al. 2007). Phragmoplasts are often misplaced and wavy, resulting in incomplete cell plate deposition, multiple nuclei and enlarged cells (Bannigan et al. 2007). In addition, Kinesin-5s in tobacco and carrot decorate phragmoplast microtubules, especially at the plus ends (Asada et al. 1997; Barroso et al. 2000). Kinesin-5 motors may contribute to phragmoplast assembly by bundling and sliding overlapping microtubules at the midzone, similar to its function in the spindle apparatus.

KCBP in the Kinesin-14 family also plays a role in phragmoplast formation. When KCBP is artificially activated by blocking its self-inhibitory domain using antibodies, phragmoplast formation is significantly delayed (Vos et al. 2000). In addition, both ATK1 and KCH in the Kinesin-14 family are localized to the phragmoplast (Liu et al. 1996; Bowser and Reddy 1997; Xu et al. 2007). However, whether this localization is functionally relevant is unknown.

The role of kinesins in phragmoplast function

Phragmoplast microtubules provide tracks for the delivery of Golgi-derived vesicles to the phragmoplast midzone for cell plate formation. Kinesins are hypothesized to be involved in the transportation of these vesicles. PAKRP2 in the Kinesin-10 family decorates the phragmoplast microtubules in a punctate pattern (Lee et al. 2001), suggestive of such a function. With the motor domain located at the N-terminus, PAKRP2 is presumed to be a plus-end directed motor, a necessary property for transporting vesicles to the phragmoplast midzone. A null mutant of PAKRP2 has

not been isolated, suggesting that the function of PAKRP2 is essential (Lee et al. 2001). However, it remains to be determined whether PAKRP2 is an active motor and whether it transports vesicles along phragmoplast microtubules.

As the phragmoplast expands outwards, the microtubules in the center are disassembled. HIK and TES in the Kinesin-7 family are important for this process. HIK is localized at the phragmoplast midzone (Nishihama et al. 2002). In the *hik* mutant, phragmoplast microtubules in the center persist, resulting in incomplete cell plate formation and multinucleate cells (Strompen et al. 2002). Recent studies have shown that HIK is important for phragmoplast expansion by depolymerizing microtubules in the center through a MAPK pathway. In *Arabidopsis*, HIK binds and thus activates ANP1, 2 and 3 (MAPKKK), which then activates ANQ1 (MAPKK) and subsequently MPK4 (MAPK) (Takahashi et al. 2010; Komis et al. 2011). Some of the targets of the MAPK pathway are the microtubule-bundling proteins MAP65-1, MAP65-2 and MAP65-3, all of which can be phosphorylated by MPK4 in vitro (Sasabe et al. 2011). The phosphorylated MAP65 proteins are thought to dissociate from phragmoplast microtubules, thus promoting their depolymerization. Consistent with this interpretation, *anp2anp3* and *mpk4* mutant has excessive underphosphorylated MAP65 and extensively bundled microtubules (Beck et al. 2011). TES shares about 57% amino acid sequence identity with HIK and it functions redundantly with HIK in both male and female gametophytic cytokinesis, probably through a similar mechanism (Tanaka et al. 2004; Oh et al. 2008).

CONCLUSIONS AND FUTURE DIRECTIONS

Many plant kinesins are known to contribute to the organization and/or function of the various plant microtubule arrays. However, the molecular mechanisms by which these kinesins fulfill their functions are largely unknown. Visualization of kinesins at high resolution in living cells and identification of their interacting proteins/cargoes is needed to fill this gap in our knowledge. Another outstanding question relates to the regulation of kinesin activity. How the activity of plant kinesins is regulated in space and time is largely a mystery, with the notable exception of KCBP, which is known to be regulated

by calcium-calmodulin (Deavours et al. 1998) and a second calcium-binding protein called KIC (Reddy et al. 2004). Regulation of motor activity is particularly important to understand how multiple kinesins work collectively to shape microtubule array organization.

As mentioned in the introduction, the Kinesin-7 and Kinesin-14 families are extensively expanded in land plants. The available studies show that some of these kinesins are indeed performing new functions to meet plant-specific needs. For example, HIK and TES in the Kinesin-7 family function in phragmoplast expansion and KCA1 and KCA2 in the Kinesin-14 family are involved in chloroplast movement. However, some of the other plant Kinesin-7 and Kinesin-14 members are expected to perform functions that are normally attributed to these kinesin families. For example, at least one plant Kinesin-7 member is expected to function in linking spindle microtubules to kinetochores since this is critical for attaching chromosomes to the spindle. In addition, as discussed previously, the ATK1 and ATK5 members of the Kinesin-14 family play a role in mitotic and meiotic spindle assembly, similar to the *Drosophila* NCD kinesin.

The functions of nearly 65% of the *Arabidopsis* kinesins remain unexplored. Therefore, a lot remains to be learned about plant kinesins. It is of particular interest to determine if kinesins function in the disassembly of the various microtubule arrays and to identify plant kinesins that function in chromosome condensation, capture, alignment and segregation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURES

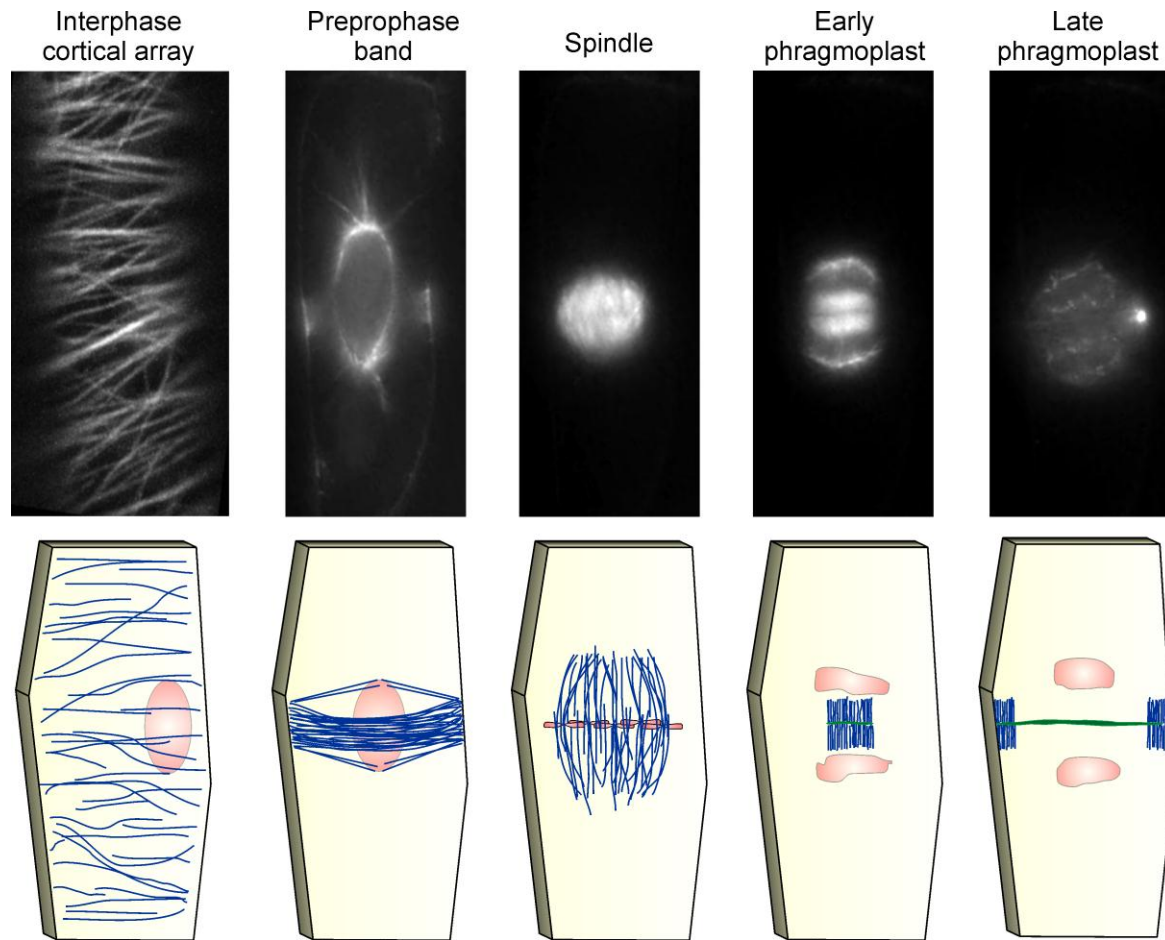


Figure 1: Plant microtubule arrays at various stages of the cell cycle. Images on the top show the major plant microtubule arrays visualized using GFP-labeled tubulin in tobacco BY2 cells. The key morphological features of these arrays are diagrammed in the cartoons below each image. In the cartoons, the nucleus is shown in red, microtubules are shown in blue and the cell plate is shown in green.

Table 1: *Arabidopsis* kinesins and their localization to the various plant microtubule arrays

Kinesin family	Gene ID	Other names	Cortical microtubule array [†]	Preprophase band [†]	Spindle [†]	Phragmoplast [†]	
Kinesin 1 (n=1)	At3g63480#						
Kinesin 2							
Kinesin 3							
Kinesin 4 (n=3)	At5g47820	FRA1	(Zhong et al. 2002)				
	At3g50240						
	At5g60930*						
Kinesin 5 (n=4)	At2g28620*	AtKRP125c=AtKIN5c	(Bannigan et al. 2007)	(Bannigan et al. 2007)	(Bannigan et al. 2007)	(Bannigan et al. 2007)	
	At2g37420*	AtKRP125a=AtKIN5a					
	At2g36200*	AtKRP125b=AtKIN5b					
	At3g45850	AtKIN5d=AtF16L2					
Kinesin 6 (n=1)	At1g20060						
Kinesin 7 (n=15)	At1g18370* At3g43210*	HIK=NACK1 TES=NACK2				(Nishihama et al. 2002; Strompen et al. 2002; Takahashi et al. 2010; Komis et al. 2011)	
	At1g21730 At4g39050	MKRP1 MKRP2	(Itoh et al. 2001)				
Kinesin 7 (n=15)	At2g21300 At4g38950 At3g51150# At5g66310 At4g24170 At5g42790# At3g12020 At5g06670 At2g21380# At1g59540*# At3g10180						
	Kinesin 8 (n=2)	At1g18550 At3g49650					
	Kinesin 9						
	Kinesin 10 (n=3)	At4g14330*	AtPAKRP2				(Lee et al. 2001)
		At5g02370*					
		At5g23910*					
	Kinesin 11						
	Kinesin 12 (n=6)	At4g14150* At3g23670*#	AtPAKRP1 AtPAKRP1L				(Lee and Liu 2000; Pan et al. 2004; Lee et al. 2007)
		At3g17360* At3g19050	POK1 POK2		(Walker et al. 2007; Xu et al. 2008)		
		At3g44050* At3g20150*					
Kinesin 13 (n=2)	At3g16630	Kinesin 13A	(Lu et al. 2005; Wei et al. 2009)				
	At3g16060*	Kinesin 13B					
Kinesin 14 (n=21)	At5g10470# At5g65460	KCA1=KAC1 KCA2=KAC2	(Suetsugu et al. 2010)	(Vanstraelen et al. 2006b)		(Vanstraelen et al. 2006b)	
	At4g21270*	ATK1=KATA	(Liu et al. 1996)	(Marcus et al. 2003; Liu et al. 1996)	(Liu et al. 1996; Chen et al. 2002; Marcus et al. 2003)	(Liu et al. 1996)	
	At4g05190	ATK5	(Ambrose et al. 2005)		(Ambrose et al. 2005; Ambrose and Cyr 2007)	(Ambrose et al. 2005)	
	At5g65930#	KCBP	(Oppenheimer et al. 2005)	(Bowser and	(Bowser and	(Bowser and	

			al. 1997; Mathur and Chua 2000)	Reddy 1997)	Reddy 1997; Vos et al. 2000)	Reddy 1997; Vos et al. 2000)
	At3g44730	KP1=AtKIN14h	(Ni et al. 2005) (Yang et al. 2011)			
	At5g27000	K C H	ATK4=KATD	(Frey et al. 2010) (Xu et al. 2007)		(Xu et al. 2007)
	At1g09170 At2g47500* At1g63640# At5g41310 At3g10310*					
	At4g27180 At5g54670	ATK2=KATB ATK3=KATC				
	At1g18410 At1g73860 At1g72250*# At2g22610*# At5g27550* At1g55550					
	At5g27950	Tobacco TBK5 counterpart	(Matsui et al. 2000; Goto and Asada 2007)			
Ungrouped (n=3)	At3g54870 At1g01950#	ARK1 =MRH2= AtKINUc ARK2=AtKINUb	(Yang et al. 2007; Sakai et al. 2008)			
	At1g12430#	ARK3=AtKINUa		(Malcos and Cyr 2011)		

† Microtubule array localization is based on either direct cell biological evidence or inferred from genetic analysis.

* indicates that the kinesin is upregulated during mitosis (Vanstraelen et al. 2006a)

indicates that the kinesin is predicted to have multiple isoforms due to alternative splicing (Based on the TAIR10 annotation)