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Kinesin-Related Proteins in Plant Cytokinesis

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ABSTRACT

Cytokinesis in higher plant cells is mediated by the phragmoplast. The framework of the phragmoplast consists of two anti-parallel sets of microtubules with their plus ends facing each other at or near the division site. During cytokinesis, the phragmoplast microtubule array undergoes dynamic reorganization from a solid cylinder to a hollow array. Concomitant with the microtubule reorganization, Golgi-originated vesicles are rapidly transported along microtubules toward their plus ends to give rise to the centrifugally growing cell plate. Kinesin-related motor proteins play crucial roles in microtubule reorganization and vesicle transport. To date, a number of plant proteins in the kinesin superfamily have been localized to the phragmoplast, and possibly exert roles in cytokinesis. Plus end-directed motors in the BIMC subfamily play a role in sliding anti-parallel microtubules apart to establish the phragmoplast microtubule array, while microtubule minus end-directed Ncd/Kar3-like motors in the C-terminal motor subfamily likely act antagonistically to balance the force generated by the BIMC-like kinesins. The novel C-terminal motor kinesin KCBP probably regulates microtubule organization and cross-linkage of the microtubule minus ends in a Ca^{++}/calmodulin-dependent manner. By acting at or near the plus ends of the microtubules, the *Arabidopsis* phragmoplast-associated kinesin-related protein AtPAKRP1 likely plays a role in establishing and/or maintaining the organization of phragmoplast microtubules. We anticipate more phragmoplast-associated motors to be revealed in the next few years as the completed *Arabidopsis* genome contains more than 45 genes encoding kinesin-related proteins. Orchestrated forces generated by different motors are required for microtubule-dependent activities to take place in the phragmoplast.

Key words: Cytokinesis; Kinesin-related proteins; Microtubules; Motor protein; Phragmoplast; Plant

INTRODUCTION

Cytokinesis in higher plant cells is mechanistically different from that in animal cells (Staehelin and Hepler 1996). In animal cells, cytokinesis takes place via a constriction process with the landmark of an actomyosin contractile ring (Robinson and Spudich 2000). In higher plants, however, cytokinesis is carried out in a centrifugal manner when cells physically partition segregated genomes and the cytoplasm (Smith 1999). General aspects of cell plate formation and regulation of cytokinesis have been summarized in several review articles published recently (Nacry and others 2000; Otegui and Staehelin 2000; Scheres and Benfey 1999; Smith 1999; Staehelin and Hepler 1996). Here we intend to collectively discuss several kinesin motor proteins found in the phragmoplast.

The phragmoplast, an apparatus executing cytokinesis in higher plant cells, is a highly organized structural complex with microtubules, actin mi-

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crofilaments, and membranous vesicles derived from endoplasmic reticulum and the Golgi apparatus (Staehelin and Hepler 1996). Arranged in an anti-parallel pattern, two sets of microtubules are the framework of the phragmoplast. These microtubules are oriented perpendicularly to the division plane with their plus ends pointed at each other at or near the future division site. The function of these microtubules is to allow vesicles to be transported toward their plus ends (Yasuhara and others 1993). The vesicles contain materials including callose and xyloglucans (Samuels and others 1995; Sonobe and others 2000), and their fusion gives rise to the cell plate, a physical divider between two daughter cells. Actin microfilaments in the phragmoplast also comprise two anti-parallel sets with their barbed ends pointed towards the division plane (Kakimoto and Shibaoka 1988). Unlike microtubules, however, they do not overlap at the division site (Zhang and others 1993). Actin microfilaments have been implicated in linking the expanding phragmoplast/cell plate to the predetermined cortical division site (Valster and Hepler 1997). Formation of the cell plate requires orchestrated reorganization of microtubules, microfilaments, and vesicles during the progression of cytokinesis.

Microtubule dynamics in the phragmoplast has been elegantly shown in living cells by fluorescent analog histochemistry (Zhang and others 1990, 1993), and by the green fluorescent protein (GFP) technique (Granger and Cyr 2000). A comparison of microtubule reorganization during phragmoplast development in plant cells and midbody development in animal cells is shown in Figure 1. Phragmoplast microtubules are derived from interzonal microtubules of the anaphase spindle. Initially, the interzonal microtubules appear in loose fibers running between two sets of segregated chromosomes. These microtubules then coalesce near the middle region, and new microtubules are assembled adjacent to the preexisting ones. This microtubule array aggregates into a cylindrical shape while the distal ends of microtubules (near the daughter nuclei) gradually depolymerize. During early stages of phragmoplast development, the interzonal microtubules have mixed polarities (Euteneuer and others 1982). Later on, the microtubule polarities are sorted out by a microtubule-sliding mechanism (Hogan and Cande 1990). The phragmoplast now has two mirror sets of microtubules with their plus ends overlapping near the future cell plate position (Euteneuer and others 1982). New microtubules continue to polymerize at the periphery of the phragmoplast, which results in the expansion toward the cell periphery.

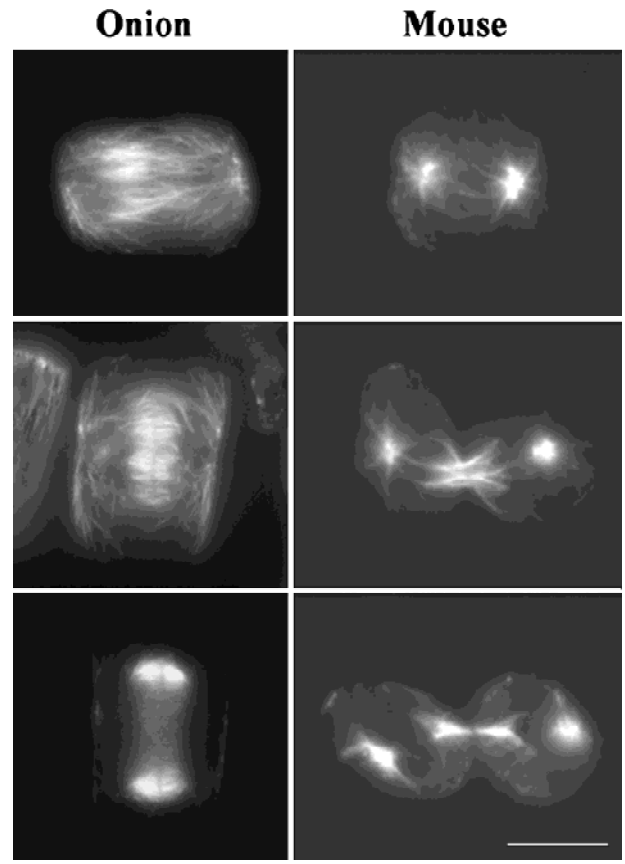

Figure 1. A comparison of microtubule organization in onion root tip cells (left column) and cultured mouse erythrocytes (right column). Cell have been stained with an anti-α-tubulin monoclonal antibody and visualized with an epifluorescence microscope. Top row: early telophase cells; middle row: early cytokinetic cells; bottom row: cells undergoing late cytokinesis. Scale bar: 10 μm.

While microtubule polymerization takes place at the phragmoplast periphery, microtubules depolymerize in the center of the phragmoplast. In *Tradescantia* stamen hair cells, depolymerization initiates when the phragmoplast microtubule array occupies approximately two-thirds of the cell diameter (Valster and Hepler 1997). The depolymerization event is concomitant with the build-up of the cell plate starting from the center. Therefore, the phragmoplast microtubule array appears as a collar once the cell plate is formed in the center of a dividing cell (Valster and Hepler 1997). Upon the fusion of the expanding cell plate with the parental cell membrane, microtubules in the phragmoplast completely depolymerize and cortical microtubules emerge (Hasezawa and others 2000).

Microtubule-based motor proteins, the kinesins and dyneins, play critical roles in microtubule reorganization and vesicle transport (Goldstein and Philp 1999). They are mechanochemical ATPase enzymes that utilize energy released from ATP hydrolysis to conduct motile activities along microtubules. Their ATPase activity can be activated upon binding to microtubules. Only kinesins are discussed in this review. Conventional kinesin was first identified as a force-generating protein in the squid giant axon (Vale and others 1985). Since then, proteins related to kinesin have been identified among various other eukaryotic organisms. These proteins share a conserved motor domain of approximately 350 amino acids with the conventional kinesin (Vale and Fletterick 1997). They are collectively called kinesin-related proteins (KRPs), and together with the conventional kinesin they are classified as members of the kinesin superfamily. The motor domain contains a catalytic core and a neck region. The catalytic core has an ATP-binding site and a microtubule-binding site, and is responsible for ATP hydrolysis and force generation. The neck region functions in the directionality and the amplification of motions (Case and others 1997, 2000; Endow and Higuchi 2000). Like conventional kinesin, KRPs often contain coiled-coil domains for oligomerization (mostly dimerization). Following the coiled-coil domains is the tail domain, which often has a globular structure. The tail domain specifies motor activities, for example, for targeting to their cargoes. The variations in the neck and tail domains determine differences in the structure and function among kinesins and KRPs (Goldstein and Philp 1999). To date, more than 200 KRP genes/proteins have been isolated from different eukaryotic organisms (http://www.blocks.fhcrc.org/~kinesin/MotorSeqTable.html). Depending on the location of the motor domain in individual polypeptides, kinesin motors are often classified as N-terminal motor KRPs, C-terminal motor KRPs, and central motor KRPs. Like the conventional kinesin, N-terminal motor KRPs generally are microtubule plus end-directed motors; all C-terminal motor KRPs tested so far are minus end-directed motors (Goldstein and Philp 1999). However, the location of the motor domain does not determine the directionality (Stewart and others 1993). As a matter of fact, the motor domain has an intrinsic property of being able to move toward the microtubule plus end (Endow and Waligora 1998). The neck domain of C-terminal motor KRPs overrides the intrinsic directionality to allow these motors moving toward the microtubule minus end (Endow and Waligora 1998; Henningson and Schliwa 1997). Because homologous motor proteins can be identified in different organisms, kinesin and KRPs can be divided into more than nine different subfamilies (Kim and Endow 2000). Kinesin and KRP subfamilies are named either according to the founding members in the subfamilies or according to the structures of motor holoenzymes (Kim and Endow 2000).

A number of KRPs have been identified in several angiosperm species (Asada and Collings 1997; Cai and others 2000). According to its genomic sequence, the *Arabidopsis thaliana* genome contains more than 45 *KRP* genes. To date, KRPs from three different subfamilies have been localized to the phragmoplast. They are BIMC, C-terminal motor KRPs, and AtPAKRP1 subfamilies. Table 1 compares these motors. A review of structures and functions of these different KRPs is presented here.

Members of the BIMC Subfamily: The Tobacco TKRP125 Protein and Its Homologues in Carrot and *Arabidopsis*

The founding member of this subfamily, BIMC, was identified in the filamentous fungus *Aspergillus nidulans* a decade ago (Enos and Morris 1990). A mutation in the corresponding *bimC* gene blocks nuclear division at M phase, with bim standing for *b*lock-*i*n-*m*itosis. Homologs of BIMC are found among all eukaryotic organisms so far examined, indicating that they are evolutionarily conserved. These KRPs have a common tripartite structure: an N-terminal motor domain, coiled-coils, and a globular tail domain. A conserved "BIMC box" sequence in the tail domain is found among these KRPs (Figure 2) (Asada and others 1997; Blangy and others 1995). Phosphorylation of a threonine residue in the "BIMC box" is required for the motor to associate with the mitotic spindle in animals (Blangy and others 1995; Sawin and Mitchison 1995). However, the fission yeast homolog Cut7 functions independently of the phosphorylation event (Drummond and Hagan 1998), indicating a divergence of functional regulation during evolution. Hydrodynamic analysis indicates that these KRPs are in homotetrameric forms under native conditions (Cole and others 1994; Gordon and Roof 1999). Shown by ultrastructural analysis, these motors have a bipolar structure with two motor domains at both ends in a dumbbell-like appearance (Gordon and Roof 1999; Kashina and others 1996). Therefore, motors in the BIMC subfamily are often referred to as bipolar kinesins (Kashina and others

Table 1. A Comparison of Phragmoplast-Localized KRPs from Three Different Subfamilies

KRPs	Size (kDa)	Motor location	Velocity (μm/min)	Localization	Possible role(s)	Ref
BIMC-like KRPs	~125	N-terminus	1.28, +	Concentrated along interzonal Mts and phragmoplast Mts	Sliding of anti-parallel Mts	1–3
TKRP125			ND, + (?)			
DcKRP120-2			ND, + (?)			
AtBIMCa/b/c						
Ncd/Kar3-like KRPs	~85	C-terminus	ND, – (?)	Concentrated along interzonal Mts and phragmoplast Mts	Brake of anti-parallel Mt sliding, parallel Mt transport	4–7
AtKatA/B/C						
AtKCBP	~140	C-terminus	8.1, –	Concentrated toward Mt minus ends in spindle and phragmoplast	Stabilizing spindle and phragmoplast Mts	8–12
AtPAKRP1	~145	N-terminus	ND, + (?)	At or near plus ends of phragmoplast Mts	Maintaining Mt interdigitation	13

ND, not determined; Mts, microtubules. References: 1, Asada and Shibaoka 1994; 2, Asada and others 1997; 3, Barroso and others 2000; 4, Mitsui and others 1993; 5, Mitsui and others 1994; 6, Mitsui and others 1996; 7, Liu and others 1996; 8, Reddy and others 1996; 9, Song and others 1997; 10, Bowser and Reddy 1997; 11, Smirnova and others 1998; 12, Vos and others 2000; 13, Lee and Liu 2000.

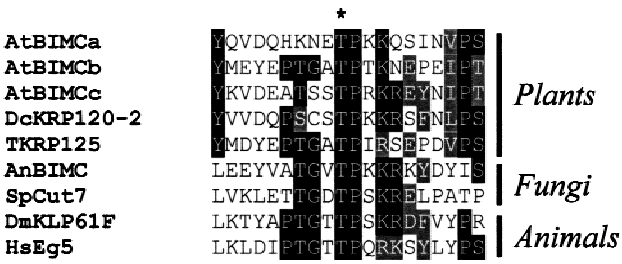

Figure 2. Sequences of the "BIMC box" in BIMC-like kinesins from plants, fungi, and animals. The threonine residue marked by the star (*) is phosphorylated by $p34^{cdc2}$ **during cell division. AtBIMCa/b/c are from** *Arabidopsis thaliana;* DcKRP120-2 is from *Daucus carota;* TKRP125 is from *Nicotiana tabacum;* AnBIMC is from *Aspergillus nidulans;* SpCut7 is from *Schizosacchromyces pombe;* DmKLP61F is from *Drosophila melanogaster;* HsEg5 is from *Homo sapiens.*

1996). All examined members of this subfamily demonstrate a microtubule plus end-directed motor activity with a velocity of 1–3 μm/min (Asada and Shibaoka 1994; Barton and others 1995; Cole and others 1994; Gheber and others 1999).

Both fungal and animal BIMC-like motors localize to the mitotic spindle with a preference to the microtubule-overlapping region in anaphase spindles (Hagan and Yanagida 1992; Sharp and others 1999a). Genetic data and antibody microinjection results indicate that the BIMC KRPs are required for maintaining the bipolar structure of mitotic spindles (Enos and Morris 1990; Heck and others 1993; Saunders and Hoyt 1992; Sawin and others 1992; Sharp and others 1999b). Such a role was clearly shown in an *A. nidulans* strain carrying the temperature-sensitive *bimC4* mutation and a GFP-α-tubulin fusion (YR Lee and B Liu unpublished data). Upon a shift from permissive temperature to restrictive temperature, the mitotic spindles were broken in the middle rendering a microtubule array of two half spindles with two juxtaposed spindle poles. In the mitotic spindle, BIMC-like KRPs cross-link interpolar anti-parallel microtubules *in vivo,* and slide them apart (Figure 3) (Sharp and others 1999a). They are also concentrated along midbody microtubules in animal cells, implying a similar role of sliding anti-parallel microtubules in the midbody as well (Sharp and others 1999a; Whitehead and Rattner 1998).

In the phragmoplast, microtubule polymerization takes place continuously at the plus ends located at or near the division site (Asada and others 1991; Vantard and others 1990). Using phragmoplasts isolated from synchronized tobacco BY-2 cells, it was found that newly polymerized microtubule segments were pushed away from their overlapping region toward daughter nuclei in an energy (ATP or GTP)-dependent manner (Asada and others 1991). A 125 kDa polypeptide, which bears a microtubule plus end-directed microtubule-translocating activity, was isolated from the tobacco phragmoplasts (Asada and Shibaoka 1994). The amino acid sequence of this TKRP125 protein indicates that its N-terminus resembles the kinesin motor domain (Asada and others 1997). The polypeptide also contains a "BIMC box" consensus sequence (Figure 2). Proteins related to TKRP125 have been isolated from carrot suspension cells (Barroso and others 2000;

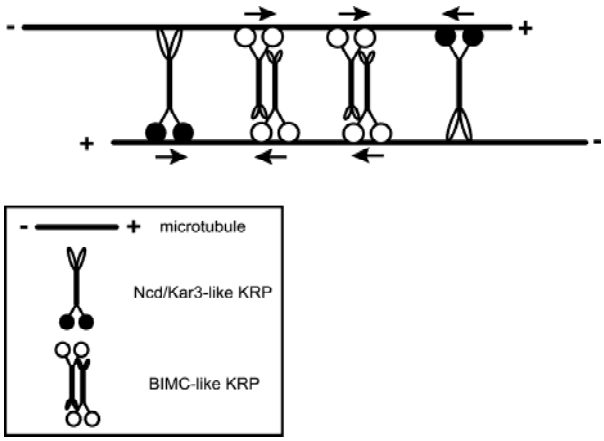

Figure 3. Diagram of antagonistic interaction between a BIMC-like kinesin and a Ncd/Kar3-like KRP along anti-parallel microtubules. While the BIMC-like kinesin pushes anti-parallel microtubules apart, the Ncd/Kar3 KRP holds them together. Arrows indicate the directions of motor movement. The microtubule plus end is marked as "+", and minus end as "–".

Chan and others 1996). Using antibodies raised against these polypeptides, such proteins have been shown to decorate interphase cortical microtubule array, the preprophase band, spindle, and the phragmoplast by immunofluorescence microscopy (Asada and others 1997; Chan and others 1996). The localization is especially conspicuous along interzonal microtubules in spindles at late anaphase. Recently, it has been shown that carrot DcKRP120-2 strongly localizes to the phragmoplast midline (Barroso and others 2000).

In the *A. thaliana* genome, three *BIMC*-like genes have been identified, and their products have been designated as AtBIMCa, AtBIMCb, and AtBIMCc (YR Lee and B Liu unpublished data). They share approximately 40% identity to each other at the amino acid sequence level, and all have 29% identity to the *Aspergillus* BIMC. AtBIMCa and AtBIMCc share approximately 40% identity to TKRP125, and AtBIMCb shares 70% identity with TKRP125, implying that it is the *Arabidopsis* ortholog of TKRP125. Sequences resembling the "BIMC box" have been identified from all three predicted sequences (Figure 2). It is not clear why a plant needs three different motors of a single subfamily.

The C-Terminal Motor KRP Subfamily Members: KatA, KatB, KatC, and KCBP

Ncd/Kar3-like KRPs

The budding yeast *Kar3* gene and the fly *Ncd* (Non-claret disjunctional) gene encode the first known members of the C-terminal motor KRPs (Endow and others 1990; Meluh and Rose 1990). Ncd/Kar3-like KRPs have a nucleotide-independent microtubule-binding domain at the N-terminus besides a nucleotide-dependent microtubule-binding site in the motor domain located at the C-terminus. Multiple coiled-coil domains are present in the central region. Although it is generally predicted that these KRPs form homodimers with the coiled-coils, the budding yeast Kar3 protein forms a heterodimer with the microtubule-associated protein Cik1p (Barrett and others 2000). Unlike conventional kinesin, C-terminal motor KRPs are microtubule minus end-directed motors with velocities of 4–6 μm/min (McDonald and others 1990; Middleton and Carbon 1994; Walker and others 1990). The unique neck sequence in C-terminal motor KRPs overrides the directionality of the motor domain to allow them to become minus end-directed motors (Endow 1999).

A polymerase chain reaction-based screening has revealed three *A. thaliana* genes encoding polypeptides of the C-terminal motor subfamily: KatA, KatB, and KatC (Kat for *k*inesin of *A. thaliana*) (Mitsui and others 1993, 1994). Approximately 30% amino acid identity can be found among each of these three predicted polypeptide sequences and the fly Ncd protein. KatA is 56% identical to either of KatB or KatC, and KatB and KatC share 83% identity. Although not tested, it will not be surprising if the N-terminus of these proteins has microtubule-binding activity. These *Arabidopsis* KRPs also have conserved overall structure properties with Ncd/Kar3-like KRPs from other organisms. Therefore, they probably bear a microtubule minus end-directed motor activity.

In synchronized tobacco BY-2 cells, homologs of KatB and KatC accumulate during M phase (Mitsui and others 1996). Antibodies raised against these two proteins localize to the mitotic apparatus and the phragmoplast (Mitsui and others 1996). Use of antibodies raised against two peptides in the motor domain of KatA reveals that KatA is concentrated along interzonal microtubules of the anaphase spindle and along phragmoplast microtubules (Liu and others 1996). Among the Ncd/Kar3-1ike KRPs from other organisms, the budding yeast Kar3 protein and the *A. nidulans* KLPA protein localize to spindle pole bodies, the microtubule-organizing centers in these organisms (Manning and others 1999; Saunders and others 1997; B Liu unpublished data). However, *Drosophila* Ncd, *Xenopus* XCTK2, and mouse CHO2 localize along spindle microtubules (Hatsumi and Endow 1992; Kuriyama and others 1995; Walczak and others 1997). It is also interesting that, although Kar3 specifically destabi-

lizes microtubules at their minus ends, the tail microtubule-binding domain of Ncd stabilizes microtubules *in vitro* (Endow and others 1994; Huyett and others 1998; Karabay and Walker 1999a, 1999b).

The Kar3 protein was first suggested to be required for nuclear migration during karyogamy in budding yeast, but not for mitosis (Meluh and Rose 1990). In *A. nidulans,* deletion of the *klpA* gene alone did not show a noticeable phenotype (O'Connell and others 1993). However, the null *klpA1* mutation suppresses the otherwise lethal *bimC4* mutation implying that the KLPA protein acts antagonistically with the BIMC protein. Similar effects can be found in both budding yeast and fission yeast (Hoyt and others 1993; Pidoux and others 1996). Recently, antibody microinjection experiments have further indicated that a similar antagonistic interaction between BIMC-like KRPs and Ncd/Kar3-1ike KRPs exists in animal cells (Mountain and others 1999; Sharp and others 1999b).

We anticipate an antagonistic interaction between plus end-directed BIMC-like KRPs and minus end-directed Ncd/Kar3-like KRPs in anti-parallel phragmoplast microtubules as both are concentrated along interzonal microtubules of the anaphase spindle and phragmoplast microtubules (Figure 3). A balance of such antagonistic forces could be important for a smooth transition from microtubules with mixed polarities to two anti-parallel sets of microtubules.

A Novel Calmodulin-binding C-terminal Motor KRP: KCBP

The *k*inesin-like *c*almodulin-*b*inding *p*rotein, KCBP, was isolated using biotinylated calmodulin as a bait to screen an *A. thaliana* expression library (Reddy and others 1996). Besides the common feature found among Ncd/Kar3p-like KRPs, three additional domains are present: a myosin tail homolog domain, a talin-like domain, and a C-terminal calmodulin-binding domain (Narasimhulu and Reddy 1998; Reddy and Reddy 1999). In an *in vitro* microtubule motility assay, KCBP was shown to have a minus end-directed motor activity with a velocity of 8–10 µm/min (Song and others 1997). The motor-microtubule interaction and motor activity are inhibited upon binding to Ca^{++}/calmodulin, which is unique to KCBP (Deavours and others 1998; Narasimhulu and others 1997; Song and others 1997). Therefore, the motor activity can be activated using an antibody against the calmodulin-binding domain, as the association with the antibody prevents the interaction between KCBP and Ca^{++}/calmodulin from taking place (Narasimhulu and others 1997).

Using the antibody against the calmodulin-binding domain, KCBP was localized to the preprophase band, spindle, and the phragmoplast in plant suspension cells in an immunofluorescence study (Bowser and Reddy 1997). In the phragmoplast, the KCBP immunofluorescence signal was especially pronounced towards microtubule minus ends. In *Haemanthus* endosperm cells, a KCBP homolog associates with kinetochore fibers during metaphase, then accumulates to the spindle poles during late anaphase, and finally associates with phragmoplast microtubules (Smirnova and others 1998). KCBP's association with anaphase spindle poles and minus ends of phragmoplast microtubules suggests that it may be involved in organizing microtubule minus ends.

The same antibody was used in microinjection experiments to reveal the consequence of the constitutive activation of KCBP (Vos and others 2000). The antibody introduction induced the formation of aberrant phragmoplasts, and delayed the completion of cytokinesis significantly. No effect was found on the progression of anaphase, but cells injected prior to nuclear envelope breakdown had an early nuclear envelope breakdown and were subsequently arrested at prometaphase. It has been proposed that KCBP's activity is down-regulated in the phragmoplast by a rise of Ca^{++} concentration in the phragmoplast region. The up-regulation of its activity after nuclear envelope breakdown and during anaphase may contribute to converging microtubule minus ends in the spindle poles (Vos and others 2000).

In an independent genetic analysis, it has been shown that the *Arabidopsis zwichel* gene is allelic to the *KCBP* gene, and the null *zwichel* mutation only affects trichome branching (Oppenheimer and others 1997). No abnormality in cell division has been observed in mutant plants. It will be interesting to test whether KCBP is functionally redundant with other C-terminal motor KRPs during cell division in plants.

A Novel Phragmoplast-Associated KRP: AtPAKRP1

AtPAKRP1, *A. thaliana p*hragmoplast-*a*ssociated *k*inesin-*r*elated *p*rotein *1*, is an N-terminal motor KRP (Lee and Liu 2000). Because it bears a neck sequence conserved among plus end-directed kinesin/KRPs, AtPAKRP1 is suggested to be a plus end-directed motor, although its *in vitro* motility has not been shown successfully. Following a neck domain, AtPAKRP1 has coiled-coil domains and sequences with no obvious motifs.

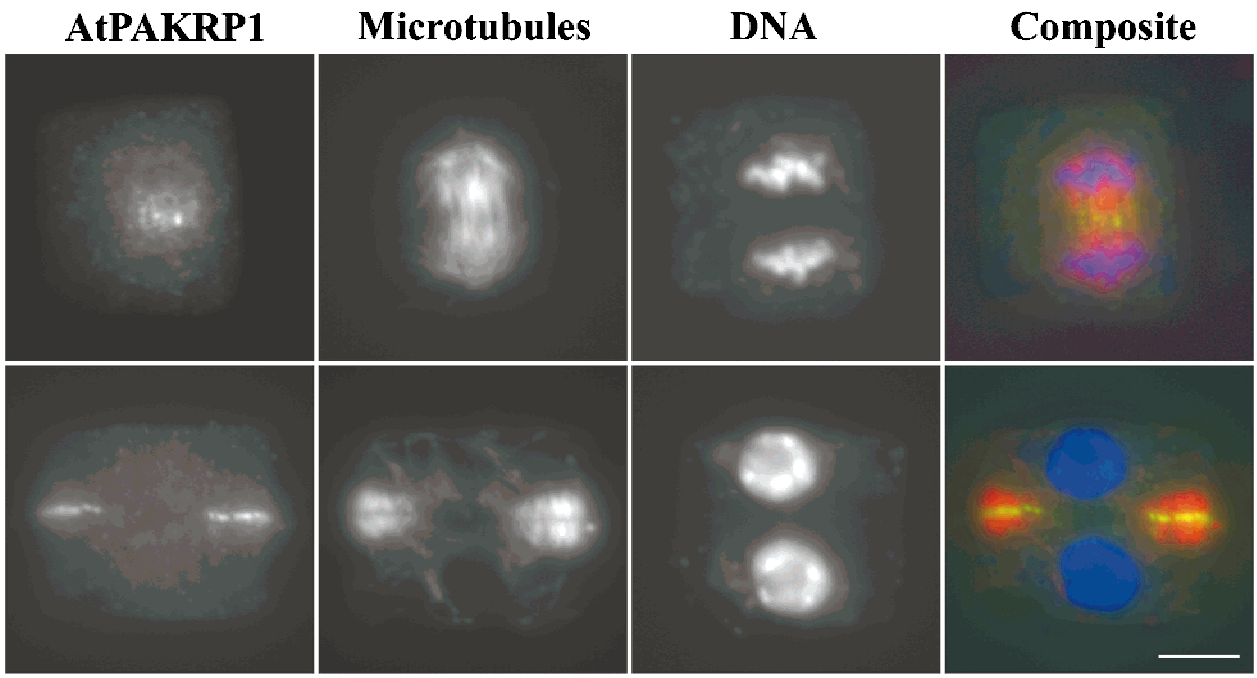

Figure 4. Triple labeling of AtPAKRP1, microtubules, and DNA in *Arabidopsis* root cells entering telophase (top row) and undergoing cytokinesis (bottom row). Color composite images are shown in the far right column, with AtPAKRP1 shown in green, microtubules in red, and DNA in blue. Scale bar: 5 μm. Reproduced from Lee and Liu (2000), with permission from Elsevier Science.

In an immunofluorescence study, AtPAKRP1 did not have a distinct localization pattern prior to late anaphase (*Lee and Liu 2000*). When chromosomes almost reach the spindle poles, AtPAKRP1 appears along interzonal microtubule bundles in the late anaphase spindle. At this stage, AtPAKRP1 localizes to a rather wide region near the middle of these interzonal microtubules. Once the polarities of these interzonal microtubules are sorted out, presumably by one or more BIMC-like KRPs, AtPAKRP1 is much more restricted in the middle of the microtubule bundles running between the two phragmoplast halves. Later on, AtPAKRP1 is restricted at or near the plus ends of the interdigitating phragmoplast microtubules (Figure 4). This localization pattern persists until the phragmoplast microtubules are fully depolymerized. AtPAKRP1 localization in the phragmoplast is dependent on the integrity of these microtubules, as microtubule depolymerization by the anti-microtubule agent colchicine can abolish its localization at the division site (Lee and Liu 2000).

The function of the tobacco homolog of AtPAKRP1 was tested in BY-2 cells (Lee and Liu 2000), using anti-AtPAKRP1 antibodies or truncated AtPAKRP1 fusion proteins as inhibitors. After the inhibitors were loaded into glycerin-permeablized cells, phragmoplast microtubules were disorganized in a significant proportion of the cells. They lose their anti-parallel pattern and became randomly arranged. Therefore, AtPAKRP1 is suggested to be a microtubule motor protein that maintains the integrity of phragmoplast microtubules by keeping microtubule plus ends in position while new microtubule segments are added to the ends (Figure 5). Such activity could be required after BIMC-like KRP(s) complete the microtubule-sliding duty. The establishment and maintenance of the phragmoplast microtubule array allow vesicles to be delivered to the right place.

Recently, we have identified another phragmoplast-specific N-terminal motor KRP in *A. thaliana*, AtPAKRP2. Preliminary immunofluorescence results indicate that it associates with phragmoplasts in a distinct manner from that of AtPAKRP1 (YR Lee and B Liu unpublished results). AtPAKRP2 appears in a punctate manner near the division site. Additionally, punctate signals can be observed along phragmoplast microtubules. This localization pattern coincides with theoretic motor(s) for transporting Golgi-derived vesicles to the division site, although the AtPAKRP2 function has yet to be determined.

In a recent study of the MAP kinase signaling pathway, two KRPs act as activators for the MAP

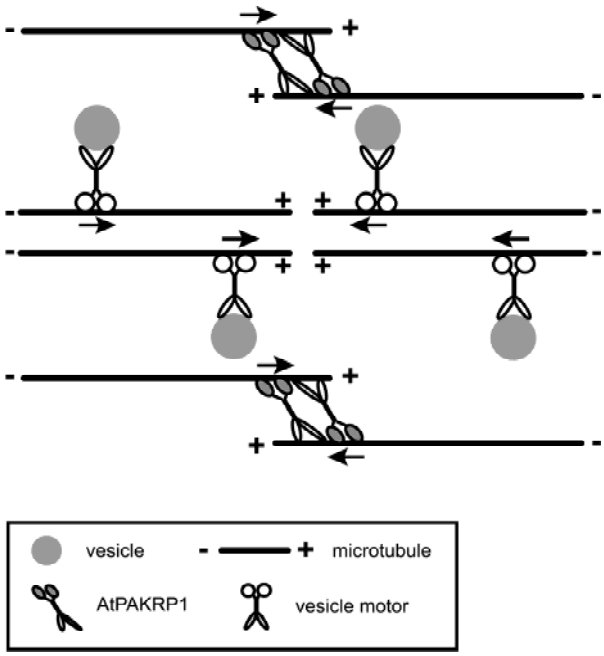

Figure 5. Model of how two KRPs might be acting in the phragmoplast. AtPAKRP1 acts at or near the plus ends of interdigitating microtubules. While the overall microtubule array is maintained by AtPAKRP1, Golgi-derived vesicles are being transported along non-interdigitating microtubules towards the division site by plus end-directed motors. Vesicle motors are yet to be identified. Arrows indicate the directions of motor movement. The microtubule plus end is marked with "+", and minus end with "–".

kinase kinase kinase (MAPKKK) NPK1 (Machida and others 1998). Although the article did not reveal the identities of these KRPs, it was mentioned that all these proteins had been localized to the division site.

CONCLUSION

Phragmoplast-mediated cytokinesis is clearly dependent on a number of plus end- and minus end-directed microtubule-based motors. In an oversimplified version, we suggest that BIMC-like KRPs initiate the sliding of interzonal microtubules. The Ncd/Kar3-like KRPs act antagonistically against the BIMC-like KRPs to balance forces along microtubules. Once phragmoplast microtubules emerge in an anti-parallel pattern, AtPAKRP1 is activated to stabilize the overall organization of this mirror-image array of microtubules. The establishment and maintenance of microtubule plus ends at or near the division site is required for vesicles to be delivered accurately to form the cell plate. Vesicle transport toward the division site must be carried out by plus end-directed motors. Therefore, to ensure a smooth progression of cytokinesis, activities of different microtubule-based motors must be sequentially up-regulated and down-regulated to create and balance forces required for each specific motion along phragmoplast microtubules.

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