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INVITED REVIEW

Evaluating the microtubule cytoskeleton and its interacting proteins in monocots by mining the rice genome

Longbiao Guo[1,2,†], Chin-Min Kimmy Ho[2,†], Zhaosheng Kong[2,†], Yuh-Ru Julie Lee[2,†], Qian Qian[1] and Bo Liu[2,*]

[1]*State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310006, China and* [2]*Department of Plant Biology, University of California, Davis, CA 95616, USA*

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• *Background* Microtubules (MTs) are assembled by heterodimers of α- and β-tubulins, which provide tracks for directional transport and frameworks for the spindle apparatus and the phragmoplast. MT nucleation and dynamics are regulated by components such as the γ-tubulin complex which are conserved among eukaryotes, and other components which are unique to plants. Following remarkable progress made in the model plant *Arabidopsis thaliana* toward revealing key components regulating MT activities, the completed rice (*Oryza sativa*) genome has prompted a survey of the MT cytoskeleton in this important crop as a model for monocots.
• *Scope* The rice genome contains three α-tubulin genes, eight β-tubulin genes and a single γ-tubulin gene. A functional γ-tubulin ring complex is expected to form in rice as genes encoding all components of the complex are present. Among proteins that interact with MTs, compared with *A. thaliana*, rice has more genes encoding some members such as the MAP65/Ase1p/PRC1 family, but fewer for the motor kinesins, the end-binding protein EB1 and the mitotic kinase Aurora. Although most known MT-interacting factors have apparent orthologues in rice, no orthologues of arabidopsis RIC1 and MAP18 have been identified in rice. Among all proteins surveyed here, only a few have had their functions characterized by genetic means in rice. Elucidating functions of proteins of the rice MT cytoskeleton, aided by recent technical advances made in this model monocot, will greatly advance our knowledge of how monocots employ their MTs to regulate their growth and form.

Key words: Cytoskeleton, kinesins, microtubules (MTs), microtubule-associated proteins (MAPs), motors, rice, *Oryza sativa*.

INTRODUCTION

The 25-nm tubular structure of microtubules (MTs) was first described in the monocot *Phleum pratense* (Timothy-grass) over four decades ago (Ledbetter and Porter, 1963). Since then, MTs have been implicated in almost every single aspect of intracellular activities, from cell division to cell morphogenesis in plants, as well as among other eukaryotic organisms. Assembled by the heterodimers of α- and β-tubulin GTPases in a head-to-tail fashion, MTs exhibit their characteristic dynamic instability phenomenon *in vitro* and *in vivo* (Baskin, 2000). The polarity of MTs is defined with a more dynamic (faster growing and shortening) plus end and less dynamic minus end. The terminal sub-unit at the minus end is α-tubulin, and at the plus end is β-tubulin. MTs often exhibit a rapid catastrophic process resulting from forming a cap of GDP-β-tubulin following GTP hydrolysis, which precedes a slower rescue process to recruit tubulin dimers containing GTP-bound β-tubulin. This phenomenon is known as dynamic instability, which is observed more frequently at the plus end than at the minus end.

In plants, one of the most noticeable features of the dynamic MT network is reflected by the four MT arrays exhibited by mitotic cells in somatic tissues. They are the parallel cortical MTs, the pre-prophase band (PPB), the barrel-shaped mitotic apparatus and the phragmoplast. The rapid turnover of these arrays was first observed in live cells by microinjection of fluorescently labelled tubulins, and later by green fluorescent protein (GFP) fusion proteins with α-tubulin or an MT-binding motif (Zhang *et al.*, 1990; Marc *et al.*, 1998; Ueda *et al.*, 1999; Granger and Cyr, 2000; Kumagai *et al.*, 2001). Besides the dynamic instability property intrinsic to MTs, their rapid turnover in plant cells must be modulated by MT-associated proteins (MAPs) whose activities may be regulated in a cell cycle-dependent manner. Progress made in the past few years has resulted in the identification of proteins which decorate all arrays or a particular array, mainly in *A. thaliana*. A number of these proteins are either remotely related to MAPs in organisms of other kingdoms, or specific to flowering plants. Unfortunately, very little progress has been made in characterizing proteins which are associated with MTs in rice and other monocots.

In the past years, fascinating discoveries have been made to allow MTs to be connected with various aspects of plant growth and development. One of the remaining questions is how plant cells remodel their MTs in response to hormones such as auxins and brassinosteroids. Recently, a previously identified auxin-inducible gene has turned out to encode a MAP which may participate in spatial regulation of cell division (Buschmann *et al.*, 2006). Another quest for plant MAPs has resulted in a phospholipase D isoform as a potential linker between cortical MTs and the plasma membrane

[†]These authors contributed equally to this work.
* For correspondence. E-mail: bliu@ucdavis.edu

(Gardiner *et al.*, 2001). In addition, external cues such as salt stress also trigger responses of MT reorganization (Shoji *et al.*, 2006; Wang *et al.*, 2007*a*). Now, there is no doubt that plant cells need MAPs for MT-based activities (Lloyd and Hussey, 2001). It is foreseeable that mining plant genomes and proteomes by various approaches will soon result in the identification of more plant MAPs that mediate various growth responses.

The excitement generated by functional studies of plant MTs and MAPs has been expressed in several review articles (Hussey and Hawkins, 2001; Sedbrook, 2004; Hamada, 2007; Kaloriti *et al.*, 2007; Mineyuki, 2007). However, most if not all published studies have been carried out in dicot systems such as *A. thaliana* and tobacco BY-2 cells. A proteomic attempt showed promise in identifying proteins interacting with tubulin in rice (Chuong *et al.*, 2004). Following in the footsteps of the initial description of MTs in monocots, the sequenced rice genome has prompted a survey of the inventory of the MT cytoskeleton and its interacting proteins in this model species of Graminaceae. The sequence analysis reported here was largely carried out by referring to two annotation systems: the Rice Genome Annotation Project (RGAP, http://rice.plantbiology.msu.edu/) with calls noted as LOC_Osxxgxxxxx, and the Rice Annotation Project (RAP, http://rapdb.dna.affrc.go.jp/) with calls noted as Osxxgxxxxxxx. Proteins discussed in this article are listed in Table 1. By summarizing the rice MT cytoskeleton based on the best current understanding of plant MTs, it is anticipated that in coming years additions of novel rice proteins will be made to the inventory or rice MAPs. It is hoped that this survey will help integrate functions of the proteins of the rice MT cytoskeleton with historical observations on MT-based cellular processes, e.g. cell division, in monocot systems such as onion and spiderwort.

TABLE 1. *Proteins discussed in this review*

Protein family	Members in rice
Tubulin	OsTUA (3), OsTUB (8), OsTUG (1)
Tubulin-folding factors	Tubulin-specific chaperones A–E (1 each), OsARL2 (1)
γ-Tubulin ring complex	OsGCP2–4 (1 each), OsGCP5 (2), OsGCP6 (1), OsNEDD1/GCP-WD (1)
Aurora kinase	OsAUR1 (1), OsAUR2 (1)
TPX2/WVD2	OsWDL (6)
MAP65/Ase1p	OsMAP65 (11)
XMAP215/TOG	OsMOR1 (1)
MAP70	OsMAP70 (4)
AIR9	OsAIR9 (1)
TOR1/SPR2	OsTOR1/SPR2 (4)
EB1	OsEB1a (1), OsEB1b (1)
SPR1	OsSPR1 (4)
CLASP	OsCLASP (3)
TAN	OsTAN (1)
TON1	OsTON1 (2)
TON2	OsTON2 (1)
Katanin	OsKSS1/SGL1 (p60) (1), OsKLS (p80) (3)
PLDδ	OsPLDδ (1)
MAP190	OsMAP190 (1)
Kinesin	OsKinesin (52)

Numbers in parentheses represent how many similar proteins are encoded by the rice genome.

THE TUBULIN FAMILY PROTEINS

Early persistent investigations have resulted in the conclusion that plant tubulins are encoded by multigene families, e.g. there are six α-tubulin, nine β-tubulin and two γ-tubulin genes in the model organism *A. thaliana* (Kopczak *et al.*, 1992; Snustad *et al.*, 1992; Liu *et al.*, 1994). Although it is always tempting to postulate that different isoforms of tubulins may take on different tasks, to date experimental data have not been able to prove this assumption despite ample evidence of tissue-specific expression patterns of tubulin genes (Eun and Wick, 1998; Goddard *et al.*, 1998). When polymerization-competitive bovine brain α- and β-tubulins were delivered by microinjection into stamen hair cells of the spiderwort *Tradescantia virginiana*, they entered polymerization/depolymerization cycles and were incorporated into various arrays of MTs (Zhang *et al.*, 1990). Hence, different isoforms of tubulins are believed to be functionally equivalent, and when expressed in the same cells they exhibit functional redundancy, as shown by the two γ-tubulin genes in *A. thaliana* (Pastuglia *et al.*, 2006).

Prior to the completion of the rice genome, the quest for rice tubulin genes had already uncovered all α- and β-tubulins (Kang *et al.*, 1994; Qin *et al.*, 1997; Yoshikawa *et al.*, 2003). There are three α-tubulin genes (*OsTUA*) and eight β-tubulin genes (*OsTUB*) in rice. Expression analysis indicated that tissue-specific expression was found among individual members of these tubulins (Qin *et al.*, 1997; Yoshikawa *et al.*, 2003). For example, *OsTUB8* was specifically expressed in anthers (Yang *et al.*, 2007). This fact mirrors the finding that *AtTUA1* and *AtTUB9* are preferentially expressed in anthers in *A. thaliana* (Carpenter *et al.*, 1992; Cheng *et al.*, 2001).

Plant γ-tubulin was first detected in all MT arrays and found to be pronounced toward minus ends of MTs in the onion *Allium cepa* 15 years ago (Liu *et al.*, 1993). Plant γ-tubulin and its associated proteins are not restricted to the MT minus end, and often appear on the wall of extant MTs to initiate the formation of MT branches (Murata *et al.*, 2005). Genetic approaches have confirmed that γ-tubulin is essential for MT organization in *A. thaliana* (Binarova *et al.*, 2006; Pastuglia *et al.*, 2006). Surprisingly, there is only a single γ-tubulin gene (*OsTUG*) in the rice genome. The protein encoded by this LOC_Os05g06450/Os05g0156600 locus is expected to be essential for MT organization as seen for the redundant role of two γ-tubulin genes in *A. thaliana* (Pastuglia *et al.*, 2006).

Despite the significantly larger genome size of rice compared with that of *A. thaliana*, the fact that there are fewer tubulin genes in rice makes it an attractive system for future genetic manipulation of functions of individual tubulins.

PREPARING TUBULINS FOR POLYMERIZATION: TUBULIN-SPECIFIC CHAPERONES

Six tubulin-specific chaperones (A–E) and ARL2 are conserved proteins which are solely devoted to folding tubulins to make heterodimers competent for polymerization. Studies in *A. thaliana* have indicated that their functions are indispensable for MT-dependent cellular activities, and mutations often lead to embryo lethal phenotypes (Kirik *et al.*, 2002*a*, *b*; Steinborn *et al.*, 2002; Tzafrir *et al.*, 2002).

In the rice genome, single genes encode tubulin-specific chaperones A–E and ARL2: LOC_Os02g57150/Os02g0816500 (113 amino acids), LOC_Os04g59560/Os04g0692100 (267 amino acids), LOC_Os02g35110/Os02g0557000 (353 amino acids), LOC_Os10g36490/Os10g0508500 (1140 amino acids), LOC_Os05g01500/Os05g0105300 (433 amino acids) and LOC_Os02g22140/Os02g0327100 (186 amino acids), respectively. Similarly to their counterparts in other organisms, chaperones B and E contain a CAP-Gly domain toward their N-termini, which is the key element of the MT-binding domain in proteins such as the dynactin component $p150^{Glued}$ and CLIP170. Whether the CAP-Gly motif of these two proteins binds MTs *in vivo* awaits further examination. The ARL2 protein is a small GTPase, and is known as an ADP-ribosylation factor.

AT THE MT MINUS END: THE γ-TUBULIN RING COMPLEX

Unlike α- and β-tubulins whose heterodimers act as bricks of the MT cylinder, γ-tubulin forms a ring complex (γTuRC) with five other accessory proteins in metazoans (Wiese and Zheng, 2006). The proteins of the complex are often abbreviated as GCPs (γ-tubulin complex proteins) or Grips (γ-tubulin ring proteins), which typically decorate the MT minus end. The accessory proteins are structurally related, and all contain five homologous regions or two grip motifs, with regions I and II for motif 1 and regions III–V for motif 2 (Gunawardane *et al.*, 2000; Murphy *et al.*, 2001). The most critical players of the γTuRC are γ-tubulin plus Spc97p/GCP2 and Spc98p/GCP3, which are conserved among all eukaryotes and form the γ-tubulin small complex (γTuSC), which is the core of the large γTuRC (Wiese and Zheng, 2006). The budding yeast *Saccharomyces cerevisiae* only produces γTuSC (Wiese and Zheng, 2006). The γ-tubulin complex plays an essential role in MT nucleation. Recently, it has been found that a WD40 repeat-containing protein NEDD1 or GCP-WD interacts with γTuRC, and is essential for spindle assembly, probably due to its role in recruiting γTuRC to the centrosome, the MT-organizing centre (MTOC) in animal cells (Gunawardane *et al.*, 2003; Haren *et al.*, 2006; Lüders *et al.*, 2006; Manning and Kumar, 2007).

Homologues of GCP2-6 and NEDD1/GCP-WD have been identified among land plants by mining the available plant genomes (Murata *et al.*, 2007). Among them, the GCP2/Spc97p and GCP3/Spc98p homologues have been proved to form a soluble complex with γ-tubulin, and decorate the nuclear envelope (Erhardt *et al.*, 2002; Seltzer *et al.*, 2007).

The rice genome contains single genes for homologues of GCP2/Spc97p, GCP3/Spc98p, GCP4 and GCP6: LOC_Os04g42330/Os04g0501700, LOC_Os09g27370/Os09g0446200, LOC_Os05g06260/Os05g0154500 and LOC_Os04g47906/Os04g0566800, respectively. However, two genes encode homologues of GCP5, i.e. LOC_Os12g41290/Os12g0606100 and LOC_Os02g32340/Os02g0523300, with homology as high as 90 % identity at the amino acid level. This scenario is almost identical to that in *A. thaliana* as a model dicot also containing two GCP5-encoding genes: At1g80260 and At1g20570. In addition, homologues of GCP2-4 and GCP6, with >80 % amino acid sequence identity with those rice counterparts, are encoded by single genes in *A. thaliana* (Murata *et al.*, 2007).

A single putative NEDD1/GCP-WD homologue was identified by analysing the rice genome. This hypothetic protein encoded by the LOC_Os09g09470/Os09g0267500 locus is 65 % identical to the protein encoded by the At5g05970 locus in *A. thaliana*. The rice protein has the highest homology with human NEDD1 toward the N-terminal WD40 repeats. It is unclear whether the plant homologues function like the mammalian NEDD1/GCP-WD in the recruitment of the γ-tubulin complex. Given the fact that the spindle pole body functions as a centrosome-like primary MTOC, to date no such WD40 repeat-containing proteins have been identified as a NEDD1/GCP-WD homologue in fungi. It is rather intriguing whether the plant counterpart functions in the same way as the animal protein. More specifically, it would be critical to examine whether it is involved in MT nucleation on the wall of extant MTs.

THE MITOTIC KINASE AURORA

The Aurora family enzymes are serine/threonine kinases with molecular weights of approx. 30–40 kDa. In animals and fungi, Aurora kinases act in discrete regions of the spindle apparatus, such as the centrosome, kinetochores and the spindle midzone (Carmena and Earnshaw, 2003). The frog Aurora A isoform Eg2 directly binds to MTs *in vitro*, implying a direct interaction between the kinase and proteins associated with MTs *in vivo* (Roghi *et al.*, 1998). The animal Aurora A appears at the centrosome and MTs toward spindle poles, and activates MAPs and kinesin motors essential for MT nucleation and organization (Marumoto *et al.*, 2005). Because beads coated with Aurora A are able to promote spindle assembly in the absence of the centrosome or chromosomes, Aurora A has earned the nickname of 'guardian of poles' (Tsai and Zheng, 2005). The animal Aurora B forms a complex with the inner centromere protein (INCENP), survivin and borealin, and the complex appears at the centromere before the onset of anaphase and in the spindle midzone and the midbody after anaphase (Ruchaud *et al.*, 2007). They are referred to as components of the chromosomal passenger complex (CPC) because of their dynamic behaviour during mitosis. Aurora B substrates also include MAPs and kinesins, but they are different from those of Aurora A because of different intracellular localizations.

Three Aurora kinases of two classes are encoded by the *A. thaliana* genome. AtAUR1 and AtAUR2 form the first class, and AtAUR3 the second, and all of them were predominantly expressed in tissues enriched with dividing cells (Demidov *et al.*, 2005). Driven by the 35S promoter, their cDNAs were expressed in fusions with the GFP-coding sequence in cultured tobacco BY-2 cells (Demidov *et al.*, 2005; Kawabe *et al.*, 2005). Both AtAUR1–GFP and AtAUR2–GFP fusions decorated the nuclear envelope and structures resembling mitotic spindles, and the AtAUR1–GFP signal also conspicuously appeared at the site of the forming cell plate during cytokinesis. AtAUR3–GFP, however, exhibited a dot-like pattern on chromosomes, probably at the centromere/kinetochore region. Overexpression of AtAUR3 in BY-2 cells has profound effects, causing alteration

of the distribution of the MT nucleation factor γ-tubulin, partial disassembly of spindle MTs and misorientation of the cell division plane (Kawabe *et al.*, 2005).

Rice expresses only two distinct Aurora kinases, OsAUR1 (LOC_Os01g09580/Os01g0191800) of 292 amino acids and OsAUR2 (LOC_Os03g55620/Os03g0765000) of 279 amino acids, belonging to the first and second classes mentioned above, respectively. Similarly to AtAURs, sequence comparison shows no preference of homology for OsAURs to either animal Aurora A or Aurora B. Thus, novel activities of plant Aurora kinases are expected.

THE TPX2 HOMOLOGUE AND RELATED WVD2 PROTEINS

In mammals, Aurora A is targeted to the centrosome and spindle MTs by the MAP TPX2 (Kufer *et al.*, 2002). TPX2 also plays a critical role in directing the kinesin XKLP2 to the centrosome in the frog *Xenopus* (Wittmann *et al.*, 2000). Among TPX2 proteins identified in different organisms, a signature motif in the MT-binding site has been identified as pfam06886 with the KLEEK penta-peptide (http://pfam.sanger.ac.uk/).

In *A. thaliana*, an expressed protein of 758 amino acids encoded by the At1g03780 locus has been identified as a putative TPX2 homologue, which bears a region resembling the signature motif (Perrin *et al.*, 2007). Several other expressed proteins related to this homologue also share this feature, but with significantly smaller sizes of approx. 200–300 amino acids. One of them is the WVD2 (WAVE-DAMPENED 2) protein which acts as an MT-bundling and stabilizing factor (Yuen *et al.*, 2003). WVD2 and seven related WDL1–7 proteins all contain the pfam06886 motif (Yuen *et al.*, 2003; Kaloriti *et al.*, 2007; Perrin *et al.*, 2007). When WVD2 or its homologues are expressed constitutively at elevated levels, cortical MTs are often bundled and stabilized, resulting in reduced anisotropic cell elongation, and consequently short and thick stems and roots (Perrin *et al.*, 2007). Based on sequence comparison, it was determined that at least 14 proteins bear the TPX2 pfam06886 motif in *A. thaliana* (data not shown). Among them, WVD2 and WDL1 are able to bind directly to MTs *in vitro* and *in vivo*, indicating that they are authentic MAPs (Perrin *et al.*, 2007). It would not be surprising if other proteins in this group of 14 turn out to be true MAPs as well because of the presence of the KLEEK penta-peptide inside the signature motif.

In the rice genome, the LOC_Os07g32390/Os07g0507200 locus encodes a protein sharing 45 % amino acid sequence identity with that of At1g03780. While there is little if any doubt that rice and arabidopsis TPX2 homologues are indeed MAPs, it would be intriguing to determine whether either or both of them function in targeting any Aurora kinase or kinesin to the spindle or spindle poles. Based on the criterion of the presence of the penta-peptide KLEEK as in WVD2 and WDLs, six putative proteins related to WVD2 were identified. They are LOC_Os02g10690/Os02g0200800, LOC_Os03g58480/Os03g0799100, LOC_Os06g40450/Os06g0606800, LOC_Os07g08790/Os07g0185500, LOC_Os11g36340/Os11g0571900 and LOC_Os11g38010/Os11g0592600, which could be considered to encode OsWDL proteins. Two additional loci, LOC_Os03g11400/Os03g0212600 and LOC_Os09g13650/Os09g0307300, may also encode MAPs because the deduced sequences contain the pfam06886 motif with an incompletely conserved KLEEK region.

In contrast to the TPX2 homologue which may be specifically involved in MT organization in spindles, WVD2 and WDL proteins may play roles in modulating the cortical MT array to facilitate anisotropic cell expansion. We are eager to learn the function of the rice TPX2 homologue, and the physiological significance of having so many WDL isoforms in rice and *A. thaliana*.

MEDIATING MT–MT INTERACTIONS: SPLENDID MEMBERS OF THE MAP65/ASE1P/PRC FAMILY

Early heroic endeavours in isolating plant MAPs resulted in clear candidates purified from cultured carrot cells (Cyr and Palevitz, 1989). Whether plant MAPs could be purified to homogeneity was no longer questioned after MAP65 was purified from tobacco BY-2 cells (Jiang and Sonobe, 1993). It was not realized at the time that similar proteins could be found in organisms across eukaryotic kingdoms. The yeast homologue of MAP65 was identified as a protein critical for anaphase B spindle elongation, thus named as Ase1p for anaphase spindle elongation 1 protein (Pellman *et al.*, 1995). The mammalian homologue PRC1 (protein regulating cytokinesis 1) was identified as an important MAP playing a regulatory role in cytokinesis (Jiang *et al.*, 1998).

The initial cloning of tobacco MAP65 cDNA was followed by revealing nine genes encoding MAP65-like proteins in *A. thaliana* (Smertenko *et al.*, 2000; Hussey *et al.*, 2002). Surprisingly, different *A. thaliana* MAP65 isoforms exhibit dramatically different localization patterns when expressed in fusions with GFP in BY-2 cells (Van Damme *et al.*, 2004*b*). While MAP65-1 and -5 fusions decorate cortical MTs, MAP65-4 only appears on MTs in the spindle pole region during mitosis (Van Damme *et al.*, 2004*a*, *b*). MAP65 isoforms also differentially decorate phragmoplast MTs, with MAP65-3 appearing conspicuously near the MT plus end (Van Damme *et al.*, 2004*a*). Most surprisingly, endogenous MAP65-6 is associated with mitochondria, probably implying its role in mediating the interaction between the organelle and MTs (T. Mao *et al.*, 2005). Biochemical studies conclude that MAP65-1 and probably other isoforms form homodimers of approx. 25 nm to cross-link MTs, suggesting a role in bundling antiparallel MTs in the phragmoplast mid-zone (Smertenko *et al.*, 2004; Wicker-Planquart *et al.*, 2004). Activities of MAP65 are regulated by phosphorylation, especially by cyclin-dependent kinase (Cdk) during the cell cycle (G. Mao *et al.*, 2005; Sasabe *et al.*, 2006; Smertenko *et al.*, 2006). It has also been found that the MT-bundling activity of tobacco MAP65-1 is downregulated when phosphorylated by a cytokinesis-specific mitogen-activated protein kinase cascade to allow phragmoplast MTs to be disassembled (Sasabe and Machida, 2006; Sasabe *et al.*, 2006). Genetic evidence pinpoints AtMAP65-3/PLE for its critical role in cytokinesis (Müller *et al.*, 2004; Caillaud *et al.*, 2008). Because multiple endogenous MAP65 proteins are present at the plus end of phragmoplast MTs, as revealed by GFP

TABLE 2. *List of MAP65 family proteins in rice*

RGAP IDs	RAP IDs
LOC_Os01g49200	Os01g0685900*
LOC_Os02g03400	Os02g0126300
LOC_Os02g48830	Os02g0720200
LOC_Os03g13460	Os03g0237600
LOC_Os03g50970	Os03g0719000
LOC_Os05g33890	Os05g0409400
LOC_Os05g47970	Os05g0552900
LOC_Os06g20370	Os06g0308300
LOC_Os06g40840	Os06g0611000
LOC_Os08g41890	Os08g0531100
LOC_Os09g27700	Os09g0450300

IDs were assigned by RGAP and RAP, respectively.
* Known as MAP65-3.

fusions and immunolocalization, it remains to be understood how their activities are coordinated spatially and temporally.

Eleven rice genes were identified as encoding MAP65 homologues (Table 2). It appears that an additional member has been added to each of the two clades containing AtMAP65-1/AtMAP65-2 and AtMAP65-6/AtMAP65-7 (Fig. 1). The homology between the hypothetical OsMAP65-3 (LOC_Os01g49200/Os01g0685900) and AtMAP65-3 is 48 % identity/64 % similarity at the amino acid level. Because of the aforementioned role of AtMAP65-3 in cytokinesis, it was important to test whether OsMAP65-3 behaved similarly during mitotic cell division. When the endogenous OsMAP65-3 protein was probed with isoform-specific antibodies, it appeared near the plus end of the phragmoplast MTs during cytokinesis (Fig. 2). Hence, the result has suggested that OsMAP65-3 may play a similar role to that of AtMAP65-3. A recent genetic analysis indicated that insertional mutations at the OsMAP65-3 locus caused dwarf and low-fertility phenotypes (Miyao *et al.*, 2007). Whether the phenotype is a result of a cytokinesis defect awaits further examination of the mutants. It would be rather interesting to dissect distinct developmental roles of these 11 MAP65 isoforms in rice.

The yeast protein Ase1p is a substrate of the Aurora B-type kinase Ipl1p, and its phosphorylation is critical for spindle assembly (Kotwaliwale *et al.*, 2007). It is intriguing whether a similar phosphorylation event regulates the activity of OsMAP65-3 and/or other isoforms.

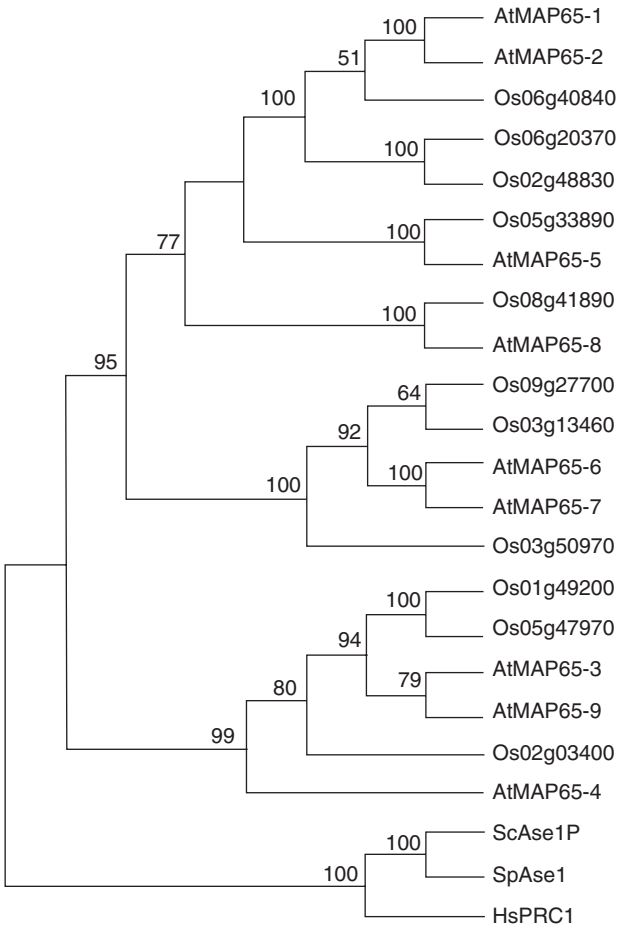

FIG. 1. Neighbor–Joining tree of MAP65 family proteins. Phylogenetic relationships among the MAP65 members of rice (Os), *A. thaliana* (At), the budding yeast *Saccharomyces cerevisiae* (ScAse1p), the fission yeast *Schizosaccharomyces pombe* (SpAse1) and human (HsPRC1) are shown. For rice proteins, shown in this figure were IDs annotated by RGAP omitting LOC_. The AtMAP65 members were assigned according to published nomenclature (Hussey and Hawkins, 2001). The GenBank accession numbers for the others are: NP_116582 for ScAse1p, CAC21482 for SpAse1 and AAC02688 for HsPRC1. Bootstrap values at the branches represent the percentages obtained in 1000 replications. Only values >50 % are presented.

PROMOTING MT ASSEMBLY AT THE PLUS END: XMAP215/TOG FAMILY PROTEINS

The founding member of this family, XMAP215, was first identified more than two decades ago as a MAP which could promote centrosome-nucleated MT assembly (Gard and Kirschner, 1987*a*, *b*). A decade later, a genetic screen recovered the homologous Stu2p as an essential MAP required for spindle assembly in budding yeast (Wang and Huffaker, 1997). The outstanding feature of this family of proteins is the presence of HEAT (huntingtin, elongation factor-3, A sub-unit of PR65, Tor-kinase) repeats scattered across the polypeptides (Gard *et al.*, 2004). The protein functions as an MT polymerase by adding new tubulin dimers to the plus end while moving with the growing plus end (Howard and Hyman, 2007).

The plant gene homologous to XMAP215 was first isolated when examining the *mor1* mutant defective in MT organization in *A. thaliana* (Whittington *et al.*, 2001). Allelic mutations also cause defects in cytokinesis during microgametophyte formation (Twell *et al.*, 2002). Detailed examinations of the *mor1* mutant suggest that the MOR1 protein decorates all MT arrays during mitosis, and is essential for various aspects of MT organization during cell division, in addition to ensuring the integrity of cortical MTs (Eleftheriou *et al.*, 2005; Kawamura *et al.*, 2006).

Unlike in *A. thaliana*, the *O. sativa* genome contains two linked genes, LOC_Os01g60040/Os01g0816400 and LOC_Os01g60050/Os01g0816500, which are expressed for MOR1/XMAP215-related proteins of 1998 and 326 amino acids, respectively. It seems that the protein encoded by the

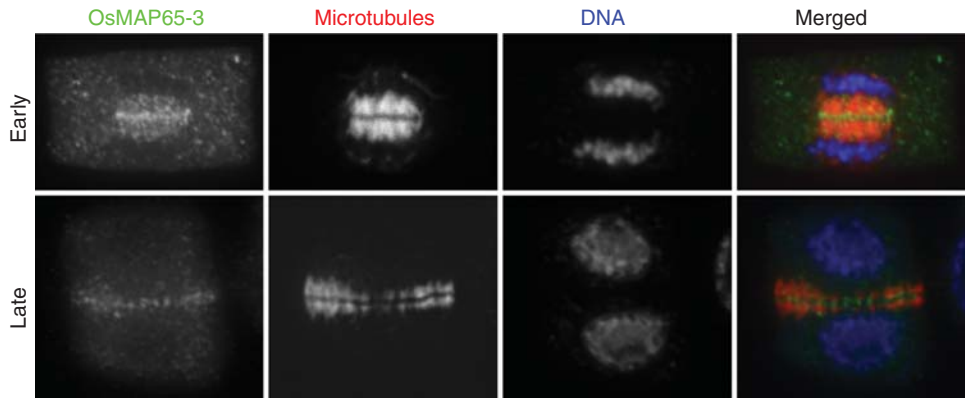

FIG. 2. Immunolocalization of OsMAP65-3 in developing phragmoplasts. OsMAP65-3 was detected by an isoform specific antibody, MTs by an anti-α-tubulin antibody, and DNA by DAPI (4′,6-diamidino-2-phenylindole). Cells at an early and late stage of cytokinesis are shown. OsMAP65-3 appeared in a broader distribution pattern in the early phragmoplast, and then became more concentrated in the phragmoplast midline in the late phragmoplast. In the merged image, OsMAP65-3 was pseudocoloured in green, MTs in red and DNA in blue.

first gene is an apparent orthologue of MOR1 due to its similarity in size to AtMOR1 of 1978 amino acids. The protein encoded by the latter shows >60 % sequence identity with the N-terminal approx. 300 amino acid region of OsMOR1. Hence, it is rather mysterious whether this smaller protein acts as a MAP *in vivo*.

In the frog *Xenopus*, XMAP215 is a substrate of Aurora A, and its phosphorylation is critical for its function in MT nucleation (Giet *et al.*, 2002). Again, possible Aurora phosphorylation events might play critical roles in regulating functions of XMAP215 homologues in rice and other plants.

A PLANT-SPECIFIC MAP FAMILY: THE MAP70 PROTEINS

The aforementioned penta-peptide KLEEK can also be found in a family of plant-specific MAPs known as MAP70s (Korolev *et al.*, 2007). However, this penta-peptide does not fall in the determined MT-binding site of AtMAP70 (Korolev *et al.*, 2005). It is possible that the N-terminal regions of AtMAP70s may constitute another MT-binding pocket which cannot be simply revealed by a truncated fusion. It has been shown by both overexpression and RNA interference (RNAi)-mediated gene silencing that the most abundantly expressed isoform AtMAP70-5 plays a role in anisotropic cell expansion in stems (Korolev *et al.*, 2007).

While there are five MAP70 isoforms in *A. thaliana*, four were detected in rice by sequence comparison (Korolev *et al.*, 2005). Among them, proteins encoded by LOC_Os02g50320/Os02g0736100, LOC_Os06g14080/Os06g0251700 and LOC_Os12g44340/Os12g0640900 are closely related to each other and to AtMAP70-1–4. However, the fourth isoform, encoded by LOC_Os03g11650/Os03g0215700, is more divergent.

WHEN AUXIN MEETS MTS: AIR9

AIR9, previously known as an auxin-induced gene in root cultures, was rediscovered as a gene encoding a 187 kDa MAP (Buschmann *et al.*, 2006). Its N-terminal basic and serine-rich domain forms an MT-binding site. Besides decorating cortical MTs, the PPB and the phragmoplast when AIR9 was expressed in a GFP fusion, the fusion protein was inserted at the cortical site previously occupied by the PPB during cytokinesis followed by an inward expansion (Buschmann *et al.*, 2006).

A single rice gene LOC_Os07g05470/Os07g0148800 encodes an AIR9-homologous protein which shares 70 % amino acid sequence identity only within the A9 repeats. However, this predicted rice protein lacks the short basic and serine-rich region, and the following leucine-rich repeats found toward the N-terminus of AIR9. Therefore, it is questionable whether this much smaller rice protein indeed acts as a MAP, and whether it plays an essential role in plant cell division and development.

ANOTHER PLANT-SPECIFIC MAP FAMILY: TORTIFORLIA 1 (TOR1)/SPIRAL 2 (SPR2) AND RELATED PROTEINS

Two independent quests for genes whose mutations were responsible for the helical growth phenotype resulted in the identification of the TOR1/SPR2 protein as another plant-specific MAP of 864 amino acids (Buschmann *et al.*, 2004; Shoji *et al.*, 2004). TOR1/SPR2 and five homologous proteins in *A. thaliana* contain multiple HEAT repeats. Three lines of evidence support TOR1/SPR2 being an authentic MAP (Buschmann *et al.*, 2004; Shoji *et al.*, 2004). First, GFP fusion proteins decorate MT arrays during mitosis when ectopically expressed. Secondly, bacterially expressed and purified proteins co-sediment with MTs *in vitro*. Thirdly, a tobacco TOR1/SPR2 homologue was detected in the MAP fraction from BY-2 cells (Shoji *et al.*, 2004). The *tor1* and *spr2* mutations cause cortical MTs to form left-handed helices, leading to defects in directional cell elongation and right-handed helical growth in longitudinally expanding organs (Buschmann *et al.*, 2004; Shoji *et al.*, 2004).

The closest rice TOR1/SPR2 homologue is encoded by the LOC_Os07g33630/Os07g0520400 and LOC_Os09g38710/Os09g0560000 loci, with >36 % amino acid sequence identity. Two other genes, LOC_Os02g50640/Os02g0739900 and

LOC_Os06g13600/Os06g0244700, encode proteins with 27 and 26 % identity to TOR1/SPR2, respectively. Remaining questions include how the endogenous protein acts on cortical MTs to regulate their organization and underlying cellulose microfibril deposition in rice.

MT PLUS END-TRACKING PROTEINS: +TIPS

Among proteins decorating MTs, the most spectacular ones are those which specifically accumulate at the plus end of growing MTs. Collectively, they are named as MT plus end-tracking proteins, commonly known as +TIPs. When labelled with fluorescent proteins such as GFP, +TIPs often exhibit a comet-like dynamic localization pattern. The +TIPs include diverse proteins with no sequence homology to each other, and range from proteins as small as 30 kDa to as large as the cytoplamic dynein complex of >1000 kDa (Akhmanova and Hoogenraad, 2005). Besides dynein, several MT motor kinesins have also been determined to be +TIPs (Wu *et al.*, 2006). The tip-tracking mechanisms include treadmilling for some +TIPs and surfing for others (Asbury, 2008).

Two classes of plant +TIPs, EB1 proteins and SPIRAL 1 (SPR1) proteins, are discussed here.

EB1

As the founding member of +TIPs, EB1 (end-binding protein 1) was discovered as a binding partner of the tumour suppressor protein APC (adenomatous polyposis coli) (Su *et al.*, 1995). EB1 is present among all examined eukaryotic organisms, and acts as the heart for mediating other +TIPs to interact with MT plus ends (Lansbergen and Akhmanova, 2006). This small protein of approx. 300 amino acids contains a CH (calponin homology) domain at the N-terminus required for MT binding and an EB1 signature motif toward the C-terminus. In animal cells, the function of EB1 for promoting MT polymerization and stability is absolutely essential for MT organization during interphase and for spindle assembly (Rogers *et al.*, 2002).

In *A. thaliana*, three EB1 isoforms in two classes have been identified (Chan *et al.*, 2003; Mathur *et al.*, 2003; Dixit *et al.*, 2006; Bisgrove *et al.*, 2008). AtEB1a and AtEB1b are 78 % identical to each other, and 49 % identical to AtEB1c (Bisgrove *et al.*, 2008). The endogenous AtEB1 proteins preferentially decorate MT plus ends in various MT arrays during mitosis, and GFP fusions of AtEB1a and AtEB1b clearly reveal comet-like localization (Chan *et al.*, 2003; Mathur *et al.*, 2003; Dixit *et al.*, 2006; Bisgrove *et al.*, 2008). AtEB1a–GFP also appears at the MT-nucleating sites in spindles and phragmoplasts (Chan *et al.*, 2005). AtEB1c resides in the nucleus during interphase, and associates with spindle and phragmoplast MTs, preferentially towards the plus end, during mitosis (Dixit *et al.*, 2006; Bisgrove *et al.*, 2008). A T-DNA insertional mutation at the *EB1c* locus confers hypersensitivity to the MT-poisoning agent oryzalin, and the sensitivity is enhanced when additional *EB1* genes are compromised, indicating some overlapping functions among isoforms (Bisgrove *et al.*, 2008). Although the AtEB1 proteins play a role in cellular activities required for responses to touch and gravity, they do not seem to be essential, which is different from their animal counterparts. If plant EB1 plays a role in mitosis, such a role is probably redundantly covered by other proteins acting on MTs.

Only two rice genes are found to encode EB1: LOC_Os04g54940/Os04g0642100 (*OsEB1a*) and LOC_Os10g35580/Os10g0498900 (*OsEB1b*). OsEB1a and AtEB1a are closely related, and have 65 % sequence identity; while OsEB1b and AtEB1c are closely related, and have 59 % identity. Thus, rice produces one EB1 protein for each class. However, it is unclear how the rice EB1 proteins behave in living cells.

SPR1

In analysing *A. thaliana* mutants exhibiting altered cell expansion resulting in twisted growth as described above for *spr2*, two independent efforts were converged at the *SPR1* gene encoding a 119-amino acid protein (Nakajima *et al.*, 2004; Sedbrook *et al.*, 2004). The reports also concluded that endogenous as well as a functional SPR1–GFP fusion protein localized to cortical MTs and mitotic arrays. SPR1–GFP often appeared in a comet-like pattern, reminiscent of a +TIP (Sedbrook *et al.*, 2004). Five other proteins are encoded by the *SPR1*-like *SP1L1–5* genes, and their more conserved N- and C-terminal regions are connected by less conserved central regions (Nakajima *et al.*, 2006). The MT association is conferred by the combination of both conserved regions (Nakajima *et al.*, 2004). The twisted growth pattern caused by the *spr1* mutation can be greatly enhanced by mutations in *SP1L* genes, indicating redundant functions among these homologous proteins (Nakajima *et al.*, 2006).

An intriguing sequence pattern among SPR1 proteins is the presence of two almost identical peptide stretches at the N- and the C-terminus, 'GGGQSSLGYLF' and 'GGGSSLDYLF', respectively. Such a feature is repeated among four small proteins of 102–128 amino acids encoded by the LOC_Os03g30430/Os03g0417800, LOC_Os04g48870 (not annotated by RAP), LOC_Os11g41150/Os11g0629400 and LOC_Os12g31780/Os12g0502000 loci in the rice genome. These proteins probably play a constitutive role in regulating MT dynamics in rice based on the conserved sequence. Further genetic studies would allow a conclusion to be drawn as to whether the loss of rice SPR1-like proteins could cause a twisted growth phenotype.

The CLASP homologue: not a CLIP170-binding protein

Another often talked about +TIP in animal cell is CLASP (CLIP170-associated protein). CLIP170 (cytoplasmic linker protein 170) is a MAP which plays a role in cell migration and axon guidance (Galjart, 2005). CLASP proteins share limited similarity with XMAP215/TOG by having multiple HEAT repeats and an N-terminal TOG domain. The *A. thaliana* genome contains the single At2g20190 gene encoding a CLASP homologue (Ambrose *et al.*, 2007; Kirik *et al.*, 2007). Although AtCLASP appears preferentially toward the MT plus end, its localization pattern is clearly different from that of EB1 or SPR1 as AtCLASP also decorates cortical MTs in a punctate manner besides a comet-like appearance (Kirik *et al.*, 2007). A mitotic function of

AtCLASP is expected as: (*a*) AtCLASP is associated with the PPB and the phragmoplast; and (*b*) T-DNA insertions at the corresponding locus cause drastic reductions in the mitotic index and mitotic zone in the root (Ambrose *et al.*, 2007; Kirik *et al.*, 2007). Consequently, a significant reduction of overall vertical growth is demonstrated by the mutant plants, which also exhibit cell swelling when anisotropic expansion is required.

Surprisingly, the rice genome contains three genes encoding proteins highly homologous to AtCLASP: LOC_Os04g42840/Os04g0507500, LOC_Os02g40430/Os02g0617300 and LOC_Os02g57120/Os02g0816300. The outstanding questions about these rice CLASPs are whether they are authentic MAPs, whether they interact with EB1 as their animal counterparts and whether they are involved in responding to internal and external cues to modulate MT behaviour.

Because there is no CLIP170 homologue found in plants according to sequence homology, an obvious question is what partner(s) the plant CLASP may have, if any.

TANGLED 1: A CELL DIVISION SITE MARKER

Four decades ago, the PPB was discovered in wheat as an MT-based band present at the cell cortex prior to nuclear envelope breakdown, which forecasts the future division plane (Pickett-Heaps and Northcote, 1966*a*, *b*). Since then, this remarkable landmark has been inspiring plant cell biologists to search for the footprint left by the PPB at the cortical site. Besides the aforementioned AIR9 protein, the TANGLED (TAN) protein has also generated great enthusiasm as an indicator marking the site once occupied by the PPB (Walker *et al.*, 2007). An earlier study indicated that the maize TAN protein acted as a MAP *in vitro* (Smith *et al.*, 2001). A fluorescent fusion protein of AtTAN decorated the PPB, and remained at the cell division site as discontinuous dots encircling the cell cortex throughout the rest of mitosis (Walker *et al.*, 2007). The rice genome contains a single locus encoding an apparent TAN orthologue: LOC_Os02g26140/Os02g0459300. The hypothetically deduced polypeptide of 415 amino acids has 68 and 42 % sequence identities with the maize and arabidopsis orthologues, respectively. How TAN interacts with MTs and influences cell division plane determination remains to be determined.

TON1 AND TON2: PROTEINS REQUIRED FOR PPB FORMATION

Earlier genetic screens recovered *ton* and *fass* mutants bearing a phenotype of an extremely compressed apical–basal axis reflected by a maximum plant height of a few centimetres (Traas *et al.*, 1995; McClinton and Sung, 1997). When mutant cells undergo mitosis, MTs fail to be organized into the PPB array, but the spindle and phragmoplast arrays can still be formed. The *TON1* locus contains two tandem genes encoding redundant TON1a and TON1b with 85 % sequence identity, which decorate cortical MTs and PPB (Azimzadeh *et al.*, 2008). TON1 is related the animal centrosomal protein FOP, and interacts with the calcium-binding protein centrin. It was proposed that TON1 is an MT-organizing factor at the cell cortex, reminiscent of FOP at the centrosome (Azimzadeh *et al.*, 2008). The loci of LOC_Os11g01170/Os11g0102600 and LOC_Os12g01170/Os12g0102200 encode identical TON1 proteins in rice.

The TON2/FASS protein is a member of the B″ family of protein phosphatase 2A (PP2A) regulatory sub-units, and interacts with the AtAα regulatory sub-unit (McClinton and Sung, 1997; Camilleri *et al.*, 2002). The involvement of PP2A suggests that the phosphorylation status of cytoskeletal proteins at the cell cortex is critical for MT organization into the PPB array. The rice genome contains a single *TON2* gene LOC_Os05g05710/Os05g0149800. It would be rather interesting to identify the substrate of PP2A at the cell cortex at early stages of mitosis.

MT-SEVERING AND -DESTABILIZING FACTORS: KATANIN AND BEYOND

In animal cells, the rapid shortening of cytoplasmic MTs when cells enter mitosis is brought about by the katanin complex as an MT-severing ATPase (McNally and Vale, 1993). The complex contains a 60 kDa catalytic sub-unit and an 80 kDa accessory sub-unit. The plant homologue of the katanin catalytic sub-unit, AtKTN1/KSS1 (katanin or katanin small sub-unit), was first identified as a component responsible for ordered deposition of cellulose microfibrils, while it was also detected by direct sequence comparison (Burk *et al.*, 2001; McClinton *et al.*, 2001). Its MT-severing activity has been verified *in vitro* and *in vivo* (Burk and Ye, 2002; Stoppin-Mellet *et al.*, 2002, 2006). Surprisingly, overexpression of the native AtKTN1 protein randomized cortical MTs but did not fragment them, and consequently altered the deposition of cellulose microfibrils (Burk *et al.*, 2007). Other genetic analyses on various aspects of plant development have agreed that katanin plays a critical role in regulating the organization of cortical MTs, which is essential for anisotropic cell expansion in both arabidopsis and rice (Bichet *et al.*, 2001; Webb *et al.*, 2002; Bouquin *et al.*, 2003; Komorisono *et al.*, 2005). It has also been found that the loss of katanin caused upregulation of the expression of gibberellin (GA) biosynthesis genes in arabidopsis and rice (Bouquin *et al.*, 2003; Komorisono *et al.*, 2005). Because GA plays a critical role in regulating plant height, this phenomenon of altered gene expression could be interpreted as a feedback regulation resulting from the dwarf phenotype exhibited by the katanin mutants. The OsKSS1 encoded by the *SGL1* gene is located at LOC_Os01g49000/Os01g0683100.

The 80 kDa accessory sub-unit (KLS for katanin large sub-unit) contains a WD40 domain, and plays a role in targeting the catalytic sub-unit to the centrosome in animal cells (McNally *et al.*, 2000). There are at least four p80 homologues encoded by the *A. thaliana* genome, but their physiological roles remain to be characterized (Bouquin *et al.*, 2003). Based on the presence of the N-terminal WD40 repeats and the C-terminal AtKTN1/KSS1-interacting domain, three loci in the rice genome encode homologues of this sub-unit: LOC_Os04g58130/Os04g0677700, LOC_Os01g57210/Os01g0780400 and LOC_Os10g35200/Os10g0494800.

Besides katanin, a plant-specific 18 kDa protein known as MAP18 has been documented as a destabilizing factor on cortical MTs (Wang *et al.*, 2007*b*). This protein may be another

regulator of the dynamic MT network at the cell cortex during rapid cell expansion in *A. thaliana*. However, there is no obvious homologue of MAP18 in rice. It remains unclear whether other monocot species have MAP18 homologues.

In animal cells, members of the Kinesin-13 sub-family are critical MT depolymerases regulating dynamics of MT ends (Howard and Hyman, 2007). However, similar functions have not been observed in plant cells (Lu *et al.*, 2005).

EMERGING NOVEL PLANT MAPS: PLD/P90, MAP190 AND RIC1

A few novel MAPs have been isolated by various approaches. They have been implicated in regulating MT organization at various stages of cell growth.

PLDδ

In a quest for MAPs that were associated with the plasma membrane in tobacco BY-2 cells, a 90 kDa protein was isolated and turned out to be the phospholipase PLDδ (Marc *et al.*, 1996; Gardiner *et al.*, 2001). Endogenous PLDδ indeed decorates cortical MTs and MT arrays during mitosis in tobacco cells. In the rice genome, there are at least 13 genes encoding forms of PLDα, PLDβ, PLDγ and PLDδ. Among them, LOC_Os09g37100/Os09g0543100 encodes PLDδ.

MAP190

MAP190 was isolated from BY-2 cells as a putative MAP which also interacted with actin microfilaments *in vitro* (Igarashi *et al.*, 2000). The protein contains a calcium-binding EF-hand motif toward the C-terminus. Besides decorating mitotic MT arrays, it also appeared in the nucleus. The rice LOC_Os06g36710/Os06g0562700 locus encodes a protein related to tobacco MAP190.

RIC1

RIC (ROP-interactive CRIB motif-containing protein) proteins contain the CRIB (Cdc42/Rac-interactive binding) motif, and specifically interact with the GTP-bound form of the small GTPase ROP (Wu *et al.*, 2001). Among RICs identified in *A. thaliana*, RIC1 is associated with cortical MTs in leaf pavement cells, and acts as an MT-stabilizing factor (Fu *et al.*, 2005). While in rice there are a number of genes encoding proteins containing the CRIB motif, none of them seems to be an apparent RIC1 orthologue. Because RIC1 plays a critical role in morphogenesis of leaf epidermal cells in *A. thaliana*, the absence of a rice orthologue may suggest that RIC1 probably is associated with a signalling route specific to dicots. Specific signalling routes may be part of the molecular mechanisms specifying different appearances in leaf epidermal cells in dicots and monocots.

THE KINESIN SUPERFAMILY

Among all cytoskeletal protein families, kinesins constitute the largest family, especially in flowering plants (Lee and Liu, 2004). Kinesins belong to one of the three classes of cytoskeleton-based motor proteins, and use the energy of ATP hydrolysis to move along MT tracks. Members of the kinesin superfamily contain a highly conserved approx. 350 amino acid catalytic core bearing ATPase activity and a nucleotide-dependent MT-binding domain. Kinesins often exhibit a tripartite structure, with the motor domain of the catalytic core plus the neck generating directional forces, a coiled-coil stalk for dimerization, and diverse tail domains for recognizing their cargoes. The 'neck' domain, a 34 amino acid peptide for plus end-directed kinesins or a 14 amino acid peptide for minus end-directed kinesins, determines the directionality of the motors (Endow, 1999). Kinesins are classified according to the sequence homology in their motor domains, and have 14 designated sub-families and some orphans (Lawrence *et al.*, 2004). Except for members of Kinesin-5 and a few members of Kinesin-14 which have conserved non-motor sequences and mitotic functions, most plant kinesins are distinctly divergent from their animal and fungal counterparts when their non-motor domains are examined (Lee and Liu, 2007). Based on earlier drafts of the rice genomes of *indica* and *japonica*, >40 genes have been shown to encode kinesins in rice (Richardson *et al.*, 2006). Rice kinesins distribute across most of the 14 sub-families, except for Kinesin-2, -3, -9 and -11 (Richardson *et al.*, 2006). According to the genome annotation of the cultivar *japonica*, there are at least 52 kinesins in rice (Table 3). While a few kinesins have been experimentally investigated in *A. thaliana* and other dicot species such as tobacco and cotton, very limited information has been obtained about rice kinesins.

A previous sequence analysis has pinpointed distinct domains found among the rice kinesins, suggesting their distinct functions (Richardson *et al.*, 2006). Here some interesting features of rice kinesins in three expanded sub-families are summarized. Others are listed in Table 3.

Kinesin-7

The founding member of this sub-family is CENP-E, a kinesin which appears at the kinetochore region during mitosis in mammals, and interacts with mitotic checkpoint proteins for its role in mitotic progression (Yen *et al.*, 1992; Vos *et al.*, 2006). Kinesin-7 is expanded in flowering plants. There are at least ten members in rice (Table 3). Among them, two have been studied by genetic and biochemical means.

The *dwarf bamboo shoot 1* (*dbs1*) mutation at the *OsNACK1* (LOC_Os01g33040/Os01g0513900) locus encoding a Kinesin-7 member causes severe dwarfism in rice (Sazuka *et al.*, 2005). OsNACK1 is an apparent orthologue of NACK1 in arabidopsis and tobacco. NACK1 governs the localization of a mitogen-activated protein kinase cascade to the phragmoplast midzone, and plays a critical role in MT turnover in the phragmoplast (Nishihama *et al.*, 2002; Sasabe and Machida, 2006). The *dbs1* mutant exhibits cell wall stubs in rapidly dividing cells, reflecting defects in cytokinesis, a phenotype similar to that in *A. thaliana* (Nishihama *et al.*, 2002; Strompen *et al.*, 2002; Sazuka *et al.*, 2005).

The kinesin K16 encoded by the LOC_Os02g53520/Os02g0775400 locus, another rice Kinesin-7 member, has

TABLE 3. *List of kinesin family proteins in rice*

RGAP	RAP	Sub-family	Known as
LOC_Os01g14090	Os01g0243100	14	
LOC_Os01g15540	Os01g0260100	14	
LOC_Os01g33040	Os01g0513900	7	OsNACK1
LOC_Os01g42070	Os01g0605500	8	
LOC_Os01g43580	Os01g0625200	13	
LOC_Os01g54080	Os01g0744000	14	
LOC_Os02g01180	Os02g0101800	6	
LOC_Os02g13570	Os02g0229500+		
LOC_Os02g13580	Os02g0229600	14	
LOC_Os02g28850	Os02g0489800	12	Kinesin-12B
LOC_Os02g43050	Os02g0644400	7	
LOC_Os02g43130	Os02g0645100	7	
LOC_Os02g50910	Os02g0742800	4	
LOC_Os02g53520	Os02g0775400	7	K16
LOC_Os02g56540	Os02g0810200	UG	PAKRP2
LOC_Os03g02290	Os03g0114000	14	
LOC_Os03g05820	Os03g0152900	UG	
LOC_Os03g17164	Not annotated	5	
LOC_Os03g18980	Os03g0301800	14	
LOC_Os03g39020	Os03g0587200	12	
LOC_Os03g53920	Os03g0750200	12	
LOC_Os03g56260	Os03g0773600	8	
LOC_Os03g64415	Os03g0862200	14	
LOC_Os04g28260	Os04g0350300	12	PAKRP1, Kinesin-12A
LOC_Os04g30720	Os04g0375900	10	
LOC_Os04g45580	Os04g0538800	7	
LOC_Os04g53760	Os04g0629700	14	
LOC_Os04g57140	Os04g0666900	14	KCBP
Not annotated	Not annotated	14	OsKCH1
LOC_Os05g02670	Os05g0117798	5	
LOC_Os05g06280	Os05g0154700	13	Kinesin-13A
LOC_Os05g33030	Os05g0397900	14	
LOC_Os05g38480	Os05g0459400	10	
LOC_Os05g44560	Os05g0521300	14	
LOC_Os06g04560	Os06g0137100	UG	
LOC_Os06g11380	Os06g0217600	14	
LOC_Os06g36080	Os06g0554700	14	
LOC_Os07g01490	Os07g0105700	14	
LOC_Os07g44400	Os07g0638000	12	
LOC_Os08g02380	Os08g0117000	1	
LOC_Os08g43400	Os08g0547500	7	
LOC_Os08g44420	Os08g0558400	5	
LOC_Os09g02650	Os09g0114500	4	
LOC_Os09g25380	Os09g0421200	7	
LOC_Os09g35890	Os09g0528000	7	
LOC_Os10g36880	Os10g0512800	7	
LOC_Os11g35090	Os11g0552600	7	
LOC_Os11g37140	Os11g0581000	12	
LOC_Os11g42800	Os11g0648100	14	
LOC_Os11g44880	Os11g0672400	14	
LOC_Os12g36100	Os12g0547500	14	
LOC_Os12g39980	Os12g0590500	12	
LOC_Os12g42160	Os12g0616000	14	

IDs of these proteins were assigned by RGAP and RAP, respectively. Documented members are listed with their known names. Note that one of the Kinesin-14 members has been annotated into two loci by RGAP and RAP, and OsKCH1 on chromosome IV was not annotated by both. Orphan kinesins were noted as UG for ungrouped.

been studied biochemically for its motor activity compared with that of a conventional kinesin from brain (Umeki *et al.*, 2006). It exhibits a weaker interaction with MTs and a lower affinity for nucleotides. How this motor operates *in vivo* is unclear.

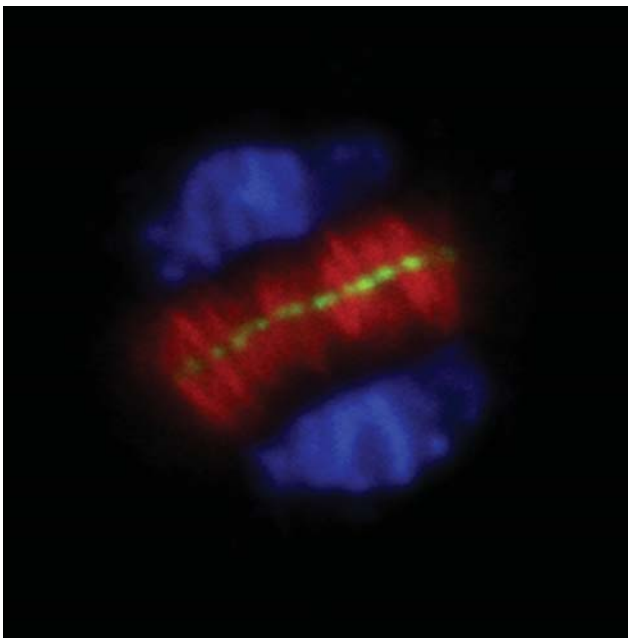

FIG. 3. Immunolocalization of OsKinesin-12A in the phragmoplast. OsKinesin-12A was labelled with an antibody raised against a truncated version of the protein, MTs by an anti-α-tubulin antibody, and DNA by DAPI (4′,6-diamidino-2-phenylindole). OsKinesin-12A was pseudocolored in green, MTs in red, and DNA in blue. OsKinesin-12A exclusively decorated the plus end of phragmoplast MTs.

Kinesin-12

While there are six members of the Kinesin-12 sub-family in *A. thaliana*, seven have been identified in rice (Table 3). According to the neck sequences present in these proteins, they are most probably plus end-directed motors. OsKinesin-12A/PAKRP1 encoded by the LOC_Os04g28260/Os04g0350300 locus was detected in rapidly dividing cells, and exclusively appeared at the plus end of phragmoplast MTs (Fig. 3). Three closely related kinesins are encoded by the rice genome at the loci of LOC_Os02g28850/Os02g0489800, LOC_Os03g39020/Os03g0587200 and LOC_Os11g37140/Os11g0581000. Their apparent orthologues in *A. thaliana* exhibit redundant functions as only the absence of both AtKinesin-12A and AtKinesin-12B leads to the loss of the bipolar organization pattern of phragmoplast MTs in developing pollen grains (Pan *et al.*, 2004; Lee *et al.*, 2007). Similar functional redundancy is expected for the rice orthologues.

Two members of the Kinesin-12 sub-family have been implicated in orienting the phragmoplast during cytokinesis in arabidopsis, and thus were named POKs (phragmoplast orienting kinesins) (Müller *et al.*, 2006). Similarly, two apparent POK orthologues are encoded by the LOC_Os07g44400/Os07g0638000 and LOC_Os12g39980/Os12g0590500 loci.

An additional Kinesin-12 member is encoded by the LOC_Os03g53920/Os03g0750200 locus, which is most closely related to the kinesin encoded by At3g44050 in *A. thaliana*. Neither has been reported in the literature.

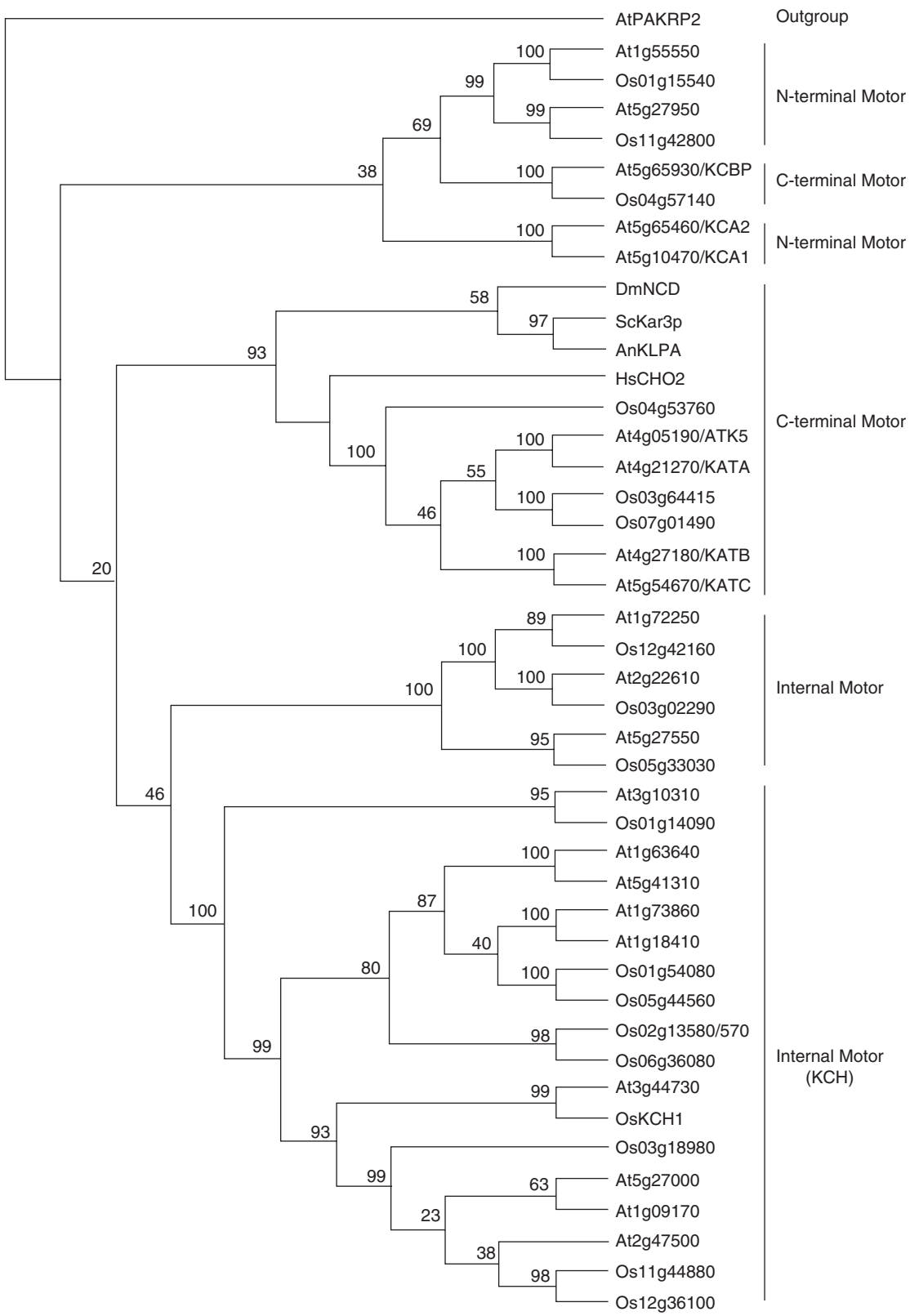

FIG. 4. Neighbor–joining tree of members of the Kinesin-14 subfamily. Phylogenetic relationships among Kinesin-14 members of rice (Os), *A. thaliana* (At), and representatives from the budding yeast *S. cerevisiae* (ScKar3p), the filamentous fungus *Aspergillus nidulans* (AnKLPA), the fly *Drosophila melanogaster* (DmNCD) and human (HsCHO2) are presented. AtPAKRP2 was used as an outgroup kinesin. For rice proteins, shown in this figure were IDs annotated by RGAP omitting LOC_. Documented plant kinesins are listed with their abbreviated names following the locus calls. Among Kinesin-14 members, those having their motor domains located in the N-terminus, the C-terminus, and the middle are highlighted as N-terminal motor, C-terminal motor, internal motor, and KCHs as specialized internal motor kinesins. Bootstrap values at the branches represent the percentages obtained in 1000 replications.

Kinesin-14

All Kinesin-14 members are minus end-directed motors as they all contain the conserved neck domain determining such directionality. This sub-family has been expanded the most compared with other sub-families of plant kinesins. This may reflect the fact that flowering plants do not make the minus end-directed motor dynein. Several distinct subgroups can be distinguished within this subfamily (Fig. 4). Among them, apparent orthologues of the yeast Kar3p decorate the MT-overlapping region in mitotic spindles and the phragmoplast, and play an evolutionarily conserved role in organizing MTs in spindles and the phragmoplast during mitosis and meiosis (Liu *et al.*, 1996; Chen *et al.*, 2002; Marcus *et al.*, 2003; Ambrose *et al.*, 2005). Four Kar3p-like kinesins, namely KATA/ATK1, KATB, KATC and ATK5, are made in *A. thaliana*. The rice genome has only two genes, LOC_Os04g53760/Os04g0629700 and LOC_Os07g01490/Os07g0105700, encoding proteins which resemble these four kinesins. These two rice kinesins are more closely related to KATA/ATK1 and its paralogue ATK5 than to the KATB and KATC paralogues. Interestingly, rice does not have apparent orthologues of KATB and KATC.

A calmodulin-binding kinesin has been added to the inventory of Kinesin-14 in photosynthetic organisms (Reddy *et al.*, 1996; Richardson *et al.*, 2006). This so-called KCBP contains a Ca^{2+}/calmodulin-binding peptide toward its very C-terminus, and an extended coiled-coil domain between the N-terminal nucleotide-independent MT-binding domain and the motor domain. As in *A. thaliana*, a single KCBP is encoded by the LOC_Os04g57140/Os04g0666900 locus in the rice genome. Detailed studies in *A. thaliana* and other organisms indicate that KCBP functions in MT organization/stability in interphase and mitotic cells, and consequently in cell morphogenesis (Oppenheimer *et al.*, 1997; Reddy and Day, 2000; Vos *et al.*, 2000; Preuss *et al.*, 2003).

Minus end-directed kinesins in fungi and animals in the Kinesin-14 sub-family typically have the motor domain located toward their C-termini. One surprise is that the plant Kinesin-14 sub-family contains members having their motor domains located toward the N-terminus or in the middle (Day *et al.*, 2000). Among those with N-terminal motors, arabidopsis At1g55550 and At5g27950 have apparent rice orthologues of LOC_Os01g15540/Os01g0260100 and LOC_Os11g42800/Os11g0648100, respectively. Overexpression of the tobacco orthologue of At5g27950 causes cortical MTs to be converted into a radial array emanating from a single perinuclear focus (Goto and Asada, 2007). Unfortunately, *in vivo* functions of these kinesins are unknown. Two other closely related arabidopsis N-terminal motor Kinesin-14s are KCA1 and KCA2. As targets of Cdk, they appear close to the plasma membrane, demarcating the division site in a cell cycle-dependent manner (Vanstraelen *et al.*, 2004, 2006). However, rice does not have an apparent KCA1/KCA2 orthologue.

Another unique feature is that a plant-specific group of Kinesin-14 distinguish themselves from other members of Kinesin-14 for having a CH domain towards the N-terminus of the polypeptide. These distinctive kinesins have been named KCH, for kinesin with CH domain, which confers binding to F-actin *in vitro* (Preuss *et al.*, 2004). The motor domain is located in the middle of the KCH kinesins. At least eight rice genes encode KCHs. Because of its unique actin-binding capability, they could probably be the long sought after dynamic linkers between MTs and F-actin in flowering plants. Unfortunately, this potential function for the founding member KATD and other KCHs has not been tested by genetic means in *A. thaliana* (Tamura *et al.*, 1999). It is foreseeable that arrays such as the PPB and the phragmoplast, where MTs closely interact with F-actin in a cell cycle-dependent manner, would need KCHs for reorganization of F-actin in response to rapid remodelling of MTs.

PERSPECTIVES

Very little has been learnt about the MT cytoskeleton in rice. The completed rice genome has presented an inventory of MT-interacting proteins whose functions are largely unknown. Functional characterization of these proteins will reveal MT-based molecular mechanisms that regulate critical cellular events that may be common to plants or unique to monocots. Rapidly growing resources such as the pools of T-DNA and transposon insertion mutants, and ample collections of cDNA clones are making rice an unmatchable model crop for studies aimed at improving Graminaceous crops (Jung *et al.*, 2008). The recent introduction of another monocot model, *Brachypodium distachyon*, will help make great leaps toward understanding the growth and form of monocots (Opanowicz *et al.*, 2008). By writing this review, we would like to invite colleagues to investigate the rice MT system for its role in growth and development in this remarkable crop, and to extend the studies to other monocot species such as *B. distachyon*.

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