

# Large-scale Identification of Tubulin-binding Proteins Provides Insight on Subcellular Trafficking, Metabolic Channeling, and Signaling in Plant Cells\*<sup>§</sup>

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Microtubules play an essential role in the growth and development of plants and are known to be involved in regulating many cellular processes ranging from translation to signaling. In this article, we describe the proteomic characterization of *Arabidopsis* tubulin-binding proteins that were purified using tubulin affinity chromatography. Microtubule co-sedimentation assays indicated that most, if not all, of the proteins in the tubulin-binding protein fraction possessed microtubule-binding activity. Two-dimensional gel electrophoresis of the tubulin-binding protein fraction was performed, and 86 protein spots were excised and analyzed for protein identification. A total of 122 proteins were identified with high confidence using LC-MS/MS. These proteins were grouped into six categories based on their predicted functions: microtubule-associated proteins, translation factors, RNA-binding proteins, signaling proteins, metabolic enzymes, and proteins with other functions. Almost one-half of the proteins identified in this fraction were related to proteins that have previously been reported to interact with microtubules. This study represents the first large-scale proteomic identification of eukaryotic cytoskeleton-binding proteins, and provides insight on subcellular trafficking, metabolic channeling, and signaling in plant cells. *Molecular & Cellular Proteomics* 3:970–983, 2004.

The cytoskeleton is the single most important structure that contributes to the highly ordered organization of the eukaryotic cell. It provides a framework for cell division and the trafficking of organelles and macromolecules, and also serves to regulate important cellular processes such as signaling, translation, and metabolism. The cytoskeleton plays a key role in a number of plant-specific processes, such as assisting in the formation of the cell plate, regulating cell-to-cell movement, and influencing the direction of cell elongation (1). A role

for the microtubule (MT)<sup>1</sup> component of the cytoskeleton in many of these processes has been demonstrated, and a number of MT-binding proteins that are responsible for regulating these events have been identified.

Plant MTs are assembled into four distinct arrays during the cell cycle (2). Three of these arrays—the interphase cortical array, the pre-prophase band, and the phragmoplast—have no counterpart in animal cells. The cortical MT array has been linked to the regulation of cellulose microfibril deposition and, hence, a role in cell expansion, while the pre-prophase band and the phragmoplast have important roles in the positioning and synthesis of the new cell plate in dividing cells. The fourth array, the spindle, has an evolutionarily conserved role in the segregation of chromosomes during cell division. The organization and dynamics of MTs in these arrays depend on the activity of various MT-associated proteins (MAPs). Several plant MAPs have been identified, including the 65-kDa MAPs, MAP 190, and MOR1 (3). These proteins are important in cross-bridging MTs, linking MTs with actin filaments, and stabilizing MTs. Many other proteins are known also to bind to MTs but do not function as MAPs. These proteins, often called MT-interacting proteins (MIPs) (2, 4), likely bind to MTs as a mechanism to regulate their own activity, to direct their subcellular localization, or as a concentrating mechanism at specific locations within the cell. The interaction of these proteins with MTs is often transient, making it difficult to visualize their interaction with MTs *in situ* (5). The large surface area provided by the MT network, and the cytoskeleton as a whole, likely serves as a matrix for the binding of hundreds of proteins to an extent that is dependent on cell type and environmental conditions (6).

Several approaches have been used to identify plant MT-binding proteins. These have included biochemical purification methods, mutant screens, and the identification of plant homologs to animal MT-binding proteins using antibody

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<sup>1</sup> The abbreviations used are: MT, microtubule; CLASP, cytoplasmic linker protein-associating protein 1; EF-1 $\alpha$ , elongation factor 1- $\alpha$ ; MAP, microtubule-associated protein; MFP, multifunctional protein; MIP, microtubule-interacting protein; PABP, polyadenylation-binding protein; RNP, ribonucleoprotein; TCP-1, T-complex protein-1.

cross-reactivity and sequence database mining (7–10). One of the first biochemical purification approaches involved the polymerization of endogenous plant tubulin in the presence of taxol, followed by pelleting of the resulting MTs and their associated proteins. This procedure was often followed by rounds of MT assembly and disassembly to enrich the MT-binding proteins (9, 11). The addition of taxol to assist in the stabilization of plant MTs was necessary due to the low concentration of endogenous tubulin in plant cells. The addition of high concentrations of neuronal MTs has been used as an alternative to facilitate the recovery of MT-binding proteins (7). Modifications to these techniques have been aimed at eliminating problems associated with the tough cell wall and lytic vacuoles in plant cells, and the development of “gentle” extraction buffers that maintain cytoskeleton integrity has made the plant cytoskeleton purification procedure more efficient (12).

MT-binding proteins have also been successfully purified from both animal and plant cells using tubulin affinity chromatography techniques. Two studies demonstrated that the SDS-PAGE profiles of plant proteins that eluted from tubulin and MT affinity chromatography columns were qualitatively similar (5, 13), and essentially all proteins that bound to the tubulin affinity columns subsequently co-sedimented with MTs in pelleting assays (14). This indicates that tubulin affinity chromatography can be used to successfully purify MT-binding proteins. Indeed, tubulin affinity chromatography has been used to identify three authentic plant MT-binding proteins—elongation factor-1 $\alpha$  (EF-1 $\alpha$ ) (15), the peroxisomal multifunctional protein (5), and phospholipase D (14)—as well as additional authentic and putative MT-binding proteins from both plant and animal cells (16–18).

Although numerous reports on the identification of cytoskeleton-binding proteins have been published, only a few of these reports have described the identification of multiple proteins of this type. For instance, in neutrophil cells the purification of a detergent-resistant membrane cytoskeleton led to the identification of 19 cytoskeleton-associated proteins involved in signaling (19). Similarly, a study of the actin cytoskeleton in thrombin-stimulated human platelet cells identified 27 actin-binding proteins from a detergent-resistant cell extract (20). These types of studies have also been performed in plants. Using a detergent-resistant cytoskeleton/protein body fraction from rice and maize endosperm cells, 15 and 5 cytoskeleton-associated proteins were identified, respectively (21, 22). Another plant study identified 8 proteins from a detergent-resistant protein fraction from pea stems (12). Here we report the identification of 122 proteins that were purified from an *Arabidopsis* cell suspension culture extract using tubulin affinity chromatography. Most, if not all, of these proteins possessed MT-binding activity *in vitro*, and almost one-half of these proteins were related to proteins that were previously shown to interact with MTs. The identification of these tubulin-binding proteins provides insight on the function of specific MT/protein interactions in plant cells.

## EXPERIMENTAL PROCEDURES

**Growth of *Arabidopsis* Suspension Cells**—*Arabidopsis* suspension cells were cultured in Murashige and Skoog medium (pH 5.8, M-6899; Sigma, St. Louis, MO) containing 3% (w/v) sucrose and supplemented with 1 mg/liter 1-naphthaleneacetic acid and 1 mg/liter kinetin (23). The cells were incubated at 23 °C in the dark and subcultured every 7 days by pipetting the suspension (one-tenth of the final volume) into fresh medium. Cells were harvested 3 days after subculturing, and the cell paste was frozen in liquid nitrogen and stored at –80 °C.

**Preparation of the Protein Extract**—Eighty grams of *Arabidopsis* cell culture were ground to a fine powder in liquid nitrogen, and 40 ml of cold extraction buffer (200 mM HEPES, pH 7.6, 10 mM MgSO<sub>4</sub>, 10 mM EGTA, 20 mM DTT, 2 mM PMSF, and 5  $\mu$ g/ml each of leupeptin, pepstatin, and aprotinin) was then added, followed by further grinding. After filtering through two layers of Miracloth (Calbiochem, La Jolla, CA), the extract was centrifuged for 30 min at 48,000  $\times$  *g* at 4 °C in an ultracentrifuge (model L7; Beckman, Fullerton, CA), and the supernatant was collected and centrifuged for 90 min at 100,000  $\times$  *g* at 4 °C to remove cellular debris and insoluble proteins. The supernatant from the 100,000  $\times$  *g* centrifugation step was then filtered through a 0.45- $\mu$ m nylon filter.

**Tubulin Affinity Chromatography and MT Co-sedimentation Assays**—A bovine brain tubulin column with a 5-ml bed volume was prepared as described previously (5). A BSA control column was also prepared with an equivalent amount of protein as the tubulin column and using the same coupling procedure. Cell extract containing ~150 mg of protein was loaded onto the columns at a rate of 0.5 column volumes per hour at 4 °C. The columns were washed thoroughly with 20 column volumes of washing buffer (50 mM HEPES, pH 7.5, 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 10% glycerol, 50 mM KCl, 0.5 mM DTT, and 2.5  $\mu$ g/ml each of leupeptin, pepstatin, and aprotinin), and proteins that bound to the columns were eluted with washing buffer containing 500 mM KCl. The protein was acetone precipitated in preparation for gel electrophoresis, or desalted and concentrated using a microfiltration apparatus (Centricon, Millipore, Bedford, MA) for co-sedimentation experiments.

MT co-sedimentation assays were performed as described previously (5). Briefly, 5  $\mu$ g of MTs and 2  $\mu$ g of tubulin-binding proteins were incubated either alone or together in 20 mM HEPES, pH, 7.5, 50 mM KCl, 1 mM EGTA, 0.1 mM GTP, 1 mM DTT, and 2.5  $\mu$ g/ml each of leupeptin, pepstatin, and aprotinin at 24 °C for 30 min and then centrifuged at 100,000  $\times$  *g* for 15 min in a benchtop ultracentrifuge (model TL-100; Beckman). The supernatant was carefully removed and the pellet was gently washed and then solubilized in SDS-PAGE gel-loading buffer, and the supernatants and pellets were then analyzed by SDS-PAGE.

**Gel Electrophoresis**—SDS-PAGE, two-dimensional NEPHGE, and immunoblot analysis were performed as described previously (5, 21). Protein gels were stained with either Coomassie Brilliant Blue stain (R-250) or by silver staining (Silver Stain Plus; Bio-Rad, Hercules, CA). For immunoblot experiments, rice multifunctional protein (MFP) and EF-1 $\alpha$  antisera were both used at a dilution of 1:1,000, and horseradish peroxidase-conjugated anti-rabbit secondary antibodies (Sigma) were used at a dilution of 1:3,000.

**Mass Spectrometry**—Coomassie blue-stained protein spots were excised from the two-dimensional gel and analyzed individually by LC-MS/MS at the University of Victoria Genome BC Proteomics Centre. Protein samples were “in-gel” digested with trypsin in 50 mM ammonium bicarbonate overnight at 37 °C, followed by extraction of peptides with 10% formic acid. Samples were pumped onto a 300- $\mu$ m inner diameter  $\times$  1-mm PepMap C18 5- $\mu$ m, 100-A nano precolumn (LCPackings/Dionex, Sunnyvale, CA) to concentrate and desalt the peptide mixture before MS analysis. The peptides were

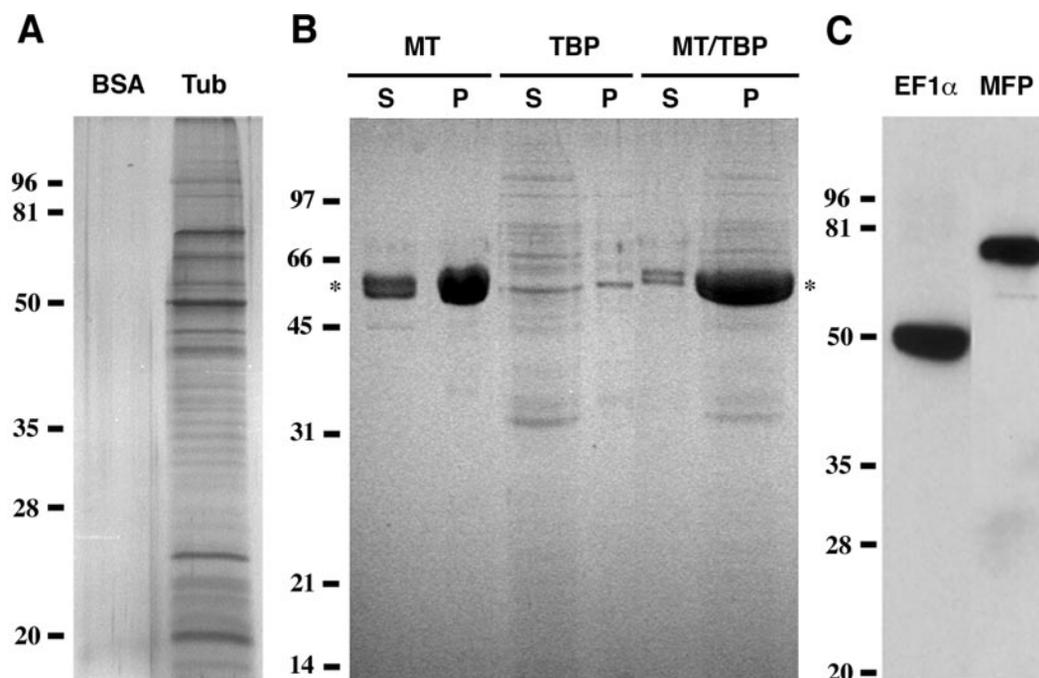


FIG. 1. Affinity purification of *Arabidopsis* cell culture tubulin-binding proteins. A, silver-stained SDS-PAGE gel of *Arabidopsis* cell culture proteins that bound to the tubulin affinity column (*Tub*). A BSA column served as a negative control for identifying any nonspecific protein binding (BSA). B, Coomassie blue-stained SDS-PAGE gel of the soluble (S) and pellet (P) fractions from MT co-sedimentation assays of the *Arabidopsis* tubulin-binding protein fraction. The  $\alpha/\beta$  tubulin dimer is marked by an asterisk. C, an SDS-PAGE gel blot of the tubulin-binding proteins probed with a rice polyclonal EF-1 $\alpha$  and MFP antisera. Molecular mass standards (in kDa) are indicated to the left of the panels.

then separated on a 75- $\mu$ m inner diameter  $\times$  15-cm PepMap C18 3- $\mu$ m, 100-A column (LCPackings/Dionex), followed by MS/MS on a PE Sciex QStar Pulsar I Q-TOF mass spectrometer (Sciex, Thornhill, Ontario, Canada). Proteins were identified using the MASCOT search program (version 1.9; Matrix Science, London, United Kingdom) with a confidence limit value set at  $<0.05$ , where  $P$  is the probability that the observed match is a random event. The comprehensive Mass Spectrometry Protein Sequence Database (MSDB) containing 1,165,316 protein entries was searched, and the oxidation of methionine was selected as a variable modification.

## RESULTS

**Purification of Tubulin-binding Proteins**—Tubulin affinity chromatography was used to purify tubulin-binding proteins from an *Arabidopsis* cell suspension culture. Previously, we demonstrated that bovine brain tubulin affinity columns were as effective in purifying rice tubulin-binding proteins as were columns generated with rice tubulin as the ligand (5). In addition, tubulin and MT affinity chromatography of plant extracts resulted in the purification of proteins that had SDS-PAGE profiles that were qualitatively similar (5, 13). The ease of purifying large amounts of bovine brain tubulin and the enhanced stability of tubulin columns over MT columns prompted us to use bovine tubulin affinity columns in this study, as in our previous study (5).

The *Arabidopsis* protein fraction that bound to the bovine tubulin column was analyzed by SDS-PAGE (Fig. 1A). Numerous proteins were identified in this fraction, and the number

and pattern of proteins observed was consistent with that of a similar fraction purified from rice and carrot cells (5, 13). In contrast to the tubulin-binding protein fraction, no proteins were evident in the eluate from a BSA control column (Fig. 1A). The BSA column served as an appropriate control column to identify any proteins that interacted solely by nonspecific ionic interactions, because the isoelectric points of BSA ( $pI = \sim 4.7$ ) and tubulin ( $pI = \sim 4.6$ ) are very similar. When the tubulin-binding protein fraction was used in MT co-sedimentation assays, most, if not all, of the proteins in this fraction co-sedimented with MTs (Fig. 1B). This was a similar observation to what has been reported previously (14) and is consistent with reports demonstrating that tubulin and MT affinity column eluates had similar SDS-PAGE protein profiles (5, 13). Additional evidence for the presence of MT-binding proteins in the tubulin-binding protein fraction came from the immunological detection of the known MT-binding proteins EF-1 $\alpha$  (15) and the peroxisomal MFP (Ref. 5 and S. D. X. Chuong, R. T. Mullen, and D. G. Muench, unpublished observations), as shown in Fig. 1C.

Two-dimensional NEPHGE analysis of the tubulin-binding protein fraction followed by Coomassie blue staining identified  $\sim 100$  prominent proteins spots (Fig. 2). Close examination of these gels indicated that there were many additional, less abundant proteins in this fraction. The two-dimensional gel profiles were consistent between independently purified

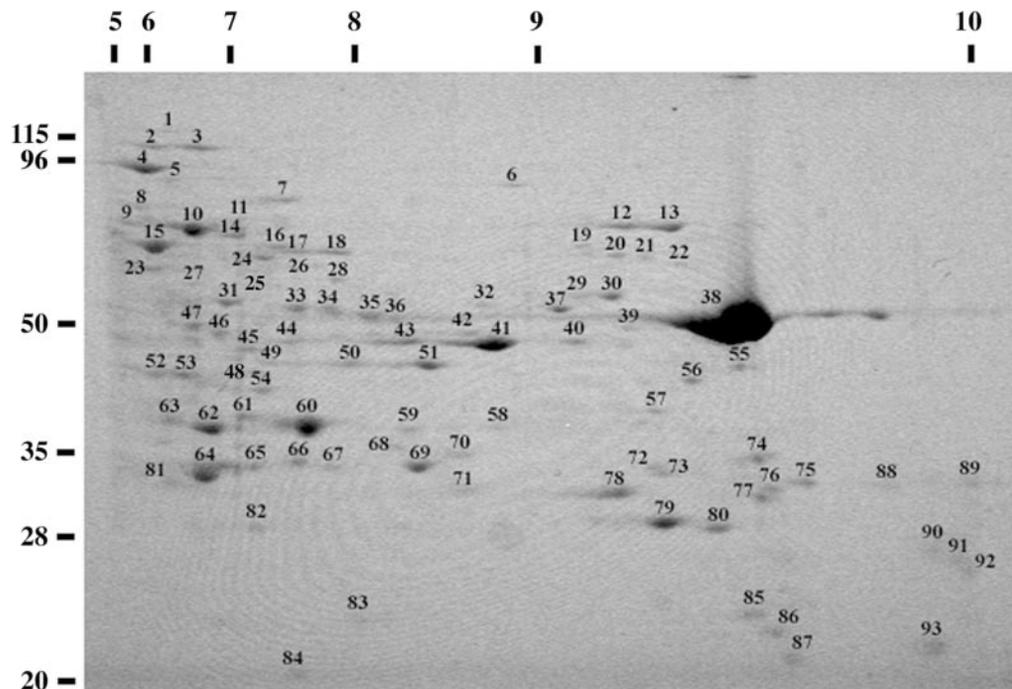


FIG. 2. **Two-dimensional NEPHGE of the tubulin-binding protein fraction.** Two hundred fifty micrograms of the tubulin-binding protein fraction was separated by two-dimensional NEPHGE and stained with Coomassie blue. Protein spots with relatively high staining intensity were candidates for MS analysis and were given a reference number as shown. The approximate pH value at specific positions of the first-dimension gel is indicated at the *top* of the gel. Molecular mass standards (in kDa) are indicated to the *left* of the gel.

tubulin column eluate fractions, showing only differences in spot intensity rather than in spot number or position (data not shown). These quantitative differences were likely the result of variation in the amount of protein loaded onto the columns. When a high concentration of protein was used, proteins with a higher affinity for tubulin could effectively outcompete other proteins for tubulin-binding sites. For instance, the relative abundance of EF-1 $\alpha$  was variable between column purifications, as observed by comparing the relative intensities of the abundant protein in spot 38 in Fig. 2 with the less obvious corresponding band at the 50-kDa molecular mass marker position in the tubulin-binding protein fraction lane in Fig. 1A. We observed previously that the tubulin-binding protein fraction was composed mostly of basic proteins, as determined by NEPHGE (5). This was also the case here; therefore, our first dimension, nonequilibrium tube gels were run so as to observe the proteins within the pH 6–10 range of the gel to allow for better separation of proteins (Fig. 2). Due to the basic properties of these proteins, the NEPHGE system was optimal as it preserved the solubility characteristics of the protein fraction, whereas these proteins formed a precipitate in standard, fixed pH IEF gels strips. One disadvantage of using the NEPHGE system was that some smearing occurred at the acidic end of the first-dimension gels, often causing protein spots to overlap in that region of the gel (Fig. 2).

**LC-MS/MS Protein Identification**—Ninety-three protein spots containing the highest abundance of protein were identified on the two-dimensional gel (Fig. 2). Several of these

proteins appeared to migrate as probable isoform pairs (for example, spots 2 & 3 and 12 & 13), and in these instances only a single member of each pair was selected for LC-MS/MS analysis. The large cluster of heavily stained proteins that migrated at the pH 9.3 position in the first-dimension gel and had a molecular mass of 50 kDa was assigned a single number (spot 38). A total of 86 of these spots were chosen for LC-MS/MS analysis. Sufficient protein was present in each spot to allow for the use of a single gel for protein spot excision and subsequent LC-MS/MS analysis. Protein spots were carefully excised from the gel, and tryptic peptides that were liberated from each spot were analyzed by LC-MS/MS. High-confidence peptide identity data ( $p < 0.05$ ) was obtained from 74 of the 86 spots analyzed. An average of 5.0 peptides were identified for each protein, with a range of 1–22 peptides identified per protein. The high confidence limit settings that were used in the analysis of the peptide data coupled with the identification of multiple peptides for most of the proteins allowed for the unambiguous identification of individual proteins using this technique.

The MS analysis of single gel spots often identified peptides that corresponded to more than one protein, indicating that proteins within this fraction often migrated to the same location in the gel, or there was occasionally some overlap of spots due to protein streaking in the gel. In total, 122 proteins were identified, and these were grouped according to their predicted functions (Tables I–VI, Fig. 3). The experimentally determined molecular mass of each protein was generally

TABLE I  
 Proteins similar to known MT-associated proteins that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	$M_r$ , theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
23	59.8 (62)	Q9SF16	Putative TCP-1 $\eta$ subunit (4)	(28, 102)
40	159 (48)	Q8RWY6	Hypothetical protein with homology to CLASP1/ORBIT (3)	(24)
47	62.9 (50)	G96703	Similar to myosin heavy chain related protein/kinectin/centriolin (4)	(103–105)
57	40.0 (39)	T48161	Heat shock protein 40 (2)	(28)
58	36.4 (38)	Q9SA50	Hypothetical protein with domain similar to myosin heavy chain/dynein/kinesin (4)	(105)
60	36.4 (37)	Q9SA50	Hypothetical protein with domain similar to myosin heavy chain/dynein/kinesin (4)	(105)
82	70.0 (29)	Q9LHA8	70-kDa heat shock protein (5)	(28)

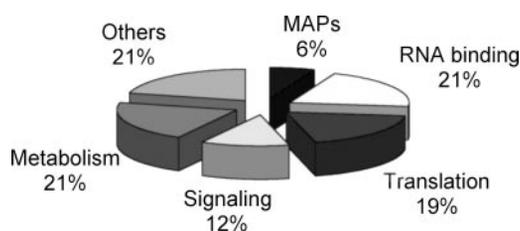


FIG. 3. Distribution of tubulin-binding proteins according to their proposed function.

consistent with the predicted value of the corresponding protein from the database (Tables I–VI). The discrepancy between the experimental and theoretical values observed for some proteins could be explained by proteolytic degradation of polypeptides during sample preparation, or variability arising from alternate splicing of mRNAs. Individual peptide sequences and their probability scores are listed in Supplementary Tables I–VI. Each of these peptides was confirmed as a tryptic digest product based on the presence of flanking arginine or lysine residues.

**Microtubule-associated Proteins**—Table I lists the tubulin-binding proteins that were identified as MAP-like proteins. One hypothetical protein (spot 40) possessed 42 and 44% amino acid similarity to the human cytoplasmic linker protein-associating protein 1 (CLASP1) and its *Drosophila* homolog ORBIT, respectively. This protein functions in spindle development as well as other roles in MT organization in animal cells (24, 25). HSP70, a well-characterized MAP (26–28), was also identified in this study (spot 82), as was its co-factor, HSP40 (spot 57). This chaperone complex is believed to regulate MT dynamics (28). In addition, the  $\eta$  subunit of the T-complex protein-1 (TCP-1) chaperone was identified (spot 23) and may assist the TCP-1 complex in the folding of  $\alpha$ - and  $\beta$ -tubulin and in regulating MT nucleation (28, 29).

The remaining proteins listed in Table I contained domains that showed moderate to high amino acid similarity to motor proteins. Spot 47 contained a protein with 76% amino acid similarity to a myosin heavy chain-like protein from *Arabidopsis*, as well as 59% similarity to an 80-aa region from a human centriole-binding protein (centriolin), and 45% similarity in a

200-aa region with a chicken kinesin-binding protein (kinectin). A protein that was present in spots 58 and 60 contained an amino-terminal domain of  $\sim$ 100 aa that has 52–57% similarity to mouse myosin heavy chain, human kinesin, and fungal dynein.

**RNA-binding Proteins**—A large number of proteins identified in the tubulin-binding protein fraction are well-characterized RNA-binding proteins or are hypothetical proteins containing RNA-binding domains (Table II). Among these were a SNC/Tudor domain protein (spot 3), a polyadenylation-binding protein (PABP; spot 10), and several RNA helicases (spots 10, 15, 22, 23, 30, 32, 37). Homologs of these proteins have been shown to associate with MTs, either directly or indirectly. For example, PABP co-purified with MTs from sea urchin embryos and HeLa cells (30, 31), and a human RNA helicase (Vasa) interacts with a MT-binding protein that regulates MT nucleation (32). A protein similar to the SNC/Tudor domain-containing protein has also been identified in cytoskeleton fractions from rice and pea (33, 34). The SNC domain is involved in single-stranded nucleic acid binding, while the Tudor domain has been suggested to function in RNA trafficking/processing (34, 35). This protein is involved in seed storage protein mRNA localization to subdomains of the endoplasmic reticulum in rice endosperm cells (T. W. Okita, personal communication).

A number of the RNA-binding proteins identified here are predicted to have a nuclear localization. For instance, nucleolin (spots 8, 14) functions in the synthesis, assembly, and maturation of ribosome components and appears to shuttle between the nucleus and cytosol (36). Also identified here was a protein similar to the La protein (spot 20) that functions in the nucleus to stabilize small RNAs, such as tRNA, and also shuttles between the nucleus and the cytosol (37, 38). Spots 54, 64, 79, and 80 contained proteins identified as components of small nuclear ribonucleoproteins (snRNPs), a protein class that functions in splicing. Although there are no reports of snRNPs having MT binding activity, two of these proteins (spots 54, 64) have sequence conservation (50% similarity) with fungal  $\beta$ -transducins, a WD-40-containing protein that binds to MTs in sea urchin cells (39). A transcriptional co-

TABLE II  
 Proteins with RNA binding activity that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	<i>M<sub>r</sub></i> , theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
3	108 (107)	AAL57629	SNC/Tudor domain protein (19)	(33, 34)
8	58.8 (78)	A96527	Probable nucleolin protein (4)	(106)
10	67.6 (71)	Q8LA13	ATP-dependent RNA helicase (7)	(32)
10	72.7 (71)	C96534	Poly(A) binding protein (8)	(30, 31)
14	58.8 (68)	A96527	Probable nucleolin protein (4)	(106)
15	65.8 (66)	Q9LU46	DEAD-box RNA helicase (4)	(32)
20	55.8 (65)	Q94K80	Hypothetical RNA binding protein with homology to La protein (6)	
22	53.1 (63)	Q8W4R3	DEAD box RNA helicase (6)	(32)
23	66 (62)	Q8LA13	ATP-dependent RNA helicase (3)	(32)
30	65.4 (56)	AAM13255	DEAD box RNA helicase (16)	(32)
32	55.6 (54)	B96593	Ethylene response RNA helicase (5)	(32)
37	55.6 (52)	Q9C718	Ethylene-response RNA helicase (22)	(32)
41	38.9 (47)	AAL09735	Nuclear RNA-binding protein A-like protein (16)	
42	38.9 (49)	Q8LB56	Nuclear RNA-binding protein A-like protein (6)	
43	38.9 (46)	G714444	Nuclear RNA-binding protein (3)	
44	41.7 (48)	Q94AC3	Hypothetical protein with RRM domain (1)	
51	38 (44)	Q8LDQ7	Nuclear RNA-binding protein A-like protein (10)	
53	45 (43)	B96600	Protein with PPR domain involved in RNA stability (3)	
54	37.9 (42)	C84870	Probable small nuclear RNP (2)	
64	28.1 (32)	S30580	U2 small nuclear RNP A (3)	
72	33.5 (33)	Q8RWN5	RNA-binding protein-like (1)	
79	26.2 (29)	Q8LB63	Putative small nuclear RNP U2B (2)	
80	26.2 (28)	C84706	Small nuclear RNP U2B (3)	
90	25.9 (27)	Q9C7C3	Hypothetical protein with KH domain (4)	(107)
91	25.7 (26)	Q8L773	Transcription coactivator-like protein with RRM domain (2)	(108)
93	25.3 (21)	Q8LDY1	Hypothetical protein with RRM domain (3)	(107)

activator protein with an RNA-recognition motif was also identified (spot 91) and has high sequence similarity to the mammalian ALY protein that is involved in RNA export from the nucleus (40, 41).

**Proteins Involved in Translation**—It is now well established that the cytoskeleton has an important role in regulating translational efficiency (1, 42). Several proteins that are known to function in this process were identified in the tubulin-binding protein fraction (Table III). As demonstrated previously using rice and carrot cell extracts (5, 13) and here by protein gel blot analysis (Fig. 1C) and LC-MS/MS (Table III), EF-1 $\alpha$  (spot 38) was an abundant protein in the tubulin-binding protein fraction. Several other elongation and initiation factors were also identified, some of which have been shown to bind to MTs and other cytoskeletal structures in previous studies (Table III) (12, 43–46). These included EF-2 (spot 4) and subunits of the eukaryotic translation initiation factors eIF3 and eIF4 (spots 7, 18, 24, 27, 50, 54, 70). Spot 30 contained a hypothetical protein with 56% amino acid similarity throughout its sequence to the PINHEAD/ZWILLE AND ARGONAUTE1 proteins that are involved in vegetative development and are members of a family of proteins that includes eIF2C (47). Additional evidence for the role of cytoskeletal elements in translation comes from reports that aminoacyl-tRNA synthetases and ribosomes can associate with the cytoskeleton (21, 31, 48–51). Several aminoacyl-tRNA synthetases were

identified here (spots 5, 8, 9, 23, 85), as were various isoforms of large and small ribosomal subunits (spots 75, 86 to 91, 93). These ribosomal proteins may function to anchor ribosomes to MTs (51) or may have functions that extend beyond translation (52).

**Proteins Involved in Signaling**—Table IV lists a number of potential cell-signaling proteins that were identified in the tubulin-binding protein fraction. These included several protein kinases, including casein kinase I and II (CK; spots 68, 73), a cyclin-dependent protein kinase (CDK; spot 39), and several other serine/threonine kinases (spots 5, 15, 70, 84). In addition to the protein kinases, a hypothetical protein that contains a protein phosphatase 2A (PP2A) catalytic domain (spot 32) and a PP2A regulatory subunit (spot 33) were also identified. Other proteins in the tubulin-binding protein fraction with probable signaling roles included a leucine-rich repeat (LRR) containing protein (spot 22) involved in disease-resistance signaling (53). This protein shows 55% amino acid similarity to a *Drosophila* protein, FLIGHTLESS, which is thought to link the cytoskeleton to signal transduction pathways, and which binds to MTs and actin filaments (54). A hypothetical protein was identified (spot 44) that contains a SWI/SNF domain first identified in yeast (55). This domain is important in regulating transcriptional activity by remodeling chromatin, and it also localizes to the kinetochores of mitotic chromosomes where the SWI/SNF complex may interact with

TABLE III  
Proteins involved in translation that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	<i>M<sub>r</sub></i> theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
4	94 (94)	AAK96653	EF-2 (9)	(109)
5	89.8 (86)	T05247	Methionyl-tRNA synthetase (4)	(48)*
7	82.9 (80)	Q8H179	eIF4-like protein (17)	(44)
8	77.2 (78)	Q8LBY0	Glycyl tRNA synthetase (4)	(48)*
9	77.3 (72)	O04630	Threonyl tRNA synthetase (6)	
18	59.3 (65)	Q9M7E8	eIF4B (8)	(44)
23	60.8 (62)	T48004	Multifunctional aminoacyl-tRNA synthetase-like protein (4)	(48)*
24	57.7 (64)	Q9LIN5	eIF4B (8)	(44)
27	57.6 (58)	Q9SQK7	eIF4B (5)	(44)
30	129.1 (56)	AAF24586	Hypothetical protein similar to eIF2C/ARGONAUTE1/PINHEAD/ZWILLE (1)	(109)
38	49.8 (50)	AY048275	EF-1 $\alpha$ (11)	(15)
39	49.8 (50)	Q8GTY0	EF-1 $\alpha$ (3)	(15)
50	39.2 (45)	Q9C8J1	eIF4A (5)	(110)*
54	46.7 (42)	Q8LBZ6	eIF4A (6)	(110)*
70	32.6 (34)	Q9C5Y9	eIF3G (5)	(45)
75	27.5 (32)	Q9FJA6	Ribosomal protein S3 (4)	(21)*
85	20 (23)	AAM15519	Tyrosyl tRNA synthetase (4)	(48)
86	22.9 (22)	P51427	Ribosomal protein S5-2 (4)	
87	22 (21)	Q8LCC2	Ribosomal protein L9 (5)	
88	27 (32)	T45927	Ribosomal protein S3a (7)	(21)*
89	30.9 (33)	AY085726	Ribosomal protein S2 (3)	
90	24.5 (27)	Q8VZB9	Ribosomal protein L10a-1 (3)	
91	27.9 (26)	Q940T2	Ribosomal protein L7 (4)	
93	22 (21)	Q8LD03	Ribosomal protein L7 (2)	

\* Proteins described in these references were identified from a detergent-treated fraction containing total cytoskeleton proteins.

TABLE IV  
Proteins involved in signaling that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	<i>M<sub>r</sub></i> theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
5	65.8 (86)	B84833	Hypothetical protein with serine-threonine kinase domain and HEAT repeat (2)	
15	79.0 (66)	T05148	Serine/threonine protein kinase homolog (1)	
22	64.0 (63)	Q9FHL8	Putative disease resistance protein family with LRR domain (5)	(54)
32	27.7 (54)	C96606	Hypothetical protein with PP2A domain (2)	(85, 86)
33	45.0 (53)	Q94A11	PP2A regulatory chain (1)	(85, 86)
39	72.4 (50)	Q8W2N0	Cyclin-dependent kinase CDC2C (1)	(80)
44	42.7 (48)	Q8LFA6	Hypothetical protein with SWI/SNF subunit domain (2)	
45	46.5 (47)	T46063	Hypothetical protein with SEC14 lipid-binding domain (4)	
64	35.7 (32)	AAF78369	G-protein $\beta$ -subunit/contains WD-40 repeat (4)	(57)
65	35.8 (33)	Q8LAF2	G-protein $\beta$ -subunit/contains WD-40 repeat (2)	(57)
68	47.1 (35)	Q8H120	Casein kinase II $\alpha$ chain 2 (5)	(84)
70	36.2 (34)	Q9FNL4	Serine/threonine protein kinase (1)	(87)
73	33.5 (33)	E85088	Casein kinase I-like protein (1)	(82)
75	64.8 (32)	Q8W4K6	Hypothetical protein with GTPase-activating domain(2)	(111)
84	87.0 (21)	Q9C833	Serine/threonine protein kinase (2)	(87)

MTs (56). Two  $\beta$ -subunits of G-proteins were identified in this fraction (spots 64, 65), and were previously shown to bind to MTs in mammalian cells (57). Proteins with probable roles in G-protein activation were also identified and included a hypothetical protein with a SEC14 lipid-binding domain known to associate with G-protein subunits (spot 45) and a hypothetical protein with a GTPase-activating domain (spot 75).

*Proteins Involved in Metabolism*—A large number of proteins in the tubulin-binding protein fraction were identified as metabolic enzymes (Table V). Five of these enzymes (spots 15, 23, 31, 35, 67/69) are involved in catalyzing reactions in folate-dependent pathways of one-carbon metabolism (58), and five others (spots 13, 50, 51, 64/81, 82) have roles in fatty acid metabolism in the peroxisome (59). Evidence for perox-

TABLE V  
 Proteins involved in metabolism that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	<i>M<sub>r</sub></i> , theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
8	73.4 (78)	T06034	Phosphoenolpyruvate carboxykinase (8)	(5)
13	77.8 (72)	T08956	AIM protein (peroxisomal MFP) (8)	
15	67.8 (66)	C96541	10-Formyltetrahydrofolate synthetase (7)	
23	64.9 (62)	Q8RWT5	Phosphoribosylaminoimidazole carboxamide formyltransferase (2)	
28	59.2 (59)	T52611	Glucose-6-phosphate 1- dehydrogenase (7)	(62)
31	51.7 (55)	O23254	Serine hydroxymethyl- transferase (14)	
33	58.0 (53)	T06104	2-Dehydro-3-deoxyphospho- heptonate aldolase (2)	
35	50.7 (52)	T05362	Glycine hydroxymethyl- transferase (3)	
47	52.9 (50)	Q9FWA3	6-Phosphogluconate dehydrogenase (5)	
50	47.6 (45)	T46895	Acyl-CoA dehydrogenase (2)	
51	48.6 (44)	Q9S7M3	3-Ketoacyl-CoA thiolase (11)	
53	47.3 (43)	T04668	Phosphoserine transaminase (7)	
53	43.3 (43)	Q8H1Y0	Pyruvate dehydrogenase E1 $\alpha$ -like subunit IAR4 (3)	
57	34.4 (39)	T10203	Protein from the alcohol dehydrogenase family (6)	
59	36.7 (38)	H84898	3-Methyl-2-oxobutanoate hydroxy-methyl-transferase (4)	
60	36.7 (37)	O82357	3-Methyl-2-oxobutanoate hydroxy-methyl-transferase (9)	
61	37.4 (39)	T47945	3-Methyl-2-oxobutanoate hydroxy-methyl-transferase (2)	
62	36.9 (38)	Q8LAS0	Glyceraldehyde-3-phosphate dehydrogenase C subunit (8)	
64	31.7 (32)	Q9LDF5	3-Hydroxyacyl-CoA dehydrogenase-like protein (5)	
66	37.5 (34)	G84616	Glyoxosomal malate dehydrogenase (6)	
67	31.6 (33)	Q8LEA3	5,10-Methylenetetrahydrofolate dehydrogenase (4)	
69	31.6 (33)	Q8LEA3	5,10-Methylenetetrahydrofolate dehydrogenase (13)	
81	31.7 (32)	Q9LDF5	3-Hydroxyacyl-CoA dehydrogenase-like protein (3)	
81	36.5 (32)	T46192	Acetylglutamate kinase-like protein (2)	
82	29.9 (29)	Q9FHR8	Enoyl CoA hydratase (3)	

isomal enzyme interactions with MTs has been reported previously (5, 16, 60, 61). The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also identified in this fraction (spot 62) and has been previously shown to have MT-binding activity (22, 62, 63). Additional proteins were identified that function in other metabolic pathways, including the pentose phosphate pathway (spots 31, 47), phosphate metabolism (spot 33), amino acid biosynthesis (spots 53, 81), the tricarboxylic acid cycle (spot 53), anaerobic glycolysis (spot 57), and pantothenate biosynthesis (spots 59–61).

**Other Proteins with Known or Unknown Functions**—The tubulin-binding proteins listed in Table VI likely participate in cellular activities other than those described above. These proteins have a wide range of functions, and at least one of these proteins has previously been reported to bind to MTs. Spot 81 contained a regulatory subunit of the 26S proteasome, a complex that was shown previously to bind to multiple MT arrays in plants (64). Other interesting proteins were identified that have not been shown previously to bind to MTs; however, their predicted functions implicate a possible MT association. For instance, the sorting nexin-like protein (spots 45, 46) is involved in vesicle trafficking in both plants and animals and has a role in axonal guidance, a form of cell movement requiring cytoskeletal rearrangements (65). Actin-11 (spot 4), a unique and ancient actin subclass in the *Arabidopsis* actin family that has a divergent sequence from

other actins, was also identified in this study. This protein likely participates in specific developmental roles (66) and may function to link actin filaments and MTs together. Several proteins were identified that possessed DNA-binding domains (spots 8, 46/47, 56, 84), including the transcription factor Scarecrow. Transcription factor activity is known to be regulated in some instances by its shuttling into and out of the nucleus in a MT-dependent manner, as was reported for the mammalian tumor suppressor protein p53 (67). A group of proteins that were unexpectedly represented in the tubulin-binding protein fraction were those involved cell wall modification and included  $\beta$ -galactosidase (spot 6), xylosidase (spot 19), pectate lyase (spot 58), xyloglucan endo-1,4- $\beta$ -D-glucanase (spot 71), and endoxyloglucan glycosyl-transferase (spot 78).

#### DISCUSSION

Here we report the identification of 122 tubulin-binding proteins that were purified from *Arabidopsis* suspension cells using tubulin affinity chromatography, a procedure that has been used previously to purify *bona fide* MT-binding proteins (5, 13–15, 17). The following observations indicated that tubulin affinity chromatography also served as an effective method for the purification of MT-binding proteins in the present study. First, antibodies raised against two known MT-binding proteins, EF-1 $\alpha$  (15) and the peroxisomal MFP (5),

TABLE VI  
Proteins with other known or unknown functions that were identified in the *Arabidopsis* cell culture tubulin-binding protein fraction

Spot no.	$M_r$ theoretical (experimental)	Accession no.	Identity (no. of peptides matched)	References supporting a MT interaction
4	41.9 (92)	P54396	Actin 11 (3)	
6	93.6 (86)	CAB64737	$\beta$ -Galactosidase (11)	
8	79.0 (78)	Q9LDX1	Hypothetical protein with XS zinc finger nucleic acid binding domain (4)	
19	83.5 (68)	Q9FGY1	Xylosidase (4)	
20	63.1 (65)	Q9FK40	Histone acetyltransferase (5)	
23	56.9 (62)	G86349	Hypothetical protein with homology to auxilin, trichohalin, and has a DnaJ domain (3)	
45	46.5 (47)	Q9FG38	Sorting nexin-like protein (7)	
46	46.5 (48)	Q9FG38	Sorting nexin-like protein (9)	
46	44.0 (48)	T51151	Nuclear DNA-binding protein G2p (7)	
47	44.0 (50)	T51151	Nuclear DNA-binding protein G2p (7)	
50	42.5 (45)	T05870	Cell division protein pelota (3)	
56	169.4 (42)	Q9LNX6	Scarecrow-like transcription factor (2)	
56	49.0 (42)	Q9FF81	Vacuolar protein sorting 36 (2)	
58	46.0 (38)	Q9LTZ0	Putative pectate lyase 11 precursor (4)	
59	35.7 (38)	Q8W457	Hypothetical protein with cysteine	
65	36.5 (33)	T52133	Potassium channel protein, b subunit (5)	
67	34.3 (33)	B96602	Hypothetical protein with homology to myosin heavy chain (3)	
70	51.8 (34)	Q93Y09	Serine carboxypeptidase II (4)	
71	32.8 (31)	C49539	Xyloglucan endo-1,4- $\beta$ -D-glucanase (4)	
76	33.6 (31)	Q8LB34	Phosphate-induced protein 1 (2)	
77	172 (30)	T06677	Protein with Bromo adjacent homology domain (1)	
78	32.8 (30)	Q39099	Endoxyloglucan glycosyl- transferase (12)	
81	33.5 (32)	Q8LD11	26S proteasome non-ATPase regulatory subunit (6)	(64)
84	22.3 (21)	BT004166	Protein with single-stranded DNA-binding domain (2)	
84	20.6 (21)	Q8RXZ8	Hypothetical protein (2)	

cross-reacted with proteins of the expected size on protein gel blots containing the tubulin-binding proteins (Fig. 1C). The presence of these two proteins was confirmed by LC-MS/MS analysis (results from spots 38 and 13 in Tables III and V, respectively). Second, it appears that most, if not all, of the proteins that bound to the tubulin affinity column, pelleted with MTs in co-sedimentation assays (Fig. 1B). A similar observation was made previously (14) and is consistent with observations that SDS-PAGE profiles of proteins that bound to tubulin and MT affinity columns were qualitatively similar (5, 13). Third, 43% of the proteins identified in this study (52/122) are related to proteins previously reported to have an association with MTs (see references in Tables I–VI). These observations gave us confidence that the proteins identified here are authentic MT-binding proteins.

The tubulin-binding proteins that were identified in this study were grouped according to their predicted primary function as either MAPs, RNA-binding proteins, translation factors, metabolic enzymes, signaling proteins, or proteins with other functions (Tables I–VI, Fig. 3). Although we expected to identify a broad range of protein types based on the identities of previously characterized plant MT-binding proteins (1, 2, 10), certain groups of these proteins would not be expected to bind to MTs because of their known subcellular location. In these cases, the association of these proteins with

MTs may play a role in their trafficking within the cell. Alternatively, these proteins may possess multiple functions and carry out the additional function(s) in the cytosol in association with MTs. A good example of a group of tubulin-binding proteins identified here that is known to be localized to a particular membrane-bound organelle were the peroxisomal matrix enzymes. Five of these enzymes catalyze reactions in fatty acid  $\beta$ -oxidation (Table V, spots 13, 50, 51, 64/81, 82), and one functions in the glyoxylate cycle (malate dehydrogenase; spot 66). Each of these enzymes possesses a putative amino- or carboxyl-terminal signal sufficient to target the protein to the peroxisome matrix (68). Peroxisomal matrix proteins are imported into the peroxisome in their fully folded conformation and, therefore, have the potential to bind to MTs prior to their import. We have performed extensive studies on the MT binding activity of the rice peroxisomal MFP (5) and have demonstrated that it does indeed bind to MTs *in vivo* (S. D. X. Chuong, R. T. Mullen, and D. G. Muench, unpublished observations). We proposed previously that the binding of MFP to MTs may be important in facilitating its import into the peroxisome (69). The fact that at least six peroxisomal matrix enzymes were present in the tubulin-binding protein fraction in this study (Table V), and one reported in another study (16), supports a general role for MTs in peroxisomal matrix protein trafficking in plant cells.

The identification of numerous cytosolically localized metabolic enzymes in the tubulin-binding protein fraction (Table V) is consistent with reports suggesting that sequential enzymes from individual metabolic pathways can cluster on the cytoskeleton to assist in the efficient and regulated control of flux through those pathways in a process called metabolic channeling (70). For instance, five enzymes that catalyze coupled reactions in the folate-dependent pathways of one-carbon metabolism (58) were identified as components of the tubulin-binding protein fraction (Table V, spots 15, 23, 31, 35, 67/69). This suggests that these cytosolic enzymes bind to MTs individually or as a complex to facilitate channeling of intermediates through these pathways. An interaction between homologs of some of the one-carbon metabolism enzymes that were identified here has been implicated in facilitating metabolic channeling in mammalian cells (58, 71, 72). Our data suggests that any metabolic channeling that results from such an interaction would also require the binding of this complex to the MT matrix in order to stabilize these interactions. The identification of other classes of metabolic enzymes in this study (Table V) indicates that MTs may have an important role in regulating the activity of many metabolic processes.

Interestingly, several enzymes that were identified in the tubulin-binding protein fraction have cell wall modification activities (spots 6, 19, 58, 71, 78), including endo-1,4- $\beta$ -glucanase and endoxyloglucan glycosyltransferase (Table VI). These enzymes are known to have a cooperative role in the modification of cell wall architecture that is required for cell elongation and fruit ripening (73). The complex organization of the cell wall requires a highly coordinated activation of wall-loosening proteins to achieve precise control of wall disassembly. Perhaps an interaction occurs between some of these proteins to form a multicomponent system of enzymes that functions to channel substrates in the modification and degradation of xyloglucans. This would be analogous to the plant cell wall-degrading "cellulosome" of certain bacteria (74). An endo-1,4- $\beta$ -glucanase that contains a transmembrane domain is localized to the plasma membrane and Golgi in expanding cells of tomato (75) and could potentially interact with MTs through its cytosolic domain. An interaction between endo-1,4- $\beta$ -glucanase and MTs across the plasma membrane, perhaps as a component of an enzyme complex in the cell wall, could function to precisely regulate the activity of these wall-loosening enzymes. Several examples of linker proteins that are components of the cytoskeleton-plasma membrane-cell wall continuum in plants have been identified and appear to mediate communication across the plasma membrane (76).

In plant cells, the cortical MT network lines the plasma membrane and responds to a diverse array of extracellular stimuli (77–79). Several putative signaling proteins that were identified in the tubulin-binding protein fraction (Table IV) have been shown previously to interact with MTs directly or indi-

rectly through their association with MAPs and play an important role in regulating the cell cycle, cytoskeletal dynamics, and stress responses. For instance, a cyclin-dependent kinase binds to MTs in the spindle and phragmoplast in plant cells (80), casein kinases regulate MT organization by interacting with MAPs (81–84), and PP2A assists in regulating MT organization (85). MAPs are a target of kinases and phosphatases, and their modification by phosphorylation/dephosphorylation can result in changes in MT organization and dynamics, as well as the activation of signaling cascades (86–90). In plants, rearrangement of cortical MTs is known to occur in response to various signals, including cold and aluminum stress, wounding, and pathogen attack (91–93). The putative signaling proteins identified in the tubulin binding protein fraction may be involved in the transduction of signals generated from abiotic and biotic stresses such as these, or in response to light or developmentally regulated signals (95). As very little is known about cytoskeleton-associated proteins and their role in plant-specific signaling pathways, these will serve as useful target proteins in future signaling studies.

Many proteins identified in this study have predicted roles in RNA binding and translation (Tables II and III, Fig. 3). Proteins that function in translation have been reported to bind to the cytoskeleton (21, 42), and several of these proteins were identified here, including translation initiation and elongation factors, aminoacyl-tRNA synthetases, and ribosomal proteins. This concentration of the translational machinery on the cytoskeleton may function to increase the efficiency of translation, or serve as a mechanism to regulate translation rate (42). Some of the RNA-binding proteins identified in this fraction may function to anchor RNAs to MTs in a specific or general manner in order to facilitate translation, as has been described previously (95, 96). Alternatively, these proteins may be involved in trafficking mRNAs from the nucleus to regions of the cell where they are to be translated. RNA localization is well characterized in many species and functions to contribute to the localized synthesis of protein at distinct subcellular regions (97). The initial step in RNA trafficking involves nuclear export with the assistance of nuclear/cytosolic shuttling proteins. These shuttling proteins can remain as a component of RNP particles that move specific RNAs throughout the cell along MTs (98). The transcriptional co-activator protein identified here (spot 91) is homologous to the animal nuclear RNA export factor ALY, which has a general role in RNA export from the nucleus and in the initial stage of localization of specific RNAs (41, 99). The identification of several types of RNA-binding proteins and translation factors in the tubulin-binding protein fraction is consistent with reports describing the existence of highly organized and efficient RNA localization and translation machineries that operate in association with the cytoskeleton (42).

A group of proteins that represented a relatively small percentage of the total proteins in the tubulin-binding protein fraction were the MAPs (Table I). Proteins such as EF-1 $\alpha$  and

GAPDH also have MAP activities (13, 100) and could have been included in this group; however, these were grouped according to their originally documented functions (Tables III and V). The absence in this fraction of well-characterized plant MAPs, such as the 65-kDa MAPs, MAP190, or MOR1 (3), may have been due to the fact that the tubulin column does not bind these proteins with high affinity. The 65-kDa MAPs and MAP 190 were originally purified by pelleting endogenous taxol-stabilized plant MTs after rounds of polymerization and depolymerization (11, 102). Perhaps these proteins were in low abundance in our tubulin-binding protein fraction and were not visible on the Coomassie blue-stained gel, and therefore were not selected for MS analysis. The presence in this fraction of MAPs that are common in eukaryotes, such as molecular chaperones, CLASP, and putative motor proteins, indicates that at least some MAPs are enriched using this procedure.

The identity of the proteins reported in this study supports previous evidence suggesting that MTs serve as a matrix where proteins can bind and carry out numerous functions within the cell, and provides insight on the mechanisms responsible for macromolecule trafficking, metabolic channeling, and signaling in plant cells. Additional, more direct methods will be required to confirm that a *bona fide* interaction occurs between these proteins and MTs, as it is possible for some proteins that their interaction with tubulin *in vitro* occurs as a result of nonspecific binding during the chromatography procedure. Also, some of the proteins identified here may associate indirectly with MTs by interacting with other MT-binding proteins as a component of a protein complex.

There were many additional, less abundant proteins in the tubulin-binding protein fraction that were not identified in this study. Future cytoskeleton studies from plants and other eukaryotic species using similar and different methods of purification will be important in identifying additional MT-binding proteins, as well as proteins that bind to the actin component of the cytoskeleton. Technological advances that eliminate the need for gel electrophoresis and allow for the identification of low-abundance proteins will also accelerate the characterization of the cytoskeleton-binding proteome.

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## REFERENCES

- Davies, E., Fillingham, B. D., and Abe, S. (1996) The plant cytoskeleton, in *The Cytoskeleton*, Vol. 3 (Hesketh, J. E., and Pryme, I. F., eds) pp. 405–449, JAI Press, Greenwich, CT
- Lloyd, C., and Hussey, P. (2001) Microtubule-associated proteins in plants—Why we need a MAP. *Nat. Rev. Mol. Cell. Biol.* **2**, 40–47
- Hussey, P. J., Hawkins, T. J., Igarashi, H., Kaloriti, D., and Smertenko, A. (2002) The plant cytoskeleton: Recent advances in the study of the plant microtubule-associated proteins MAP-65, MAP-190 and the *Xenopus* MAP215-like protein, MOR1. *Plant Mol. Biol.* **50**, 915–924
- Maccioni, R. B., and Cambiazo, V. (1995) Role of microtubule-associated proteins in the control of microtubule assembly. *Physiol. Rev.* **75**, 835–864
- Chuong, S. D. X., Mullen, R., and Muench, D. G. (2002) Identification of a rice RNA- and MT-binding protein as the multifunctional protein (MFP), a peroxisomal enzyme involved in the  $\beta$ -oxidation of fatty acids. *J. Biol. Chem.* **277**, 2419–2429
- Janmey, P. A. (1998) The cytoskeleton and cell signaling: Component localization and mechanical coupling. *Physiol. Rev.* **78**, 763–781
- Cyr, R. J., and Palevitz, B. A. (1989) Microtubule-binding proteins from carrot 1. Initial characterization and microtubule bundling. *Planta* **177**, 245–260
- Whittington, A. T., Vugrek, O., Wei, K. J., Hasenbein, N. G., Sugimoto, K., Rashbrooke, M. C., and Wasteneys, G. O. (2001) MOR1 is essential for organizing cortical microtubules in plants. *Nature* **411**, 610–613
- Vantard, M., Schellenbaum, P., Fellous, A., and Lambert, A. M. (1991) Characterization of maize microtubule-associated proteins, one of which is immunologically related to tau. *Biochemistry* **30**, 9334–9340
- Gardiner, J., and Marc, J. (2003) Putative microtubule-associated proteins from the *Arabidopsis* genome. *Protoplasma* **222**, 61–74
- Jiang, C.-J., and Sonobe, S. (1993) Identification and preliminary characterization of a 65 kDa higher-plant microtubule-associated protein. *J. Cell Sci.* **105**, 891–901
- Davies, E., Stankovic, B., Azama, K., Shibata, K., and Abe, S. (2001) Novel components of the plant cytoskeleton: A beginning to plant ‘cytomics’. *Plant Sci* **160**, 185–196
- Durso, N. A., and Cyr, R. J. (1994) A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1 $\alpha$ . *Plant Cell* **6**, 893–905
- Marc, J., Sharkey, D. E., Durso, N. A., Zhang, M., and Cyr, R. J. (1996) Isolation of a 90-kD microtubule-associated protein from tobacco membranes. *Plant Cell* **8**, 2127–2138
- Durso, N. A., Leslie, J. D., and Cyr, R. J. (1996) In situ immunocytochemical evidence that a homolog of protein translation elongation factor EF-1 $\alpha$  is associated with microtubules in carrot cells. *Protoplasma* **190**, 141–150
- Harper, J. D. I., Weerakoon, N. D., Gardiner, J. C., Blackman, L. M., and Marc, J. (2002) A 75-kDa plant protein isolated by tubulin-affinity chromatography is a peroxisomal matrix enzyme. *Can. J. Bot.* **80**, 1018–1027
- Balaban, N., and Goldman, R. (1992) Isolation and characterization of a unique 15 kilodalton trypanosome subpellicular microtubule-associated protein. *Cell Motil. Cytoskeleton* **21**, 138–146
- Jablonsky, P., Elliott, J., and Williamson, R. (1993) Purification of a mung bean protein binding to microtubules through two defined sites in the carboxy-terminal domain of  $\beta$ -tubulin. *Plant Sci.* **94**, 35–45
- Nebi, T., Pestonjamas, K. N., Leszyk, J. D., Crowley, J. L., Oh, S. W., and Luna, E. J. (2002) Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. *J. Biol. Chem.* **277**, 43399–43409
- Gevaert, K., Eggermont, L., Demol, H., and Vandekerckhove, J. (2000) A fast and convenient MALDI-MS based proteomic approach: Identification of components scaffolded by the actin cytoskeleton of activated human thrombocytes. *J. Biotechnol.* **78**, 259–269
- Wu, Y., Muench, D. G., Kim, Y.-T., Hwang, Y.-S., and Okita, T. W. (1998) Identification of polypeptides associated with an enriched cytoskeleton-protein body fraction from developing rice endosperm. *Plant Cell Physiol.* **39**, 1251–1257
- Azama, K., Abe, S., Sugimoto, H., and Davies, E. (2003) Lysine-containing proteins in maize endosperm: A major contribution from cytoskeleton-

- associated carbohydrate-metabolizing enzymes. *Planta* **217**, 628–638
23. Mathur, J., and Koncz, C. (1998) Callus culture and regeneration. *Methods Mol. Biol.* **82**, 31–34
  24. Mathe, E., Inoue, Y. H., Palframan, W., Brown, G., and Glover, D. M. (2003) Orbit/Mast, the CLASP orthologue of *Drosophila*, is required for asymmetric stem cell and cystocyte divisions and development of the polarised microtubule network that interconnects oocyte and nurse cells during oogenesis. *Development* **130**, 901–915
  25. Inoue, Y. H., do Carmo Avides, M., Shiraki, M., Deak, P., Yamaguchi, M., Nishimoto, Y., Matsukage, A., and Glover, D. M. (2000) Orbit, a novel microtubule-associated protein essential for mitosis in *Drosophila melanogaster*. *J. Cell Biol.* **149**, 153–166
  26. Garnier, C., Barbier, P., Gilli, R., Lopez, C., Peyrot, V., and Briand, C. (1998) Heat-shock protein 90 (hsp90) binds in vitro to tubulin dimer and inhibits microtubule formation. *Biochem. Biophys. Res. Commun.* **250**, 414–419
  27. Czar, M. J., Welsh, M. J., and Pratt, W. B. (1996) Immunofluorescence localization of the 90-kDa heat-shock protein to cytoskeleton. *Eur. J. Cell Biol.* **70**, 322–330
  28. Liang, P., and MacRae, T. H. (1997) Molecular chaperones and the cytoskeleton. *J. Cell Sci.* **110**, 1431–1440
  29. Brown, C. R., Doxsey, S. J., Hong-Brown, L. Q., Martin, R. L., and Welch, W. J. (1996) Molecular chaperones and the centrosome. A role for TCP-1 in microtubule nucleation. *J. Biol. Chem.* **271**, 824–832
  30. Kraemer, D., and Blobel, G. (1997) mRNA binding protein mrnp41 localizes to both nucleus and cytoplasm. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 9119–9124
  31. Hamill, D., Davis, J., Drawbridge, J., and Suprenant, K. A. (1994) Polyribosome targeting to microtubules: Enrichment of specific mRNAs in a reconstituted microtubule preparation from sea urchin embryos. *J. Cell Biol.* **127**, 973–984
  32. Shibata, N., Tsunekawa, N., Okamoto-Ito, S., Akasu, R., Tokumasu, A., and Noce, T. (2004) Mouse RanBPM is a partner gene to a germline specific RNA helicase, mouse vasa homolog protein. *Mol. Reprod. Dev.* **67**, 1–7
  33. Abe, S., Sakai, M., Yagi, K., Hagino, T., Ochi, K., Shibata, K., and Davies, E. (2003) A Tudor protein with multiple Snc domains from pea seedlings: Cellular localization, partial characterization, sequence analysis, and phylogenetic relationships. *J. Exp. Bot.* **54**, 971–983
  34. Sami-Subbu, R., Choi, S. B., Wu, Y., Wang, C., and Okita, T. W. (2001) Identification of a cytoskeleton-associated 120 kDa RNA-binding protein in developing rice seeds. *Plant Mol. Biol.* **46**, 79–88
  35. Amikura, R., Hanyu, K., Kashikawa, M., and Kobayashi, S. (2001) Tudor protein is essential for the localization of mitochondrial RNAs in polar granules of *Drosophila* embryos. *Mech. Dev.* **107**, 97–104
  36. Borer, R. A., Lehner, C. F., Eppenberger, H. M., and Nigg, E. A. (1989) Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* **56**, 379–390
  37. Wolin, S. L., and Cedervall, T. (2002) The La protein. *Annu. Rev. Biochem.* **71**, 375–403
  38. Bachmann, M., Pfeifer, K., Schroder, H. C., and Muller, W. E. (1989) The La antigen shuttles between the nucleus and the cytoplasm in CV-1 cells. *Mol. Cell. Biochem.* **85**, 103–114
  39. Li, Q., and Suprenant, K. A. (1994) Molecular characterization of the 77-kDa echinoderm microtubule-associated protein. Homology to the  $\beta$ -transducin family. *J. Biol. Chem.* **269**, 31777–31784
  40. Zhou, Z., Luo, M. J., Straesser, K., Katahira, J., Hurt, E., and Reed, R. (2000) The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* **407**, 401–405
  41. Palacios, I. M. (2002) RNA processing: Splicing and the cytoplasmic localisation of mRNA. *Curr. Biol.* **12**, R50–52
  42. Jansen, R.-P. (1999) RNA-cytoskeletal associations. *FASEB J.* **13**, 455–466
  43. Gavrillova, L. P., Rutkevitch, N. M., Gelfand, L. P., Stahl, U., Bommer, A., and Bielka, H. (1987) Immunofluorescent localization of protein synthesis components in mouse embryo. *Cell Biol. Int. Reports* **11**, 745–752
  44. Bokros, C. L., Hugdahl, J. D., Kim, H. H., Hanesworth, V. R., van Heerden, A., Browning, K. S., and Morejohn, L. C. (1995) Function of the p86 subunit of eukaryotic initiation factor (iso)4F as a microtubule-associated protein in plant cells. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7120–7124
  45. Hasek, J., Kovarik, P., Valasek, L., Malinska, K., Schneider, J., Kohlwein, S. D., and Ruis, H. (2000) Rpg1p, the subunit of the *Saccharomyces cerevisiae* eIF3 core complex, is a microtubule-interacting protein. *Cell Motil. Cytoskeleton* **45**, 235–246
  46. Palecek, J., Hasek, J., and Ruis, H. (2001) Rpg1p/Tif32p, a subunit of translation initiation factor 3, interacts with actin-associated protein Sla2p. *Biochem. Biophys. Res. Commun.* **282**, 1244–1250
  47. Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M. K. (1999) The PINHEAD/ZWILLE gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the ARGONAUTE1 gene. *Development* **126**, 469–481
  48. Mirande, M., Le Corre, D., Louvard, D., Reggio, H., Paillicie, J. P., and Waller, J. P. (1985) Association of an aminoacyl-tRNA synthetase complex and of phenylalanyl-tRNA synthetase with the cytoskeletal framework fraction from mammalian cells. *Exp. Cell Res.* **156**, 91–102
  49. Dang, C. V., Yang, D. C. H., and Pollard, T. D. (1983) Association of methionyl-tRNA synthetase with detergent-insoluble components of the rough endoplasmic reticulum. *J. Cell Biol.* **96**, 1138–1147
  50. Melki, R., Kerjan, P., Waller, J. P., Carlier, M. F., and Pantaloni, D. (1991) Interaction of microtubule-associated proteins with microtubules: Yeast lysyl- and valyl-tRNA synthetases and tau 218–235 synthetic peptide as model systems. *Biochemistry* **30**, 11536–11545
  51. Davies, E., Comer, E. C., Lionberger, J. M., Stankovic, B., and Abe, S. (1993) Cytoskeleton-bound polysomes in plants. III. Polysome-cytoskeleton-membrane interactions in corn endosperm. *Cell Biol. Int.* **17**, 331–340
  52. Starkey, C. R., and Levy, L. S. (1995) Identification of differentially expressed genes in T-lymphoid malignancies in an animal model system. *Int. J. Cancer* **62**, 325–331
  53. Dixon, M. S., Hatzixanthis, K., Jones, D. A., Harrison, K., and Jones, J. D. (1998) The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* **10**, 1915–1925
  54. Davy, D. A., Campbell, H. D., Fountain, S., de Jong, D., and Crouch, M. F. (2001) The flightless I protein colocalizes with actin- and microtubule-based structures in motile Swiss 3T3 fibroblasts: Evidence for the involvement of PI 3-kinase and Ras-related small GTPases. *J. Cell Sci.* **114**, 549–562
  55. Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60
  56. Xue, Y., Canman, J. C., Lee, C. S., Nie, Z., Yang, D., Moreno, G. T., Young, M. K., Salmon, E. D., and Wang, W. (2000) The human SWI/SNF-B chromatin-remodeling complex is related to yeast rsc and localizes at kinetochores of mitotic chromosomes. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13015–13020
  57. Wu, H. C., Huang, P. H., and Lin, C. T. (1998) G protein  $\beta$  subunit is closely associated with microtubules. *J. Cell. Biochem.* **70**, 553–562
  58. Chen, L., Chan, S. Y., and Cossins, E. A. (1997) Distribution of folate derivatives and enzymes for synthesis of 10-formyltetrahydrofolate in cytosolic and mitochondrial fractions of pea leaves. *Plant Physiol.* **115**, 299–309
  59. Hooks, M. A. (2002) Molecular biology, enzymology, and physiology of  $\beta$ -oxidation, in *Plant Peroxisomes: Biochemistry, Cell Biology and Biotechnological Applications* (Baker, A., and Graham, I. A., eds) pp. 19–55, Kluwer Academic Publishers, Norwell, MA
  60. Balaban, N., and Goldman, R. (1990) The association of glycosomal enzymes and microtubules: A physiological phenomenon or an experimental artifact? *Exp. Cell Res.* **191**, 219–226
  61. Kueng, V., Schleppei, K., Schneider, A., and Seebeck, T. (1989) A glycosomal protein (p60) which is predominantly expressed in procyclic *Trypanosoma brucei*: Characterization and DNA sequence. *J. Biol. Chem.* **264**, 5203–5209
  62. Somers, M., Engelborghs, Y., and Baert, J. (1990) Analysis of the binding of glyceraldehyde-3-phosphate dehydrogenase to microtubules, the mechanism of bundle formation and the linkage effect. *Eur. J. Biochem.* **193**, 437–444
  63. Walsh, J. L., Keith, T. J., and Knull, H. R. (1989) Glycolytic enzyme interactions with tubulin and microtubules. *Biochim. Biophys. Acta* **999**, 64–70
  64. Yanagawa, Y., Hasezawa, S., Kumagai, F., Oka, M., Fujimuro, M., Naito, T., Makino, T., Yokosawa, H., Tanaka, K., Komamine, A., Hashimoto, J.,

- Sato, T., and Nakagawa, H. (2002) Cell-cycle dependent dynamic change of 26S proteasome distribution in tobacco BY-2 cells. *Plant Cell Physiol.* **43**, 604–613
65. Worby, C. A., Simonson-Leff, N., Clemens, J. C., Kruger, R. P., Muda, M., and Dixon, J. E. (2001) The sorting nexin, DSH3PX1, connects the axonal guidance receptor, Dscam, to the actin cytoskeleton. *J. Biol. Chem.* **276**, 41782–41789
66. Huang, S., An, Y. Q., McDowell, J. M., McKinney, E. C., and Meagher, R. B. (1997) The *Arabidopsis* ACT11 actin gene is strongly expressed in tissues of the emerging inflorescence, pollen, and developing ovules. *Plant Mol. Biol.* **33**, 125–139
67. Giannakakou, P., Sackett, D. L., Ward, Y., Webster, K. R., Blagosklonny, M. V., and Fojo, T. (2000) p53 is associated with cellular microtubules and is transported to the nucleus by dynein. *Nat. Cell Biol.* **2**, 709–717
68. Mullen, R. T. (2002) Targeting and import of matrix proteins into peroxisomes, in *Plant Peroxisomes* (Baker, A., and Graham, I. A., eds) pp. 339–383. Kluwer Academic Publishers, Norwell, MA
69. Muench, D. G., and Mullen, R. T. (2003) Peroxisome dynamics in plant cells: A role for the cytoskeleton. *Plant Sci.* **164**, 307–315
70. al-Habori, M. (1995) Microcompartmentation, metabolic channelling and carbohydrate metabolism. *Int. J. Biochem. Cell Biol.* **27**, 123–132
71. Schirch, V., and Strong, W. B. (1989) Interaction of folylpolyglutamates with enzymes in one-carbon metabolism. *Arch. Biochem. Biophys.* **269**, 371–380
72. Strong, W. B., and Schirch, V. (1989) In vitro conversion of formate to serine: Effect of tetrahydroteroyl polyglutamates and serine hydroxymethyltransferase on the rate of 10-formyltetrahydrofolate synthetase. *Biochemistry* **28**, 9430–9439
73. Rose, J. K., and Bennett, A. B. (1999) Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening. *Trends Plant Sci.* **4**, 176–183
74. Doi, R. H., and Tamaru, Y. (2001) The Clostridium cellulovorans cellulosome: an enzyme complex with plant cell wall degrading activity. *Chem. Rec.* **1**, 24–32
75. Brummell, D. A., Catala, C., Lashbrook, C. C., and Bennett, A. B. (1997) A membrane-anchored E-type endo-1,4- $\beta$ -glucanase is localized on Golgi and plasma membranes of higher plants. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 4794–4799
76. Baluska, F., Samaj, J., Wojtaszek, P., Volkmann, D., and Menzel, D. (2003) Cytoskeleton-plasma membrane-cell wall continuum in plants. Emerging links revisited. *Plant Physiol.* **133**, 482–491
77. Gardiner, J. C., Harper, J. D. I., Weerakoon, N. D., Collings, D. A., Ritchie, S., Gilroy, S., Cyr, R. J., and Marc, J. (2001) A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. *Plant Cell* **13**, 2143–2158
78. Nick, P., Bergfeld, R., Schafer, E., and Schopfer, P. (1990) Unilateral reorientation of microtubules at the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. *Planta* **181**, 162–168
79. Hush, J. M., Hawes, C., and Overall, R. L. (1990) Interphase microtubule re-orientation predicts a new cell polarity in wounded pea roots. *J. Cell Sci.* **96**, 47–61
80. Weingartner, M., Binarova, P., Drykova, D., Schweighofer, A., David, J. P., Heberle-Bors, E., Doonan, J., and Bogre, L. (2001) Dynamic recruitment of Cdc2 to specific microtubule structures during mitosis. *Plant Cell* **13**, 1929–1943
81. Sapir, T., Cahana, A., Seger, R., Nekhai, S., and Reiner, O. (1999) LIS1 is a microtubule-associated phosphoprotein. *Eur. J. Biochem.* **265**, 181–188
82. Yang, P., and Sale, W. S. (2000) Casein kinase I is anchored on axonemal doublet microtubules and regulates flagellar dynein phosphorylation and activity. *J. Biol. Chem.* **275**, 18905–18912
83. Behrend, L., Stoter, M., Kurth, M., Rutter, G., Heukeshoven, J., Deppert, W., and Knippschild, U. (2000) Interaction of casein kinase 1 delta (CK1 $\delta$ ) with post-Golgi structures, microtubules and the spindle apparatus. *Eur. J. Cell Biol.* **79**, 240–251
84. Schaerer-Brodbeck, C., and Riezman, H. (2003) Genetic and biochemical interactions between the Arp2/3 complex, Cmd1p, casein kinase II, and Tub4p in yeast. *FEMS Yeast Res.* **4**, 37–49
85. Hiraga, A., and Tamura, S. (2000) Protein phosphatase 2A is associated in an inactive state with microtubules through 2A1-specific interaction with tubulin. *Biochem. J.* **346**, 433–439
86. Camilleri, C., Azimzadeh, J., Pastuglia, M., Bellini, C., Grandjean, O., and Bouchez, D. (2002) The *Arabidopsis* TONNEAU2 gene encodes a putative novel protein phosphatase 2A regulatory subunit essential for the control of the cortical cytoskeleton. *Plant Cell* **14**, 833–845
87. Gundersen, G. G., and Cook, T. A. (1999) Microtubules and signal transduction. *Curr. Opin. Cell Biol.* **11**, 81–94
88. Pedrotti, B., Francolini, M., Cotelli, F., and Islam, K. (1996) Modulation of microtubule shape in vitro by high molecular weight microtubule associated proteins MAP1A, MAP1B, and MAP2. *FEBS Lett.* **384**, 147–150
89. Hoshi, M., Ohta, K., Gotoh, Y., Mori, A., Murofushi, H., Sakai, H., and Nishida, E. (1992) Mitogen-activated-protein-kinase-catalyzed phosphorylation of microtubule-associated proteins, microtubule-associated protein 2 and microtubule-associated protein 4, induces an alteration in their function. *Eur. J. Biochem.* **203**, 43–52
90. Lindwall, G., and Cole, R. D. (1984) Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J. Biol. Chem.* **259**, 5301–5305
91. Sivaguru, M., Pike, S., Gassmann, W., and Baskin, T. I. (2003) Aluminum rapidly depolymerizes cortical microtubules and depolarizes the plasma membrane: Evidence that these responses are mediated by a glutamate receptor. *Plant Cell Physiol.* **44**, 667–675
92. Abdrakhamanova, A., Wang, Q. Y., Khokhlova, L., and Nick, P. (2003) Is microtubule disassembly a trigger for cold acclimation? *Plant Cell Physiol.* **44**, 676–686
93. Kobayashi, Y., Kobayashi, I., Funaki, Y., Fujimoto, S., Takemoto, T., and Kunoh, H. (1997) Dynamic reorganization of microfilaments and microtubules is necessary for the expression of non-host resistance in barley coleoptile cells. *Plant J.* **11**, 525–537
94. Nick, P. (1999) Signals, motors, morphogenesis—The cytoskeleton in plant development. *Plant Biol.* **1**, 169–179
95. Wickham, L., Duchaine, T., Luo, M., Nabi, I. R., and DesGroseillers, L. (1999) Mammalian stauin is a double-stranded-RNA and tubulin-binding protein which localizes to the rough endoplasmic reticulum. *Mol. Cell Biol.* **19**, 2220–2230
96. DeFranco, C., Chicurel, M. E., and Potter, H. (1998) A general RNA-binding protein complex that includes the cytoskeleton-associated protein MAP1A. *Mol. Biol. Cell* **9**, 1695–1708
97. Okita, T. W., and Choi, S. B. (2002) mRNA localization in plants: Targeting to the cell's cortical region and beyond. *Curr. Opin. Plant Biol.* **5**, 553–559
98. Brumwell, C., Antolik, C., Carson, J. H., and Barbarese, E. (2002) Intracellular trafficking of hnRNP A2 in oligodendrocytes. *Exp. Cell Res.* **279**, 310–320
99. Kataoka, N., Diem, M. D., Kim, V. N., Yong, J., and Dreyfuss, G. (2001) Magoh, a human homolog of *Drosophila* mago nashi protein, is a component of the splicing-dependent exon-exon junction complex. *EMBO J.* **20**, 6424–6433
100. Sirover, M. A. (1999) New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. *Biochim. Biophys. Acta* **1432**, 159–184
101. Igarashi, H., Orii, H., Mori, H., Shimmen, T., and Sonobe, S. (2000) Isolation of a novel 190 kDa protein from tobacco BY-2 cells: Possible involvement in the interaction between actin filaments and microtubules. *Plant Cell Physiol.* **41**, 920–931
102. Himmelspach, R., Nick, P., Schafer, E., and Ehmann, B. (1997) Developmental and light-dependent changes of the cytosolic chaperonin containing TCP-1 (CCT) subunits in maize seedlings, and the localization in coleoptiles. *Plant J.* **12**, 1299–1310
103. Kumar, J., Yu, H., and Sheetz, M. P. (1995) Kinectin, an essential anchor for kinesin-driven vesicle motility. *Science* **267**, 1834–1837
104. Gromley, A., Jurczyk, A., Sillibourne, J., Halilovic, E., Mogensen, M., Groisman, I., Blomberg, M., and Doxsey, S. (2003) A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. *J. Cell Biol.* **161**, 535–545
105. Schliwa, M., and Woehlke, G. (2003) Molecular motors. *Nature* **422**, 759–765
106. Hollander, B. A., Liang, M. Y., and Besharse, J. C. (1999) Linkage of a nucleolin-related protein and casein kinase II with the detergent-stable photoreceptor cytoskeleton. *Cell Motil. Cytoskeleton* **43**, 114–127
107. Havin, L., Git, A., Elisha, Z., Oberman, F., Yaniv, K., Schwartz, S. P.,

- Standart, N., and Yisraeli, J. K. (1998) RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. *Genes Dev.* **12**, 1593–1598
108. Qutob, M. S., Bhattacharjee, R. N., Pollari, E., Yee, S. P., and Torchia, J. (2002) Microtubule-dependent subcellular redistribution of the transcriptional coactivator p/CIP. *Mol. Cell. Biol.* **22**, 6611–6626
109. Heuvelink, J., Pieper, F., Ramaekers, F., Timmermans, L., Kuijpers, H., Bloemendal, H., and van Venrooij, W. J. (1989) Association of mRNA and eIF-2 $\alpha$  with the cytoskeleton in cells lacking vimentin. *Exp. Cell Res.* **181**, 317–330
110. Howe, J. G., and Hershey, J. W. B. (1984) Translational initiation factor and ribosome association with the cytoskeletal framework fraction from HeLa cells. *Cell* **37**, 85–93
111. Pay, A., Resch, K., Frohnmeyer, H., Fejes, E., Nagy, F., and Nick, P. (2002) Plant RanGAPs are localized at the nuclear envelope in interphase and associated with microtubules in mitotic cells. *Plant J.* **30**, 699–709