Like the skeleton of any vertebrate animal, the cytoskeleton plays a major role in determining the three-dimensional form of a cell. However, in plants the cytoskeleton is probably more analogous to a scaffold, being directly involved in the establishment of cell morphology and less involved in the maintenance of cell shape once the relatively rigid cell walls are in place. The term "skeleton" unfortunately tends to connote a rigid, somewhat permanent structure, but it is clear that much of the cytoskeleton is dynamic and polymorphic. Microtubules in particular are continually being disassembled, assembled, and rearranged into new configurations or arrays as a cell progresses through division and differentiation.

The cellulosic wall of a typical plant cell largely precludes cell migration, which plays a central role in animal development. Thus, morphogenesis in plants is governed primarily by the processes that establish the planes of cell divisions and the axes of cell expansions, and components of the cytoskeleton play important roles in both processes. In addition, the plant cytoskeleton is directly involved in the intracellular transport of a wide variety of macromolecules, vesicles, organelles, and other macromolecular structures essential to plant growth and development.

All three major classes of cytoskeletal filaments—microtubules, intermediate filaments, and microfilaments—are present in plant cells. However, our discussion will be restricted to the microtubule-based components of the plant cytoskeleton. First, we will describe the major microtubule arrays present in plant cells and discuss their known and/or predicted functions. Then we will focus on (a) recent information about the large tubulin gene families in plant cells and their possible significance, (b) questions about MTOCs in plants and the probable nucleation of microtubule assembly by γ-tubulin, and (c) the roles of MAPs in plant cells. Finally, we emphasize that a clear picture of plant cell growth and differentiation will become available only when we understand the coordinated interactions between the cytoskeleton, the cell nucleus, the nuclear envelope, the ER, the plasma membrane, and the cell wall. At present there is too little definitive information about most of these important interactions to warrant further discussion here.

**Microtubule Components of the Plant Cell Cytoskeleton**

Russell H. Goddard, Susan M. Wick, Carolyn D. Silflow, and D. Peter Snustad*


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**Microtubule Composition and Structure**

Microtubules are hollow filaments about 24 nm in diameter that are assembled from heterodimers containing one α-tubulin polypeptide and one β-tubulin polypeptide, each with a mol wt of approximately 50,000. The tubulin heterodimers align head-to-tail to form protofilaments, which are associated side-by-side to form a hollow cylinder. Most microtubules contain 13 protofilaments, but variations occur in specific microtubules of some species. The head-to-tail arrangement of the heterodimers generates an inherent structural polarity that is reflected in a polarity for microtubule assembly. The addition of tubulin dimers is favored at the end designated the plus (+) end.

Assembly of microtubules in vivo is typically initiated at specific sites (MTOCs), and the minus (−) end of each microtubule is associated with the MTOC. At free plus ends, some microtubules are subject to stochastic, rapid disassembly, whereas others continue to assemble; this process is termed dynamic instability. The half-life for individual microtubules within an array may be on the order of a few minutes or less. Heterogeneity at microtubule plus ends may result from differences in the size of a stabilizing cap of GTP dimers, possibly resulting from transient interactions of other cellular factors with microtubule ends (reviewed by Caplow, 1992).

The tubulin domains involved in polymerization, nucleotide binding, and probably interactions with other proteins have been highly conserved throughout evolution, and as a result tubulins from plants, protists, fungi, and animals will co-polymerize in vitro. Also, several antibodies exhibit cross-reactivity with tubulins from a wide variety of divergent species (see reviews by Silflow et al., 1987; Morejohn, 1991; Fosket and Morejohn, 1992). Despite this overall conservation of tubulin structure, plant tubulins differ markedly from animal tubulins in their susceptibility to and binding of antimicrotubule drugs and herbicides such as colchicine and oryzalin (Morejohn, 1991). Now that the sequences of a fairly large number of plant α- and β-tubulins are available (Fosket and Morejohn, 1992; Kopczak et al., 1992; Snustad et al., 1992; Villemur et al., 1992), the molecular bases for these differences should be established in the near future.

**Abbreviations:** MAP, microtubule-associated protein; MTOC, microtubule organizing center; PPB, preprophase band.

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A PROGRESSION OF MICROTUBULE ARRAYS
DURING THE MITOTIC CELL CYCLE

Several distinct arrays of microtubules form transiently as a plant cell proceeds through a mitotic cell cycle: the most prominent are the interphase cortical array, PPB, spindle, and phragmoplast. During preprophase and cytokinesis there are also microtubule arrays associated with the nuclear perimeter, in addition to the PPB and the phragmoplast. All of these arrays except the mitotic spindle are unique to plant cells. As a cell progresses through the cell cycle, the microtubules of one stage gradually are replaced by or rearranged into those of the next stage. Immunofluorescence photographs of the major microtubule arrays of plant cells are shown in Figure 1, along with a schematic diagram emphasizing the structure of each array.

The Interphase Microtubule Array

During interphase, cortical microtubules are found roughly parallel to each other and are oriented perpendicular to the primary axis of cell expansion (Fig. 1, A and B) (reviewed by Williamson, 1991). Considerable evidence indicates that in most cells the cortical microtubules are involved in the orientation of cellulose microfibrils during the synthesis of new cell walls (reviewed by Giddings and Staehelin, 1991) and thus play a major role in establishing the axis of cell elongation. Recently, Hush and Overall (1992) demonstrated that...
rearrangement of microtubules, but not actin microfilaments, precedes the reestablishment of cell polarity after wounding of pea root cells. Moreover, perturbation of microtubule organization in the wounded root cells prevented the reestablishment of cell polarity, demonstrating that functional rearrangement of microtubules is essential to this process.

PPB

Prior to mitosis, the cortical interphase array is gradually replaced by the PPB, which at its most developed stage is a densely packed ring of cortical microtubules that encircles the nucleus (Fig. 1, C and D) and accurately predicts the final location of the nascent cell plate formed during cytokinesis (reviewed by Wick, 1991). During PPB development, numerous microtubules are found between the nucleus and the cell cortex and at the nuclear surface. This latter array eventually takes on the orientation and form of the prophase spindle, with the number of spindle microtubules increasing at the expense of a dwindling PPB array. The appearance of the PPB prior to cytokinesis and its location within the cell suggest that factors involved in directing or joining the nascent cell plate to the division site along the parental cell wall at cytokinesis are deposited or modified at this site during the lifetime of the PPB. Support for this has come from studies on the moss Adiantum, where experimental elimination of the PPB resulted in altered phragmoplast expansion and cell plate orientation (Mineyuki et al., 1991a).

Recent evidence obtained by labeling cells with an antibody that recognizes a motif common to numerous kinases (Mineyuki et al., 1991b) or with an antibody specific to the maize analog of p34<sup>cdc2</sup> protein kinase (Colasanti et al., 1993) indicates that one or more cell cycle regulatory kinases are associated with the PPB, at least transiently. These results suggest that phosphorylation of cortical or plasma-membrane proteins at the PPB site could be involved in marking the division site. Also, the division site has been shown to remain devoid of actin during mitosis and cytokinesis, whereas the rest of the cortex contains abundant actin (Cleary et al., 1992; Liu and Palevitz, 1992). Other details about the molecular mechanism(s) by which the PPB marks the position at which the cell plate will fuse with the parent cell walls during cytokinesis remain unknown.

Despite the paucity of information about this molecular "memory," it is clear that the PPB assembles after DNA replication in the cell cycle, somehow defines the orientation, location, and the shape of the new cell plate, and disappears by the time of nuclear envelope breakdown during mitosis. Its apparent importance during the plant cell cycle is limited to cells that have cellulosic walls and that are involved in tissue or organ morphogenesis: no PPB is present during mitosis in cells such as endosperm cells without rigid walls or in cells undergoing meiotic divisions.

The Mitotic Spindle

The mitotic spindle is a complex, highly organized apparatus (Fig. 1, E and F) that gradually replaces the PPB. It consists of bundles of kinetochore microtubules, as well as other microtubules that may or may not end at one or the other spindle pole region. Whereas an animal cell spindle is sharply focused onto the centriole at each spindle pole, there are no centrioles in plant cells, and plant spindle poles are broader, usually consisting of numerous microtubule foci (see reviews by Lambert et al., 1991; Smirnova and Bajer, 1992). Because mitotic spindles are responsible for the accurate transmission of complete sets of chromosomes to all of the somatic cells of the mature plant, the importance of this microtubule array is self-evident.

The Phragmoplast

When the daughter chromosomes have separated and kinetochore bundles of microtubules have disappeared, the phragmoplast microtubule array begins to appear at or near the equatorial plane of the spindle. The phragmoplast consists of a double ring of microtubules with the microtubules of one ring antiparallel to and interdigitating with those of the other ring (Fig. 1, G and H). Current evidence suggests that the plus ends of the microtubules lie in the overlap zone. The phragmoplast appears to be directly involved in the transport of vesicles containing material for new cell plate synthesis. As previously stated, the final orientation and location of the cell plate reflect those of the preceding PPB at the onset of mitosis.

The phragmoplast forms at late telophase and may incorporate the interzonal microtubules of the telophase spindle at its onset, but quickly nucleates new microtubules at the edge of the forming cell plate as it expands centrifugally (Vantard et al., 1990). It appears that normal growth of this array requires highly dynamic microtubules, because if taxol is used to prevent microtubule depolymerization in tobacco BY-2 cells, vesicles will accumulate at the forming cell plate but the centripetal expansion of the phragmoplast will nearly cease (Yasuhara et al., 1993).

A method to synchronize tobacco BY-2 cells growing in culture has allowed large numbers of staged cells to be harvested and used for the isolation of phragmoplasts (Yasuhara et al., 1992). EM of the isolated phragmoplasts reveals numerous vesicles that appear to be bound to the microtubules by cross-bridges (Yasuhara et al., 1993). This might indicate that a plus-end-directed motor protein such as kinesin is involved in the translocation of vesicles along these microtubules to the phragmoplast midzone, where the cell plate is forming. However, to date there is no evidence to confirm the occurrence of a kinesin-like motor protein in phragmoplasts. There is evidence for a mechanochemical enzyme in phragmoplasts that hydrolyzes GTP and transports microtubules that are on a substrate such as a glass slide toward their minus ends (equivalent in directionality to a motor on microtubules that transports vesicles to the microtubule plus end) (Asada et al., 1991). These observations fit the view that the phragmoplast functions in the transport of raw materials for cell plate formation to the site of wall assembly.

UNIQUE MICROTUBULE ARRAYS IN SPECIALIZED CELLS

In addition to the common microtubule arrays present in most dividing plant cells, there are a number of unique arrays
found in specialized plant cells. For instance, an array of microtubules radiating from the nucleus is present in plant cells lacking walls, such as endosperm cells. In the male gametophyte, the pollen tube cortical microtubule array is axial to the direction of pollen tube growth, and there are extensive bundles of microtubules within the generative cell and its two products, the elongated sperm cells, which are moving toward the tip of the growing pollen tube (see reviews by Palevitz and Tiezzi, 1992; Pierson and Cresti, 1992). Also, developing stomatal guard cells and differentiating tracheary elements are examples of cell types in which cortical microtubules are found transiently in unique arrangements.

**LARGE TUBULIN GENE FAMILIES IN PLANTS**

Although reliable data are available on tubulin gene number for only three plant species, these data suggest that plants contain more expressed tubulin genes than animals (Fosket and Morejohn, 1992; Kopczak et al., 1992; Snustad et al., 1992; Villemur et al., 1992). The small genome of Arabidopsis contains at least 6 expressed α-tubulin genes (Kopczak et al., 1992) and 9 expressed β-tubulin genes (Snustad et al., 1992), and all 15 of these genes have been cloned, sequenced, and analyzed for patterns of transcript accumulation by using 3′ noncoding gene-specific hybridization probes. This represents more expressed α- and β-tubulin genes than are present in the human genome, which is 30 times larger. The large genomes of maize and soybean apparently contain even larger tubulin gene families (Fosket and Morejohn, 1992; Villemur et al., 1992), raising the question of why plants contain so many tubulin genes.

Two major hypotheses have been put forward to explain the existence of multiple tubulin genes in eukaryotes. Fulton and Simpson (1976) proposed that the tubulins encoded by different genes were functionally divergent, with different tubulin isoforms performing different biological functions. Raff (1984) proposed that the different tubulin gene products might be functionally equivalent, but that tubulin gene families have evolved to permit individual genes to acquire distinct regulatory elements so that they can respond differentially to various demands for tubulin during development. In animals, both hypotheses are correct; some tubulin isoforms are functionally divergent, whereas others are functionally equivalent but exhibit very different patterns of expression during development.

To date, there is no evidence for functionally divergent tubulin isoforms in any plant. However, there is extensive evidence for differential expression of plant tubulin genes in various organs and tissues during development (Han et al., 1991; Carpenter et al., 1992, 1993; Joyce et al., 1992). Although tubulin genes are sometimes thought to be “house-keeping” genes and presumed to be expressed constitutively, this is not the case for the plant tubulin genes that have been studied to date. Rather, each gene has been found to exhibit its own unique spatial and temporal pattern of expression during plant growth and development, with some genes being expressed only briefly in a few cell types and other genes being expressed throughout much of development in most, but not all, tissues of the plant. Thus, differential regulation of tubulin genes during development appears to be an important feature of plant tubulin gene families.

In addition to the developmentally regulated expression of tubulin genes discussed above, some plant tubulin genes are regulated by environmental changes. The soybean Sb1 β-tubulin gene is up-regulated in hypocotyls in response to growth in the dark (Han et al., 1991), and the Arabidopsis TUB8 β-tubulin gene is sharply down-regulated at low temperature (4°C as compared with normal growth at 25°C; Chu et al., 1993). In oats, β-tubulin gene transcript levels increased over 5-fold in excised internode segments within 6 h after treatment with GA3 and before significant elongation had occurred (Mendu and Silflow, 1993).

Perhaps these environmental effects on tubulin gene expression provide hints as to why there appear to be more expressed tubulin genes in plants than in the animals studied to date. A key difference between plants and terrestrial animals is that plants are sessile, whereas animals are motile. Plants cannot come in from the cold or thermoregulate, nor can they evade antagonistic environmental conditions such as darkness, strong winds, or drought; their survival depends on their ability to adapt in place to changes in the environment. As seen by comparing the morphologies of plants grown in warm versus cold environments, of light-grown versus etiolated seedlings, or of plants grown in still air versus strong winds, the adaptations plants make to these alternative environmental conditions include major changes in morphology, and such changes in morphology are mediated, at least in part, by changes in the cytoskeleton. Thus, perhaps it should not be surprising that some plant tubulin genes are regulated in part by environmental factors, and perhaps plants contain more tubulin genes to facilitate this additional type of regulation.

Although there is no evidence to date of functional diversity among different tubulin gene products in plants, one type of functional diversity seems likely. The function of microtubules clearly involves their interaction with a variety of MAPs. It may be that tubulin isoforms have diverged because of their co-evolution with tissue- and cell-specific MAPs (Lewis and Cowan, 1988); this possibility should be evaluated critically as information on plant MAPs accumulates in the future.

**MTOCs AND γ-TUBULIN**

Microtubule function in cells depends on precise control over the spatial and temporal organization of multiple microtubule arrays. Although centrosomes (centrioles together with surrounding amorphous material) function as MTOCs in cells of animals and many protists, no centriole is present in plant cells. A prominent nucleating center in plants is thought to be the nuclear surface (Lambert, 1993). In nonflowering plants exhibiting monoplastic cell division, the plastid apparently can serve as a MTOC as well, but in hepatics the organizing center is more closely associated with the nuclear envelope (see Brown and Lemmon, 1992). The sites of nucleation and organization of cortical microtubules of interphase and PPB arrays have been the subject of considerable controversy, but no clear and consistent answer has yet emerged.
Microtubule Components of the Plant Cell Cytoskeleton

An important question regarding MTOCs in plant cells is whether they contain proteins homologous to proteins in the pericentriolar regions of cells with centrioles. Although there have been several cases of immunological cross-reactivity by antibodies that recognize components of animal pericentriolar material, the best evidence that similar proteins may be functioning in microtubule organization in plants and animals comes from recent work with antibodies to γ-tubulin. γ-Tubulin is a highly conserved component of MTOCs that is thought to be involved in microtubule nucleation (Oakley, 1992).

Antibodies to γ-tubulin were found to bind to a 58-kD polypeptide in protein blots from flowering plants and to decorate plant microtubule arrays when used in immunofluorescence studies (Liu et al., 1993). Although γ-tubulin is restricted to discrete microtubule nucleating centers in animal and fungal cells, anti-γ-tubulin binding in plant cells was found in a punctate pattern throughout the interphase cortical array, the PPB, and most of the phragmoplast; antibody also accumulated around the nucleus and formed a polar cap from which the spindle microtubules radiated (Liu et al., 1993). Further studies on the function and behavior of γ-tubulin in plants should lead to a better understanding of plant MTOCs.

**PLANT MAPS**

For microtubules to perform their designated functions, they must be able to interact with other components of the cell, including other microtubules, microfilaments, organelles, the plasma membrane, and perhaps a variety of other macromolecules and macromolecular structures. These key interactions are facilitated by a large variety of MAPs. Of the different classes of MAPs, the two most widely studied (in animal cells) are fibrous MAPs such as tau and MAP2, which provide stability to microtubules and cross-link them to other structures, and energy-transducing MAPs such as dynein and kinesin, which perform transport functions along microtubules. Electron micrographs of plant cytoplasm have shown the presence of structures that cross-link microtubules and link microtubules to membranes and vesicles (Lancelle and Hepler, 1992; Jiang and Sonobe, 1993; Yasuhara et al., 1993), suggesting the presence of plant MAPs.

Early attempts to characterize plant MAPs by biochemical techniques resulted in the identification of proteins that qualified as MAPs by some, but not all, criteria. Cyr and Palevitz (1989) isolated carrot proteins that bound to microtubules and affected their assembly, cold stability, and bundling in vitro. Schellenbaum and co-workers (see Schellenbaum et al., 1993, and refs. therein) identified a protein that is immunologically related to tau in a MAP-enriched fraction from maize culture cells. In addition, three of the putative MAPs from maize promoted assembly of microtubules from purified maize tubulin or brain tubulin (Schellenbaum et al., 1993).

Recently, tubulin that polymerizes in the absence of added microtubule-stabilizing agents was obtained from cultured tobacco BY-2 cells, and the microtubules polymerized from these crude fractions were found to have bridge-like structures between adjacent microtubules. Examination of purified fractions of putative MAPs from the crude isolate showed that only one, a 65-kD polypeptide, exhibited microtubule-bundling activity and formed cross-bridges between microtubules similar to those observed in the crude fraction. Also, antibodies raised against the 65-kD polypeptide co-localized with microtubules throughout the cell cycle in cultured tobacco cells (Jiang and Sonobe, 1993, and refs. therein).

As for energy-transducing MAPs in plants, immunoreactive homologs of mammalian kinesin have been reported in tobacco pollen tubes (Tieghi et al., 1992). The presence of kinesin-like proteins in plants also is supported by the identification of genes in Arabidopsis thaliana that predict gene products with extensive homology to the kinesins and kinesin-like proteins of Drosophila and yeast (Mitsui et al., 1993). Although these results are promising, plant MAPs remain poorly characterized and a significant amount of work remains to identify and characterize their properties. However, it seems reasonable to predict that a wide variety of plant MAPs will be identified and characterized in the next few years.

**CONCLUSIONS AND FUTURE DIRECTIONS**

The microtubule components of the plant cell cytoskeleton have long been known to be intimately involved in many aspects of growth and differentiation. Within the last few years, a complex picture of the genetic control of tubulins in plants has begun to unfold, with the individual members of large tubulin gene families exhibiting unique spatial and temporal patterns of expression. The large tubulin gene families in plants facilitate not only the tissue-specific patterns of expression associated with differentiation, but also regulation by external factors that lead to changes in morphology as plants adapt to new environmental conditions.

In the next few years we can look forward to the discovery and characterization of a plethora of plant MAPs, both fibrous and motor MAPs. One can probably expect that these MAPs will be encoded by gene families that are as large as, or perhaps larger than, the tubulin gene families. We can anticipate that these MAP gene products will be synthesized in a tissue-specific fashion, and thus a complete understanding of tubulin-MAP functions will require a detailed map of the expression patterns of both the tubulin genes and the MAP genes. A question of obvious importance is whether individual MAPs interact specifically with individual tubulin gene products or equally with all tubulin isoforms present in a given cell. One prediction seems safe: Our understanding of the plant cytoskeleton will grow rapidly in the next few years, but the picture will also become more complex as the number of macromolecules involved increases dramatically.

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