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Of Microtubules and MAPs

Many aspects of the behavior of plant cells—indeed, of all eukaryotic cells—are influenced by microtubules and other components of the cytoskeleton, which give cells form and structure as well as providing for the mechanics of intracellular transport and cell division. Microtubule assembly is regulated in time and space, so that the appropriate microtubule arrays form when and where they are needed. Although in vitro, tubulin heterodimers are able to self-assemble in the absence of other proteins, microtubules formed in vivo include not only tubulin but also microtubule-associated proteins, or MAPs, which regulate their assembly kinetics and functions. MAPs enhance both the rate of nucleation, the process by which microtubules are “seeded,” and the rate at which microtubules elongate; they also mediate the interactions of microtubules with other cellular components (Wiche et al., 1991; Fosket and Morejohn, 1992). No plant MAPs have yet been identified. Although plant proteins have been isolated that bind to plant microtubules in vitro, whether these actually function as MAPs in vivo is not yet known.

Even though plant and animal tubulins are very well conserved, plants contain several unique microtubule arrays, whose formation and function are most likely the result of the activity of plant-specific MAPs. Among the plant-specific microtubule arrays is the cortical array, which is thought to determine the orientation of cellulose microfibrils in the wall; the preprophase band (PPB), a ring of microtubules that forms transiently during preprophase and early prophase and that predicts the division plane; and the phragmoplast, two overlapping sets of microtubules that run perpendicular to the division plane and that are involved in transporting new cell wall material to the forming cell plate (for reviews, see Lloyd, 1991).

To understand how these structures are assembled and function, it will be essential to identify the MAPs with which they are associated. Even in the absence of bona fide plant MAPs, however, it is possible to begin to characterize some aspects of plant microtubule–MAP interactions, as Hugdahl and coworkers show on pages 1063–1080 of this issue. Using a mammalian brain MAP, MAP2, they have been able to probe the MAP binding sites of maize microtubules and to examine the effects of MAP binding on microtubule structure and polymerization.

These workers used two basic approaches to study the effects of MAP2 on maize microtubules: adding MAP2 to microtubules preformed in the absence of MAP2 and polymerizing tubulin in the presence of MAP2. The first type of experiment showed, among other things, that MAP2 binds in a saturable fashion to maize microtubules. Its binding also influences the structures of preformed maize microtubules, indicating that it affects dimer–dimer interactions. However, MAP2 dissociates more readily from MAP2-saturated maize microtubules than from MAP2-saturated brain microtubules upon transfer into MAP-free buffer. Because electrostatic interactions are components of MAP–microtubule binding, raising the salt concentration causes further MAP dissociation; again, it takes less salt to induce dissociation from maize microtubules than from brain microtubules. From their observation that about half the bound MAP2 dissociates from maize microtubules in the absence of added salt and that most of the rest dissociates in the presence of high salt, Hugdahl and coworkers conclude that MAP2 binds to two different classes of binding site on maize microtubules, one low affinity and one higher affinity.

The second type of experiment showed that adding MAP2 to unpolymerized maize tubulin heterodimers induces them to polymerize; it also reduces the critical concentration (C_c) of the tubulin concentration below which polymerization will not occur, although less than it reduces the C_c for brain tubulin. Interestingly, in the course of these experiments, the authors found that in the absence of added MAP, the C_c for maize tubulin at 25°C is lower than that of brain tubulin at 37°C—that is, that maize tubulin’s polymerization domain is more efficient than that of brain tubulin. This suggests that the stability of plant microtubules at temperatures that would destabilize microtubules from homeothermic organisms is due, at least in part, to properties of plant tubulin itself.

Although MAP2 induces maize tubulin to polymerize, the structures that form are aberrant helical ribbons rather than normal microtubules. These structures contain fewer protofilaments than normal microtubules, and the filaments are curved rather than straight. The authors propose that these structures arise both because MAP2 enhances the rate of elongation more than the rate of nucleation and because it induces the formation of abnormally curved prenuclei. Elongation of these incomplete, abnormal protifilament nuclei results in helical ribbon structures. Polycations induce brain tubulin to assemble into similar structures, suggesting that MAP2 may act like a polycation in its interaction with maize tubulin, that is, that the interaction is of partial specificity.

The results of Hugdahl and coworkers thus illuminate several properties of maize microtubules and their interactions with MAPs. First, like animal microtubules, maize microtubules contain binding sites for MAPs, although proof that these sites bind plant MAPs in vivo will have to await the isolation of true plant MAPs. Moreover, there are two classes of MAP2 binding site, which hints that plants contain at least two classes of MAP. Finally, MAP2’s promotion of abnormal microtubule formation suggests that proper microtubule assembly requires that MAPs bind with...
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high specificity to tubulin. This in turn implies that there are significant differences between the plant tubulin binding domains of the plant MAPs and those of MAP2. Circumstantial, although negative, evidence that this is indeed the case comes from the fact that no one has yet been able to isolate a plant MAP using the polymerase chain reaction with primers to sequences highly conserved in animal MAPs.

Animal MAP activity is, in some cases, known to be regulated by post-translational events such as phosphorylation (Wiche et al., 1991), and this will most likely hold true for plant MAPs as well. For example, two spindle-associated mammalian MAPs, MAP4 and MAP1B, are differentially phosphorylated during the cell cycle by spindle-associated p34cdc2 or an immunologically related kinase (Tombes et al., 1991); this phosphorylation presumably influences spindle structure, which in turn determines the site of the division plane (see Strome, 1993, for review). p34cdc2 was originally identified as the product of the yeast cdc2 gene and is now known to be ubiquitous in eukaryotic cells; several plant cdc2 genes have been isolated by complementing the mitotic defect of cdc2 yeast mutants (e.g., Colasanti et al., 1991; Ferreira et al., 1991; Hirt et al., 1991). Depending on which cyclin it is associated with, p34cdc2 can phosphorylate many different substrates; these phosphorylation events mediate the progression through the cell cycle (Lewin, 1990).

In animal and yeast cells, p34cdc2 is primarily nuclear during interphase. During mitosis, it becomes associated with the spindle pole bodies of fungi and the centrosomes of animal cells (e.g., Bailly et al., 1989). Plant cells contain neither centrosomes nor single, focused spindle poles, raising the question of what structure p34cdc2 is associated with in plant cells. In this issue (pages 1101-1111), Colasanti and coworkers use an antibody specific for maize p34cdc2 to show that the plant p34cdc2 kinase is associated with the PPB but not with the mitotic spindle or the spindle poles. Localization of p34cdc2 or a related protein to the PPB was also observed by Mineyuki et al. (1991), who used a less specific antibody to a conserved p34cdc2 sequence motif that is found in p34cdc2-related proteins as well as in p34cdc2 itself. The presence of p34cdc2 at the PPB raises the possibility that even though plants lack centrosomes, one function of p34cdc2 in plants, as in animals, is to determine the division plane.

Colasanti and coworkers' analysis showed that during interphase, plant p34cdc2 is mainly nuclear, just as it is in animal and fungal cells. After the PPB forms, p34cdc2 begins to appear in the PPB region, but only in 10% or so of the PPB-containing cells. The authors suggest that the inconsistent PPB staining may indicate that p34cdc2 association with the PPB is transient. Once the nuclear membrane breaks down and the spindle forms, p34cdc2 is present throughout the cytoplasm; during telophase, as the daughter nuclei form, p34cdc2 again becomes concentrated in the nucleus. A similar staining pattern is seen in dividing cells of the developing stomatal complex; in these cells, as in root tip cells, some of the cells in late G2/M show colocalization of p34cdc2 with the mature PPB.

To ask whether the presence of p34cdc2 is required for the formation of the PPB or whether the reverse might be the case, the authors treated maize root tip cells with oryzalin, a microtubule depolymerizing agent (Hugdahl and Morejohn, 1993). Oryzalin completely eliminated PPBs in cells that were about to divide (i.e., that were in early prophase) and that normally would have had a well-formed PPB. Several hundred such cells were stained with p34cdc2 antibody, and p34cdc2 was never detected in the region where the PPB should have been. This finding suggests that rather than defining the location of the PPB, p34cdc2 is brought to this location by the PPB.

If p34cdc2 does not specify where the PPB will form, what is its function? One possibility is that phosphorylation of its cortical substrate is the event that 'imprints' in cellular memory the location of the PPB once that structure disappears. This substrate may be a MAP, but it is just as likely that it is some other type of molecule, perhaps a protein that interacts with F-actin, which is also associated with the PPB in some cell types (Mineyuki and Palevitz, 1990). An alternative possibility is that PPB-associated p34cdc2, rather than imprinting the division plane, phosphorylates a MAP that is involved in spindle formation.

A better understanding of plant MAPs and how they influence microtubule function may eventually help explain several unique aspects of plant mitosis, not only the imprinting of the division plane by the PPB but also the plasticity of the plant mitotic apparatus, a phenomenon that Palevitz discusses in a review article on pages 1001–1009 of this issue. As Palevitz points out, the inability of plant cells to move relative to one another or to change shape means that the plant mitotic apparatus often adopts a variety of deformed or irregular configurations. For example, the spindles of guard mother cells are often oblique or S-shaped. Most remarkably, in the division of the generative cell of the spiderwort, Tradescantia virginiana, which occurs within the confines of the growing pollen tube, the chromosome pairs are arrayed throughout the length and depth of this long, thin cell rather than on a planar metaphase plate. In some cases, as Palevitz discusses, the morphology of the mitotic apparatus is determined epigenetically, by mechanical constraints imposed by the cell wall; in other cases, there is evidence that the morphology may be a result of genetic programming.

What endows the plant mitotic apparatus with such plasticity? One obvious difference between plant and animal mitotic apparatuses lies in the structures of their spindle poles. The plant spindle does not focus at a single point, as does the animal spindle; instead, the plant spindle pole is broad and, frequently, multipolar. This may give the kinetochore fibers of the plant spindle some 'positional free-
"other. In other words, the plant spindle may actually comprise multiple spindles that move chromosomes in a partially independent fashion. Nevertheless, there are also extensive linkages between the kinetochore fibers of plant spindles, and these may help coordinate the movement of the partially separable spindles.

Understanding the basis for the plasticity of the plant mitotic apparatus will be aided by learning about the proteins, including MAPs, that associate with the plant spindle poles. Palevitz points out that γ-tubulin, a member of the tubulin superfamily, has recently been found in plants. In animals and fungi, γ-tubulin, which is localized to microtubule organizing centers, is thought to function in microtubule nucleation, anchoring, and polarity determination (Oakley, 1992). Plant γ-tubulin has also been found to colocalize with plant microtubule organizing regions, although it appears to be more broadly distributed than animal γ-tubulin (Liu et al., 1993). In addition, γ-tubulin is, like p34cdc2, present at the PPB, raising the possibility that γ-tubulin might be involved in PPB nucleation or imprinting. Plants presumably contain other centrosomal protein homologs as well, even though these proteins are not organized into classic animal-type centrosomes, and the analysis of the distributions and functions of these plant proteins should also shed light on the mechanisms that underlie the plasticity of the plant spindle.

Taken together, the papers in this issue provide a clear demonstration that plant microtubules have the capacity to act in very different fashions than do animal and yeast microtubules, despite their biochemical and structural conservation. These differences most likely represent the activities of the proteins that organize both the assembly of microtubules and their interactions with other cellular constituents, although the existence of multiple tubulin isoforms raises the possibility that differences in tubulin structure account for some of the differences as well. Further study of both plant microtubules and MAPs should one day help us to understand how the microtubule arrays unique to plants function: why the plant spindle is so flexible and how, despite the flexibility of the spindle, the PPB is able so accurately to predict the placement of the future cell wall.

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REFERENCES

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