Microtubule arrays play critical roles in intracellular organization and cell division in all eukaryotes. During cell division, microtubule arrays guide mitotic spindle and phragmoplast assembly and function. In animal cells, interphase microtubule arrays perform a number of functions, including transport and positioning of organelles, such as vesicles, granules, and mitochondria; they form the structural core of cilia and flagella; and they provide support for overall cell shape. In plants, intracellular motility is based predominantly on arrays of actin filaments, rather than microtubule-based structures, and the cortical microtubule array has a primary function in cell growth and elongation, guiding the deposition of new cellulose microfibrils as they emerge from the cellulose synthase complex embedded in the plasma membrane (Wasteneys, 2002).

Microtubules are cylinders composed of linear chains (protofilaments) of α- and β-tubulin dimers. The α- and β-tubulin subunits heterodimerize in a head-to-tail fashion, giving rise to polarity that plays a crucial role in the function of the microtubule array. Heterodimers are oriented with β-tubulin pointing toward the faster polymerizing plus end and α-tubulin pointing toward the more slowly polymerizing minus end of the microtubule. γ-Tubulin is related to α- and β-tubulin but does not heterodimerize with either of these subunits. In animal and fungal cells, γ-tubulin functions as a microtubule nucleating factor that is localized in the microtubule organizing center (the centrosome in animal cells and the spindle pole body in yeast). Microtubule assembly in animal cells requires the function of a centrosome, an organelle consisting of a pair of centrioles surrounded by a complex collection of proteins known as the pericentriolar material (PCM). During interphase, the minus (slow-growing) ends of microtubules are embedded in the PCM and the plus (fast-growing) ends project outwards into the cytoplasm (or during mitosis, into the spindle apparatus). In animal cells, γ-tubulin forms ring structures in the PCM that are associated with the minus ends of newly polymerized microtubules. The outer diameter of the γ-tubulin complex is similar to the outer diameter of a microtubule, and this is thought to provide a base for the assembly of α/β-tubulin dimers (Moritz et al., 1995; Zheng et al., 1995). In addition to the well-established role in microtubule nucleation, γ-tubulin has been found to function in the coordination of late mitotic events (Hendrickson et al., 2001) and to have a microtubule-independent function in mitotic checkpoint control (Prigzhina et al., 2004).

Plant microtubule arrays are distinct from those of animals and fungi in that new microtubules do not arise from discrete organizing centers, such as the centrosome or the spindle pole body, but rather are nucleated from dispersed sites across the cell cortex (Shaw et al., 2003). Wasteneys (2002) proposed that microtubules might be nucleated from dispersed sites along existing microtubules from complexes containing γ-tubulin. Such complexes might be generated from microtubule minus ends by the microtubule-severing activity of katanin. Organization of the cortical microtubule array occurs concurrent with or after microtubule nucleation and depends on intermicrotubule interactions and the activity of other proteins, such as MICROTUBULE ORGANIZATION1 (Wasteneys, 2002). Dixit and Cyr (2004) showed that when the growing plus ends of cortical microtubules encounter existing cortical microtubules, their behavior is modified depending on the angle at which they encounter each other. Shallow-angle encounters promote microtubule coalignment and stabilization, whereas steep-angle encounters are more likely to result in microtubule depolymerization, and this behavior contributes to the highly ordered configuration of the microtubule array.

Plant γ-tubulin has been shown to have microtubule nucleating activity in vitro (Drykova et al., 2003; Horio and Oakley, 2003), but until recently, very little experimental evidence of its in vivo function has been reported. It has been shown that γ-tubulin is localized in dispersed sites along microtubule arrays in plant cells (Liu et al., 1994; Panteris et al., 2000), which is also consistent with the idea that nucleation occurs at these dispersed sites. Recent work has now confirmed a central role for plant γ-tubulin in the acentrosomal nucleation and organization of microtubule arrays in plants (Murata et al., 2005). In this issue of The Plant Cell, this conclusion is strengthened and extended by the work of Pastuglia et al. (pages 1412–1425) and by a related study from Binarová et al. in the May issue (pages 1199–1212), who together present genetic evidence of the role of γ-tubulin in Arabidopsis.

Murata et al. (2005) showed that microtubule nucleation in plant cells requires both existing microtubules and the presence of γ-tubulin. Using α-tubulin fused with green fluorescent protein expressed in tobacco cell cultures, the authors first showed that new microtubules are initiated from existing microtubules, forming a branched structure. Key experiments were then performed in a cell-free system consisting of plant cortical microtubules (plasma membrane “ghosts” isolated from tobacco cell culture), cytosolic extracts, and purified tubulin. The branch points where new microtubules form contain γ-tubulin, and microtubule nucleation was prevented by the addition of a specific antibody that inhibited γ-tubulin activity. These authors proposed that a cytoplasmic γ-tubulin complex shuttles between the cytoplasm and the sides of cortical microtubules and has nucleation activity only when bound to the microtubules.

Binarová et al. (2006) used RNA interference (RNAi) to knock down expression of the two γ-tubulin genes in Arabidopsis, TUBG1 and TUBG2, confirming that γ-tubulin is essential for acentrosomal
cells with partially depleted γ-tubulin was associated with the absence of microtubules and lethality at the cotyledon stage. Some plants showed a partial loss of γ-tubulin, allowing for a more detailed investigation of various cellular processes. They found that cells with partially depleted γ-tubulin could progress through mitosis, but cytokinesis was severely impaired. In addition, a gradual depletion of γ-tubulin over the course of 10 to 15 d showed effects on cell file organization, anisotropic and polar tip growth, and stomatal patterning. These results provide important information on the role that microtubules play in cell patterning and morphogenesis. The authors speculate that some of these effects might point to functions of γ-tubulin that are independent of microtubule nucleation. It is also possible that these events are dependent on microtubule nucleation (e.g., the continual formation of microtubule arrays in growing cells), since the cortical microtubule array is known to exert a major influence over cell shape. To test the hypothesis would require, for example, an assessment of these processes following disruption of microtubule nucleation in the presence of functional cytoplasmic γ-tubulin or the isolation of an allele that is not disrupted in microtubule nucleation but nonetheless shows defects in other cellular processes, as further discussed below.

Like Binarová et al. (2006), Pastuglia et al. conducted genetic loss-of-function studies in Arabidopsis, but in this case using T-DNA insertional mutants of the two γ-tubulin genes TUBG1 and TUBG2. These authors made use of one TUBG1 and two TUBG2 insertion lines and crossed these to create double mutant lines. All three single mutants were fully fertile and were indistinguishable from the wild type in their growth and development, suggesting functional redundancy between the two genes. By contrast, the double mutants showed severely abnormal phenotypes. One of the double mutants (tubg1-1 tubg2-1) exhibited lethality at the gametophyte stage and was presumed to represent a null mutant combination, whereas the other (tubg1-1 tubg2-2) was likely a leaky allele combination that allowed synthesis of residual γ-tubulin sufficient to sustain embryogenesis and early seedling development. Disruption of both γ-tubulin genes in the severe tubg1-1 tubg2-1 mutant caused aberrant spindle and phragmoplast structures and altered nuclear division in gametophytes. The tubg1-1 tubg2-2 combination of alleles affected late seedling development, ultimately leading to lethality 3 weeks after germination. The authors used a green fluorescent protein marker in the partially viable tubg1-1 tubg2-2 mutant combination to follow the effects of γ-tubulin depletion on microtubule array dynamics in dividing cells. They found that the duration of the cell cycle in the double mutant was longer than normal and was highly variable, which may reflect the defects in cytokinesis observed by Binarová et al. (2006) in the RNAI mutant plants. These results emphasized the central role of γ-tubulin in the formation and organization of microtubule arrays in Arabidopsis.

The results of Pastuglia et al. together with those of Binarová et al. (2006) provide solid genetic support for the conclusion that γ-tubulin plays an essential role in acentrosomal microtubule nucleation in plants. Future work likely will focus on identification and function of γ-tubulin-interacting proteins, both in microtubule nucleating branch points and in the cytoplasm, and possible roles for γ-tubulin that are independent of microtubule nucleation. In this regard, Prigozhina et al. (2004) showed that γ-tubulin in Aspergillus nidulans has a microtubule-independent function in mitotic checkpoint control. This conclusion was based on the analysis of a mutant allele of the A. nidulans γ-tubulin gene mipA that did not inhibit mitotic spindle formation but led to severe disruption of late mitotic events in that nuclei reentered interphase without completing mitosis successfully. Thus far, this type of checkpoint defect has not been noted for any of the disruptions in Arabidopsis γ-tubulin genes. Binarová et al. observed defects in late mitosis in cells with reduced γ-tubulin, most commonly misoriented phragmoplasts and cell division planes during cytokinesis. This highlights an important function on mitotic spindle poles and cell plate formation, both of which are likely dependent on microtubule nucleation events.

The A. nidulans mutant allele (mipA159) was described as a cold-sensitive allele created by replacement of adjacent Asp-159 and Arg-160 residues with Ala. The mutant allele was originally isolated using the innovative approach of charged-to-Ala scanning mutagenesis (Jung et al., 2001), based on the principle that charged regions tend to be on the outside of proteins and that replacement with Ala can alter regions important for protein interactions without causing gross structural changes. Hendrickson et al. (2001) used a similar approach to analyze γ-tubulin function in Saccharomyces pombe, pointing to roles in the coordination of postmetaphase events, anaphase, and cytokinesis. It might be of interest to create similar constructs using the Arabidopsis TUBG1 coding sequence, which encodes a number of conserved charged amino acids, including Asp-159 and Arg-160. Such constructs might be tested by expression in a heterologous system and, possibly, in the putative null tub1-1 tub2-1 double mutant (depending on several factors, such as whether it resulted in a conditional mutation and whether an appropriate promoter could be chosen to rescue sterility in the double mutant).

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