Supplemental and Online Materials

**Plant and animal microtubule dynamics.** The halftime to fluorescence recovery in the plant cell cortical array is approximately four times faster (this study, 8) than for animal interphase arrays (SI). Yet, our measurements of single microtubule dynamics showed that the total subunit turnover per unit time, the dynamicity, is approximately the same between the two systems (4.9±1.6 and 4.5 ± 2.8 (SI)). Further, we found that the growth and shortening velocities of plant microtubules are actually slower than those reported for animal cells by a factor of two (SI). An examination of polymerization patterns at both microtubule ends reveals a possible explanation for these apparently conflicting measurements. While plant microtubule ends grow and shorten at about half the velocity of animal microtubules (SI), they have a similar dynamicity because they exhibit dynamic behavior far more often (90% in plants vs 35% in animals (SI)). In treadmilling, plant microtubules the lagging ends also contribute to dynamicity. Plant microtubules are slowly growing and shortening almost constantly, whereas activity in animal arrays is concentrated in fewer but faster bursts.

In both plant and animal interphase microtubules, subunit addition occurs primarily at the leading ends. However, in treadmilling plant microtubules subunit loss occurs not at one end, but at both ends. Because the two systems have similar rates of subunit gain and loss, distributing loss over two ends results in a larger bias towards net subunit gain at the plant leading ends. In the FRAP experiment, the shape of the recovery curve is strongly influenced by the initial period of recovery, which is solely due to polymerization. The
large bias towards polymerization at the leading end of the plant microtubules will accelerate the FRAP recovery rate when compared to the non-treadmilling animal system. The minus end of the plant microtubules does not directly affect the fluorescence recovery because the slow depolymerization only eliminates bleached polymer over the duration of the experiment. In sum, the FRAP experiments highlight the bias toward polymerization at the leading end of the treadmilling plant microtubule and do not suggest a dramatic difference in the actual dimer flux rates or microtubule turnover between animal and plant interphase arrays.

**Origin of cortical microtubules.** Nucleation and tethering of microtubules at a central organizing center in animal and yeast cells creates an astral interphase array with well-defined polarity. By contrast, the cells of higher plants lack a discrete microtubule organizing organelle, such as the centrosome or spindle pole body, and do not contain cytoplasmic dynein, thought to be important for gathering and tethering the minus ends. Several lines of evidence show that nucleation of plant interphase microtubules occurs at the nuclear surface (S2). We present evidence in this work for additional nucleation of interphase microtubules at the plant cell cortex. Lengthwise attachment of new, intact microtubules from the cytosol or the introduction of new microtubules to the cortical array from trans-vacuolar strands was not observed in this study. We conclude that the majority of the new microtubules in the cortex are likely born at the cortex and not transferred from other sites such as the nuclear surface. While several sites of multiple microtubule initiations were found, in no case did the minus ends remain tethered together to form a polarized, astral array. Polymers either depolymerized to extinction or
were released from their cortical initiation sites. Release could conceivably occur by dissolution of the initiation complex or through cleavage near the minus end by a katanin-like protein ($S3,S4$).

**Polymer gain at the lagging end.** Release of the microtubule from the initiation site resulted in a free minus end that exhibited some capacity for dimer addition. The majority of lagging end growth events was within the measurement error for the experiment and in no case did we observe a persistence of growth events leading to elongation of more than a micrometer. These observations suggest that polymerization is not strongly promoted at the lagging ends and that lagging end growth does not contribute significantly to minus end dynamics.

**Polarity of the cortical array.** The leading ends of adjacent microtubules often were oriented in opposite directions (Movie S3), showing that cortical microtubules are not organized in a uni-polar fashion but can have opposing polarity in the same array. Homogeneous recovery of fluorescence in photobleaching experiments also revealed that the organization of the plant cortical array is not highly polarized (Movie S5). This lack of polarity is observed even in cells where the microtubules are dramatically co-aligned, showing a net transverse orientation relative to the long axis of the cell. The bi-directionality of polymers in the highly ordered cortical array suggests that net polarity in the array is not required for array organization or function. Further, tethering microtubules to the cell cortex requires a mechanism that can recognize microtubules in a variety of orientations.
**Methods and Materials**

Fusions between EYFP (Clontech) and *Arabidopsis* tubulin isoforms were created by amplification of AtTub3A and AtTub1A from a pooled *Arabidopsis* cDNA library using primer pairs homologous to the first 22 base pairs of the tubulin open reading frame and to the first 22 base pairs of the untranslated sequence immediately following the stop codon. Methyl-dCTP replaced dCTP during amplification to block cleavage of EcoR1 and HindII sites in the amplified products. Amplified sequences were cloned into the EcoR1 and HindIII sites proximal to the 35S promoter in the pEGAD plant expression vector (S5) using palindromic double-stranded linkers (S5). All constructs were verified by sequence analysis and introduced into *Arabidopsis* Col 0 by *Agrobacterium*-mediated transformation (strain GV3101) (S5). T1 transgenic plants were characterized for GFP expression and the quality of microtubule labeling. Selected plants were allowed to self-pollinate to yield T2 seed for analysis.

A concern when introducing a large molecule like GFP to mark a protein complex is that the presence of the label may interfere with normal cell function. In a recent study, Rusan et al analyzed a similar GFP-alpha tubulin fusion protein in animal tissue culture cells and found that the dynamic behavior of microtubules marked by expression of the GFP fusion protein did not differ significantly from those marked by injecting dye-conjugates of tubulin (S1). Likewise, our FRAP analysis of cortical array dynamics in *Arabidopsis* plants expressing GFP-tubulin agrees remarkably well with measurements...
made in *Tradescantia* cells injected with dye-conjugated tubulin. Expression of the fusion proteins in *Arabidopsis* plants did not result in any obvious developmental or cellular abnormalities in the plants that were analyzed, suggesting that microtubule function in these plants is normal in most important respects. However, these transgenic plants did tend to grow slightly more slowly than wildtype individuals (unpublished observations) and also displayed a modest sensitivity to the microtubule destabilizing drug oryzalin. At 175 nM, a sub-threshold dose that has no measurable effect on wildtype plants, the transgenics show approximately a 15-20% decrease in root length as compared to wildtype. There is no evidence for cell swelling at this concentration (A. Paredez, personal communication).

*Arabidopsis* seeds were refrigerated at 4°C for 2-3 days then germinated on Murashige-Skoog (MS) agar at 23°C under constant light. At 3-4 days, seedlings were transferred to large coverslips, mounted in MS media, and stabilized by an overlying coverslip held in place with silicon vacuum grease. Most confocal images were acquired with a BioRad 1024 confocal head mounted on a Nikon TMD 200 inverted microscope equipped with a 60x 1.2 n.a. water immersion objective lens. Imaging was typically performed at 3% laser power with 2-5 second intervals between images for a total duration of 3-6 minutes. FRAP experiments were performed on a Zeiss 510 confocal microscope using a 60x, 1.2 n.a. multi-immersion objective. The position of free microtubule ends was recorded by hand after image scaling and contrast enhancement with the assistance of dedicated software routines developed in the MATLAB (v6.2) computing environment. Velocities and dynamicity were determined from the original position coordinates. Transition rates
and percent time in phases were calculated from velocities. Kymographs and linearization of microtubules were created in MATLAB with dedicated routines. Cells for timelapse analysis were selected along the length of the hypocotyl, from petiole insertion to the root-shoot junction.

**Supplemental References**


**Movies**

Movies S1,S2 and S3. Time-lapsed images of *Arabidopsis* hypocotyl cells expressing GFP-tubulin. Evidence for the initiation of microtubules at the cell cortex (O), the formation of a microtubule bundle (B), and detachment of a microtubule from the cell cortex (D) are illustrated in each cell. Examples of nearby microtubules polymerizing in
opposite directions, within array are shown in Movie S3 (AP). The images in each sequence were acquired every 3.8 seconds. Movie 1 consists of 60 frames and Movies 2 and 3 consist of 100 frames each.

Movie S4. Photobleaching of a line across the cortical microtubule array reveals that both single microtubules and microtubule bundles move by treadmilling (33 images acquired at 8 second intervals). Photobleaching was accomplished using 4 laser scans at 100% laser power.

Movie S5. Fluorescence recovery after photobleaching (FRAP) experiments were performed using 100% laser power for 4 scans in ~10μm diameter circle. 20 images were acquired at 9 second intervals. The green circle denotes the position of the bleached area. Note the recovery of fluorescence in the bleached region shows no obvious spatial bias.