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3D Time-Lapse Microscopy Reveals Microtubule Patterning Differences Between Adjoining Cell Faces in *Arabidopsis* Cells

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The microtubule arrays in flowering plant cells organize into a variety of functional patterns at the cell cortex[1]. Absent a defined microtubule organizing center, such as the centrosome in animal cells, it is unknown how these polymer arrays achieve specific organizational states [1]. Live-cell imaging studies of microtubules on the outer (periclinal) cell face established that these polymers are nucleated on the cell cortex, remain laterally attached to the cell cortex, and exhibit a hybrid form of treadmilling motility that is strictly dependent upon net subunit addition at one end, and persistant subunit loss at the other [2]. Additional work has shown that polymers often bundle through treadmilling-dependent interactions [1] and undergo severing preferentially at sites where one polymer crossed over another [3]. How these basic microtubule behaviors are directed to create the patterns required for orchestrating cell wall construction remains a critical question in plant cell biology.

Recent investigations using plant hormones to induce microtubule pattern changes have suggested that the microtubule arrays on different faces of these large, box-shaped epidermal plant cells may be differentially regulated [4]. Quantitative observations comparing the number of microtubule ends polymerizing out of the outer periclinal array versus plus ends entering that array from the cell's anticlinal side faces revealed a >3:1 bias. Microtubule ends polymerized out of the outer periclinal array and into the lateral anticlinal arrays with no apparent barriers to polymerization at the cell face junctions for these cells [4]. Hormone treatments, used to shift the arrays into a transverse co-alignment relative to the cell growth axis, corrected the bias in edgeward polymerization trajectory to <2:1, suggesting that this bias can be regulated by the cell. We therefore hypothesize that these box-shaped plant cells can locally regulate microtubule behaviors on the spatial scale of an individual cell face.

To address this hypothesis, we are focussing on improved 3D time-lapse imaging of the microtubules in the anticlinal side faces of these plant cells. We have identified the major technical impediment to obtaining these data as the refractive index mismatch between plant cell wall and cytoplasm. The cortical microtubules lie within an optical volume shared by the cell wall refractive index (~1.41) on one side and the cytoplasmic refractive index (~1.34) on the other. Imaging axially into the specimen to follow the microtubules on the anticlinal cell face is progressively degraded by asymmetric spherical aberations dominating the image formation process. Our initial efforts to address this issue include the use of a spinning-disk confocal microscope head and glycerol immersion objectives with the immersion medium empirically determined to compensate for spherical aberrations midway down the anticlinal side walls.

Time-lapse imaging of epidermal cells in the plant cotylodon expressing an integrated GFP-tubulin transgene [2] show the marked cortical nature of the interphase microtubule arrays (Figure 1a). Using 120 axial images taken at 0.20 μm intervals to capture the majority of the cell volume at 1 min intervals, the changes in array pattern on anticlinal and periclinal cell faces were followed over a 60 min duration. The outer periclinal array contains both bundled and unbundled microtubules with no dominant directionality or orientation for the polymers (Figure 1a). Cutaways to view the cells' anticlinal side

faces revealed a strong dominant axial orienation to the polymers resulting in a 'picket fence' appearance around the cell perimeter (Figure 1c). MATLAB code was developed to extract and project the axial data in side view from hand-traced curves following the outlines of the anticlinal cell walls in the time-lapse data (Figure 1c-d). The 3D time-lapse revealed a complete absence (0 in 3 data sets for n = 12 cells; >1000 linear microns of lateral cell wall over 180 total min) of microtubules in the lateral side faces initiating at angles >45° relative to the dominant axial orientation of the microtubules. Unlike the outer periclinal arrays, these anticlinal arrays only make microtubules that are in one orientation. Unbundled microtubules polymerizing into the anticlinal arrays from the outer periclinal array were either rapidly redirected into the dominant axial alignment or lapsed into a depolymerizing phase, retreating from the anticlinal cell face. Our intial examination microtubule patterning in the arrays of the anticlinal cell faces thereofre strongly supports our hypothesis that the microtubule behaviours are locallly regulated on the spatial scale of the individual cell face.

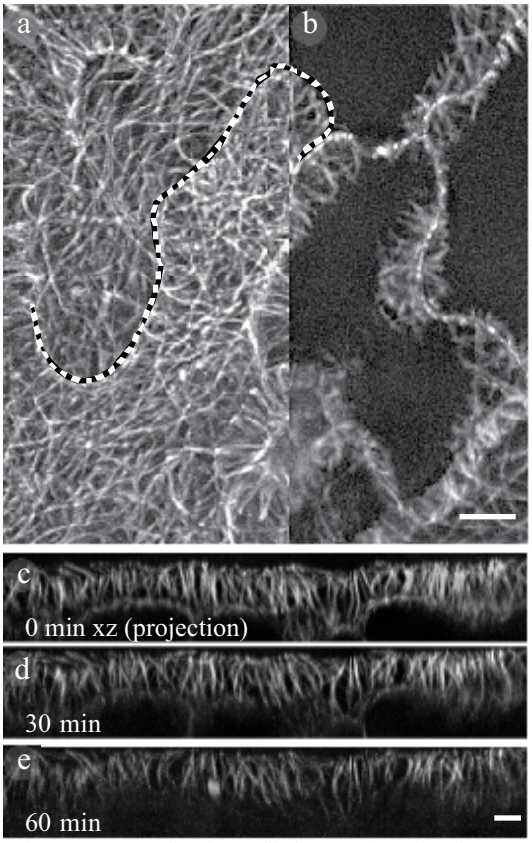

Figure 1. Spinning disk confocal microscopy of microtubule arrays in plant epidermal cells (a) with digital cutaway (b) revealing central vacuole devoid of polymers. Side-on projections of microtubule arrays (under dashed line in a and b) for 0 min (c), 30 min (d), and 60 min (e) time points showing no evidence of transversely oriented microtubules.

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