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Reorganization of the plant cortical microtubule array

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The interphase microtubule arrays in flowering plant cells assemble at the cell cortex into patterns that affect cellular morphogenesis. A decade of live cell imaging studies has provided significant information about the *in vivo* properties of the microtubule polymers. Efforts to extrapolate individual properties to larger roles in organizing or patterning the microtubule array have produced models focused on self-organization and local levels of biological control. Recent studies looking at cortical microtubule arrays as they transition from an existing pattern to a new pattern have re-emerged as a testbed for examining these models and the molecular hypotheses underpinning them. The evidence suggests that microtubule patterning is locally controlled on the scale of a cell face, using or circumventing self-organizating properties as necessary.

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Introduction

Polymer systems gain function through their organization into patterned arrays. Microtubules (MT) in cultured animal cells typically form a radial array with the minus ends anchored at the centrosome and the dynamic plus ends extending outward toward the cell cortex [1,2]. The inherent polarity of the MT polymers creates a spatially determinate matrix for the animal cell organizing the secretory system and positioning the nucleus [3]. Flowering plants evolved away from centrosome production and rarely form radial arrays [4–6]. The interphase plant MTs typically associate with the cell cortex, forming patterns that range widely from co-aligned (i.e. parallel to each other) to seemingly random. How flowering plants organize the acentriolar MT arrays into specific patterns and how those patterns contribute to cellular function remain a central question in cell biology.

Drug studies showed that plant cell growth was sensitive to MT perturbation even before interphase MTs were formally identified [7–9]. Cell biological and genetic analyses provided subsequent evidence that the MT pattern *per se* influences morphogenesis [10–13]. MTs affect morphogenesis through at least two mechanisms. Cellulose synthase complexes from the Golgi apparatus insert into the plasma membrane at positions coincident with cortical MTs [14–16]. Once inserted, the MTs can guide the cellulose synthases [17], and therein, influence the material properties of the cell wall [11]. The effect of MT pattern on morphogenesis is particularly evident for axially expanding root and hypocotyl cells. Organization of the cortical MTs into a co-aligned array, transversely oriented to the plant growth axis, results in cellulose deposition with the same patterned orientation [4,11,18]. When applied to all cells in a tissue, the material anisotropy of the walls creates an opportunity for the plant to coordinately extend cells in an axial fashion, driving morphogenesis of the tissue as a whole. This fundamental property allows acentriolar conifers and flowering plants to compete successfully for resources, extending stems into the sunlight and roots into the soil.

Plant cortical microtubule behaviors

Explaining plant MT array organization originally focused on extrapolations from animal cell biology. Models proposed MTs originating at the nucleus and sliding into position at the cell cortex [6,19]. Subsequent live-cell imaging studies revealed some significant surprises. Cortical MTs nucleate at the cell cortex [20,21] from gamma-tubulin containing complexes (GCPs) associated, in most cases, with existing MTs [20,22,23]. Polymers nucleate at a ~40° angle to the associated MT (i.e. branched nucleation) or parallel with the existing MT (i.e. intrabundle nucleation) with the relative proportions being affected by a phosphatase [20,22,23,24•]. Once nucleated, katanin proteins sever the MT minus end from the nucleation complex creating a free minus end [22]. The minus ends show slow, processive depolymerization while the plus ends exhibit dynamic instability biased toward polymer accumulation [21]. When combined, the dynamic properties of the two ends give rise to a form of polymer treadmilling where the laterally anchored MTs appear to translocate on the plasma membrane [21].

Treadmilling results in a significant amount of MT–MT interaction, often resulting in angle-dependent MT bundling [25] or a switch from growth to shortening [25,26]. MT bundling has no measurable effect on MT dynamics *in vivo* [27] but does serve to reorient MT polymerization trajectories and to increase the persistence of MT polymer along a given 'track' at the cell

cortex [27]. The longer-lived and more organized portions of the cortical MT array appear to be bundled MTs. This persistence is likely critical for connecting the pattern of MT organization to cell wall patterning because the cellulose synthase complexes move at ~1/3 the velocity of a treadmilling MT [17,21,28].

Creating models for array organization

The fixed lateral association of the MTs to the plasma membrane and the absence of intrabundle MT sliding generally ruled out sliding models for array organization [13]. More recent models have focused on taking the MT activities observed in steady-state arrays and hypothesizing ways that those behaviors could create specific array patterns [29–33]. Many of these MT activities show enticing biophysical properties, suggesting that array patterning could arise from a specific set of self-organizing properties. In this case, a self-organizing property can be defined as a globally applied rule for all cortical MTs that leads to an ordered state. For example, treadmilling MTs typically form bundles when intersecting other MTs at angles $<35^{\circ}$, suggesting that the MTs should form bundles with a more defined relationship throughout the cell [29–31,33]. In another example, shifting from MT branched nucleation to predominantly intrabundle nucleation globally would presumably bias the system to maintain an existing array pattern or at least prolong the transition to a new pattern.

An alternative to strict self-organization is the idea of local biological control. Here, the cell creates local distinctions in how the MTs behave in order to control how a pattern is formed. For example, cells programmed to enter xylogenesis express a system of proteins that create local domains to exclude MTs through induced catastrophe of growing plus ends [34••]. Here, the MT pattern is proposed to arise through local activation of a MT property rather than through a global self-organizing mechanism.

Computer simulations have generally shown that self-organizing properties alone are not sufficient to create both MT co-alignment and transverse orientation in elongated cells [29,33,35,36••]. However, combining self-organizing properties with local biological control yields plausible mechanisms for array organization. For example, coupling the bundling interactions that co-align MTs with induced catastrophes at the cell's apical and basal end faces, to eliminate longitudinal MTs, tends to produce robust transverse co-alignments *in silico* [29,35,36••].

Watching arrays reorganize into specific patterns

A growing number of new genes have been shown to be required for patterning the cortical MT array (e.g. [34••,35,37,38•]). In most cases, it is not known whether the gene product is specifically required to direct the pattern, or more generally for maintaining the MT population. In this context, it is important to emphasize that some genes likely facilitate transitioning between states, but may not be directing or 'steering' the array into a new orientation. To determine how genes function in MT array patterning, several groups have shifted emphasis away from steady-state MT arrays and initiated experiments that follow living cells over time as they organize their MTs into specifically defined array patterns. These works have generally focused either on cells rebuilding a cortical array after mitosis or on cells stimulated with light or hormones to induce array reorganization.

Live-cell imaging of the end-stage phragmoplast in *Arabidopsis* root cells suggests that MT nucleation sites are concentrated at the position of cell wall attachment, where they may preferentially initiate new cortical MTs perpendicular to the division plane [39]. A second study, examining the return of cortical MTs after division in tobacco BY-2 cultured cells did not note this phenomenon, but found a non-random initial patterning of the cortical MTs, appearing more prominently at angles 45° and 135° to the cell growth axis [40]. Both studies provide evidence that, despite the apparent absence of a defined MT organizing center in flowering plants, these cells exhibit a level of temporal and spatial control over where new MTs are created and initially directed in the interphase cortical array. A third study, examining cortical array reformation in recently divided root and leaf cells, provides evidence that plant cells can also spatially control polymer dynamics [35]. Data are presented showing an asymmetric distribution of the AtCLASP1 protein to a subset of cell edges at the outer periclinal array. The absence of that protein was reported to specifically preclude MTs from polymerizing across the transfacial cell boundary, and therefore, bias the array pattern for MTs in the opposite orientation. While CLASP's broader role(s) in MT array organization is still being defined [41], this work provides a specific proposal for how local cellular control of MT dynamics may influence global array patterning.

De-etiolation of the hypocotyl cells provides a natural means of examining MTs transitioning from a predominantly transverse co-alignment to a longitudinal co-alignment. Light from the microscope signals the cells, providing an opportunity to examine reorganization to a known pattern. In the context of mutant analysis, this assay has recently been used to show that the *wave-dampened like-3* mutant forms transverse arrays in the dark, but is retarded for re-orientation to the longitudinal pattern during de-etiolation [38•]. The light-triggered degradation of this protein may provide new clues to how cells signal for changes in MT array pattern. Recent data showing that TON2(FASS), a protein phosphatase 2A subunit, is also required for light-driven reorganization