Mechanisms of Self-Organization of Cortical Microtubules in Plants Revealed by Computational Simulations

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Microtubules confined to the two-dimensional cortex of elongating plant cells must form a parallel yet dispersed array transverse to the elongation axis for proper cell wall expansion. Some of these microtubules exhibit free minus-ends, leading to migration at the cortex by hybrid treadmilling. Collisions between microtubules can result in plus-end entainment (“zippering”) or rapid depolymerization. Here, we present a computational model of cortical microtubule organization. We find that plus-end entainment leads to self-organization of microtubules into parallel arrays, whereas catastrophe-inducing collisions do not. Catastrophe-inducing boundaries (e.g., upper and lower cross-walls) can tune the orientation of an ordered array to a direction transverse to elongation. We also find that changes in dynamic instability parameters, such as in mor1-1 mutants, can impede self-organization, in agreement with experimental data. Increased entainment, as seen in clasp-1 mutants, conserves self-organization, but delays its onset and fails to demonstrate increased ordering. We find that branched nucleation at acute angles off existing microtubules results in distinctive sparse arrays and infer either that microtubule-independent or coparallel nucleation must dominate. Our simulations lead to several testable predictions, including the effects of reduced microtubule severing in katanin mutants.

INTRODUCTION

Microtubules (MTs) are ubiquitous biopolymers that endow animal cells with structural rigidity, intracellular transport, and the ability to proliferate. Without the constraints of an MT-organizing center, the MTs at the cortex of plant cells are able to self-organize into robust yet dynamic arrays. After cell division and disassembly of the phragmoplast array, MTs form de novo on the inside of the plasma membrane, where a strong association with the cell cortex forces their dynamics to play out on an effectively two-dimensional geometry (Wasteneys, 2002). Through polymer dynamics, interactions with the membrane and microtubule-associated proteins (MAPs), and MT–MT interaction, the thousands of MTs at the cortex (Dixit and Cyr, 2004) form a strikingly parallel array transverse to, but dispersed along, the cell’s axis of elongation. This highly ordered transverse array can reform within 120 min of the initiation of MT assembly after drug-induced depolymerization (Wasteneys and Williamson, 1989a), and it is required to generate anisotropic mechanical properties of the cell wall, although the relationship between the cortical MT array and the oriented deposition of cellulose microfibrils is not yet clear (Himmelspach et al., 2003; Wasteneys, 2004; Wasteneys and Fujita, 2006). For recent reviews, see Wasteneys and Ambrose (2009), Ehhardt and Shaw (2006), Hashimoto and Kato (2006), and Wasteneys and Fujita (2006).

The study of MT organization in the absence of an MT-organizing center is important for understanding plant cell growth, but it also has implications for understanding acen-trosomal MT organization in general. Systems for which organization relies on MT dynamics in concert with activity of MT cross-linking and bundling proteins have occurred in numerous contexts. These systems include mitotic spindle organization (Burbank et al., 2007; Groen et al., 2009), astert formation (Surrey et al., 2001; Pinot et al., 2009), and organelle positioning (Malikov et al., 2005).

MTs are stiff, polar polymers composed of tubulin. One end of the polymer, known as the plus-end, randomly switches between states of rapid growth and rapid shrinkage (Mitchison and Kirschner, 1984) as well as intermittent pauses (Shaw et al., 2003) in a phenomenon known as dynamic instability. Transitions from growth to shrinkage and shrinkage back to growth are known as catastrophe and rescue, respectively. The minus-end might remain static or can slowly depolymerize (Shaw et al., 2003). Although photobleaching studies show that individual tubulin subunits remain mostly fixed relative to the cell cortex (Shaw et al., 2003), this hybrid-treadmilling mechanism (dynamic instability at the plus-end and on-average shrinkage of the minus-end) allows individual MTs to migrate as the plus-end polymerizes (on average) and the minus-end depolymerizes.

The collision of two cortical MTs may result in several possible outcomes. If the angle of collision is steep, the incident MT may switch to the shrinking state, or it may continue to grow unperturbed, crossing over the barrier MT. If the angle of collision is shallow, the incident MT may become entrained with the barrier MT, after which the plus-end grows parallel to the barrier MT, resulting in a sharp bend in the MT at the site of collision. This phenomenon is

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Abbreviations used: CIC, collision-induced catastrophe; MAP, microtubule-associated protein; MT, microtubule; γ-TuRC, γ-tubulin ring complex.
commonly referred to as “zippering” (Dixit and Cyr, 2004), but recently it has been suggested that this term should be reserved for situations in which two preformed MTs are progressively coalignled (Wasteneys and Ambrose, 2009). Here, we refer to this phenomenon as plus-end entrainment or, simply, entrainment. After an MT is entrained by another, the MTs form a bundle most likely mediated by members of the MAP65 class of MAPs, which cross-link adjacent MTs together with a spacing of 20–30 nm (Chan et al., 1999). Once bundled, MTs remain dynamic (Shaw et al., 2003), although possibly with different polymerization properties (Van Damme et al., 2004). Other collision outcomes are possible: the incident MT may buckle before the barrier (Wightman and Turner, 2007); it may cross over the barrier and continue in a perturbed direction (Hashimoto and Kato, 2006); or, it may become severed at the cross-over point with the new MT formed from the leading end undergoing a gradual reorientation via treadmilling (Wightman and Turner, 2007).

In the absence of an organizing center, nucleation of new MTs occurs throughout the cortex. As in other eukaryotes, nucleation is mediated by the γ-tubulin ring complex (γ-TuRC) (Murata et al., 2005). There is evidence that nucleation can occur in the absence of existing MTs (Wasteneys and Williamson, 1989b; Wasteneys et al., 1993; Chan et al., 2003) and also in an MT-dependent manner where γ-TuRC is distributed along extant MTs. New MTs have been reported to branch off extant MTs at a specific angle of 40° from (Wasteneys and Williamson, 1989b; Murata et al., 2005) or parallel with (Wasteneys and Ambrose, 2009) the extant MTs. One hypothesis is that the minus-end of the new MT remains statically associated with the γ-TuRC for a short time until it is severed by the MT-severing protein katanin (Wasteneys, 2002; Murata et al., 2005).

MOR1 is an MT-associated protein with the ability to alter several dynamic instability parameters, including increasing both shrinking and growing velocities. It is a homologue of XMAP215, which has MT polymerase activity in vitro (Brouhard et al., 2008). Altering these parameters has a dramatic effect on the cortical MT array. The temperature-sensitive mutant mor1-I has an organized array at permissive temperature 21°C, whereas the dynamic instability parameters are modified significantly at 31°C and the MTs become short and disorganized (Whittington et al., 2001; Kawamura and Wasteneys, 2008). Strong association of cortical MTs to the cortex is essential for a properly organized array (Dhonukshe et al., 2003; Ambrose and Wasteneys, 2008). Anchoring is believed to involve phospholipase D (Dhonukshe et al., 2003) and the CLASP protein (Ambrose and Wasteneys, 2008). Inhibiting or perturbing either of these perturbs the MT array organization. In particular, in the clasp-1 mutant in which CLASP transcripts are not present, anchor density is decreased and the distance between anchors is increased (Ambrose and Wasteneys, 2008). The free, unanchored length at the plus-end of a growing MT seems to entrain more readily in these mutants, as the free end can explore more space and be entrained with less curvature. Furthermore, the clasp-1 mutant’s array is more highly ordered (that is, with fewer deviations from the dominant orientation) than the wild-type array.

It has been widely hypothesized (Dixit and Cyr, 2004; Ehrhardt and Shaw, 2006; Wasteneys and Ambrose, 2009) that the MT–MT interactions outlined above can lead to the formation of an ordered array, where the majority of MTs point in the transverse direction relative to the major growth axis. However, this hypothesis remains to be tested theoretically. What MT–MT interactions are sufficient to lead to an organized array, and how long would this self-organization take to emerge? What are the relative importance of various aspects, such as the density and length of MTs? Computer simulations can address these questions in a way that experiments cannot. In a recent study simulating a similar system, by Baulin et al. (2007), a dominant direction indeed emerged. In their caricature of the plant system, each MT is constantly growing at one end and shrinking at the other end (at a slower rate) unless it encounters a barrier MT, in which case it pauses until the barrier MT has treadmilled out of its way. In a more closely related study by Dixit and Cyr (2004), simulations were carried out including collision-induced catastrophe (CIC) as well as plus-end entrainment (which they refer to as zippering). However, given the computational difficulty of the problem, the authors were only able to consider at most 20 MTs for 10 min, and a statistically meaningful interpretation is difficult to extract from their results.

Here, we present a computational model of cortical MTs in plants. We simulate several thousands of MTs over time scales of minutes to hundreds of minutes, including the effects of CIC, plus-end entrainment and MT-dependent nucleation. We explicitly model the mor1-I and the clasp-1 mutants of Arabidopsis thaliana and find agreement with experiments for mor1-I, but not clasp-1. Our results illustrate assumptions under which an ordered array will emerge, and assumptions under which it does not.

MATERIALS AND METHODS

Ambrose and Wasteneys (2008) and Shaw et al. (2003) report cortical MTs switching spontaneously between growth (g), pause (p), and shrinkage (s). This three-state dynamic instability model thus involves eight parameters: six transition rates between the states fij, where ij = g,p,s, and growth and shrinkage velocities vg and vs. As a simplification of this three-state model, a two-state dynamical instability model involving four parameters has been studied previously (Rubin, 1988; Dogterom and Leibler, 1993) and used extensively in cell biology (Grill and Hyman, 2005; Wollman et al., 2005; Gardner et al., 2008). The mean length (Dogterom and Leibler, 1993) and mean lifetime (Rubin, 1988) of an MT in the two-state model depend on a threshold quantity f = fgs + vs. If the quantity is positive, the MTs tend to shrink more than they grow, and the MTs will have a finite mean length and mean lifetime. Otherwise, on average, they tend to grow forever. For the three-state case, we consider an equivalent expansion in the Supplemental Material. There is an equivalent threshold quantity that determines if the MTs tend to remain finite or grow indefinitely. Note that these simplified models only consider dynamic instability: they are only valid in the absence of interactions between the MTs and any growth boundaries, and in the abundance of free tubulin. Thus, the mean length and mean lifetime should be thought of as characteristic scales that are perturbed by MT–MT interactions and the action of MAPs. Tables 1 and 2 summarize parameters from the literature (Shaw et al., 2003; Dixit and Cyr, 2004; Kawamura and Wasteneys, 2008) that we use in this article.

We assume the minus-end is either always static, or continuously shrinking with constant rate vs. Shaw et al. (2003) report MT minus-ends spending 25.3% of the time shrinking at, on average, 2.78 μm/min, and 8.4% of the time growing at 1.96 μm/min, and the remaining time (66.3%) paused. Thus, for instances in which we assume minus-ends shrink, we use an appropriately weighted average of these data: vs = 0.084 (1.96 μm/min) + 0.663 (0) = 0.53 μm/min.

When two MTs collide, the outcome depends on the angle between the incident and barrier MTs (Wasteneys and Ambrose, 2009), which we call the collision angle θ. We define the critical entrainment angle θc as follows. If θc < θp, the collision is steep and catastrophe occurs with probability pcat otherwise the incident MT crosses over the barrier MT (with probability 1 − pcat). In A. italiana, 9% of stepping MTs collide at the epidermal cells and 25% in leaf pavement cells (Wightman and Turner, 2007), whereas in tobacco BY2 cells, catastrophe results 60% of the time (Dixit and Cyr, 2004). In these studies, the angles 45° and 40°, respectively, were found to delineate the transition between entrainment or catastrophe. If θp < θc, the collision is shallow and plus-end entrainment occurs with probability pentr. After an entrainment event, the extent segment of the incident MT remains in its precollision configuration, but the plus-end continues to grow. Thus, the MT is reconnected to a line segment with a kink. In this article, we assume this phenomenological description of entrainment, neglecting fine-grain biophysical properties of the kink. The segment from the kink to the plus-end of the entrained MT is kept
a distance of $\delta = 25$ nm from the barrier MT in agreement with electron microscopy of cross-linking due to MAP65 (Chan et al., 1999).

We consider two modes of MT nucleation. The first is independent of the extent MT array. MTs of zero initial length and uniform random orientation are inserted randomly into the cortex, at rate $k_0$, in micrometers $^{-2}$ minute $^{-1}$. The second is MT-dependent nucleation, where new MTs are nucleated off extant MTs. The rate of MT-dependent nucleation will depend upon both the length of existing polymer and the number of available $\gamma$-TuRC in the cytoplasm (Murata et al., 2005). However, we assume the $\gamma$-TuRC is rate limiting and thus MT-dependent nucleation occurs at a constant rate $k_1$ in minutes $^{-1}$. Once the new MT is nucleated, its plus-end immediately begins dynamic instability, and if $\tau > 0$, the minus-end immediately begins shrinking. In reality, there is likely to be a delay before katanin severs the minus-end (Sedbrook and Kaloriti, 2008), but because an actual lag time is unknown, we consider only the effects of dynamic instability, they should be thought of as characteristic scales that are perturbed by MT–MT interactions and the action of MAPs.

### Table 1. Dynamic instability parameters from three-state models using data from Kawamura and Wasteneys (2008) for wild type (WT) and the mor1-1 mutant and Shaw et al. (2003), and two-state models using data from Dixit and Cyr (2004)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{sp}$</td>
<td>0.20</td>
<td>0.380</td>
</tr>
<tr>
<td>$f_{ps}$</td>
<td>0.17</td>
<td>1.590</td>
</tr>
<tr>
<td>$f_{pt}$</td>
<td>2.01</td>
<td>1.400</td>
</tr>
<tr>
<td>$f_{w}$</td>
<td>1.02</td>
<td>0.700</td>
</tr>
<tr>
<td>$f_{p}$</td>
<td>1.00</td>
<td>1.990</td>
</tr>
<tr>
<td>$v_p$</td>
<td>0.31</td>
<td>0.440</td>
</tr>
<tr>
<td>$v_g$</td>
<td>3.50</td>
<td>6.500</td>
</tr>
<tr>
<td>$v_f$</td>
<td>9.00</td>
<td>12.000</td>
</tr>
</tbody>
</table>

### Table 2. Parameters used in the model in addition to the dynamic instability parameters in Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_c$</td>
<td>Critical entrainment angle</td>
<td>40°, 60°</td>
</tr>
<tr>
<td>$\theta_{bac}$</td>
<td>Branched nucleation angle</td>
<td>40°</td>
</tr>
<tr>
<td>$k_0$</td>
<td>Background nucleation rate</td>
<td>10 $\mu$m $^{-2}$ min $^{-1}$</td>
</tr>
<tr>
<td>$k_1$</td>
<td>MT-dependent nucleation rate</td>
<td>10$^3$ min $^{-1}$</td>
</tr>
<tr>
<td>$p_{cat}$</td>
<td>Probability of catastrophe upon steep collision</td>
<td>0.09–0.6</td>
</tr>
<tr>
<td>$p_{sp}$</td>
<td>Probability of entrainment upon shallow collision</td>
<td>$\sim$1</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Spacing between bundled MTs</td>
<td>25 nm</td>
</tr>
</tbody>
</table>
parameter sets taken from Kawamura and Wasteneys (2008) for plus-end dynamics and the average $v_m^p$ from Shaw et al. (2003). In Figure 2, A–C, a single dominant direction is evident with patches of deviation present. The dominant direction (red arrows) is uniformly random (data not shown) across simulations and persists for at least $10^3$ min.

The time course of some of these simulations are shown in Supplemental Movies S3–S7. Initially, several locally ordered domains emerge, grow and shrink (but never rotate, as reported in Chan et al. (2007)). By $10^3$ min, typically a single orientation dominates. However, sometimes the cortex is divided into two domains with distinct dominant orientations. These directions were never observed to differ by more than the critical entrainment angle $\theta_c$. It remains possible that one of these domains becomes globally dominant on time scales much larger than $10^3$ min.

In Figure 3, A and B, we show time series from 10 runs with wild-type 31°C parameters (blue curves). The mean lengths increase slowly. After $\sim 10^2$ min, in each simulation, the mean MT length converges to a value below $\bar{L}$, the predicted mean length in the absence of interaction (Table 1 and Eq. 8 in Supplemental Material). For comparison, single cortical MTs have been reported to be 2–4 $\mu$m when measured by transmission electron microscopy (Hardham, 1978). The number of MTs converges quickly to roughly $10^3$ (data not shown). Note that the steady-state number of MTs depended on $k_w$ and we chose $k_w = 10 \mu$m$^{-2}$ min$^{-1}$ to give $\sim 10^3$ MTs.

Using $S$ as our measure of order, we conclude that plus-end entrainment does give rise to order. This order emerges with a time scale $<10^2$ min (Figure 3B), in agreement with the observed time-to-order in vivo (Wasteneys and Williamson, 1989a). The full orientational distribution is shown in Figure 3C. Notably, without CIC in the simulations (i.e., $p_{cat} = 0$) ordering still emerged, see Figure 5A. Ordering also occurred using parameters from Shaw et al. (2003) and for the two-state model using parameters from Dixit and Cyr (2004) (data not shown).

With Kinetic Parameters Taken from the mor1-1 Mutant at 31°C, Ordered Arrays Do Not Form

In simulations of the mor1-1 mutant at 31°C, the MTs were short and therefore much lower in total polymer density. From Figure 4A, we see their average length is 0.5 $\mu$m, roughly one sixth of their mean free length in the absence of interactions. Reducing the nucleation rate increased their mean length slightly but still did not allow for ordering.

Static Minus-Ends Delay, but Do Not Prevent, Array Organization

Although three-state dynamic instability of MT plus-ends has been reported extensively (Shaw et al., 2003; Dixit and Cyr, 2004; Kawamura and Wasteneys, 2008), the hybrid treadmilling has been reported less often (Shaw et al., 2003). To explore the consequences of a freely depolymerizing minus-end, we ran simulations with static minus-ends ($v_m^m = 0$).

A typical array arising from $v_m^m = 0$ is qualitatively similar to the arrays in Figure 2B, the equivalent runs with $v_m^m = 0.53 \mu$m/min. The order parameter $S$ after $10^3$ min is, on average, also comparable (0.9 and 0.8 for $v_m^m = 0.53 \mu$m/min and $v_m^m = 0$, respectively). However, static minus-ends seemed to delay the onset of self-organization. In Figure 3B, we show the order parameter’s time evolution for both $v_m^m = 0.53 \mu$m/min (blue curves) and $v_m^m = 0$ (green curves). Although the

shown in Figure 1A. For high values of $p_{cat} = 0.9$ (shown in the figure), the MTs were too short in length and lifetime for orientation to emerge, whereas for low values of $p_{cat} = 0.1$, they simply did not interact enough. Intermediate values of $p_{cat}$ also failed to organize. However, Baulin et al. (2007) report that pause-inducing collisions alone are enough to give rise to an oriented array, a result we confirm with our simulations (see Figure 1B and Supplemental Movies S1 and S2). In the Supplemental Material, we show that the pause-inducing collision model is a limiting case of the catastrophe-inducing collision model. After conducting a random sweep of $10^3$ kinetic parameter sets, we conclude that CIC only leads to self-organization in the limit where the shrinkage rate and catastrophe rate are approximately 0 ($v_m^p f_{gs} \approx 0$) and the rescue rate is much larger than the catastrophe rate ($f_{gs} \gg f_{gs}$), consistent with Baulin et al. (2007).

Plus-End Entrainment, with or without CIC, Results in an Ordered Array

Simulations that include entrainment gave rise to significant order parameters within the first 60 min. In Figure 2, we display snapshots from the simulations for the four kinetic

![Figure 1. Simulation snapshots at t = 60 min with collision-induced catastrophe only, using parameters from wild type at 31°C (Kawamura and Wasteneys 2008) (A) and collision-induced pauses, using the single-state model of Baulin et al. (2007) (B).](image-url)
simulation with a shrinking minus-end has reached its well-ordered steady-state (within 10% of its steady-state order parameter $S$) within 20 min, it takes the simulation with static minus-ends roughly 80 min, which is 4 times longer.

**Static Minus-Ends Allow “mor1-1” to Self-Organize**

The dynamics of the minus-ends affect the average length of MTs (Table 1) in that if minus-ends are static, the MTs grow slightly longer. In simulations with kinetic parameters from the *mor1-1* mutant at 31°C, we find that static minus-ends induce a change sufficient to allow for ordering. Time series data for this kinetic parameter set are shown in Figure 4, A and B. The order parameter in Figure 4B shows that the mutant with a depolymerizing minus-end does not organize (blue curve). With static minus ends, organization is rescued (green curve). In this case, however, self-organization still takes longer than in simulations of wild-type plants with nonstatic minus ends.

**Increasing the Critical Entrainment Angle Does Not Enhance, but Rather Delays, Array Organization**

MTs in the *clasp-1* mutant described by Ambrose and Wasteneys (2008) entrain over a wider range of incident angles, with a mean entrainment angle roughly 11° larger than in wild type and demonstrate “hyperparallel” arrays, indicated by a smaller SD of MT orientations. To test whether a higher critical entrainment angle can explain the hyperparallel arrays, we ran simulations in which we increased the critical entrainment angle from $\theta_z = 40°$ to $\theta_z = 60°$, which is equivalent to increasing the mean entrainment angle by 10°.

Qualitatively, the resulting arrays seem indistinguishable from the corresponding array in Figure 1 (see Supplemental

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**Figure 2.** Collision-induced catastrophe at steep angles ($>40°$) and entrainment at shallow angles ($<40°$) for four sets of kinetic parameters from Kawamura and Wasteneys (2008) and continuously depolymerizing minus-end (Shaw et al., 2003). The top (A and B) and bottom (C and D) rows are wild-type and *mor1-1* kinetic parameters, respectively, and the left (A and C) and right (B and D) are at 21 and 31°C, respectively. New MTs are inserted randomly at a rate of $k_0 = 10 \mu m^{-2} \text{min}^{-1}$. The boundaries are periodic in both directions. After 60 min, order emerges in local domains in all cases except *mor1-1* at 31°C. The direction of the red arrow indicates the dominant direction of the MT array, whereas their lengths are proportional to the order parameter.
Movie S8). However, examining the time course of the order parameter $S$ reveals that the increased $\theta_2$ delays and slightly reduces array ordering, similar to the case of static minus-ends. The time series of ordering is shown in red in Figure 3B, with a typical angle distribution in Figure 3E. This suggests that the \textit{clasp-1} hyperparallel MT phenotype is dependent on another mechanism.

\textbf{MT-dependent Branched Nucleation Leads to Unrealistic Array Structures}

Up to this point, all MT nucleation has been assumed to occur uniformly in space and at random angles, referred to here as background nucleation. To explore the reports of MT-dependent branched nucleation, we ran simulations...
without background nucleation ($k_0 = 0$) and nonzero MT-dependent nucleation rate $k_1 = 10^3$ min$^{-1}$.

These simulations always resulted in arrays with a distinct structure reminiscent of shattered glass. As with background nucleation, one or a few dominant angles emerged locally at early times, the domains grew or shrank, and often one direction dominated globally. However, the arrays were always sparse with large gaps free of persisting MTs. A typical snapshot is shown in Figure 5C. Time courses of typical simulations are shown in Supplemental Movies S9 and S10.

Although MT-dependent branch nucleation alone leads to an unrealistic array organization, this does not necessarily mean it is not important. We ran simulations with a combination of MT-dependent and MT-independent nucleation. As MT-independent nucleation is reduced, the arrays seemed more and more sparse. A simulation with a combination of nucleation types, $k_1 = 500$ min$^{-1}$ and $k_0 = 5$ min$^{-1}$ $\mu$m$^{-2}$, is shown in Figure 5D.

Catastrophe-inducing Boundaries Are Enough to Bias the Dominant Orientation

Up to now, all simulations described were carried out on a square cortex with periodicity in both directions. That is, an MT that disappears off any edge appears from the opposite edge. When a dominant angle emerges in our simulations, it is uniformly random. In diffusely elongating plant cells, the dominant angle is transverse to the direction of elongation, indicating that there must be a symmetry-breaking mechanism that signals a preferred orientation to the MTs. One candidate for this mechanism is interaction with the apical and basal poles of the plant cell (Ehrhardt and Shaw, 2006).

Real cells have distinct faces. Side walls may allow MTs oriented transverse to the elongation axis to continue growing indefinitely, unperturbed by boundaries between faces. In the longitudinal direction, MTs can treadmill onto the cross walls but rarely do (Collings and Wasteneys, 2005). It has been suggested that the boundaries of the poles inhibit MT growth, either sterically or through MAP activity (Ehrhardt and Shaw, 2006). We represent this interaction by imposing catastrophe on any MT that collides with the top or bottom of the cylinder.

We find that this catastrophe-inducing boundary effect is enough to cause selection of the transverse angle as the dominant orientation. Snapshots from these simulations are shown in Figure 5B. At $t = 60$ min, not all nontransversely oriented patches disappear, yet the dominant transverse angle always persists.

In fact, even in the complete absence of MT–MT interactions, catastrophe-inducing boundaries at the cross-walls will lead to a certain amount of ordering. MTs transverse to the elongation axis can treadmill indefinitely, whereas those parallel to the elongation axis will quickly encounter a boundary. The ordering of MTs is therefore strongest near the cross walls and decays toward the mid-cell over a distance of roughly the mean length in the absence of interaction (see Supplemental Material for details). MT–MT interaction allows this boundary-induced orientation to propagate further into the mid-cell cortex. We also found that the introduction of catastrophe-inducing boundaries does not induce ordering in the CIC-only model described above.
DISCUSSION

Recent genetic and pharmacological experiments on cortical MTs in plants have given rise to a model for the self-organization of these MTs into a parallel array. Here, we have presented the results of large-scale simulations of a quantitative implementation of this model. We find that self-organization into a parallel array can arise from a combination of MT dynamic instability, plus-end entrainment, and, in certain cases, CIC. The arrays that arise in our simulations seem qualitatively similar to the arrays in plant cells, including the local domains of orientation that are similar to the patchwork patterns in maturing Chara cells (Wasteneys et al., 1993) and the outer epidermis of Arabidopsis hypocotyl cells (Chan et al., 2007). In addition to recapitulating wild-type behavior, our model also agrees with mutant studies (Kawamura and Wasteneys, 2008).

It has been proposed that CIC can explain ordering. Our results show that CIC is neither necessary nor sufficient for ordering when physiologically reasonable dynamic instability parameters are used. Previous modeling efforts have focused on the role of CIC in the emergence of order. Dixit and Cyr (2004) showed that CIC in combination with plus-end entrainment leads to ordering. In light of our results, we suggest ordering in their model arises due to entrainment rather than CIC. The model of Baulin et al. (2007) corresponds to a limit in which the growth rate dominates the shrinkage rate, and the rescue frequency dominates the catastrophe frequency. This model fails to self-organize when extended to a regime that matches reported kinetic parameters (Shaw et al., 2003; Dixit and Cyr, 2004; Kawamura and Wasteneys, 2008).

Plus-end entrainment with branched MT-dependent nucleation gives rise to an oriented array that seems sparse because areas of low MT content have no candidate nucleation sites, whereas areas of high MT content have many. From this, we conclude that exclusive MT-dependent branched nucleation leads to unrealistic array structures. Free nucleation seems to be necessary to explain the dispersed arrays seen in vivo. This seems to contradict the hypothesis that branched nucleation helps to disperse the array throughout the cortex (Wasteneys and Ambrose, 2009), but is consistent with the observation that during recovery from drug-induced disassembly, the initial transverse order of freely nucleated MTs is progressively lost when most subsequent MTs are produced by branch-form nucleation (Wasteneys and Williamson, 1989a). Although MT-dependent branch-form nucleation alone leads to unrealistic arrays, this does not suggest that it does not occur. As proposed by Wasteneys and Ambrose (2009), it may be specifically promoted under conditions where it is beneficial to change the predominant orientation of MTs. Recent improvements in live cell imaging has enabled the detection of microtubule-dependent nucleation that is parallel to the parent MT (see figure 1C in Ambrose and Wasteneys 2008) and this alternative form of MT-dependent nucleation might prove to be much more common than previously thought (Wasteneys and Ambrose 2009).

We find that increasing the critical entrainment angle does not enhance the array order. In fact, order is reduced slightly and delayed. This conflicts with recent experiments in which Ambrose and Wasteneys (2008) observed “hyperparallel” arrays in the clasp-1 mutant. This suggests that the CLASP protein affects array organization through more than simply modulating the critical entrainment angle.

Two of the phenomena we neglect in our simulations are increased MT stability through bundling, and severing after crossover. MTs within bundles remain dynamic, however, with slightly modified kinetic parameters (Van Damme et al., 2004). It is unknown whether this effect arises simply through the reduced collision frequency or whether it is important for MT array organization. MT severing at sites of existing crossovers, possibly mediated by katanin, also has been reported (Wightman and Turner, 2007); however, this occurs rarely as severing of elongated MTs is rare (Shaw et al., 2003).

Three novel predictions arise from this work. First, if the transverse dominant direction is selected by catastrophe-inducing boundaries at the top and bottom edges of the cell, then the time to order will increase as the cell length increases, as it takes longer for the signal to propagate inward.

The other two predictions demonstrate the paradoxical influence of static minus ends. First, if minus-ends do not become mobile in wild type (e.g., in a katanin knockout), we predict ordering to take fourfold longer. Second, we predict that a similar perturbation of the mor1-1 31°C mutant rescues ordering.

This last set of predictions illustrates one of the values of computational modeling. The subtle influence of static as opposed to mobile minus-ends, which in one case promotes and in the other inhibits organization, is essentially impossible to tease out without recourse to computational techniques.

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REFERENCES


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Supplemental Material

Three-state MTs considering only dynamic instability

In the absence of interactions, the length distribution of a population can be modeled using a partial differential equation [3]. For the two-state model,

\[
\frac{\partial}{\partial t} \begin{bmatrix} N_g \\ N_s \end{bmatrix} = A \begin{bmatrix} N_g \\ N_s \end{bmatrix} + \frac{\partial}{\partial l} \left( V \begin{bmatrix} N_g \\ N_s \end{bmatrix} \right)
\]

(1)

where \(N_g(l,t)\) and \(N_s(l,t)\) represent the density of growing and shrinking MTs of length \(l\), respectively, and

\[
A = \begin{bmatrix} -f_{gs} & f_{sg} \\ f_{gs} & -f_{sg} \end{bmatrix}, \quad V = \begin{bmatrix} -v_g & 0 \\ 0 & +v_s \end{bmatrix}
\]

(2)

represent transitions between states and advection, respectively. If new MTs are nucleated with zero length and in the growing state at rate \(\tau\), the boundary conditions are \(v_gN_g(0,t) = k\) and \(N_s(l,t) \to 0\) as \(l \to \infty\). This leads to a unique steady-state \(N_i = \alpha_i \exp(-l/\bar{l})\) where

\[
\bar{l} = \frac{v_g v_s}{f_{gs} v_s - f_{sg} v_g}
\]

(3)

as long as the denominator is positive [3]. The mean lifetime can be found by assuming the system is in steady-state, when nucleation must balance a constant death rate \(\tau^{-1}\),

\[
k = \frac{1}{\tau} \int_0^l N_g + N_s\,dl
\]

(4)

where \(\tau\) is the mean lifetime. This gives

\[
\tau = \frac{v_g + v_s}{f_{gs} v_s - f_{sg} v_g}
\]

(5)

in agreement with [4]. For the three-state model, the partial differential equations now involve \(N_i(l,t), i = (g,p,s)\) and the matrices become

\[
A = \begin{bmatrix} -(f_{gp} + f_{ps}) & f_{pg} & f_{pg} \\ f_{pg} & -(f_{pg} + f_{ps}) & f_{pg} \\ f_{gs} & f_{ps} & -(f_{sg} + f_{sp}) \end{bmatrix}
\]

(6)

and

\[
V = \begin{bmatrix} -v_g & 0 & 0 \\ 0 & 0 & 0 \\ 0 & +v_s \end{bmatrix}
\]

(7)

The mean length and mean lifetime can be found as above,

\[
\bar{l} = \frac{v_g v_s (f_{pg} + f_{ps})}{D}
\]

(8)

\[
\tau = \frac{v_s (f_{gp} + f_{pg} + f_{ps}) + v_g (f_{sp} + f_{pg} + f_{ps})}{D}
\]

(9)

where the denominator

\[
D = v_s (f_{gp} f_{ps} + f_{gs} f_{pg} + f_{gs} f_{ps}) - v_g (f_{pg} f_{sg} + f_{pg} f_{sp} + f_{ps} f_{sg})
\]

(10)

is the threshold quantity: if it is negative, the mean length and lifetime are infinite. In both the two-state and three-state models, if the minus-end shrinks at a constant velocity, we make the coordinate transformation

\[
v_g = v^p_g - v^m_g
\]

(11)

\[
v_s = v^p_s + v^m_s
\]

(12)

Relationship between two-state models and Baulin et al. [1]

To understand the difference between catastrophe-inducing collisions and pause-inducing collisions, we consider the two-state model, which has five parameters, \(v^p_g, v^p_s, f_{gs}\) and \(f_{sg}\) which all pertain to the plus-end, and \(v^m_g, v^m_s\), which pertain to the minus-end. In addition, the rate of nucleation, \(k_0\), provides an additional time scale. However, if we rescale time to be measured in units of \(T \equiv (v^p_g)^{-2/3} k_0^{-1/3}\) and length \(L \equiv (v^p_g/k_0)^{1/3}\), then the two-state model is described by four parameters,

\[
\alpha = \frac{v^p_g}{v^p_g}
\]

(13)

\[
\beta = f_{gs} T
\]

(14)

\[
\gamma = \frac{1}{f_{sg} T}
\]

(15)

\[
\delta = \frac{v^m_s}{v^p_s}
\]

(16)

(Note that scaling by \(\bar{l}\) and \(\tau\) is not appropriate here, since we are sometimes in the infinite-growth regime.)

To ensure MT nucleation can occur, \(\delta < 1\). In this parametrization, the model of Baulin et al. [1] corresponds to \(\alpha, \beta, \gamma \to 0\) and it completely described by one parameter, \(\delta\) (related to their \(\alpha\), which they set in \([0.17, 1.5]\)). The two-state parameters reported in [2] (Table 1) give \(\alpha = 1.8, \beta = 0.16, \gamma = 3.1\) and either \(\delta = 0\) (since they did not study minus-end dynamics) or \(\gamma = 0.09\) (using \(v^m_g\) from [5]).
Effects of a catastrophe-inducing boundary in the absence of MT-MT interactions

Even in the absence of any MT-MT interactions, MTs randomly nucleated on a cylindrical cortex can lead to a transverse ordering if collisions with the boundaries induce catastrophe. A MT plus-end a distance \( y \) from the boundary making an angle \( \theta \), measured from transverse the axis of the cylinder, can grow to a maximum length \( L = (L_C - y)/\sin \theta \) where \( L_C \) is the cell length. In this case, the right boundary condition on the system of partial differential equations in Eq. 1 is \( v_s N_s(L,t) = v_g N_g(L,t) \). The solution is still exponential with decay length \( \bar{l} \) but is truncated. The average length of MTs of angle \( \theta \) at height \( y \) is

\[
\langle l \rangle \propto \left(1 - e^{-y/(\bar{l} \sin \theta)}\right) \left(1 - e^{-((L_C-y)/\bar{l} \sin \theta)}\right).
\]  

From this it is straightforward to compute the order parameter \( S \). We can also compute a local order parameter \( S(y) \) that takes into account all MTs passing through a given \( y \) value (a given circumference of the cylinder). Although \( S(y) \) has no closed form, it can be computed numerically. We find that this boundary-induced ordering decays away from the boundaries towards midcell, with a decay length scale of roughly \( \bar{l} \) (data not shown).