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ABSTRACT

There is rising interest in modeling the noncentrosomal cortical microtubule cytoskeleton of plant cells, particularly its organization into ordered arrays and the mechanisms that facilitate this organization. In this review, we discuss quantitative models of this highly complex and dynamic structure both at a *cellular* and *molecular* level. We report differences in methodologies and assumptions of different models as well as their controversial results. Our review provides insights for future studies to resolve these controversies, in addition to underlining the common results between various models. We also highlight the need to compare the results from simulation and mathematical models with quantitative data from biological experiments in order to test the validity of the models and to further improve them. It is our hope that this review will serve to provide guidelines for how to combine quantitative and experimental techniques to develop higher-level models of the plant cytoskeleton in the future.

INTRODUCTION

In land plants, the interphase microtubules that associate with the plasma membrane along their lengths form wonderfully ordered arrays in the complete absence of a discrete microtubule organizing entity like the centrosome of animal cells (Figure 1A). These so-called cortical microtubules (CMTs) act as a scaffold for the directional deposition of cell wall material, which defines the axis of cell expansion and thus plant growth and development [Lucas and Shaw, 2008; Szymanski and Cosgrove, 2009]. To perform this morphogenetic function, the CMTs must organize themselves into appropriate arrays. In the absence of a central control mechanism, a self-organizational mechanism driven by the dynamics and interactions of CMTs has been proposed to play a major role in shaping CMT arrays [Dixit and Cyr, 2004a; Ehrhardt and Shaw, 2006; Wasteneys, 2002; Wasteneys and Ambrose, 2009]. To test this hypothesis, many researchers have developed computer simulation and mathematical models of CMTs based on the experimentally observed behavior of CMTs in living cells. The purpose of this review is to compare and contrast the various quantitative models of CMTs, to discuss what we have learned so far and to outline future challenges.

Properties of CMTs

The CMT arrays consist of relatively short microtubules (typically about 5-10 μm) that overlap in a staggered manner to create superstructures of cellular dimensions [Barton et al., 2008; Hardham and Gunning, 1978]. The linear arrangement of the CMTs is similar to the noncentrosomal microtubule arrays from other systems such as fission yeast and differentiated animal cells like neurons and myotubes [Bartolini and Gundersen, 2006; Keating and Borisy, 1999]. The CMT array is a highly dynamic structure. Individual CMTs turnover in a period of minutes but the overall morphology of the CMT array nonetheless persists stably over time [Hush et al., 1994; Shaw et al., 2003; Wasteneys et al., 1993]. How such a dispersed and dynamic CMT population becomes organized into particular spatial patterns has been a long-standing question.

Live-cell imaging has revealed many of the fundamental properties of CMTs, which has laid the foundation for a mechanistic understanding of CMT organization. Here, we briefly describe the basic dynamics and interactions of CMTs to provide a framework for reviewing the available

quantitative models of CMTs. CMTs originate from γ-tubulin-containing nucleation complexes that are scattered throughout the cell cortex [Erhardt et al., 2002; Liu et al., 1993; Nakamura et al., 2010; Seltzer et al., 2007]. Some of these nucleation complexes associate with the lateral walls of preexisting CMTs, resulting in branch-form microtubule nucleation [Chan et al., 2009; Murata et al., 2005; Nakamura et al., 2010; Wasteneys and Williamson, 1989a]. The CMTs are released from their nucleation sites by the activity of microtubule-severing enzymes [Nakamura et al., 2010; Nakamura and Hashimoto, 2009]. As a result, both CMT ends are free and CMTs show treadmilling-type polymer behavior due to net growth at the plus-ends and net shortening from the minus-ends [Shaw et al., 2003]. CMTs are typically tightly attached to the plasma membrane along their length, which confines them to the two-dimensional surface of the plasma membrane rather than being distributed in three-dimensional volume of the cytoplasm. The plasma membrane anchoring of treadmilling CMTs is important because it results in frequent encounters between them.

Encounters between CMTs have been observed to lead to different outcomes depending on the encounter angle [Dixit and Cyr, 2004b]. In particular, if the encounter angle is shallow (< 40º), then the plus-end of the encountering CMT almost invariably reorients and grows along the impeding CMT, resulting in CMT bundling. If the encounter angle is steep (> 40º), then the encountering CMT will either cross over unimpeded or start to depolymerize (called collision-induced depolymerization). More recently, CMTs have also been observed to be severed at certain crossover junctions followed by depolymerization from the newly created plus-end of the cut CMT [Wightman and Turner, 2007]. These properties of CMTs are consistent with a self-organizing system in which numerous dynamic, interacting parts become organized based on certain rules of interactions. A self-organization model for CMT array patterning envisions that CMT bundling directly fosters parallel CMT arrangement, whereas severing of CMTs at crossover junctions and collision-induced depolymerization of CMTs are envisioned to selectively eliminate discordant CMTs, thus maintaining parallelism (Figure 1B). This model is consistent with the observation that CMTs in cells start out in a disorganized fashion and progressively gain order over time [Dixit et al., 2006; Granger and Cyr, 2001; Wasteneys and Williamson, 1989a; Wasteneys and Williamson, 1989b; Yuan et al., 1994]. Note that this simple model explains how CMT parallelism is generated, but it does not explain how the CMT array as

a whole becomes oriented in a particular direction. Clearly, additional rules are necessary to orient the CMT array towards a particular direction.

In a cell, the frequency and outcomes of CMT interactions are governed by various microtubule-associated proteins (MAPs) that mediate CMT nucleation, polymer dynamics, bundling and severing [Wasteneys and Ambrose, 2009]. Thus, the activity of these MAPs in space and time are key control points for CMT organization. Indeed, there is ample genetic evidence which points to MAPs as key regulators of the CMT array. High-resolution, live-cell imaging of CMTs in these mutants is beginning to offer a mechanistic understanding of how the various MAPs contribute to CMT array organization. However, an integrated picture of how multiple MAP activities together contribute to CMT array organization is lacking.

The need for quantitative models of CMTs

All models are wrong, but some are useful. – George E.P. Box

As discussed above, the plant CMT cytoskeleton is a complex, dynamic and highly interactive system. Both the dynamics of individual CMTs as well as the interactions between multiple CMTs are governed by stochastic rules and processes. In addition, the interactions between CMTs are highly distributed and their impact on the angular orientation and lifetime of individual CMTs is likely to be nonlinear with respect to the encounter angle and number of microtubules. These characteristics make it difficult, if not impossible, to intuitively predict the behavior of the overall system starting with a particular recipe of participants.

Quantitative models are needed to understand the workings of such a complex system because they boil the system down to key components and the major driving forces. Quantitative models of CMTs are therefore simplifications, but this does not necessarily invalidate them. Rather quantitative models are powerful heuristic tools that help us rapidly evaluate the range of outcomes or solutions for a given set of conditions and identify plausible mechanisms for CMT organization. Quantitative models can be used to compare the relative impact of different perturbations to the CMT system (e.g., by conducting sensitivity analyses) to identify the key driving factors. In addition, quantitative models can reveal if we are missing some key

information and predict unanticipated behaviors in response to perturbations, thus spurring new research to address these issues.

Another major advantage of quantitative modeling is that it can help us understand the CMT array from a holistic viewpoint. Traditional biological investigations are reductionist in nature and provide a detailed understanding of individual components such as the various MAPs that regulate the CMT array. However, a reductionist approach by itself does not suffice to understand how multiple MAPs and other factors work together to build a functional CMT array. Quantitative models can take the information about individual components obtained from reductionist studies and integrate it to study the behavior of the CMT system as a whole.

To develop quantitative models of a particular system, one needs a quantitative understanding of the system parameters. Work from many laboratories has generated a rich set of measurements that describe the dynamic behavior of individual CMTs (e.g., the dynamic instability parameters of the plus and minus ends of CMTs) as well as spatial and temporal features of the CMT array as a whole (e.g., net polarity of the CMT array and time to array organization). Furthermore, such measurements are available for both wild-type plants and various mutants with defective CMT organization. Of course, many properties of CMTs and their arrays remain to be measured (and even to be discovered). As new measurements of the CMT system become available, they will need to be incorporated in the quantitative models. In this way, the models need to be constantly refined as our understanding of the CMT array improves. In turn, the outputs of the models will generate new hypotheses that will lead to new experiments/measurements that will serve to further improve the model. The goal of this iterative activity is to develop models that mimic reality as closely as possible and hence provide meaningful insight into the mechanisms underpinning CMT organization.

Quantitative modeling, in the form of computer simulations and mathematical models, is fast becoming an indispensable tool for investigating cellular processes [Drubin and Oster, 2010]. Like the traditional tool sets of biochemistry, genetics and cell biology, quantitative modeling has its own set of advantages and disadvantages which must be carefully considered before formulating models of a particular process. A detailed discussion of the various types of

quantitative models and their advantages and disadvantages is out of the scope of this review and we refer the reader to several excellent papers on this topic [Mogilner et al., 2006; Schilstra et al., 2008]. In general, while adopting quantitative models, there are certain trade-offs that need to be considered in terms of modeling assumptions, types of relations needed between input parameters and output measures, as well as computational time. Simulation models can replicate real systems in great detail but require longer computational time compared to mathematical models. Mathematical models often rely on stronger assumptions incorporating fewer details to be able to replicate the system in terms of mathematical equations such as differential, difference and integro-differential equations. Therefore, they generate stronger and more general results in terms of the relationships between input parameters and output measures. Mathematical models also readily give insight into the scaling behavior of system parameters. A combination of both techniques can be used to balance these aspects according to the characteristics of the specific system on hand.

MODELING APPROACHES AND RESULTS

Most of the quantitative models developed so far focus on understanding how particular CMT arrays are generated starting from a randomly oriented CMT population and focus on questions such as:

- Are simple dynamics and interactions between CMTs sufficient to result in organization? How does this self-organization occur? What are the necessary conditions for emergence of ordered CMT arrays?
- What are the effects of altering dynamics, interactions, cell boundaries and other properties on the CMT self-organization? What are the relative contributions of these different mechanisms on organization?

Recently, another line of research has focused on modeling molecular and mechanical behavior of CMTs to understand the mechanisms that govern their interactions as well as their individual tendencies for orientation based on cell geometry. In other words, these models delve into the details of events that are induced by interactions of CMTs with other CMTs and the constraints

imposed by the space that they are confined in. More specifically, the objective of such studies might be answering one or more of the following questions:

- How do interactions such as CMT bundling and collision-induced depolymerization occur?
- Why are those events dependent on the encounter angle?
- How does the anchoring of CMTs to the cell cortex occur and what are its effects on CMT interactions?
- What is the effect of the constraints posed by space and geometry on CMTs?

In this review, we refer to the first class of models as *organization-oriented* models and the second class of models as *interaction-oriented* models. We begin by reviewing organization-oriented models leaving interaction-oriented studies for later discussion.

CMT ORGANIZATION-ORIENTED MODELS

Both simulation and mathematical models have been developed to study CMT self-organization emerging due to the interactions in the system. To the best of our knowledge, the first published attempt to model CMT organization computationally is by Dixit and Cyr, 2004. They developed a Monte Carlo simulation with a limited number of CMTs in the system where the nucleation process is not considered. Their simulations show that simple rules for CMT interactions extracted from real-cell experiments can result in a parallel CMT arrangement from a randomly arranged population [Dixit and Cyr, 2004b]. They found that both bundling and collision-induced depolymerization are necessary and sufficient for CMT organization, although this conclusion might be related to the restricted size of the simulations, as stated by the authors. Indeed, more complex models distinguish between the relative significance of those two mechanisms as discussed below. Baulin et al., 2007 simulate a CMT system where they incorporate CMT nucleation and assume simple deterministic dynamics based on average CMT velocities of both plus and minus ends. They consider stalling as the only interaction mechanism, where a CMT encountering another one remains static as long as it is blocked by the barrier CMT and resumes its growth as soon as the blocking is over. Stalling is different from pausing

that is part of the normal stochastic dynamic behavior of CMTs, where the end of a CMT appears to neither grow nor shorten for a certain duration irrespective of whether the CMT has encountered another one. The simulations of Baulin et al., 2007 show that even these overly-simplified CMT dynamics and interaction mechanisms are enough to achieve parallel CMT organization [Baulin et al., 2007]. However, their model is limited in the sense that only growth-prone dynamics can be studied due to exclusion of dynamic instability. Shi and Ma, 2010 simulated CMT organization using a similar CMT interaction mechanism with stalling— that they call steric interactions— with dynamic instability modeled at both ends according to a GTP-cap model [Margolin et al., 2006]. They particularly focus on the effect of dynamicity parameters on self-organization, by scanning a wide range of parameters to locate points of transition between ordered and disordered CMT array states [Shi and Ma, 2010].

Recently, simulation models of CMTs have striven to better capture the polymerization dynamics and interactions of CMTs as they exist in plant cells. Tindemans et al., 2010 consider a two-state dynamic instability simulation together with bundling and collision-induced depolymerization interactions, complementing their mathematical model in Hawkins et al., 2010. According to the Tindemans et al simulation model, a CMT plus end is either growing or shortening at anytime, whereas the minus end is always static. Allard et al., 2010b consider a three-state dynamic instability model by incorporating the possibility of pausing at the plus end. They also capture the treadmilling mechanism by assuming that the minus end continuously shortens with an average velocity calculated according to data from real cell experiments. In Eren et al., 2010, stochastic dynamic behavior of the minus-end is considered as well as that of the plus-end. In their model, a CMT plus end stochastically switches between growth, shortening and pause states; whereas the minus end alternates between shortening and pause states. The authors also model the growing and shortening velocities as normally distributed random variables with parameters in line with the data from real cell experiments. Table 1 summarizes the modeling assumptions for the available organization-oriented models. Note that different scenarios might be tested using each model, however we roughly list the assumptions that correspond to the wild-type plant scenario for each study.

In addition to computer simulations, mathematical models have also been used to study CMT organization. In general, the mathematical models of CMT organization complement the simulation models in terms of analysis and results. In the mathematical model of Baulin et al., 2007, the impact of interactions (that result in CMT stalling) are approximated inspired by the kinetic theory of gases based on the average length, velocity and density of CMTs in the system, and accounting for the fact that CMTs with similar angles run into each other less frequently. Hawkins et al., 2010 develop a stronger mathematical model that considers CMT bundling and collision-induced depolymerization. They use this model to investigate parameter regions where CMT array organization is possible. In a subsequent study, the authors show that the predictions of this mathematical model agree well with their simulations, although the performance deteriorates for simulations that include bundling [Tindemans et al., 2010]. Similarly, Shi and Ma, 2010 combined both mathematical and simulation models to generate a phase diagram that relates regions of CMT dynamics and density parameters to array organization, although their CMT interaction mechanism is quite simplistic, similar to Baulin et al., 2007.

Relative contribution of bundling vs. catastrophe-inducing collisions

The necessity of CMT interactions for parallel array organization is commonly agreed upon among different modeling studies [Allard et al., 2010b; Baulin et al., 2007; Dixit and Cyr, 2004b; Eren et al., 2010; Hawkins et al., 2010; Tindemans et al., 2010]. However, among the studies that consider both bundling and collision-induced depolymerization interactions, there are controversial results regarding the relative contribution of these two mechanisms. Tindemans et al., 2010 conclude that collision-induced depolymerization is sufficient to induce organization even in the absence of bundling, in line with their mathematical model in Hawkins et al., 2010. In their model, bundling has only a minor contribution on organization. In contrast, Allard et al., 2010b find bundling as the main contributor of organization and conclude that collision-induced depolymerization is neither necessary nor sufficient to organize CMTs into parallel arrays. Eren et al., 2010 also show that bundling has a more significant contribution on CMT organization compared to collision-induced depolymerization, although in their simulations both mechanisms operating together resulted in better ordered arrays than either mechanism alone.

This controversy might be due to different choice of dynamicity parameters and assumptions, which is not addressed thoroughly in any of the studies. The simulation model of Tindemans et al., 2010 considers the region of bounded growth only, where CMTs have finite length even in the absence of interactions. In addition, both their mathematical and simulation models assume that CMT minus ends are static. It is possible that the impact of bundling on array organization is underestimated due to a combination of these factors. Specifically, a static minus end assumption would be expected to disfavor the bundling mechanism as it does not allow depolymerization of segments of CMTs that are not bundled [Tindemans et al., 2010]. In addition, shortening-prone plus-end CMT dynamics in such a setting would hypothetically make the bundling process pretty much reversible, thus reducing its contribution to array organization. In contrast, Allard et al., 2010b might be overlooking the indirect effect of collision-induced depolymerization on organization by regulating the CMT density in the system especially for the unbounded growth dynamics. Although their inputs seem to include both growth-prone and shortening-prone dynamicity parameters, the mean CMT length seems to stay bounded in all simulations that they present. They conclude that an extensive random sweep of dynamicity parameters shows that collision-induced depolymerization is only effective in the limit where the shortening rate and catastrophe rate are approximately zero and the rescue rate is much larger than the catastrophe rate, which approximately corresponds to the model developed by Baulin et al., 2007. However, in Eren et al., 2010 the authors show that collision-induced depolymerization results in a certain amount of parallel CMT organization even though it is much less pronounced than the organization seen with bundling alone. Their simulations also show that the CMTs become much longer and crowded in the absence of interactions, suggesting that collision-induced depolymerization helps stabilize the system by keeping the CMT length and number at a controlled level, thus indirectly facilitating organization. Nonetheless, this prediction also relies on a limited parameter set and requires more thorough analysis to capture counter-effects of different mechanisms and assumptions.

Effect of dynamicity parameters on organization

Shi and Ma, 2010 is the only study which thoroughly analyzes the effects of dynamicity parameters on CMT organization. They classify the CMT behavior into three phases: isotropic state, where the CMTs are disorganized with roughly uniform orientation, nematic I state where

long CMTs are distributed in a narrow orientation (high level of organization), and nematic II state where short CMTs are distributed in a broad orientation (lower level of organization). They explore the CMT phase behavior at a wide range of dynamicity parameters and find that CMT dynamics has a major impact on array organization. They obtained similar results with their mathematical model. However, as we mentioned earlier, their interaction mechanism includes only stalling behavior, which does not capture the range of CMT interactions that occur in cells. As stated in their paper, self-organization is generated by a competition between CMT interactions and growth dynamics, which emphasizes the need to consider both mechanisms and their counter-effects in as much detail as possible.

In some studies, microtubule length is kept under control (i.e., bounded) by considering only shortening-prone dynamics or setting a maximum value for individual CMT lengths explicitly. Other studies consider growth-prone dynamics where the CMT lengths are allowed to increase unboundedly. To the best of our knowledge, the only study that explicitly considers this issue is the paper by Ambrose et al., 2011, in which the authors state that their results are robust to the dynamicity parameters being either in the bounded or unbounded region. Recently, Mourao et al., 2011, simulated the effects of both microtubule dynamicity parameters and nucleation rate on the mean microtubule length and number of microtubules in a centrosomal system. The authors found that both microtubule dynamics and nucleation parameters contribute to array morphology by affecting the size of the free tubulin subunit pool. Although this model is for centrosomal systems without the interactions seen in the CMT system, it sets a good example for the development of CMT models that incorporate tubulin subunit concentration, nucleation sites and their relation to the dynamicity parameters.

Quantification of CMT array organization

Another reason for discrepancies between the conclusions reached by different models might be related to different metrics used for quantifying CMT organization. Baulin et al., 2007 use a *cost function* that measures the overall proximity to the dominant angle based on the cosine of angle differences. The dominant angle is derived quantitatively by maximizing this cost function. They also introduce an alternative version of this metric where the contribution of each CMT is weighted by its length. Shi and Ma., 2010 employ a method that is based on computation of

eigenvalues of a standard nematic order matrix [Chaikin and Lubensky, 1995]. Hawkins et al., 2010 and Tindemans et al., 2010 employ another nematic liquid crystal order parameter based on the orientation and length densities of CMTs. Allard et al., 2010b uses a modified version of the *cost function* in Baulin et al., 2007, that represents the difference between projected polymer length in the dominant direction and its perpendicular direction. Despite the diversity of methodologies used to measure CMT organization, it is worth noting that Allard, 2010 found that the metrics used by Shi and Ma., 2010, Tindemans et al., 2010, Allard et al., 2010b and Baulin et al., 2007 are equivalent. Finally, Eren et. al., 2010 used a distinct metric based on Shannon's entropy formula [Shannon, 1948] to quantify the diversity level of the angle distributions of CMTs.

All of the available metrics for measuring CMT organization rely on the orientation of the CMTs in the models. These metrics need to be applied to data obtained from real cells to determine if they can robustly distinguish between different stages/types of CMT organization seen in plants. It is also important to note that while CMT orientation is a major aspect of CMT array organization, other features such as the CMT density, length distribution, polarity and extent of bundling are also likely to be important attributes of CMT array organization in real cells. Therefore, additional metrics of CMT organization, which capture these features, will need to be developed in the future.

Effects of boundary conditions on orienting the CMT array

The CMT modeling studies reveal that there is heuristically no need for a complicated system to get parallel arrangement of CMTs. However, these mechanisms fail to explain how cells orient the whole array in a particular orientation. The net orientation of the CMT array in a cell can change depending on developmental and environmental cues. For example, in rapidly elongating cells of the root, the CMT array is typically arranged transverse to the cell elongation axis. When these cells stop elongating, the CMT array is typically longitudinally or obliquely arranged with respect to the long axis of the cell.

One potential mechanism to orient the entire CMT array in the cell is introducing non-periodic boundaries on two opposing edges of the cell. In particular, if a CMT encounters one of those

edges, it switches from growth to shortening. Allard et al., 2010b show that this mechanism of catastrophe-inducing boundaries is sufficient to bias the dominant orientation. They observe that even in the complete absence of CMT interactions, those boundaries lead to a certain amount of ordering near the edges. CMT interactions allow the boundary-induced orientation to propagate further into the center. Eren et al., 2010 developed three-dimensional simulations, where the top and bottom surfaces of cylinders are modeled as catastrophe–inducing boundaries. This scenario consistently results in transverse CMT arrays. These authors also performed control simulations with reflective boundaries that do not trigger CMT shortening but rather let the CMT to continue its growth from the diametrically opposite point of the same end wall. Based on this, they analyzed the effect of CMT interactions without any interference from the boundaries as well as the relative contribution of bundling and collision-induced depolymerization under the two different boundary conditions. Overall, they conclude that having all the mechanisms present results in better organization of CMT arrays.

A recent paper by Ambrose et al., 2011, conducts an extensive study on the effects of different edge behavior induced by CLASP protein on CMT orientation. Using live-cell imaging, they observed that CMTs orient parallel to sharp edges that lack CLASP, as those edges result in catastrophe of the CMTs that run into them [Ambrose et al., 2011]. Based on these observations, they developed a three-dimensional simulation in which they modeled CMTs in polyhedral cells, which better approximates the geometry of plant cells. The effects of CLASP as a regulator of CMT catastrophe were modeled at different cell edges. In addition to employing variable catastrophe probabilities among different edges, they also analyzed non-uniform behavior along an edge, such as permitting passage through only the center. Overall, their simulations show that differential catastrophe-inducing boundaries are sufficient to bias CMT array orientation [Ambrose et al., 2011]. These data provide a molecular mechanism for the establishment of cell edges as either permissive or catastrophe–inducing boundaries based on the localization of CLASP at these edges. Developmental regulation of CLASP localization to certain cell edges provides a potential mechanism to go from unorganized CMTs to transverse CMT arrays and even for remodeling of transverse arrays to longitudinal arrays.

Besides CLASP, another factor that can affect CMT behavior at specific regions of the cell cortex is the *Arabidopsis* MIDD1 protein [Oda et al., 2010]. MIDD1 localizes to particular domains of the plasma membrane in differentiating xylem cells and promotes CMT depolymerization specifically within these domains. Thus, unlike CLASP, the presence of MIDD1 at the cell cortex leads to the selective loss of CMTs from these sites. Gene expression data suggests that MIDD1 is specific to xylem cells. However, analogous mechanisms might be operating in other plant cells to destabilize CMTs along particular cell edges and/or cortical surfaces, which might contribute to the spatial orientation of the CMT array.

In addition to biochemical factors, mechanical forces have been proposed to play a role in orienting CMTs along a particular direction [Green and King, 1966]. Recently, elegant studies using laser ablation and external force application to the shoot apical meristem have provided experimental support for the idea that CMT orientation is responsive to mechanical stress fields [Hamant et al., 2008]. Interestingly, the polar localization of the auxin transporter PIN1 is also highly responsive to mechanical forces and is tightly coupled to CMT orientation [Heisler et al., 2010]. While the CMT orientation does not depend on auxin transport [Heisler et al., 2010], these data suggest that the effect of mechanical forces on CMT orientation might also boil down to boundary conditions since mechanical forces might influence the localization of proteins along particular cell edges/faces that in turn orient the CMT array.

Microtubule-dependent nucleation and array organization

As noted in the introduction, CMTs are nucleated from multiple sites at the cell cortex. Some of these CMTs originate in a microtubule-independent manner while others originate from the sides of existing CMTs. In the latter case, the newly formed CMT grows either at an acute angle to the mother CMT (called branch-form nucleation) or parallel to the mother CMT [Ambrose and Wasteneys, 2008; Chan et al., 2009; Murata et al., 2005; Wasteneys and Williamson, 1989a; Wasteneys and Williamson, 1989b]. The simulations of Allard et al., 2010b considered only branch-form nucleation and implemented it with and without microtubule-independent nucleation. In the simulations of Eren et al., 2010, both types of microtubule-dependent nucleation were modeled along with microtubule-independent nucleation according to the proportions reported from plant cells [Chan et al., 2009]. Both studies report that inclusion of

branch-form nucleation in addition to microtubule-independent nucleation does not have a significant effect on the degree and rate of CMT organization. However, branch-form nucleation by itself results in unrealistic CMT organization with highly sparse arrays [Allard et al., 2010b], consistent with the suggestion that branch-form nucleation hinders the ability of a CMT array to generate parallel order [Wasteneys and Ambrose, 2009; Wasteneys and Williamson, 1989b].

A recent study by Deinum et al., 2011 more completely analyzes the effects of branch-form nucleation on CMT organization by considering different branching processes and dynamicity parameters. In these simulations, CMT dynamicity is again limited to shortening-prone dynamics. The authors keep the overall nucleation rate constant, while the fraction of microtubule-dependent nucleation increases as a function of the total CMT length in the system. Under these conditions, all CMT nucleations are microtubule-independent at the beginning of the simulations and the ratio of microtubule-dependent nucleation keeps increasing as the system becomes more crowded. Their results show that microtubule-dependent nucleation improves parallel CMT organization and widens the range of parameters for which organization occurs [Deinum et al., 2011]. In particular, they found parallel CMT nucleation to have a strong impact on CMT array organization. In general, greater co-alignment of newly nucleated CMTs to their mother CMT was found to enhance parallel array organization as expected. In their simulations, the authors found that branch-form nucleation had only a modest effect on enhancing array organization, consistent with the results of Allard et al., 2010b and Eren at al., 2010 discussed above. Deinum et al., 2011 note that the main contribution of branch-form nucleation was to result in spatially more homogeneous arrays than achieved by parallel nucleation alone. Together these findings are consistent with experimental observations which show that branch-form nucleation correlates with an increase in CMT spatial density and not parallel organization [Wasteneys and Williamson, 1989b].

Factors that affect array polarity

In addition to ordering into parallel arrays and the overall orientation of CMTs, another characteristic of CMT organization is polarity, which is a measure of similarity of the growth direction of CMTs. Early electron microscopic imaging of CMTs suggested that adjacent CMTs may share directionality in certain cells [Hardham and Gunning, 1978]. However, hook

decoration of CMTs indicated that CMTs have mixed polarity [Tian et al., 2004]. More recently, live-cell imaging revealed that well-ordered CMT arrays can have one or more domains of net polarity, with the bulk of the CMTs facing one direction within these domains [Chan et al., 2007; Dixit et al., 2006]. In contrast, other researchers have found little net polarity in CMT arrays [Shaw and Lucas, 2011]. Polarized CMT organization might be a specialized or transitional event that occurs at certain stages of array organization. In Eren et al., 2010, they showed that it is not possible to obtain CMT array polarity in simulations with only microtubule-independent nucleation. However, when their simulations implemented microtubule-dependent CMT nucleation together with microtubule-independent nucleation, the probability of observing net polarity in ordered CMT arrays significantly increased, regardless of the boundary conditions.

Factors that result in CMT array skewing

Some CMT mutants show twisted growth and have skewed cell files in which the CMT arrays are oriented obliquely with respect to the cell elongation axis. The simulations of Eren et al., 2010 showed that changing the microtubule polymer dynamics as experimentally observed in *Arabidopsis tua4*$^{S178\Delta}$ and *tua5*^{D251N} twisted growth mutants [Ishida et al., 2007] was not sufficient to induce skewed arrays. Inspired by the conceptual framework for the role of CMT nucleation, and branch-form nucleation in particular, in CMT array orientation [Wasteneys and Ambrose, 2009], Eren et al., 2010 used their three-dimensional simulations with non-periodic boundaries to test several scenarios with branch-form nucleation and boundary conditions such as increasing or decreasing the mean branch angle, introducing a bias for branching from one side of the mother CMT, and assigning only one of the end walls of the cylinder as a non-periodic boundary. They found that changing the mean branching angle on either side of the mother CMT and boundary conditions were particularly effective, although none of these mechanisms resulted in consistent skewing for all the simulations or fixed-handed skewing. The only scenario that resulted in consistent skewing without losing well-ordered CMT arrays was an abrupt switching from regular nucleation to branch-form nucleation after the formation of an ordered transverse array [Eren et al., 2010]. As mentioned above, Deinum et al., 2011 simulated a related situation in which CMT nucleation continuously transitions from exclusively microtubule-independent to greater microtubule-dependent nucleation with increasing CMT density. This scenario did not result in array skewing in their simulations [Deinum et al., 2011].

However, it is not easy to exactly compare these two studies as the nucleation scenarios, boundary conditions and parameter ranges differ significantly between them. An important next step to resolve the controversy between these two studies is to experimentally determine the CMT nucleation pattern in mutants with skewed CMT arrays. Evidence from the *Arabidopsis spiral3* mutant does correlate skewed CMT arrays with an increase in the mean angle during branch-form CMT nucleation [Nakamura and Hashimoto, 2009]. However, this analysis needs to be extended to other twisted growth mutants to determine the universality of this observation.

CMT INTERACTION-ORIENTED MODELS

In addition to the efforts to discover the mechanisms underpinning CMT self-organization by using simulation and mathematical models, there have been some recent studies that address the CMT interactions themselves. The paper by Allard et al., 2010a is the first attempt to model CMT interactions at a molecular level isolated from the rest of the system. These authors first model CMT anchoring as a Poisson process in space, where the distance between anchors on a CMT is exponentially distributed. As discussed in the introduction, anchoring of CMTs to the plasma membrane is a major constraint that drives interactions between CMTs. This anchoring model is later used to study the interactions between CMTs based on the competition between cross-linker-based CMT bundling, CMT flexural rigidity, and CMT polymerization [Allard et al., 2010a]. Probabilities for collision-induced depolymerization vs. crossover are derived using a dimer-level model incorporating the linear elastic rod energy of CMTs. Under low CMT anchoring conditions, this model results in a limited collision-induced depolymerization probability, similar to the experimental observations in *Arabidopsis* petiole cells [Wightman and Turner, 2007]. The authors suggest that tighter CMT anchoring to the plasma membrane might explain the higher probability for collision-induced depolymerization observed in tobacco BY-2 cells [Dixit and Cyr, 2004b]. Based on these data, regulation of CMT anchoring to the plasma membrane is an important mechanism for controlling array organization. However, this model fails to explain the angle-dependence of collision-induced depolymerization observed experimentally in Dixit and Cyr, 2004b. The effect of the encounter angle is considered while modeling the bundling mechanism. By calculating the energies associated with bundling and cross-over events, the authors determine bundling probabilities as a function of the encounter

angle. Their results show that bundling probability decreases monotonically with collision angle, in line with the experimental data in Dixit and Cyr, 2004b.

There are also studies that explore CMT organization by focusing solely on the mechanical properties of microtubules, particularly their elasticity. Cosentino Lagomarsino et al., 2007 studied microtubules grown within microfabricated chambers of cellular dimensions and characterized their organization based on microtubule length, elasticity and the geometric constraints imposed by the chamber. They compare the bending energies implied by transverse vs. longitudinal orientations and estimate the preferred orientation with respect to the microtubule length and cell size. Their results show that longitudinal helices are favored for long filaments and large aspect ratios of the cell, whereas transverse helices may be favored for shorter filaments [Cosentino Lagomarsino et al., 2007]. However, the minimal energy configuration is found to be neither a helix nor a transverse array, but rather an oscillating one where the microtubules cross back and forth between the two end walls. This result holds regardless of the boundary conditions of the end walls. Overall, the authors conclude that microtubule elasticity and cell geometry fail to explain the typical CMT transverse orientation, indicating the need for active mechanisms for the emergence of transverse CMT organization [Cosentino Lagomarsino et al., 2007]. One possible active mechanism for generating transverse CMT arrays in plant cells is by localizing CLASP to specific cell edges [Ambrose et al., 2011]. In general, incorporation of the mechanical aspects of CMTs into organization-oriented models might provide further insights that neither type of modeling currently provides.

CONCLUSIONS AND PRESPECTIVES

Theories pass. The frog remains. – Jean Rostand

In this review, we have highlighted how quantitative models can help us understand the process of CMT organization. The power of these models lies in their simplification of the complex CMT system— each model is developed from first principles and using well-defined assumptions and input parameters. The simplified and explicit depiction of the CMT system allows the models to explore underlying mechanisms in ways that are not possible to do experimentally. Of course, it is essential to experimentally test the validity of the assumptions as

well as the veracity of the predictions derived from the models. In other words, models are like theories— they are useful only if they are supported by actual data. Therefore, an important next step is to test the "first generation" of simulation and mathematical models via biological experiments. The current crop of quantitative models is a starting point. These models serve as a source of new hypotheses and not as a source of answers. Some of the key hypotheses derived from modeling studies are: (i) Bundling drives parallel CMT organization; and (ii) Branch-form nucleation and boundary conditions are key parameters that specify array orientation. Diligent and iterative testing of the models against quantitative data gathered from real cells is necessary to continually improve them and thus enhance their usefulness.

Work is needed to measure properties of CMTs such as the frequency and pattern of crossover-based CMT severing, the CMT nucleation pattern over time and the strength of attachment of CMTs to the plasma membrane. Parameterization of these and other factors will help reduce assumptions in models and make them closer to reality. Other properties such as the density and length of CMTs in cells need to be measured as these characteristics can be used to constrain the parameters in quantitative models to obtain more realistic outputs. Bigger challenges relate to measuring and formulating the effect of mechanical forces on CMTs and relating CMT organization to the deposition of cell wall material. In the longer term, models of CMTs will also need to take the impact of multicellularity into account, for example the role of tissue context and hormone gradients on CMT organization.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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FIGURES

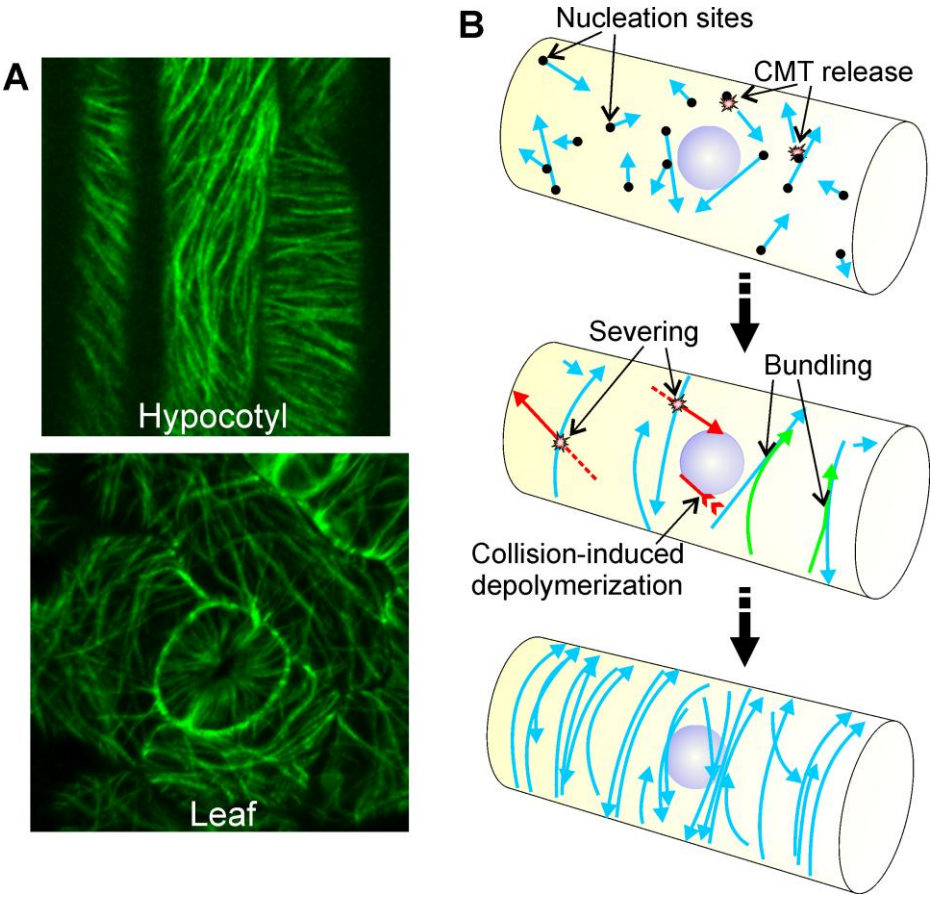

Figure 1: Patterns of the plant CMT cytoskeleton. (A) Images of CMTs labeled by GFP-tagged tubulin in hypocotyl and leaf epidermal cells of *Arabidopsis* plants. (B) A self-organizational scheme for CMT patterning. New CMTs grow from multiple sites scattered at the cell cortex, sometimes from the sides of existing CMTs. After initiation, CMTs detach from the nucleation sites and show treadmilling-driven movement. Encounters between treadmilling CMTs result in different outcomes such as bundling, collision-induced depolymerization and crossover-based severing. Together, these disparate CMT activities determine the pattern of the CMT array in ways that remain unknown.