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Modelling the role of microtubules in plant cell morphology

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Normal plant growth requires the anisotropic expansion of cells and the proper orientation of their divisions. Both are controlled by the architecture of the cortical microtubule array. Cortical microtubules interact through frequent collisions. Several modelling studies have shown that these interactions can be sufficient for spontaneous alignment. Further requirements to this self-organization are the homogeneous distribution of microtubule density and reliable control over the array orientation. We review the contribution of computer simulations and mathematical modelling on each of these challenges.These models now provide a good understanding of the basic alignment mechanism and will continue to be very useful tools for investigating more advanced questions, for example how microtubule severing contributes to alignment and array reorientation.

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Microtubules and plant cell morphology

One of the key challenges a plant cell faces is to control its morphology. In order to contain the turgor pressure it needs to build a mechanically robust cell wall. At the same time it needs to be able to grow and divide within the constraints of the surrounding tissue to which it is closely coupled through direct physical contacts. This requires control both of the direction of growth and the orientation of the division plane. A major role in all these processes is played by microtubules (MTs). These 25 nm wide tubular protein filaments are ideally suited to coordinate these tasks in the cell. They can be up to tens of micrometers long, allowing them to probe and adapt to the geometry of the cell, their intrinsic dynamical instability mechanism allows them to be reconfigured, they are structurally polarized which makes them a substrate for directed transport, either passively in the form of tip-tracking proteins or actively by motor proteins [1], and finally, they are able to exert both pushing and pulling forces which are for example, employed in positioning and shaping chromosomes, organelles and other endosomes [2].

MTs are able to coordinate anisotropic cell expansion at least in part through their interactions with the cellulose synthase complexes that deposit the main structural component of the cell wall: the cellulose microfibrils. They facilitate the positioning of new cellulose synthase complex insertion into the plasma membrane, and guide their subsequent motion, hence determining the location and direction in which the cellulose fibers are deposited [3,4]. The direction of expansion is selected by setting up the orientation of the so-called cortical array (CA), consisting of MTs tightly associated to the membrane, which on average align in a direction perpendicular to the growth axis.

In preprophase the MTs in the CA condense into a single ring-like structure surrounding the nucleus, the so-called preprophaseband (PPB), in an as yet not fully elucidated process (for a review see [5]). The PBB inherits the orientation of the CA that preceded it. In turn, the location and orientation of the PPB reliably marks the location and orientation of the future division plane, thus bringing the latter under indirect MT control.

Clearly, unravelling the complex spatio-temporal processes that underpin these various functional roles of MTs is a challenging task. We are dealing with a spatially extended, multiparticle (a fully developed CA contains 10^2 to 10^3 MTs), dynamical system. It is precisely here that mathematical and computational modelling can play a key role. These approaches allow the integration of all the relevant quantitative and qualitative data into predictive models, which explicitly embody current mechanistic hypotheses. Such models can be used to freely, and moreover cheaply, explore the consequences of both the underlying assumptions as well as (changes in) the specific values of parameters used. This confers two advantages: (i) understanding the behaviour of the model and its limitations leads to a better understanding of the process itself and (ii) using the model to predict the outcome of specific interventions, realizable through genetic, mechanical and/or chemical manipulation, allows the optimal rational design of crucial experiments aimed at either falsifying or corroborating the underlying hypotheses, with potentially significant savings in effort and cost.

Models of cortical microtubule organization

We focus on three issues addressed by modelling studies of plant cortical MT organization in turn, touching upon

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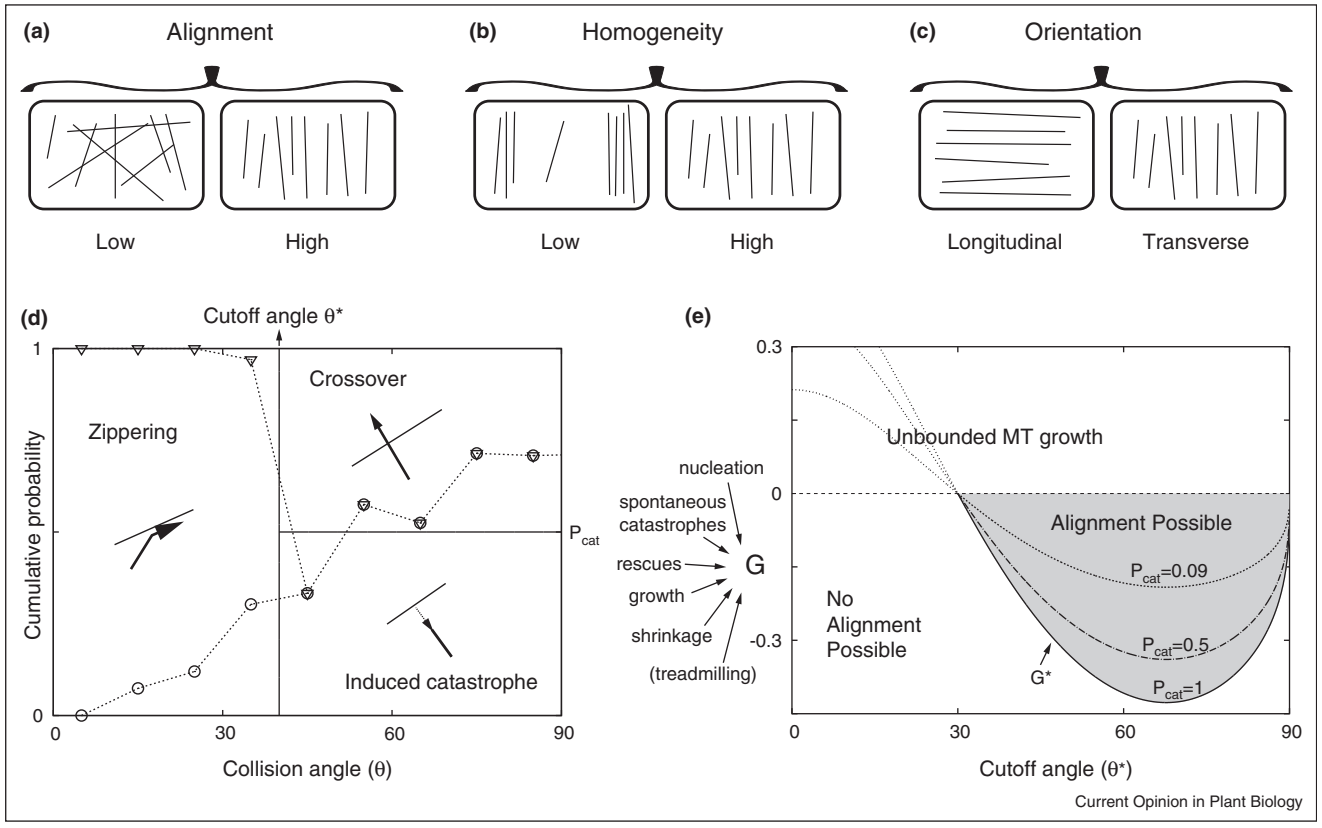
Figure 1



(a)–(c) Features of CMT self-organization: alignment (a), array homogeneity, that is, how evenly the MTs are distributed over the cell surface (b), and orientation of the array (c). **(d)** The stylized interaction function typically used in current CMT simulations: for collision angles below the cutoff angle $\theta^* = 40^\circ$ zippering, that is, continued growth along the obstructing microtubule, occurs exclusively. For larger angles, either induced catastrophes (with probability P_{cat}) or cross-overs (with probability $1 - P_{cat}$) occur. The original Dixit and Cyr [6] data are indicated with dotted lines and symbols: the fraction of induced catastrophes below the circles, that of cross-overs above the triangles and that of zippering in-between. **(e)** Theoretically predicted regime of the control parameter G, the cutoff angle θ^* and the induced catastrophe probability P_{cat} that allows for spontaneous alignment (gray area), based on [10] and interaction function D. G is a single control parameter that combines all parameters describing the MT's dynamic instability (growth, shrinkage, rescues and spontaneous catastrophes) and the nucleation rate [10]. If $G > 0$, MT growth is unbounded. As the amount of tubulin in the cell is limited, (near) steady state arrays cannot be described from this parameter regime. If $G < G^*$, the isotropic state is stable and hence no spontaneous alignment is possible. Values of $G^* < G < 0$ thus remain for physiologically relevant spontaneous alignment (gray area). Note that this regime increases with increasing P_{cat}, but that for this type of stylized interaction function the minimal cutoff angle for any alignment is always $\theta^* = 30^\circ$, independent of P_{cat}.

the alignment mechanism, the spatial homogeneity of the array, and the control of array orientation, as indicated in Figure 1a–c.

Alignment

In their seminal paper on encounters between cortical MTs Dixit and Cyr [6] already suggested that the 3 possible collision outcomes, termed zippering, induced catastrophe and cross-over, could result in spontaneous alignment. These interactions and their probability of occurrence are recapitulated in Figure 1d, and together constitute what we call an interaction function. This later inspired several groups to test the plausibility of this claim using computer simulations. First Baulin *et al.* [7] (but also see [8]) presented evidence that collisions between a growing MT and an obstructing one, leading to temporary stalling of the former could lead to ordering, but their model unfortunately lacked both the typical MT dynamic instability and the zippering interaction. In 2010, in short succession, three papers [9–11] presented simulations that fully implemented both proper microtubule dynamics and an interaction function based on Ref. [6]. Hawkins *et al.* [12] also presented a theoretical framework for analyzing the steady states of these systems.

All the groups involved study essentially the same model, with almost identical interaction functions (Figure 1d) and agree on the main issue: under certain conditions collisions between dynamical MTs, confined to the essentially 2D surface of the cytoplasmic face of the plasma membrane, can lead to spontaneous alignment. They did, however, differ on the relative importance of

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zippering and induced catastrophes (see also [13]). Both [9,11] give evidence that suggests zippering in the absence of induced catastrophes is a *sufficient* cause of ordering, and that induced catastrophes by themselves either are unlikely to cause ordering [9] at physiological values of the MT kinetic parameters. By contrast, our theory [12] predicts that induced catastrophes are both a *necessary* and a *sufficient* requirement for ordering, with the latter claim validated by simulations in the absence of zippering presented in [10], which *quantitatively* coincide with the theoretical predictions.

To put these differences into perspective, we first introduce the theoretically derived control parameter G, which summarizes the importance of the interactions between the MTs ([10], see also Figure 1e). G depends in a non-trivial manner on all the parameters that govern the dynamical behaviour of individual MTs, that is, growth and shrinkage speed, catastrophe-frequencies and rescue frequencies and the nucleation rate. If G is smaller than a critical value G^* (Figure 1e), which depends on the interaction function (Figure 1d), the interactions between the MTs are too rare to overcome the intrinsic disorder in the system. When G is larger than the critical value the interactions dominate and spontaneous alignment occurs.

The value of G is also useful for evaluating whether MT growth is bounded ($G < 0$) or unbounded ($G > 0$), see Figure 1e [10]. Unbounded growth is inconsistent with achieving a steady state, and *in vivo* cannot occur because the pool of available tubulin is limited. We therefore take the position that at the present state-of-the-art a meaningful analysis can only be carried out for $G < 0$ [10,12,14].

Beyond that we take a 'global' approach by investigating the whole $G < 0$ range. This can give a complete overview of the model's behaviour, because G collapses all dynamic instability parameters to a single number and thus greatly reduces computational efforts. This makes the analytical theory a very powerful tool, as also exploited in [14].

Refs. [9,11], on the other hand, adopt a 'local' position, focusing on a few specific experimentally determined parameter sets. However, the natural default of these parameter sets, measured in wild type *A. thaliana* at 21 °C, would predict unbounded MT growth. This issue is dealt with by confining simulations to shorter time scales [9,11], where the unavoidable build-up of MT density remains limited, or by employing a parameter set measured at 31 °C, which does produce bounded growth [9].

Although a consistently measured data set may seem a 'gold standard' for testing a model's performance vis-a-vis the *in vivo* situation, its uncritical use, however, bypasses a number of key issues. First of all, the measurements may be taken under different conditions than those being simulated, for example, in the initial or another transient state of CA development vs. the steady state (which could e.g. explain the unbounded growth issue). Second, the model most likely does not include all relevant components and mechanisms that occur in the real cell, and these omissions may shift the system over a boundary that separates regimes of different model behaviour. Third, even if the parameters are measured in the correct system and the model contains all relevant components, the cumulative effects of measurement errors may still shift the parameter set across a nearby behavioural boundary. That the natural CA (in the initial state) is in fact close to such a boundary is illustrated by the dramatic effect of transferring the WT plants from 21° to 31° in the original measurements, a seemingly small change with profound impact. In the light of the above, a possible explanation for the different findings could, therefore, be that the various groups are in fact studying the CA system in different regimes. In addition, the conclusions may also be affected by the use of different measures of alignment [13].

Another useful insight that follows from our analytical theory is a heuristic explanation of the alignment mechanism, which we have dubbed 'survival of the aligned' [10]. More or less parallel MTs will have few mutual collisions and will therefore have an average life expectancy close to an isolated MT. MTs, however, that are discordant with an average majority orientation, will experience frequent collisions. Effectively, they experience a much higher catastrophe probability, which reduces their expected lifetime. Consequently, out of the pool of newly nucleated MTs, the ones that conform to the majority orientation will 'survive' longest, and dominate the behaviour of the system. The intuitive understanding gained from the analytical model shows that its uses extend far beyond the simple case for which the theory was derived. It provides conceptual handles that facilitate the interpretation of more complex simulations. The analytical model, however, is limited to describing the steady state behaviour. Simulations in contrast also allow the study of the dynamic build-up of the CA, which is particularly useful for studying the specifics of array initiation after cell division [15] or array remodelling [16].

Array homogeneity

Apart from being properly aligned, one expects that the CA must also be spatially uniform (Figure 1b) in order to ensure homogeneous properties along the expanding cell faces. In mature arrays most nucleations occur from γ-TURC complexes bound to preexisting MTs, and display a characteristic mixture of parallel and 'branched' relative orientations, the latter with a median angle of 35–40° [17,18]. Our simulations [14] indicate that the branched nucleations are important for maintaining spatial dispersion, as purely parallel nucleations lead to inhomogeneous bundling. This role is also supported by the

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relatively inhomogeneous arrays of the *ton2* mutant, which has more parallel and fewer branched nucleations [19•].

Orientation

Finally, the orientation of the CA (Figure 1c) must be controlled reliably, both with respect to the cell axis, to dictate the axis of cell expansion, and with respect to the surrounding tissue, to establish proper developmental patterns. The spontaneous alignment direction is uncontrolled, leaving the boundary conditions — cell shape, special edges and faces — to uniquely select the orientation. In a seminal paper Ambrose *et al.* [20••] reported that induced catastrophes occur with a high frequency when MTs impinge on the high-curvature edges around the apical and basal cell faces. Using simulations on a cube-like geometry, they showed that high catastrophe inducing edges are able to orient the CA in the transverse direction. Moreover, they showed that CLASP is needed in order to create the non-transverse array patterns observed in young root and leaf cells, and linked this behaviour to a putative role of CLASP in facilitating the passage of MT (bundles) onto the apical and basal cell faces. A simulation showed that indeed the transverse orientation is lost when catastrophes are strongly reduced on selected sharp edges. Later simulations [21] revealed that tuning the ratio between the catastrophe probabilities between transverse and longitudinal edges provides cells with a robust switching mechanism able to turn the CA by 90°, thus potentially enabling developmentally important control over division planes.

Both simulations simply impose changes in catastrophe probabilities for different edges, without addressing the underlying mechanism. This bypasses, for example, the conundrum that if CLASP is supposed to facilitate the crossing of MT bundles over sharp edges, it first has to accumulate at these edges to enable the first MTs and bundles to cross, whereas it is reported to be localized to the crossing bundles themselves [20••]. Here there is perhaps a role for a differential response to a pre-existing symmetry breaking bias within the cell. In this light it is interesting to note that CA orientation and the polar positioning of PIN proteins is highly correlated [22,23•], suggesting that they both respond to the same polarity cues. If auxin feeds back on these polarity cues, as typically assumed in models investigating auxin and PIN patterning (see e.g. [24,25]), this offers an alternate mechanism for the coordination of CA orientation within a tissue.

Challenges ahead

Modelling has provided major insights into the basic mechanisms that determine the ability of cortical MTs to self-organize into an aligned, uniform and specifically oriented functional structure. However, in order for modelling to make quantitative predictions for specific cases, it essential that all MT parameters involved are measured in the system under study itself *and* under the relevant conditions.

Also, there is at least one crucial component of CA organization that has so far not received the systematic attention it deserves. This is the MT-severing protein katanin, which is known to release MT minus-ends from their nucleation sites [26], is implicated in proper MT organization [27,28], and has been suggested to be preferentially active at sites of MT crossovers [29]. It remains to be determined if functional katanin protein localizes to crossovers and acts as the severing agent at these locations, but we believe a properly parametrized implementation of severing at crossovers into existing CA simulations will be highly valuable in elucidating the functional relevance of these processes. It is likely that katanin is also involved in the striking global reorientation of the CA that occurs in dark-grown hypocotyl cells upon exposure to light [16], the mechanism of which is currently still an open question.

Finally, there are two plant-specific MT structures that await systematic modelling studies. The first is the preprophase band already mentioned in the introduction. The second is the phragmoplast, the structure built from the polarized MTs that originally made up the mitotic spindle, which in telophase targets vesicles to the growing cell plate [30]. This latter structure is especially challenging, for experimentalists and modellers alike, because it is dynamic, and, in contrast to the effectively 2 dimensional CA, fully 3 dimensional.

We believe extensions of the systematic modelling approaches reviewed here will play a major part in achieving mechanistic understanding of these more involved phenomena.

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