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Mechanism of dynamic reorientation of cortical microtubules due to mechanical stress

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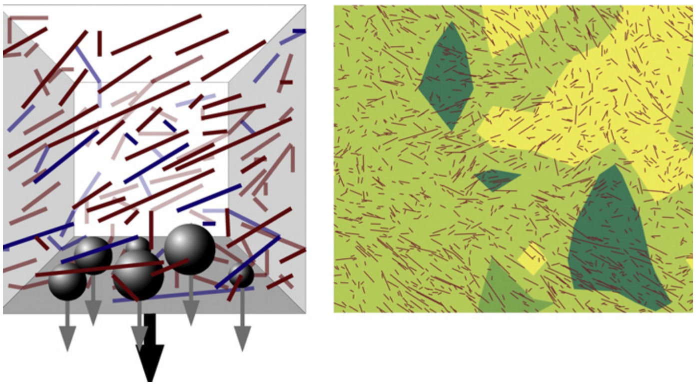
HIGHLIGHTS

- Concentration gradients and stretching may affect the orientation of microtubules
- Microtubules align perpendicular to gradients due to collision-induced orientation
- Ordered microtubule arrays can direct cellulose microfibrils assembly

GRAPHICAL ABSTRACT



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ABSTRACT

Directional growth caused by gravitropism and corresponding bending of plant cells has been explored since 19th century, however, many aspects of mechanisms underlying the perception of gravity at the molecular level are still not well known. Perception of gravity in root and shoot gravitropisms is usually attributed to gravisensitive cells, called statocytes, which exploit sedimentation of macroscopic and heavy organelles, amyloplasts, to sense the direction of gravity. Gravity stimulus is then transduced into distal elongation zone, which is several mm far from statocytes, where it causes stretching. It is suggested that gravity stimulus is conveyed by gradients in auxin flux. We propose a theoretical model that may explain how concentration gradients and/or stretching may indirectly affect the global orientation of cortical microtubules, attached to the cell membrane and induce their dynamic reorientation perpendicular to the gradients. In turn, oriented microtubule arrays direct the growth and orientation of cellulose microfibrils, forming part of the cell external skeleton and determine the shape of the cell. Reorientation of microtubules is also observed in reaction to light in phototropism and mechanical bending, thus suggesting universality of the proposed mechanism.

1. Introduction

Main ingredients of external skeleton in plant cells are cellulose microfibrils [1]. They help to maintain the shape of cells and prevent plant cells from bursting due to high internal turgor pressure [1,2].

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Cellulose microfibrils have high tensile strength and provide the cell wall mechanical strength and stiffness [2]. Since cellulose arrays are naturally anisotropic, the anisotropy of the cell growth is controlled by the orientation of the cellulose microfibril arrays [3]. In turn, the deposition of cellulose microfibrils is directed by highly aligned microtubule (MT) arrays [4], which serve as template for directed growth of cellulose microfibrils [4].

MTs are hollow cylinders of 25 nm external and 15 nm internal diameters [1] and the length ranged from few nm to several microns. They are highly dynamic by their nature [5], and MT ends are constantly switching between polymerization and depolymerization, thus making the length of MTs intermittent. This property of MTs is called dynamic instability [5–7]; a plus-end of a MT is usually more dynamic than a minus-end [7–9].

Cortical MTs in plant cells are organized in parallel arrays adjacent to the cell membrane [10]. There is an evidence [11,12] that cellulose microfibrils may be deposed in the same direction as cortical MTs during the plant growth. Depolymerizing drugs, ethylene and various agents [11,13,14] affecting orientation of MTs, also change the orientation of cellulose microfibril arrays [15]. Aligned cellulose microfibrils provide anisotropy of the cell wall, which is more rigid in the direction parallel to cellulose arrays than in perpendicular direction. This anisotropy allows to transduce the isotropic turgor pressure into a directional cell growth [11,16–18]. However, it is noteworthy that cellulose microfibril orientation in several cases was not affected by the disruption of cortical microtubules [19]. Thus, anisotropy in plant cell wall rigidity irrevocably fixes the direction of growth of the cell leading to irreversibility of the processes such as cell division and cell elongation [20,21]. We focus in particular on gravitropism and phyllotaxis, although the biochemical background is similar to other types of tropisms, such as phototropism or chemotropism [22].

Cell constituents, molecules and their aggregates, are too small to sense the gravitational field directly, however roots of many plants are able to sense the direction of gravity with the help of statocytes, specific cells located in the growing tip of roots or shoots [23]. Statocytes can efficiently perceive the direction of the gravity and direct plant growth along the gravity vector. Directed growth is observed only in the presence of gravitational or centrifugal force and disappear in the absence of gravity or when the direction of gravity is altered [2,24]. The perception of gravity in statocytes is usually attributed to amyloplasts, macroscopic, heavy organelles that sediment in a lower part of the cell [23–25] in root gravitropism and can exhibit saltatory upward movements in shoot gravitropism [3,25].

Sedimenting amyloplasts inside statocytes are probably the main driving force for root gravitropism. Since the radius of amyloplasts, r, is about few microns [26,27], their concentration corrected for buoyancy is $\sim\Delta\rho = 0.5$ g/ml [28] and $g \approx 9.8$ m/s^2, the resulting sedimentation force of one amyloplast at the bottom of the cell is $\Delta\rho g(4/3)\pi r^3 \sim 1$ pN [29]. Such force is insufficient to significantly stretch the membrane of statocyte and elongate it. However, the elongation growth occurs in epidermis, where MTs reorientation takes place. The distance between elongation zone and root cap is several mm, or ~20 cell layers [25,30,31]. Amyloplast sedimentation causes alternation of auxin flux, which causes reorientation of microtubules [32–34] supported by other signals, such as Ca^{2+} ions [35]. Most probably, gradients in hormonal fluxes change the stability of MTs depending on their direction [36–38] (Consult Fig. 1).

In turn, there is an evidence that MTs respond directly to mechanical stress of the cell [2,39]. It may be observed during phyllotaxis in shoot apical meristem [17,21,40]. This suggests that MTs might feel mechanical stress and reorient themselves inducing anisotropic cellulose deposition. Such mechanism may be also observed in the experiments with shoot apical meristem compression, laser ablations of its cells or weakening of the cell wall [17,41,42].

Here we provide a theoretical basis for MT reorientation caused by concentration gradients of chemical agents or mechanical cell elongation. Our model is based on the mechanism of collective self-orientation of cortical MTs induced by mutual collisions and re-growing of individual MTs. This mechanism was first proposed in [43]. It was shown that collisions between individual MTs may spontaneously lead to orientation domains with highly aligned MTs from initially disordered array. This simple model assumes a MT as a rigid rod that can grow at a plus-end and shorten at a minus-end, while the rate of growth at a plus-end is altered by the collisions with other MTs. It was shown that the anisotropy in the rates of growth at a plus-end due to collisions is enough to induce collective phenomena of MT self-ordering into aligned domains with preferential orientation. The selection of preferential orientation in the domains is similar to evolution selection, where MTs with “incorrect” orientation disassemble and disappear, leaving space to “correctly” aligned and thus, longer and older MTs [43].

This minimal model based on age and length discrimination was further extended and improved in consequent theoretical models [44–48]. The models consider two dimensional (2D) movements because cortical MTs in plant cells are attached to plasma membrane forming a 2D array [1,2]. Since the movements in 2D are much more restricted than in 3D, the probability of collisions is high even for relatively low concentrations. Direct observation of collisions between MTs in the cortex array and measure of collision rates [49] have shown that the probabilities of catastrophes and consequent shrinkage of MTs, rescue and continuation of growth depend on angles of collisions. Shallow angles favor continuation of growth, while perpendicular collisions may provoke catastrophe and disassembling of the MT [49]. The models based on these results [44,45] predict the self-orientation induced by collisions and show that zippering between MTs with similar orientation play an important role in onset of ordering. Another model of collision induced reorientation of MTs [47] focuses on phase transitions in MT arrays and predicts the existence of three phases: isotropic phase, weakly ordered nematic phase and highly ordered nematic phase. A similar model [46,48] explicitly includes dynamic instability of a plus-end and uses more realistic parameters for growth and shrinkage rates of MT ends. In addition, this model implements a set of rules driving MTs in case of their collisions: induced catastrophe, plus-end entrainment (zippering) and intersection are possible depending on the angle between colliding MTs [46,48]. This model predicts orientation induced by collisions and competition between domains with different orientations.

2. Materials and methods

Here we use a modified model of collision induced ordering in 2D MTs arrays that incorporates essential parts of the previous models. We model MTs as rigid rods which can switch between shrinking and growing at their plus-end [7,50]. Switching between polymerization and depolymerization on a plus-end happens according to preset catastrophe and rescue rates. In case of collision with another MT the plus-end of a MT stops growing [51]; however, it may still experience catastrophe with a preset rate and start shrinking [7]. This model is summarized at Fig. 2 and the parameters (Table 1) describing individual MT dynamics correspond to other models and to experimental data. The parameters in Table 1 are picked after several trials and to correspond the parameters used by other authors [44–48]. Each MT is characterized by its length, position and orientation. We consider that orientation is set while MT is nucleated and does not change during its lifetime. Although MTs are attached to the membrane, which is well-known to be fluid, their weight is large enough to prevent them from diffusion or rotation caused by membrane movements [5]. The only way to reorient an array of MTs is to eliminate it by complete disassembly of the MTs with “incorrect” orientation and inject new MTs with “correct”