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Review

# Environmental and Endogenous Control of Cortical Microtubule Orientation

Xu Chen,[1,*] Shuang Wu,[1] Zengyu Liu,[1] and Jiří Friml[2]

**Plant growth requires a tight coordination of cell shape and anisotropic expansion. Owing to their immobility, plant cells determine body architecture through the orientation of cell division and cell expansion. Microtubule cytoskeleton represents a versatile cellular structure essential for coordinating flexible cell morphogenesis. Previous studies have identified a large number of microtubule-associated regulators that control microtubule dynamics; however, the mechanisms by which microtubule reorientation responds to exogenous and environmental stimuli are largely unknown. In this review, we describe the molecular details of microtubule dynamics that are required for cortical microtubule array pattern formation, and recapitulate current knowledge on the mechanisms by which various environmental and endogenous stimuli control cortical microtubule reorientation.**

## Trends

Microtubule reorientation requires the activity of microtubule-associated proteins, including regulators of microtubule nucleation, severing, polymerization (and depolymerization), bundling, and interactions with cellulose microfilaments.

Predominant cortical microtubule orientations determine plant cell morphology and the direction of organ outgrowth.

In response to endogenous and environmental signals, cortical microtubules reorient and form various array patterns.

## Microtubule Dynamics during Plant Life

Plant morphogenesis requires coordination of three processes at the cellular level: cell division, cell expansion, and cell differentiation. One of the most fundamental processes of plant cells is their reproduction through cell division [1]. To adapt to developmental and environmental changes, a plant cell rapidly modifies symmetric cell division by regulating the cytoskeleton apparatus. Microtubules (MTs) organize in diverse array patterns to regulate cell division, cell expansion, and cell differentiation [2]. Corresponding to those diverse roles, plant cells develop four types of MT arrays: cortical MTs (cMTs) are mainly responsible for cell expansion; the other three types of MT arrays including the preprophase band (PPB), mitotic spindle, and phragmoplast are essential for cell division and cell differentiation [2]. Among these MTs, cMTs are well characterized and they form highly ordered parallel patterns beneath the plasma membrane. They reorient in response to external stimulation, thereby tightly correlating their orientation with subsequent changes in the axis of cell expansion and plant organ formation [2]. In this review, we describe the molecular details of MT dynamics that are required for cMT array patterns, and summarize possible mechanisms involving environmental and endogenous control of cMT orientations.

## Regulation of Microtubule Dynamics

Owing to the advancement of microscopy technologies, scientists have made great progress in understanding MT dynamics. More importantly, novel molecular components are being gradually identified, providing insights into MT dynamic behaviors, such as nucleation, growth and bundling, severing, and shrinkage as they relate to cMT reorientation (Figure 1).

[1]Haixia Institute of Science and Technology, Horticultural Plant Biology and Metabolomics Center, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[2]Institute of Science and Technology Austria (IST Austria), Am Campus 1, 3400 Klosterneuburg, Austria

*Correspondence: chenxu@fafu.edu.cn (X. Chen).

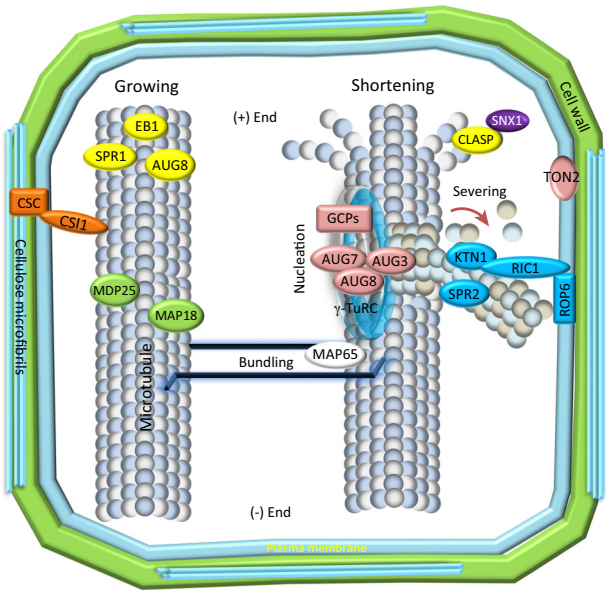


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Figure 1. Microtubule (MT) Assembly Dynamics. Processes of MT (shown as two white cylinders) organization encompass MT nucleation, polymerization, depolymerization, severing, and bundling. AUGs and GCPs accumulate at nucleation sites to mediate MT initiation. TON2 localizes on the plasma membrane and also participates in MT nucleation (labeled as pink). Once new MTs generate from their mother MTs, KTN1, which forms a complex with RIC1 and ROP6, and SPR2 are recruited to crossover sites and catalyze a severing event (labeled as blue). In the process of MT growth and shrinkage, EB1, CLASP, and SPR1 accumulate at the plus (+) end to mediate MT polymerization (labeled as yellow); MDP25 and MAP18 are involved in depolymerization (labeled as green). MTs assemble into arrays of bundled filaments in a MAP65-dependent manner (labeled as white). In addition, MTs and cellulose microfibrils are connected by CSC–CSI complexes (labeled as orange). Abbreviations: AUG, Augmin; GCP, γ-tubulin complex protein; KTN, KATANIN; RIC1, ROP-interactive CRIB motif-containing protein 1; ROP6, Rho GTPase 6; SPR2, SPIRAL2; EB1, end-binding protein 1; CLASP, CLIP-associated protein; MDP25, MT-destabilizing protein 25; MAP, MT-associated protein; CSC, cellulose synthase complex; CSI, cellulose synthase interactive.

## Microtubule Nucleation, Polymerization, and Bundling

In animal and yeast cells, MTs are nucleated from centrosome-based MT-organizing centers (MTOCs), associated with γ-tubulin and γ-tubulin complex proteins (GCPs). These components establish the 'γ-tubulin ring complex' (γ-TuRC), which serves as a template for MT initiation [3]. By contrast, plant cells lack a true centrosome; therefore, how this organization is generated in the absence of a dedicated MTOC has remained unclear. It has been suggested that plant cells contain γ-TuRC-like structures and putative MTOCs help to form well-organized cMT arrays [4]. Indeed, enhanced MTOC activity favors the formation of longitudinal cMT arrays [5].

MT nucleation sites can form three different types of MT nucleation patterns: branching nucleation, parallel nucleation, and *de novo* nucleation, which are determined by the initial branching angle of existing MTs and regulated by several enzymes [6,7]. *Arabidopsis* TON2, a putative phosphatase 2A regulatory subunit, modulates the conformation change of γ-TuRC-like structures [8]. In *ton2* mutants, branching nucleation dramatically decreases and parallel and *de novo* nucleation increase compared with wild type (WT) [8,9]. Thus, TON2 may function as a specific regulator of nucleation geometry [9].

A new model of MT dynamics called hybrid treadmilling has been proposed for plant systems: MT plus ends show polymerization-biased dynamic instability, while minus ends exhibit slow and intermittent depolymerization [10]. The newly formed MTs grow along a new trajectory, implying that changes in the growth trajectory of growing MTs are important for controlling cMT orientation. Through a copurification assay, a number of MT-associated proteins (MAPs) were found to associate with tubulin [11]. MAP65 concentrates at the plus end of MTs and inhibits MT

depolymerization [12]. CLIP-associated protein (CLASP) and end-binding protein 1 (EB1), both belonging to a particular group of MAPs (called +TIPs), also preferentially bind at the plus end of MTs and stabilize MT activity [13–15].

It was suggested that MT reorientation occurs through complete depolymerization in one orientation followed by polymerization of a new array in another orientation. However, an interesting study proposes instead that transverse-to-longitudinal reorientation of MTs contributes to an increase in discordant MTs in a nontransverse alignment, of which subsequent neighboring MTs follow [16]. Therefore, there is a stage in which different alignments of MT arrays coexist [16]. Since individual cMTs grow stochastically, growing cMTs inevitably encounter pre-existing cMTs. The response of cMTs depends on the angle of contact. When the growing plus ends hit existing cMTs at an acute angle (<40°), encountering cMTs change direction and grow coaligned with pre-existing cMTs, forming a parallel bundle. By contrast, if the plus end of cMTs encounter a steep angle (>40°), growing cMTs switch to a shrinking event. Sometimes the encountering cMTs appear unaffected and continue growing in their original trajectory [17]. Although angle-dependent cMT bundling is important for the general pattern of cMT arrangement, the underlying mechanism is still unclear.

Once those MTs assemble into arrays of bundled filaments, MT bundling occurs. MAP65-1 is an important regulator involved in MT bundling [18]. MAP65-1 inherently chooses shallow angle-encountering MTs for bundling, and the length of the rod domain of MAP65-1 determines the range of the MT bundling angle [18]. However, MAP65-1 specifically bundles antiparallel cMTs [18], suggesting other MT-bundling regulators presumably exist to participate in cMT bundling. Besides MAP65-1, the MT plus end-binding proteins known as Augmin subunit proteins (AUG) might also be involved in nucleation and bundling-mediated MT reorientation [19]. On one hand, AUG3, AUG7, and AUG8 are recruited to MT crossover sites immediately before nascent MTs branch out, subsequently allowing the docking of the γ-TuRC-like structure. On the other hand, in *aug8* mutants, MTs spent less time in the growth phase and more growing MTs underwent shrinking when the encountering MTs formed crossovers at steep angles [19]. Taken together, MT nucleation, polymerization, and bundling events play important roles in regulating MT reorientation.

## Microtubule Severing and Depolymerization

The dynamic behavior of MTs primarily depends on the regulation of subunit exchange at the ends of MT polymers. Besides MT polymerization, MT severing activity also controls MT stability [20]. MT severing is defined as a pruning mechanism whereby MTs are catalyzed by ATPase proteins at crossovers, resulting in new growing plus ends [21]. In animal cells, severing assists to establish appropriate MT arrays in neurons and meiocytes by controlling the ATPase protein KATANIN (KTN) [22]. In plant cells, MT arrays must also arrange their architecture in response to environmental and developmental changes, such as photosynthesis, nutrient acquisition, and reproduction. MT severing is the most explicitly known mechanism in plant cells to control cMT orientations [23]. Resembling the role of KTN in animal cells, the *Arabidopsis* homolog, KTN1, severs MTs in crossover sites [23]. *ktn1* mutants fail to form aligned cMT arrays [23]. By contrast, inducible overexpression of KTN1 that results in increased MT-severing activity does not enhance the order of cMT arrays, as shown in pavement cells with more fragmented, bundled cMTs [24]. These findings suggest that KTN1-dependent MT severing is necessary but not sufficient to drive cMT reorientation.

SPIRAL2 (SPR2) is another plant-specific MT-binding protein [25]. Similar to KTN1, SPR2 is enriched at newly initiated MT crossover sites. Mutations in SPR2 resulted in 'hyperaligned' cMTs in petiole cells and increased severing frequency in pavement cells as compared with WT cells [26]. The visualization of SPR2 dynamics revealed that MT severing does not occur when

SPR2 accumulates at crossovers [26]. It seems that the increased alignment of cMTs in *spr2-1* mutants is caused by a high frequency of severing events, whereby SPR2 prevents MT severing by KTN1 [26].

MT depolymerization is proposed as a mechanism of MT disassembly, although distinct from the severing process. cMTs exhibit polymerization-biased dynamic instability at one end and slow depolymerization at the other. As a result, the reorientation of cMTs could be suppressed if the dynamics of cMTs are restrained. Some MAPs, such as MAP18 and its homolog MT-destabilizing protein 25 (MDP25), disturb the rate of tubulin polymer assembly, leading to depolymerization of MTs [27,28]. Correspondingly, such suppression of cMT dynamics by MAP18 and MDP25 results in defective cMT alignment and abnormal cell morphogenesis [27,28].

## Reorientation of cMTs in Response to Endogenous and Environmental Stimuli

The studies mentioned earlier provide a basis for understanding how MT-associated regulators participate in cMT reorientation. The organization of cMTs is vital for plant growth and development. Theoretically, cMTs grow perpendicularly to the growth axis as seen in plant organ formation (Box 1 [29–36]), where transverse cMTs typically correlate with rapid cell elongation, and longitudinal arrays accompany growth inhibition [2]. Owing to a sessile lifestyle, plant cells evolved highly complex mechanisms to react to internal and external signals. Endogenous signals, such as phytohormones, as well as environmental stimuli such as light exposure, temperature, and mechanical stress force plants to adopt different growth strategies. Among them is reorientation of cytoskeletal structures.

### Auxin

Phytohormone-mediated regulation of plant architecture and cell morphology has been intensively studied for over a century. Auxin is the first identified phytohormone, initially discovered as a chemical messenger mediating the directional growth of light-stimulated coleoptiles. The effect of auxin on growth tightly correlates with cMT arrangements and depends on the developmental stage, organ, or light regime [37]. In roots and etiolated hypocotyls, endogenous or exogenous increases in auxin levels lead to a rapid rearrangement of cMTs towards a longitudinal direction, which correlates with growth inhibition [30]. By contrast, in light-grown shoots or auxin-depleted tissues, increases in auxin lead to concomitant transverse cMT reorientation and increased axial cell expansion [33]. In the shoot apical meristem (SAM) area, auxin results in disorganization of ordered circumferential cMT alignment, leading to anisotropic outgrowth [34]. However, it remains unclear whether auxin-dependent anisotropic cell expansion versus inhibition is directly caused by cMT reorientations. cMT reorientation in response to hormones does not involve

### Box 1. Cortical Microtubule Orientation in Embryogenesis and Sprout Growth

Embryogenesis is the initial developmental stage during the life cycle, encompassing several rounds of symmetric or asymmetric cell division and directional cell expansion to generate an apical–basal axis, radial cell layers, and bilateral symmetry of dicotyledonous plants. During the heart stage, most cMTs align perpendicularly with the axes of cell elongation. The proportion of transversal cMTs gradually decreases at the torpedo stage, and is further reduced to a random alignment at the cotyledon stage [29].

After germination, seedlings expand hypocotyl and root cells axially to push leaves towards sunlight and to drive the primary root into soil, respectively. In primary root cells, cMTs mostly align in a transversal pattern along transition and elongation zones [30], with some cells displaying oblique arrays just before root hair emergence [31]. In rapidly elongating cells of etiolated hypocotyl, cMTs organize in a transversal pattern and reorient to be parallel to the growth axis when growth declines [32]. When hypocotyls are exposed to light that leads to cessation of rapid growth, 15% of the cells show transversely coaligned cMTs and 40% show a so-called basket array pattern [33]. During organ formation at the SAM, cMTs are aligned in a rather disorganized pattern in the central zone and show circumferential orientation in the peripheral zone [34,35]. The leaf epidermal cells exhibit a typical jigsaw puzzle shape with indented regions and lobe-like outgrowths [36]. Correspondingly, cMTs are mainly arranged transversely in the neck regions but disorderly in the lobe regions [36].

changes in MT nucleation, implying that additional mechanisms, such as MT severing or MT stabilization, are likely involved [38]. Similarly, a recent study in root and etiolated hypocotyls cells indicated that the effect of auxin on cMT realignment requires both the canonical transport inhibitor response 1 (TIR1)/auxin-related F-box (AFB) and the auxin-binding protein 1 (ABP1)-related pathways, along with ABP1 downstream components Rho GTPases (ROP) and MT-severing ATPase KTN1 [30]. The genetic toolbox used for the postembryonic ABP1-related studies is currently under reevaluation because the originally reported embryo lethality of the *abp1* knockout alleles was caused by a mutation in a neighboring gene and the true *abp1* knockout alleles showed no embryonic defects [39,40]. Regardless of which auxin perception system is used to understand the extent of the effect of auxin on cMT rearrangement, auxin likely primarily targets cMTs to cause growth inhibition, which might depend on a KTN1-regulated MT-severing mechanism [30]. Interestingly, ROP-interactive CRIB motif-containing protein 1 (RIC1), which acts downstream of auxin signaling, colocalizes with KTN1 in a punctate manner and directly interacts with KTN1 [41]. Furthermore, RIC1 acts upstream of KTN1, promoting the detachment of branched MTs [41]. Considering that auxin activates ROP6 to modulate the association of RIC1 with MTs [42], the identification of the ROP6–RIC1–KTN1 cascade hints at the possibility that KTN1-based MT severing might be a common mechanism for cMT self-organization in response to environmental stimuli (such as light response) or endogenous signals (such as auxin response) [23,30]. For auxin-mediated growth promotion in light-grown shoots, data support the scenario that cMT rearrangement is only a consequence of auxin-mediated growth promotion (summarized in [43]), suggesting that the promotional auxin effects on light-grown shoot versus inhibitory auxin effects on root and etiolated hypocotyl is regulated by distinct cellular mechanisms [44,45] (Figure 2). How these key components, such as ROPs, KTN1, and related cMT-severing mechanisms, are involved in both auxin effects remains an exciting topic for future studies.

Other studies have tried to understand the association among directional, intercellular auxin transport and cMT arrangement. The localization of the auxin transporter PIN-FORMED1 (PIN1) [46] at the anticlinal cell walls of SAM is typically parallel to cMT alignment, correlating with PIN1 polar distribution and cMT array pattern [35]. However, chemical inhibition of PIN1-dependent auxin transport did not cause a profound change in cMT orientation, nor did MT-destabilizing herbicides significantly disrupt polar PIN1 distribution [35,47]. PIN proteins are transported through sorting nexin 1 (SNX1)-containing endosomes [48], and SNX1 vesicles are associated with MTs and bind CLASP [49], which stabilizes MT activity via its enrichment at the plus end of MTs [14]. Given the role of CLASP in maintaining MT polymer assembly [13,14], it is reasonable to speculate that polarization of PINs on the plasma membrane might depend on CLASP. However, no evidence for this hypothesis has been shown yet. Thus, the exact role of auxin in cMT arrangement, in particular for growth inhibition and promotion, remains unclear.

## Other Phytohormones

Gibberellin (GA) is another well-characterized phytohormone known to have effects on growth and cMT orientation. Application of GA to light-grown shoots increases the proportion of cells with transverse cMTs, which is further augmented by auxin cotreatment, resulting in a burst of shoot cell elongation [33]. GA might influence cMTs through the prefoldin (PFD) complex, which comprises chaperones involved in tubulin folding [50]. The PFD complex is inactive in the nucleus but can shuttle to the cytoplasm to promote tubulin folding [50–52]. Nuclear DELLA proteins, the major components of GA signaling, physically interact with PFD3 and PFD5, and thus possibly promote their nuclear localization [52]. Under low GA levels, PFDs are mostly retained in the nucleus through its interaction with nuclear-localized DELLAs. Owing to the resulting high level of inactivated PFDs, $\alpha/\beta$-tubulin heterodimer availability is severely compromised [52]. When GA levels increase, DELLAs are degraded, and PFDs are released into the cytoplasm promoting

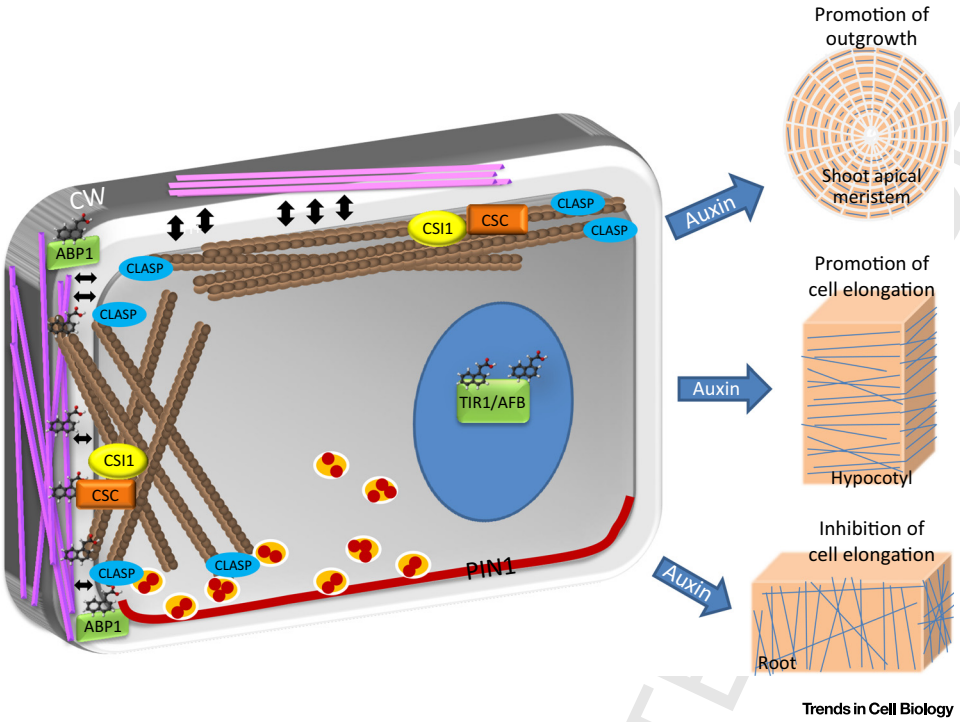


Figure 2. Speculative Scenario of the Relationship among Auxin, Cortical Microtubule (cMT) Reorientation, and Cell Growth. The plant cell wall comprises a network of stiff cellulose microfibrils (purple sticks). cMTs (brown globular sticks) form a highly ordered array beneath the plasma membrane, guiding the deposition of cellulose in the cell wall. The anisotropic cell growth between adjacent cells generates mechanical stress (black arrows) against the cell wall. In response to an auxin signal, auxin binds to nuclear-localized TIR1/AFBs and apoplast-localized ABP1, whose individual contributions to MT dynamics are still unclear. On the cellular level, activation of auxin signaling leads to the rearrangement of cMTs and cellulose microfilaments, which are connected by the CSC–CSI1 complex. On the organ morphogenesis level, high auxin levels promote the outgrowth of SAM, the elongation of the shoot cell and the inhibition of root cell growth. In addition, the polar localization of the auxin transporter PIN1 in SAM is typically parallel to cMT alignment. PIN1 is targeted to the basal side of the cell (painted red) and PIN1 proteins (red balls) are endocytosed by SNX1-mediated endocytosis (shown as orange balls). Meanwhile, CLASP localizes to the plus end of MTs and interacts with SNX1. Despite the unclear mechanism, these intriguing links suggest a possible relationship among auxin, cMT reorientation, and cell growth. Abbreviations: TIR1, transport inhibitor response 1; AFB, auxin-related F-box; ABP1, auxin-binding protein 1; CSC, cellulose synthase complex; CSI1, cellulose synthase interactive 1; PIN1, PIN-FORMED1; SAM, shoot apical meristem; SNX1, sorting nexin 1; CLASP, CLIP-associated protein.

tubulin dimerization [52]. Thus, GA may regulate cMT arrangements through the modulation of a DELLA–PFD–tubulin folding–cMT polymerization cascade (Figure 3, Key Figure).

Other phytohormones, such as brassinosteroid (BR) and ethylene, also influence both cMT orientation and subsequent cell growth [53,54]. Briefly, BR randomizes the gravitropism of etiolated hypocotyls and increases the proportion of transversely oriented MTs through MT-destabilizing protein 40 (MDP40) [53]; ethylene inhibits etiolated hypocotyl elongation through the regulation of MT stability by its associated protein WAVE-DAMPENED2-LIKE5 (WDL5) [55]. Therefore, phytohormone-based screening could be a challenging approach to identify the factors involved in plant-specific regulation of cMT organization.

## Light and Photoperiod

The circadian clock generates rhythms in response to light/dark daily cycles, regulating the rhythmic elongation of hypocotyls [56]. Etiolated seedlings exhibit elongated hypocotyls to reach upwards to the sunlight, while light exposure significantly inhibits hypocotyl growth. Correspondingly, following exposure of the etiolated seedlings to blue light, transverse MTs reorganize

## Key Figure

### Cortical Microtubule (cMT) Reorientation in Response to Endogenous and Environmental Stimuli

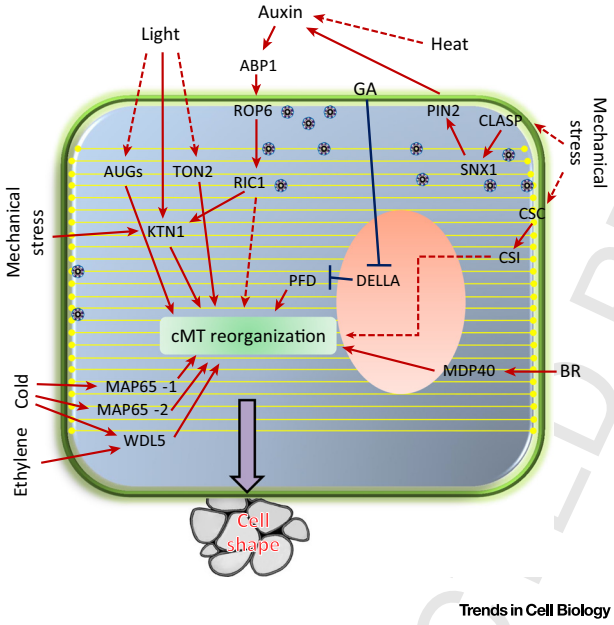


Figure 3. In response to different cues including internal signals, such as auxin, GA, BR, ethylene, as well as environmental signals, such as light, mechanical, and temperature stress, plant cells activate signal transduction cascades that lead to changes in cMT organization. Several signal transduction cascades exist: (i) auxin binds ABP1 to activate downstream ROP6–RIC1. RIC1 then directly interacts with KTN1, which probably mediates an MT-severing event; (ii) GA influences cMTs through the shuttling and relocalization of DELLA-controlled PFD; (iii) BR and ethylene regulate cMT organization through MDP40 and WDL5, respectively; (iv) blue light mediates KTN1-dependent MT severing; (v) light probably stimulates AUGs or TON2-mediated MT nucleation events; (vi) MAP65-1, MAP65-2, and WDL5 regulate cold stress-stimulated cMT reorganization; (vii) heat stress might regulate MT dynamics through auxin signaling; (viii) mechanical stress stimulates downstream KTN1, which probably influences MT severing; (ix) owing to the tight correlation between the cell wall and cMTs, mechanical stress that is generated from the rigid cell wall might regulate the signaling cascade of CSC–CSI–cMT pattern formation; and (x) at the cell edge, the CLASP–SNX1 edge complex is speculated to be an MT organizer. The reported signaling cascades are depicted by solid lines and speculated signaling cascades are connected by dotted lines. The blue circles represent endosomes, while the yellow lines represent cMTs. Abbreviations: GA, gibberellin; BR, brassinosteroid; ABP1, auxin-binding protein 1; ROP6, Rho GTPase 6; RIC1, ROP-interactive CRIB motif-containing protein 1; KTN, KATANIN; PFD, prefoldin; MDP40, MT-destabilizing protein 40; WDL5, WAVE-DAMPENED2-LIKE5; AUG, Augmin; MAP, MT-associated protein; CSC, cellulose synthase complex; CSI, cellulose synthase interactive; CLASP, CLIP-associated protein; SNX1, sorting nexin 1.

into longitudinal orientations within minutes through a process that is reliant on KTN1-dependent MT severing [23]. After blue light stimulation, pre-existing MTs and newly initiated MTs form crossovers. KTN1 then localizes at the crossover sites and subsequently participates in the removal of discordant MTs, resulting in new growing plus ends. These new growing ends initiate more plus ends, leading to an amplification of longitudinally oriented cMTs [23,57] (Figure 3).

Defects in a number of MT-associated molecular components also lead to abnormal hypocotyl elongation and reduced sensitivity to light stimulation. For example, *ton2* mutants are unable to reorganize their cMT arrays in response to light stimulation. Given the role of TON2 in MT nucleation, the arrangement of cMT arrays could depend on a balance between branching and

parallel/*de novo* MT nucleation that is mediated by TON2 [8,38]. These data suggest that additional MT-associated regulators, beyond KTN1, might be involved in light-stimulated cMT reorientation.

### Temperature Stress

When adapting to changing temperatures, plant cells trigger a cascade of cellular processes, including the reaction of MT dynamics. Cold stress has long been known to depolymerize MTs [58]. After cold treatment, cMTs were severely disrupted as shown by tubulin–GFP [59], but the same treatment had no effect on actin filaments [59]. However, our understanding of the mechanism that regulates MT dynamics in response to cold temperature is still fragmented. In plants, it was shown that MTs show more resistance to cold stress in the presence of MAP65-1 or MAP65-2 [60,61]. MAP65-1 promotes tubulin polymerization and enhances MT nucleation, while MAP65-2 bundles MTs and increases their stability [60,61]. Therefore, MAP65 may increase MT stability under cold stress [61]. In addition to MAP65 family proteins, other factors could affect MTs in response to cold temperatures. WDL5 binds to and stabilizes MTs [55]. In the absence of WDL5 protein, MT disassembly increases after cold treatment, indicating that WDL5 participates in cold-induced MT depolymerization [55]. Furthermore, because cMTs are localized underneath the membrane, membrane fluidization probably affects cMT dynamics. Phospholipids, the main components of the lipid bilayer in cell membranes, affect membrane fluidization [62]. Given that phospholipid molecules regulate the activity of MAP65-1 in MT polymerization, membrane fluidization may also participate in MT stabilization in response to cold stress [62,63] (Figure 3).

By contrast, heat stress appears to have little effect on MT organization. A number of screened temperature-sensitive mutants exhibited aberrant cell expansion and disorganized cMTs [64,65]. However, the restrictive high temperature was probably not responsible for the observed defects in cMT alignment because cMT alignment in WT cells appeared normal at the restrictive temperature. Interestingly, when seedlings are grown in light at a high temperature (29 °C), hypocotyls are dramatically more elongated compared with seedlings grown at 20 °C [66]. High temperature increases auxin levels and promotes auxin-mediated processes [66], while high endogenous auxin levels stimulate cMT reorientation [30]. These correlations suggest a potential underlying connection between heat stress and cMT alignment.

### Mechanical Stress

The rigid plant cell wall maintains proper cell shape but generates stiffness, providing directional information for plant cell growth [67,68]. The presence of a cell wall results in a force generated by anisotropic cell expansion, called mechanical stress [68,69]. In the boundary domain of SAM or after applying local forces onto SAM, cMTs tend to be parallel to the maximal direction of the stress [68]. In *ktn1* mutants, regular cMT orientation patterns are strongly altered, meristematic cells are less responsive to mechanical stress, and anisotropy cell growth is reduced [68], indicating an essential role of KTN1 in organizing cMT arrays in response to mechanical stress.

Mechanical stress is generated by differential growth direction between neighboring cells. Therefore, to coordinate the regular arrangement of adjacent plant cells, cMTs have to be organized properly in the sharp edges. CLASP preferentially accumulates at cell edges [13,14]. The specific edge distribution and the plus end-binding property of CLASP result in the suppression of an MT catastrophic event. Such anti-catastrophic activity driven by CLASP promotes the transversal arrangement of cMTs by preferential removal of longitudinal cMTs [13]. Thus, in comparison to a dominant longitudinal or mixed cMT bundling along sharp cell edges in WT cells, *clasp-1* mutants show oriented cMTs parallel to sharp edges [13]. The CLASP-edge complex is speculated to be a 'tunable' MT organizer, with an inherent flexibility to organize MT dynamics close to edges [13,70].

The presence of a cell wall is a major contributor to mechanical stress. Plastic growth of plant cells begins with the loosening of the cell wall, which is composed of a matrix of cellulose microfibrils, hemicellulose, and pectin. As the cell wall loosens in the extracellular space, communication occurs between the plasma membrane and the cell wall. As early as the 1960s, cellulose microfibrils were proposed to be deposited along cMTs [71]. Although the cellulose synthesis inhibitor isoxaben did not directly inhibit polymerization of MTs, cMT arrays were disorganized after isoxaben treatment through an unknown mechanism [72]. Until recently, MT-dependent cellulose deposition was observed *in vivo* by functional fluorescent protein tagging of cellulose synthase (CESA) proteins [73,74]. The cellulose synthase complex (CSC) is first inserted into the plasma membrane, where it catalyzes the addition of UDP-glucose to glucan chains [73,74]. The continuing catalytic activity of CSC pushes itself forward while the interaction of crystallized cellulose fibrils and other cell wall components restricts CSC dynamics [75]. Thus, we assume that CSC acts as a flexible hinge linking the horizontally aligned cellulose fibrils and the organized cMTs. This hypothesis is supported by mutants of cellulose synthase interactive 1 (CSI1/POM2), a linker protein between CESA and MTs [76,77]. Loss of function of CSI1 resulted in the separation of CESA from MTs [76]. The cMT orientation defect was also seen in another CESA interactor mutant *korrigan1* [78] and the cellulose synthase mutant *procuste1* [79], indicating that cellulose synthesis requires and also controls ordered cMT organization. Interestingly, CSCs are quickly endocytosed under abiotic stress, and subsequently recycle back to the plasma membrane when the stress is relieved [80]. During trafficking of CSCs, cMTs might act as important components involved in efficiently retaining, sorting, and/or transporting internalized CSC-containing vesicles, thus aiding in cell wall remodeling in response to various signals [80].

In summary, cMTs are essential cellular components responding to a number of internal or environmental signals by rapid reorientations, leading to developmental and morphological plasticity of the plant cells (Figure 3).

## Concluding Remarks

Compared with animal cells, plant cells have a rigid cell wall that supports the formation of various regular and sometimes extravagant shapes. These features reflect the different modes by which plants establish their body architecture from animals, especially under the influence of environmental and endogenous stimuli. Resembling MTs in animal cells, cMTs in plant cells exhibit continuous activities: nucleation, polymerization, severing, depolymerization, and bundling. To adapt to the complicated developmental and environmental changes, plants need to apply special approaches to coordinate the arrangement of the cytoskeleton pattern. However, most of our current knowledge on MT dynamics in plant cells is limited to the function of homologous regulators known from animal and yeast cells. Therefore, the plant-specific mechanisms of MT arrangements and regulations are largely not well understood. Understanding the relationship between plant-specific developmental and signaling events and MT dynamics, such as the regulation of cMT arrangements by phytohormones, is crucial to gain insight into plant-specific mechanisms (see Outstanding Questions). The recent progress in molecular genetics, biochemistry, and cell biology techniques will provide powerful tools to investigate how cMTs are precisely controlled to integrate environmental changes into appropriate modifications in growth.

## Outstanding Questions

Plant cells do not have centrosomes, and instead they develop multiple nucleation sites throughout the whole cell. How are the dispersed nucleation sites generated and what regulators are involved in plant cells?

Do all environmental and endogenous signals control cortical MT reorientation through a KATANIN1-based severing mechanism, or do other mechanisms exist?

How do discordant MTs generate at the beginning of the cortical MT reorientation process?

What is the molecular mechanism that triggers auxin-dependent rapid reorientation of cortical MTs?

Do additional mechanisms, besides cellulose synthase interactive 1, exist in plant cells to coordinate the association between cortical MTs and the cell wall?

How is the regulation of MT dynamics integrated with environmental and endogenous signaling?

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