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Microtubules and cellulose biosynthesis: the emergence of new players

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Microtubules determine the orientation of newly formed cellulose microfibrils in expanding cells. There are many hypotheses regarding how the information is transduced across the plasma membrane from microtubules to cellulose microfibrils. However, the molecular mechanisms underlying the co-alignment between microtubules and cellulose microfibrils were not revealed until the recent discovery of cellulose synthase interacting (CSI) proteins. Characterization of CSIs and additional cellulose synthase-associated proteins will greatly advance the knowledge of how cellulose microfibrils are organized.

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Introduction

Plants cells maintain a dynamic cortical microtubule (MT) array at the cell cortex that regulates cell expansion. Cell expansion is a critical determinant of plant organ size and shape. In expanding cylindrical shaped plant cells, transversely oriented cellulose microfibrils, the major load bearing components in the cell wall, restrict radial expansion and promote longitudinal expansion [1]. There has long been a debate over the role of MTs in the guidance of cellulose microfibril orientation [1–6]. Early studies suffered from the inability of observing first, the dynamic reorganization of MTs and microfibrils and second, the newest and innermost layer of cellulose microfibrils that is most influenced by the cortical MTs [7•,8•]. Moreover, the difficulty of adequately sampling dynamic MTs and cellulose microfibrils led to divergent results [9]. Although the guidance of cellulose microfibrils along cortical MTs is not observed in some cell types, like the tip growing cells of root hairs, pollen tubes, fern protonema, and certain algae, MTs do guide cellulose microfibrils in the primary walls of cells undergoing anisotropic growth and in the secondary walls of xylem cells [4,10]. This review will divulge the current understanding of the relationship between cellulose microfibrils and cortical MTs by focusing on recent work on the molecular mechanisms underlying the interaction between the integral plasma membrane cellulose biosynthetic machinery, the cellulose synthase complex (CSC), and the cortical MTs.

The dynamics of microtubule and cellulose microfibril reorientation

MTs are highly dynamic filamentous protein polymers consisting of α-tubulin and β-tubulin heterodimer subunits that undergo cycles of rapid growth and disassembly. Isolated plant tubulins were found to have a greater intrinsic dynamicity than animal tubulins [11]. Although the underlying reason for the increased dynamicity of plant MTs is not well understood, a more dynamic cytoskeleton may have evolved to help plants cope with a sessile lifestyle. Indeed, cortical MTs can change their orientation in response to a variety of signals including light, hormones, gravity, and mechanical stress. Blue light induces a complete MT reorientation from transverse to longitudinal orientation in about 10 min whereas it takes 1–2 h to induce transverse MT organization in response to hormone such as brassinolide, indole-3-acetic acid (IAA) and gibberellic acid (GA4) [12–14]. The mechanism for light-dependent and hormone-induced MT reorientation may be different, with distinct signaling pathways controlling local MT reorientation in a few cells versus large-scale MT patterning in the whole plant. The MT severing protein, katanin, has recently been implicated in blue light induced MT reorientation and another MT associated protein, SPIRAL2, cooperates with katanin to regulate cortical MT arrays in rapidly elongating cells such as leaf petiole epidermal cells [15–17].

MTs also undergo cyclic reorientation presumably following an endogenous rhythm [18]. The entire cortical MT array rotates 360° within 200–800 min in growing epidermal cells of light-grown Arabidopsis hypocotyls. This rotation appears to be associated with growth because MTs remain in oblique or longitudinal orientations when cells stop elongating. MT orientation could influence growth at least in part by altering the orientation of nascent cellulose microfibrils in the cell wall. Both light-induced and cyclic MT reorientation occurs in conjunction with a corresponding reorientation of the trajectories

of CSCs that are actively synthesizing cellulose [14,19]. Therefore, it is expected that the orientations of nascent cellulose microfibrils change accordingly. CSC trajectory rotation was blocked by drugs that either stabilize or depolymerize MTs, and resulted in the loss of polylamellate cell wall patterns [19], which is consistent with earlier observations that perturbation of MT dynamics affects wall texture [20].

Cellulose microfibrils in xylem fibers and tracheids are laid down as three consecutive layers, called S1, S2, and S3 layers. The orientation of cellulose microfibrils switches from a shallow helix in outer S1 layer (about 40–80° from the tracheid axis) to a steep helix in S2 layer (about 5–20° from the tracheid axis) and switches to a shallow helix in the S3 layer at the final stage of secondary wall formation [21]. Similar to the rotation in the orientation of the cellulose microfibrils, successive changes in the orientation of MTs were observed in differentiating conifer tracheids and in the tension wood fibers of hardwoods [22–24]. Because secondary cell wall producing tissues are buried deep within the plant, live cell imaging of CSCs and MTs during secondary wall formation has been hindered by technical difficulties. Although the *Zinnia* cell culture can circumvent some of these experimental difficulties [25], *Zinnia* cell cultures lack the genetic and molecular biology tools of Arabidopsis xylem model systems. If the live cell imaging of secondary cell wall producing cells could be improved, insight could be gained in the role of CSCs and MTs controlling the deposition of differentially oriented secondary cell wall layers. The recently developed Arabidopsis xylem system may be key in future studies of CSCs during secondary wall formation [26]. Furthermore, components involved in MT dynamics and cell wall patterning in secondary wall forming cells, such as MIDD1 and MAP70-5, are emerging [27,28].

The connection between microtubules and the cellulose synthesis complex

The combination of genetics and live cell imaging has greatly improved the understanding of how MTs guide the deposition of cellulose microfibrils. In Arabidopsis, the CSC is made of at least three different cellulose synthase (CESA) proteins in both primary (CESA1, 3 and 6 or 6-like) and secondary cell walls (CESA4, 7 and 8, Table 1). Several fluorescent protein (FP) tagged CESA isoforms (e.g. CESA7, CESA3 or CESA6) are functional in Arabidopsis as indicated by complementation of corresponding *cesa* mutants [10,14,29]. FP-CESAs have been visualized as diffraction-limited particles that move in the plane of the plasma membrane. The movement of the CESA particles is thought to be driven by cellulose synthesis with nascent cellulose microfibrils being deposited along the trajectory of CSC displacement. Formerly, it was proposed that MTs serve as boundaries within which CSCs are constrained to travel [3]. This model has been contradicted by the observation that CSCs travel along MTs [14,30]. These observations support the direct guidance model in which CSCs are attached to MTs via direct or indirect interactions, resulting in the parallel alignment between cellulose microfibrils and the underlying MTs [2].

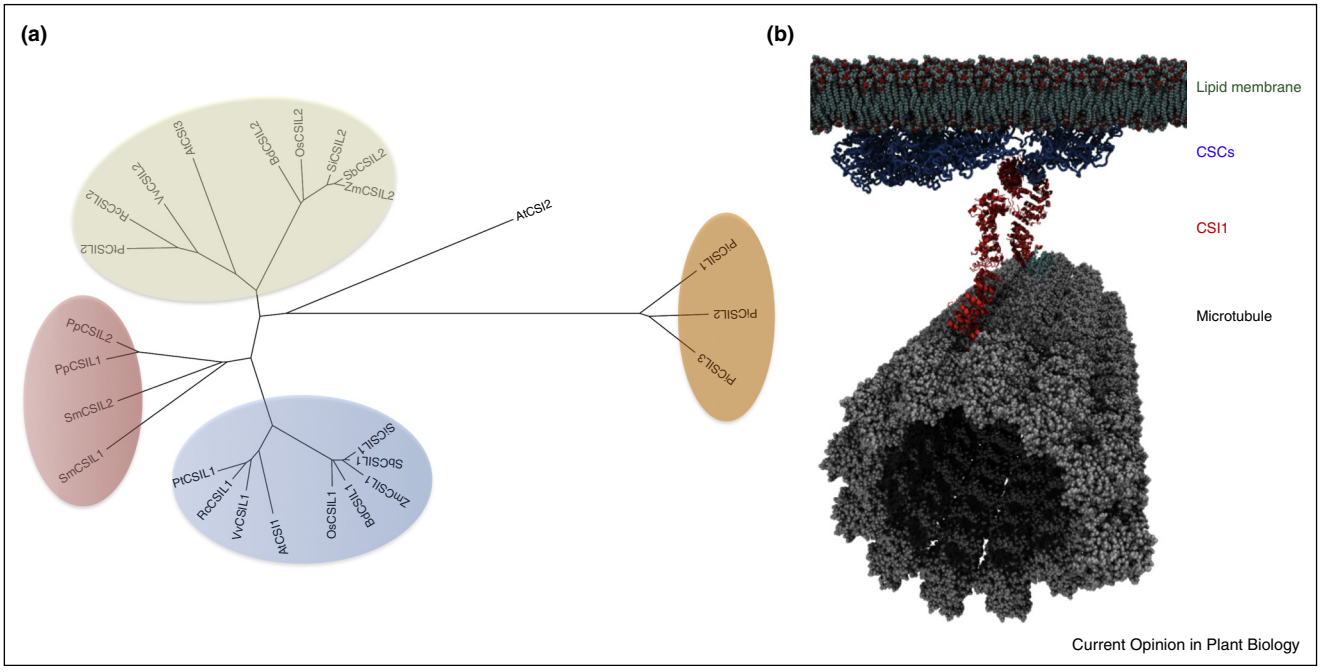
In order to address how CSCs interact with MTs, a yeast two-hybrid screen was performed to identify CESA interacting proteins. Cellulose synthase interacting protein 1 (CSI1) was shown to interact with CESA1, 3, and 6 in a yeast two-hybrid assay [31••,32•]. *In planta*, fluorescent protein-tagged CSI1 were shown to colocalize GFP-CESA3 or GFP-CESA6 that were actively traveling along MTs [31••]. CSI1 was shown to interact with MTs *in vitro* suggesting a direct association between CSI1 and MTs [33••,34]. Although the disassociation constant of CSI1 for MTs was similar to that of conventional MT associated proteins (MAPs) such as MAP65, CSI1 does not contain any conventional MT binding domains [33••]. Molecular dynamics simulations indicated that CSI1 protein folds into a long loop-like ribbon structure and developed a model in which the N-termini and C-termini interact with MTs while the central loop of CSI1 interacts with the CSC to form the link between CSCs and MTs (Figure 1) [35]. Consistent with CSI1 providing the link between CSCs and MTs, loss of CSI1 resulted in the disassociation

Table 1

Cellulose synthase complexes in primary cell wall versus secondary cell wall

Primary CSCs				Secondary CSCs			
Composition	Stoichiometry	Localization	Ref.	Composition	Stoichiometry	Localization	Ref.
Integral component							
CESA1, CESA3, CESA6 or CESA6 like	1:1:1	PM, SmaCCs/MASCs, Golgi, cell plate	[31••,48,49,54,56–58]	CESA4, CESA7, CESA8	1:1:1	PM, Golgi	[10,30,59]
KORRIGAN1	N.D.	PM, SmaCCs/MASCs, Golgi, tonoplast, cell plate	[45••,46••,60,61]	KORRIGAN1	N.D.	N.D.	
Associated protein							
CSI1, CSI3	N.D.	PM, SmaCCs/MASCs	[31••,32,33••,36•,37]	N.D.			

Figure 1



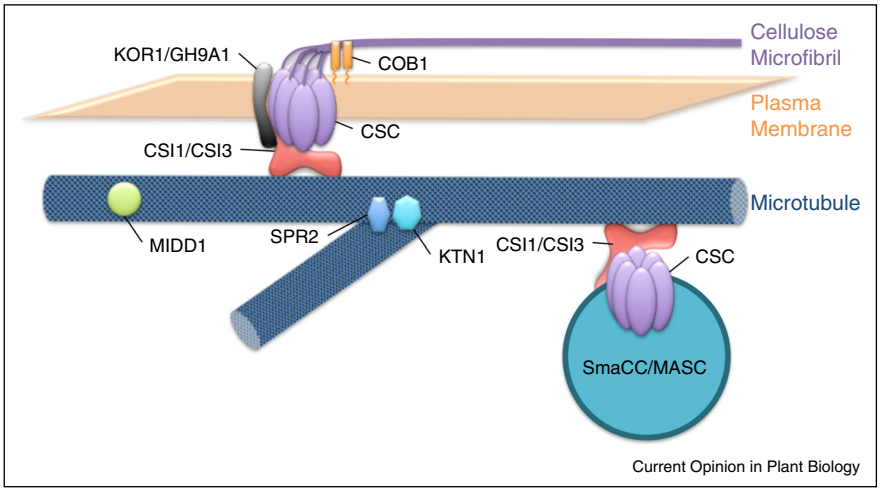
(a) Phylogenetic tree of CSI proteins in different species. Abbreviations for prefixes: At: *Arabidopsis thaliana*, Bd: *Brachypodium distachyon*, Pi: *Phytophthora infestans*, Pp: *Phycomitrella patens*, Pt: *Populus trichocarpa*, Rc: *Ricinus communis*, Sb: *Sorghum bicolar*, Si: *Setaria italica*, Sm: *Selaginella moellendorffil*, Os: *Oryza sativa*, Vv: *Vitis vinifera*, Zm: *Zea mays*. **(b)** Putative interaction model of CSI1 with CSCs and microtubules. Only cytoplasmic sides of CSCs are shown. CSI1 protein has extensive alpha-helices shown in red and C-terminus beta sheets shown in light blue. Adapted from Lei et al. [35]. Copyright American Society of Plant Biologists.

of CSCs from the underlying MTs *in planta* [33••,36•,37]. The dissociation between CSCs and MTs in *csi1* null mutants caused defects in the cell expansion of various tissues including dark-grown hypocotyls, roots, siliques, and stems [31••]. These phenotypes are likely attributed to defects exclusively in the primary cell wall because: first, CSI1 interacts with primary CESAs in a yeast two-hybrid assay; second, CSI1 is transcriptionally co-regulated with many primary CESAs including CESA1, 3, 5 and 6; third, *csi1* had no defects in the secondary cell wall that is characterized by a collapse of xylem vessels [31••,38].

In addition to CSI1, the Arabidopsis genome encodes two CSI1-like proteins, namely CSI2 and CSI3 [32•,38]. While CSI2 might be a pseudogene, CSI3 exhibits many CSI1-like characteristics including the ability to interact with CESA, to co-localize with both CSCs and MTs, and to exhibit CSC-like velocities along MTs. Unlike CSI1, CSI3 is dispensable for the co-alignment of CSCs and MTs *in planta*. Moreover, *ProCSI1::GFP-CSI3* was not able to rescue *csi1*, suggesting CSI3 is functionally not equivalent to CSI1 [32•]. The functional difference between CSI1 and CSI3 was further supported phylogenetic analysis in which CSI1 and CSI3 appear to belong to a different clade (Figure 1). Differences in the expression pattern of CSI3 and CSI1 could also suggest that CSI proteins fulfill similar functions but in different tissues. Nevertheless, CSI1 and CSI3 are present in a variety of dicots, monocots, conifers, and the moss *Physcomitrella patens*, indicating that CSI proteins might mediate the MT-cellulose interaction throughout the plant kingdom.

In addition to CSI proteins, COBRA and KORRIGAN are both proposed to be involved in the organization of cellulose microfibrils through a MT-related function. *COBRA* (*COB1*) encodes a GPI-anchored protein and localizes to MT-like structures (Figure 2) [39,40]. The dependence of COB1 localization on MTs and the disorganization of cellulose microfibrils in *cob1* led to a hypothesis that COB may be responsible for controlling microfibril orientation in a MT-dependent manner [40]. COB1 was also recently shown to bind to individual β-1,4-linked glucan chains, but *cob1* had no defect in the rate of cellulose polymerization [41•]. Brittle culm 1, a COBRA-like protein in rice, also binds crystalline cellulose and modifies cellulose crystallinity [42]. Cellulose microfibril disorganization in *cob1* might be attributed to altered cellulose crystallization. *KORRIGAN1 (KOR1)* encodes a membrane bound β-1,4 endoglucanase. Arabidopsis KOR1 and its homolog in poplar have been shown to hydrolyze carboxymethyl cellulose and amorphous cellulose *in vitro* [43,44]. Recent studies revealed that KOR1 is likely an integral part of the CSC that travels along MTs with CSCs [45••,46••]. A novel A577V missense mutation

Figure 2



New players involved in the process of cellulose synthesis along the cortical MTs. CSC: cellulose synthase complexes; COB1: COBRA1; CSI1/CSI3: cellulose synthase interacting protein 1/3; KOR1/GH9A1: KORRIGAN1/Glycosyl Hydrolase Family 9 Class A 1; SPR2: SPIRAL2; KTN1: KATANIN1; SmaCC/MASC: small CESA-containing compartments/microtubule-associated CESA compartments.

in *KOR1* abolished its endoglucanase activity and resulted in both disorganized cellulose microfibrils and MTs, which is consistent with its defect in the organization of CSCs [46••]. The concurrent defect in the organization of cortical MTs and organization of CSCs has been observed in *kor1-3* [47]. How a defect in cellulose organization affect MTs organization is unknown. It is possible that the cortical microtubule phenotype of *kor1* might be attributed to the KOR1's interaction between CSCs and MTs.

Do microtubules influence any features of cellulose in addition to orientation?

In addition to guiding the deposition of cellulose microfibrils, MTs also play a role in the intracellular trafficking of CSCs and modulating the velocity of CSCs. CESA containing compartments such as SmaCCs (small CESA compartments) or MASCs (microtubule-associated CESA compartments) are localized along MTs and exhibit intermittent instances of rapid motility that is driven by MT depolymerization [48,49]. These observations are consistent with the hypothesis that SmaCCs/MASCs are involved in assisting the delivery of CSCs along the cortical MTs. Alternatively, SmaCCs/MASCs may function as intracellular storage vesicles of internalized CSCs. The tight association between SmaCCs/MASCs and MTs suggests an important role of cortical MTs in the trafficking of CSCs. Interestingly, the formation of SmaCCs/MASCs is also dependent on intact cortical MTs. This MT-dependent trafficking of CSCs might be mediated through CSI1 since CSI1 is associated with for SmaCCs/MASCs [37]. Many important questions in the formation and function of SmaCCs/MASCs remain to be addressed.

Recent studies suggest that MT mass is inversely correlated with cellulose crystallinity and CSC velocity [50]. It is postulated that the polymerization or crystallization of cellulose microfibrils provides the force to cause CSC movement. MTs are not required to provide the forces to move CSCs as previously postulated [2]. Consistent with this idea, treatment with 200 nM of the MT depolymerizing drug, oryzalin, for over 6 h does not affect CSC motility [19]. However, prolonged treatment with 20 μM oryzalin reduces the motility of CSCs to the same extent as *csi1* [33••]. These results imply that the reduction in CSC velocity in *csi1* might be attributed to a MT-dependent mechanism. However, CSC velocities in *csi1 csi3* double mutants is reduced to a greater extent than in *csi1* alone where the CSC/MT interaction is already abolished [32•], indicating that CSI proteins may also influence CSC velocities in a MT-independent fashion.

Alternatively, MTs have been postulated to direct the formation of plasma membrane micro-domains that could influence the activities of CSCs [51]. It is possible that the organization of CSC-containing specialized lipid microdomains is dependent on MTs and that the proper function of CSCs is contingent on the integrity of each of these components [52]. Furthermore, certain aspects of the CSC motility appears to be dependent on intact MTs since the asymmetric movement of CSCs in CESA1 phosphorylation mutants is abolished when cortical MTs were removed by oryzalin [53] and the inhibition of velocity of GFP-CESA5 in dark-grown *cesa6*$^{prc1-1}$, a null allele of *cesa6*, was relieved in the presence of oryzalin [54]. However, the detailed mechanisms underlying these phenomena are still unknown.

Perspective

The emergence of new components involved in the interaction between CSC and MTs provides new opportunities to advance the knowledge of how MTs regulate cellulose biosynthesis. Additionally, genetic and cell biology studies provide evidence that components in or associated with CSCs are capable of regulating MT organization and stability [46••,47,55]. The function of cortical MTs in plant cells is certainly not limited to regulating cellulose synthesis, so the feedback between MTs and the cell wall may potentially be integrated with other MT-related functions. The unique dynamic features of MTs add another layer of complexity to the investigation of the feedback regulation between the cytoskeleton and the cell wall in plants.

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