Cellulose Biosynthesis in Higher Plants and the Role of the Cytoskeleton

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Cellulose is a polysaccharide consisting of a linear chain of β (1→4) linked D-glucose and it is the most abundant polymer on earth. As a major structural component of the cell wall, cellulose accounts for about one-third of plant mass. The regulation of cellulose biosynthesis is essential to plant development. Cellulose is synthesised by the cellulose synthase (CESA) complex in the plasma membrane. This article reviews the composition and regulation of the cellulose synthase complex with a focus on the role of cytoskeleton in higher plants. In this article, the evolving views in the field of cellulose biosynthesis are discussed and the unresolved questions, such as *in vitro* cellulose synthesis, structure of CESA and mechanism underlying microtubule–microfibril alignment hypothesis, are highlighted.

Introduction

The plant cell wall is a highly organised structure composed of many different polysaccharides, proteins and aromatic compounds. The composition of the cell wall composite varies in different stages of the life cycle. The primary cell wall begins to form during cell division and primary wall materials continue to be deposited until the cell ceases its expansion. In some cells, the secondary cell wall is formed beneath the primary cell wall after a cell is fully-grown. Cellulose is a major polymer that makes up both primary and secondary cell walls and is thought to provide the framework for the assembly of other cell wall polymers, including hemicellulose, pectin and lignin. Cellulose, in its simplest forms, is made of a linear chain of a few hundred to

a couple thousand β-1,4 linked glucose molecules. Hydrogen bonding within the same chain and with neighbouring chains results in the formation of cellulose microfibrils with high tensile strength. Cellulose microfibrils are synthesised by large membrane-bound protein complexes known as cellulose synthase complexes (CSCs). In many cells, newly formed cellulose microfibrils are in transverse orientation that is perpendicular to the growth axis. The orientation of the cellulose microfibrils is often in parallel with the underlying cortical microtubules. It has long been postulated that cortical microtubules may align the trajectory of CSCs either by direct protein-mediated interaction or by channelling the movement of CSC in the membrane (Heath, 1974; Herth, 1980). Other factors influencing the direction of cellulose deposition include the actin cytoskeleton, light, growth factors, mechanical stimuli, nutrition and cell–cell interactions. Among those factors, the role of the cytoskeleton in oriented deposition of cellulose microfibrils may be one of the best characterised. **See also**: Plant Cell Walls; Plant Cell: Overview; Plant Microtubules: Their Role in Growth and Development; Plant Cell Wall Biosynthesis; Secondary Cell Walls

## Terminal Complex is the Site of Cellulose Synthesis

Cellulose-synthesising complexes were initially named terminal complexes (TCs) as they were often observed at the end of microfibrils by electron microscopy in freeze fracture replicas. In higher plants, the TC adopts a special rosette shape, which consists of six lobes with six-fold rotational symmetry (**Figure 1a**). In mung bean hypocotyls, rosette TCs were roughly arranged in a track following the direction of a microfibril imprint (Mueller and Brown, 1980). This finding supports the postulated role of rosette TCs in cellulose microfibril formation. Since the cloning of the first cellulose synthase (CESA) gene in *Acetobacter xylinus* (Saxena *et al.*, 1990; Wong *et al.*, 1990), it has been postulated that CESAs constitute the TC. The direct proof that CESA is a component of the rosette TC came from an
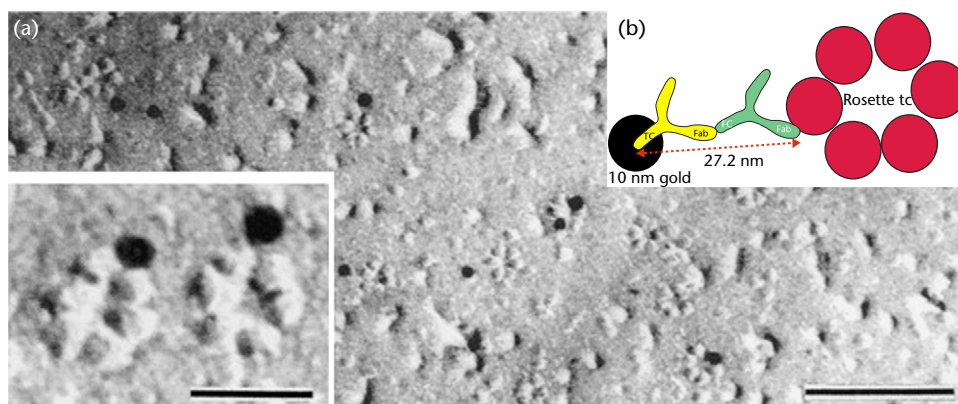
Figure 1 Immunolabelling of membrane rosettes with CESA antibodies. (a) Freeze-fractured replicas from vascular plant *Vigna angularis* were labelled with CESA antibodies. Many rosettes are labelled with antibodies. The inset shows an enlarged image of two rosettes with antibody labels. Bar=0.1 μm. Bar in inset=30 nm. (b) Scale model of primary and secondary antibody dimensions relative the 10 nm gold particle and 25 nm rosette dimensions. Adapted from Kimura *et al.* (1999). Copyright by American Society of Plant Biologists.

immunogold labelling experiment (Kimura *et al.*, 1999). The antibody against the catalytic region of CESA from cotton not only recognised protein from three genera of vascular plants including *Vigna angularis*, *Arabidopsis thaliana* and *Gossypium*, but also recognised protein from *Escherichia coli*, which suggests that the catalytic domain of CESA is highly conserved. Using a freeze-fracture labelling technique, the rosette TCs in *V. angularis* were labelled with colloidal gold bound to a goat anti-rabbit secondary antibody, which recognised antibodies that were attached to the cotton CESA antigen (**Figure 1b**). This experiment confirmed the long-held hypothesis that rosette TCs contain CESA proteins as first described by Mueller and Brown in 1980.

In vitro Cellulose Synthesis: A Difficult Approach with many Challenges

Attempts to demonstrate the *in vitro* synthesis of cellulose using biochemical approaches have been problematic. *In vitro* cellulose synthesis from membrane fractions of cotton fibres yields mostly β-1,3-glucan and little or none β-1,4-glucan (Somerville, 2006). β-1,3-glucan, structurally and functionally distinct from cellulose, is synthesised by plasma membrane-bound callose synthase and is normally found in small amounts in specialised cell types or in response to stresses such as infection or wounding. Although the reason why callose is the major product of the *in vitro* cellulose assay in cell-free extracts remains to be determined, steady progress has been made in determining suitable reaction conditions. By modifying the membrane extraction procedure to use a proper choice of detergents, *in vitro* cellulose synthesis has been tested using extract from mung bean, blackberry and suspension cell culture of hybrid aspen and tobacco (Bessueille *et al.*, 2009; Cifuentes *et al.*, 2010; Kudlicka and Brown, 1997; Lai-Kee-Him *et al.*, 2002). Compared with 4% cellulose yield from cotton

fibres, 20% and 36% cellulose yield from blackberry and tobacco BY-2 cells, respectively, represents significant advances in the field. By electron microscopy and X-ray diffraction analysis, the cellulose microfibrils synthesised *in vitro* from blackberry was determined to be identical to the native cellulose. Despite the recent progress, cellulose synthesis cannot be assayed routinely in a high-throughput manner for several reasons. First, the *in vitro* product still contains a large quantity of callose. Second, characterisation of *in vitro* product requires a complex and costly technique. Third, conditions for *in vitro* cellulose assay need to be optimised for extracts from different plant models. Recent effort has been put into *in vitro* expression of active recombinant CESA subunits. Hopefully, the determination of the three-dimensional structure of the enzyme along with progress in the *in vitro* cellulose synthesis will unravel the mysterious mechanism of cellulose synthesis.

From Genes to Rosettes: CSCs Models

The first gene isolated to encode the catalytic subunit of cellulose synthase (CESA) is from a cellulose-producing bacterium *Acetobacter xylinus*. Since then, CESA genes have been identified in many plant species. Although TC morphology in bacteria is completely different from that of higher plants, the overall gene structure of CESA is conserved from bacteria to higher plants. CESAs from bacteria and higher plants contain multiple N- and C-terminal transmembrane domains and a cytosolic catalytic domain consisting of four conserved regions (**Figure 2**). All CESAs contain the D1, D2, D3 and QXXRW signature motif, which is characteristic of processive β-glycosyltransferases and believed to be important for substrate binding and catalysis. Plant CESAs also contain a cysteine-rich zinc binding domain (Zn) at the N-terminus, a conserved region (CR-P) between U1 and U2 and a variable class specific
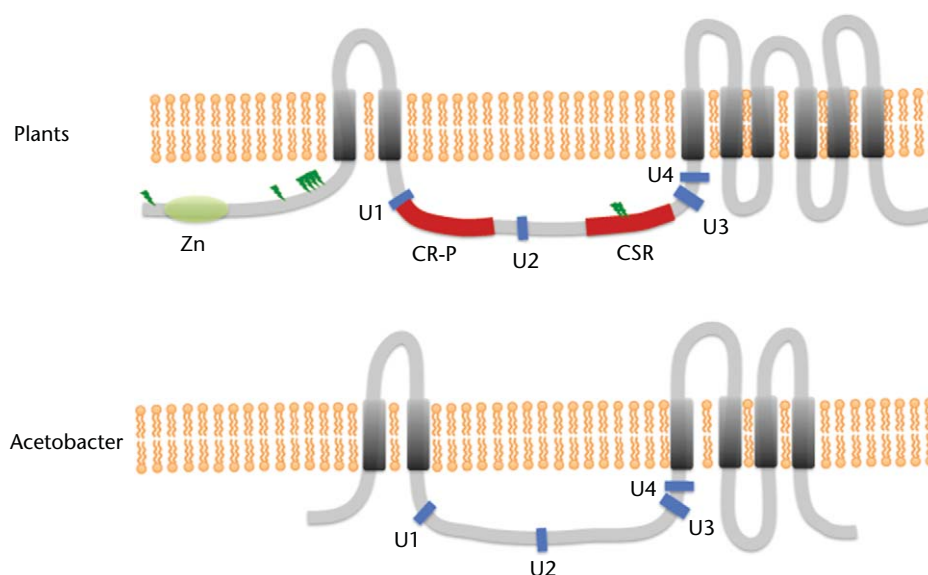
Figure 2 Comparison of predicted CESA protein structure from plants and bacteria. CESA protein from plants *A. thaliana* (O48946.1) and *Gluconoacetobacter xylinus* (CAA38487.1). The diagrams are aligned at the U4 region. Domains shown by colour blocks are: zinc-finger domain (Zn, green); transmembrane domains (TMD, black); conserved regions (U1-U4, blue); conserved region only present in plants (CR-P, red) and class-specific region (CSR, red). Phosphorylation sites (lightning mark, green).

region (CSR) between U3 and U4. The absence of Zn, CR-P and CSR in nonplant CESA proteins suggests that these regions are dispensable for catalytic activity of CESA proteins. These regions are presumably located in the cytoplasm where they may serve to interact with other proteins in the plant cell cortex. One of the hypothetical models for the topology of CESA postulates that eight transmembrane helices form a pore in the plasma membrane through which the newly synthesised glucan chain is extruded to the cell wall (Richmond and Somerville, 2000; **Figure 3a**).

To form a cellulose synthase complex (CSC), one CESA subunit must interact with other CESA subunits possibly through interactions between transmembrane helices and/or some of the cytoplasmic domains. Based on the assumptions that (1) one rosette synthesises one elementary fibril, (2) one elementary fibril contains 36-chains and (3) each CESA synthesises one chain at a time, the CSC is postulated to contain 36 CESA subunits (Doblin *et al.*, 2002; Gu and Somerville, 2010). This model is widely cited in the literature (**Figure 3a**); however, recent estimations suggest that it is more likely that only 12–18 chains are present in microfibrils in the primary cell walls of higher plants (Endler and Persson, 2011). Therefore, the number of subunits in the CSC could be 12–18 if the estimation of chain number is correct.

Tools for CSC Visualisation

Transmission electron microscopy (TEM) forms a super-resolution image from the interaction of the electrons transmitted through an ultra thin specimen. TEM has been the method of choice for ultrastructural analysis of the cell wall (Harris *et al.*, 2010). Combining freeze-fracture sample preparation or deep-etching electron microscopy, TEM was frequently used to observe the rosettes of cellulose synthase. However, TEM requires extensive sample preparation, is highly invasive and cannot be used for imaging of live samples.

With little or no sample preparation, confocal microscopy in combination with fluorescent protein (FP) fusion allow us to visualise CSCs in living cells, thus revealing dynamic localisation and/or function of CSCs. By observing fluorescent protein tagged CESA with spinning disc confocal microscopy, researchers have shown that CSCs localised at the plasma membrane and move bi-directionally at an average speed of 300–350 nm/min (Paredez *et al.*, 2006). In addition to distinct particles at the plasma membrane, CSCs also localised to donut-shaped Golgi compartments, consistent with the observation that rosettes were detected in the Golgi by freeze fracture EM (Haigler and Brown, 1986).

One inherent limitation of fluorescent microscopy is the resolution. Recent technical developments, such as stimulated emission depletion (STED) microscopy, photo-activated localisation microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), have broken the diffraction resolution barrier (Gutierrez *et al.*, 2010). Also, with the use of promising new affinity probes, a correlative imaging technique that combines fluorescence microscopy and electron microscopy is being built. With these different tools in hand, it is anticipated to resolve the molecular details of CSCs in the near future.

(a)

(b)

YFP::CESA6   RFP::TUA5   Merge
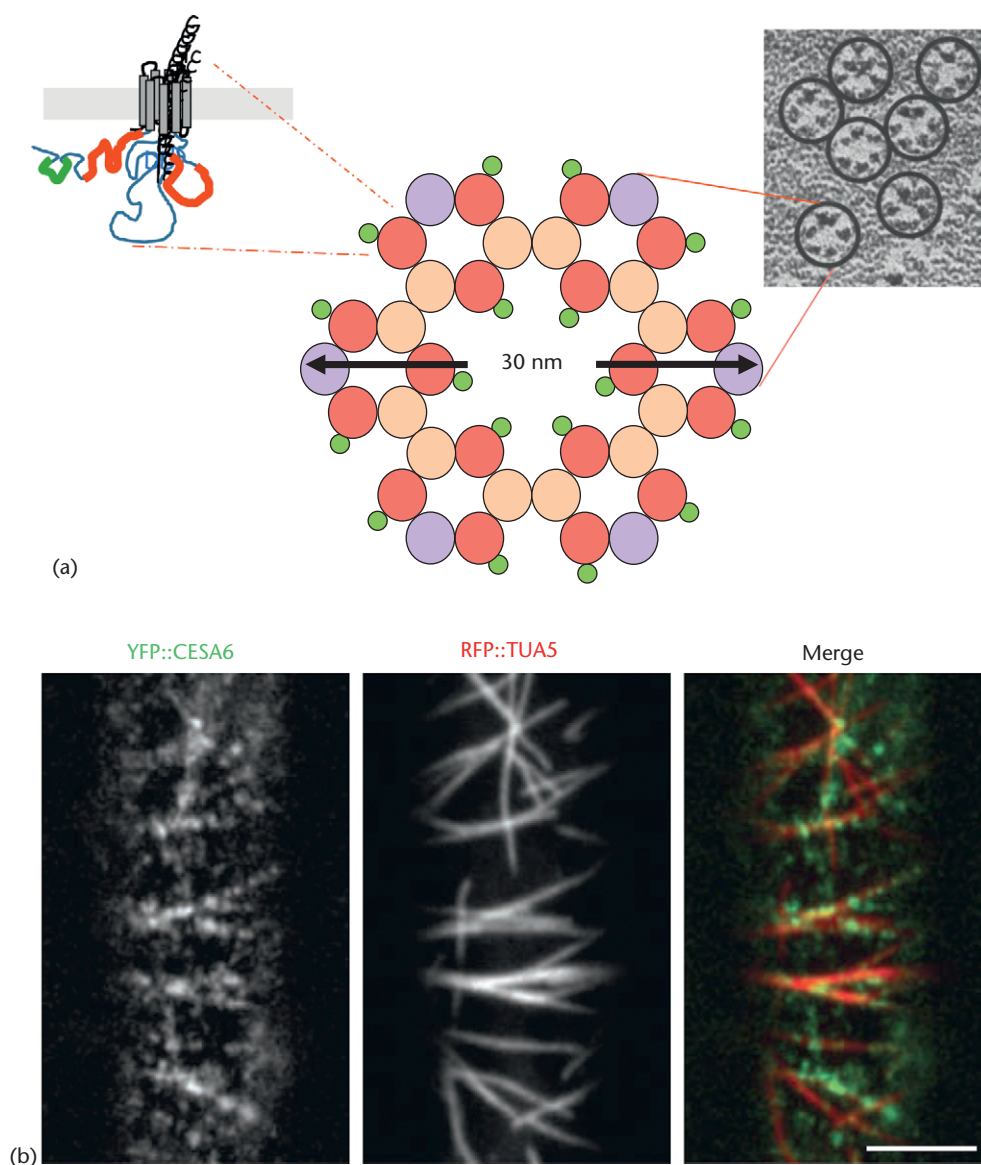
Figure 3 Visualisation of CESA complexes. (a) A heteromeric model for the CESA complexes. The rosettes (encircled for better visualisation) image is adapted from Herth (1985). In a widely cited heteromeric model, a single rosette is composed of 36 CESA subunits of three isoforms that are illustrated by three different colours (Purple, red and brown). The topology of a single CESA subunit is shown on the left, adapted from Richmond and Somerville (2000). Regions are coloured to follow those shown in **Figure 2**. (b) Co-localisation of cellulose synthase complexes and microtubules. Cellulose synthase complexes are labelled by yellow fluorescent protein (YFP) tagging of CESA6 (shown as green circles in **Figure 3a**). Microtubules are labelled by red fluorescent protein (RFP) tagging of TUA5. Merge image shows CESA complexes co-align with underlying microtubules. Bar=5 μm. Adapted from Gutierrez *et al.* (2009). Copyright by Nature Publishing Group.

Non-CESA Proteins Involved in Cellulose Synthesis

A number of proteins that affect cellulose synthesis have been identified in forward genetics mutant screens, including COBRA, KOBITO and KORRIGAN, but their exact roles in cellulose synthesis remains unknown (Lei *et al.*, 2012). The *cobra* mutation caused abnormal root cell expansion, and it also resulted in a reduction of crystalline cellulose (Schindelman *et al.*, 2001). The loss of anisotropic expansion in *cobra* mutants was accompanied by disorganisation of the orientation of cellulose microfibril, indicating COBRA has a role in orienting cellulose microfibril deposition. The COBRA gene encodes a glycophosphatidyl inositol (GPI)-anchored protein. Although possible mechanisms have been proposed, the exact role of COBRA remains elusive. Similar to *cobra*, the *kobito* mutant was defective in anisotropic expansion, proper orientation of cellulose microfibril and the amount

of crystalline cellulose (Pagant *et al*., 2002). The role of KOBITO is even more elusive because the gene codes a plant-specific protein with no homology to any known proteins. The KORRIGAN (KOR) gene encodes a membrane-localised β-1,4-glucanase (Nicol *et al*., 1998). The endoglucanase activity of KOR has been shown for Cel16, a KOR-like protein in *Brassica napus*. KOR was co-egulated transcriptionally in cells synthesising primary and secondary cell walls, and mutations in the *KOR* gene resulted in cellulose deficiency in both primary and secondary cell walls. The documented subcellular localisation of KOR includes the plasma membrane and intracellular compartments (Crowell *et al*., 2010). It is tempting to speculate that KOR might be an integral component of CSCs though no direct data supports this hypothesis.

In an attempt to identify CESA interactive proteins, the central domains of primary CESAs were used as bait in a yeast two-hybrid screen of the *Arabidopsis* complementary deoxyribonucleic acid (cDNA) library. Among several dozen putative CESA interactive proteins, cellulose synthase interactive protein 1 (CSI1) was coregulated and interacts with multiple primary CESAs (Gu *et al*., 2010). *csi1-3*, a T-DNA insertion null mutant of CSI1, displayed defects in cell expansion that are consistent with the reduction of crystalline cellulose content in those cells. Similar to CESA, CSI1 protein was present at the plasma membrane and moved bi-directionally at a speed indistinguishable from CESA. As the first non-CESA protein shown to associate with CSCs, CSI1 opens up many opportunities to explore the regulation of cellulose biosynthesis.

Cellulose Synthesis and Microtubule Cytoskeleton

Cellulose microfibrils, as the major load-bearing polymers in cell walls, are the predominant component enforcing the asymmetric cell expansion (Doblin *et al*., 2002). In growing cells, cellulose microfibrils are laid down transversely to the axis of elongation, thus forming a spring-like structure reinforcing the cell laterally and favoring longitudinal expansion. How cells establish the orientation of cellulose microfibrils is an intriguing issue. Cortical microtubules, which lie just beneath the plasma membrane, are the best candidates for participating in specifying the orientation of cellulose microfibrils because of their close vicinity to CSCs and close relation to microfibril orientation (Hepler and Newcomb, 1964; Ledbetter and Porter, 1964). In a simplified microtubule–microfibril alignment hypothesis, new microfibrils are deposited on the inner surface of the wall in a transverse orientation mirroring orientation of the underlying cortical microtubules (Green, 1962). Two hypotheses were put forward to explain the microtubule–microfibril alignment (Heath, 1974). The direct hypothesis postulates some types of direct linkage between CESA complexes and microtubules. The indirect hypothesis, also

known as the bumper model, proposes that the interaction of microtubules with plasma membrane changes membrane fluidity, thereby restricting the movement of the CSCs and there is no need for direct interaction between CSCs and microtubules. Although the precise mechanism for how microtubules might align cellulose synthesis remains unclear, the alignment hypothesis has been assessed in many higher plants including oat, *Arabidopsis*, maize and cotton (Baskin, 2001). In most cases, microtubules are parallel to microfibrils, although discrepancies have also been noted. Support of microtubule–microfibril alignment also comes from many pharmacological studies where the orientation of microfibrils was impaired when microtubules were removed using microtubule-disrupting agents (Lloyd and Chan, 2008).

A recent advance in exploring the role of microtubules in cellulose synthesis was made by *in vivo* imaging of microtubules and CSCs simultaneously. Using a transgenic line coexpressing CFP-TUA1 (labels microtubules) and YFP-CESA6 (labels CSCs), CSCs can be directly observed moving through the plasma membrane upon tracks provided by the underlying cortical microtubules (Paredez *et al*., 2006). When reorganisation of microtubule arrays was triggered by exposure to oryzalin (a microtubule-disrupting agent), the trajectories of the CESA particles changed in a correlated shift. These experiments provide convincing evidence to support the alignment model. Interestingly, complete removal of microtubules by oryzalin did not result in a random distribution of CSCs. The residual alignment of CSCs after oryzalin treatment suggests that CSCs may retain some alignment from components of the wall.

In addition to the role of guidance in the deposition of cellulose microfibrils, microtubules also have been shown to position the delivery of CSCs to the plasma membrane (Crowell *et al*., 2009; Gutierrez *et al*., 2009). YFP-CESA6 and GFP-CESA3 label at least three distinct populations including distinct particles at the plasma membrane, donut-shaped Golgi compartments and a small compartment termed SmaCCs (small CESA compartments) or MASCs (microtubule-associated cellulose synthase compartments). SmaCCs/MASCs were distinguishable from plasma membrane-localised CSCs by their focal plane (slightly below the plasma membrane), irregular speed and higher fluorescence intensities. SmaCCs/MASCs can be observed in fully elongated cells 10 mm below the apical hook where CSCs were in lower abundance. In cells actively synthesising cellulose (2-4 mm below the apical hook), SmaCCs/MASCs can be induced upon osmotic stress, protein synthesis inhibition or cellulose synthesis inhibition (Bashline *et al*., 2011). SmaCCs/MASCs associate with microtubules based on the following observations: (1) the motility of SmaCCs/MASCs was consistent with the tracking of depolymerising microtubule ends, (2) SmaCCs/MASCs moved in a linear path that coincides with microtubule trajectory, and (3) removal of microtubules affected the accumulation and motility of SmaCCs/MASCs. When cellulose synthesis was inhibited

by treatment with isoxaben, a cellulose synthesis inhibitor, or by osmotic stress, the association of SmaCCs/MASCs and microtubules was greatly enhanced. On recovery from osmotic stress, the delivery of CSCs to the plasma membrane was associated with microtubule-tethered SmaCCs/MASCs. The function of microtubule-dependent positioning of CESA delivery is speculated either to ensure efficient association of CESA with microtubules at the time of microfibril initiation or to help coordinate the location or timing of CESA delivery (Gutierrez *et al.*, 2009).

CSI1: The Missing Link between CSCs and Microtubules

Although it is generally accepted that microtubules determine the orientation of cellulose deposition, the precise molecular mechanisms linking microtubules to cellulose organisation still remain unclear. It was long proposed that a linker protein mediates the interaction between cellulose synthase complexes and microtubules (Heath, 1974). After three decades, this linker protein finally emerged. CSI1, initially identified through a yeast two-hybrid screen for

CESA interactive proteins, is linked to both CSCs and microtubules.

In seedlings coexpressing RFP-CSI1 and GFP-CESA6, CSI1 co-localised with CSCs almost completely and travelled in a nearly identical speed as that of CSCs (**Figure 4a**). When these seedlings were treated with isoxaben, both CSI1 and CSCs were depleted from the membrane, indicating that the association of CSI1 and CSCs is close and direct (Li *et al.*, 2012). To satisfy the requirements for a putative linker protein, CSI1 also needs to interact with microtubules. Indeed, CSI1 binds to microtubule *in vitro*, with a disassociation constant of $1\,\mu M$, which is similar to conventional microtubule binding proteins (MAPs). As a bona fide MAP, CSI1 also decorated microtubules *in vivo* (**Figure 4b**). The linear trajectories of CSI1 matched with tracks of microtubules nearly identically. These results convey a mechanism by which CSI1 associates with CSCs on one side and glides along the microtubules on the other. When CSI1 was missing in a *csi1* null mutant, CSCs lost their close alignment with underlying cortical microtubules (**Figure 4c–d**) and exhibited a reduction in velocity, which is consistent with the reduced crystalline cellulose content (Bringmann *et al.*, 2012; Li *et al.*, 2012). The loss of CSI1 can be phenocopied by loss of microtubules in terms of loss
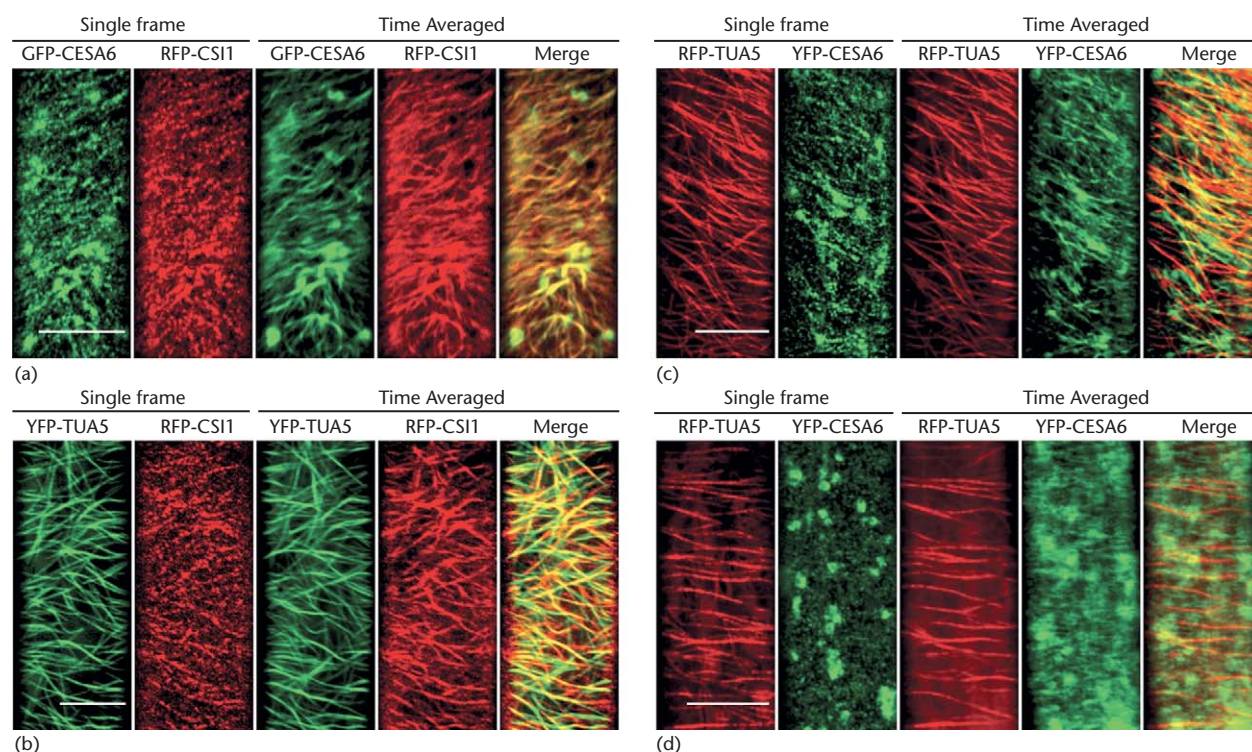
Figure 4 Co-localisation of CSI1, CESA complexes and microtubules. (a) Wild type seedlings coexpressing GFP-CESA6 and RFP-CSI1: the co-alignment of CSI1 and CESA complexes is evident in the merged time-averaged image. (b) Wild-type seedlings co-expressing YFP-TUA5 and RFP-CSI1: the co-alignment of CSI1 and microtubules is evident in the merged time-averaged image. (c) Wild type seedlings co-expressing RFP-TUA5 and YFP-CESA6: the co-alignment of CESA complexes and microtubules is evident in the merged time-averaged image. (d) In *csi1* seedlings co-expressing YFP-CESA6 and RFP-TUA5, CESA particles are randomly distributed, their time-averaged trajectories are apparently shorter and rarely co-localised with microtubules. Note that the large, roughly circular structures in the GFP-CESA6 (a) and YFP-CESA6 (c, d) images are Golgi bodies. The time-averaged images are projections of 60 frames (∼5 min) acquired at 5 s intervals. Bars=10 μm. Reproduced from Baskin and Gu (2012). Copyright by Landes Bioscience.

of anisotropic growth and reduced CESA motility, further supporting the role of CSI1 as the linker between microtubules and CSCs.

CSI1 is present in all land plants. It remains to be determined whether CSI1 represents a conserved mechanism for microfibril–microtubule alignment in other plant species. Furthermore, *Arabidopsis* encodes two CSI1-like proteins, namely CSI2 and CSI3. Though the triple mutants, *csi1csi2csi3* had an expansion defect that was greater than that of *csi1*, these plants were still viable (Bringmann *et al.*, 2012). The viability of the triple mutants might suggest that CSI proteins are dispensable for overall cellulose biosynthesis or additional proteins may be involved in microfibril–microtubule alignment.

Cellulose Synthesis and Actin Cytoskeleton

Disruption of the actin cytoskeleton by cytochalasins (an actin disrupting agent) in cotton fibres and *Zinnia* treachery elements resulted in the disorganisation of cellulose microfibrils (Seagull, 1990). Microtubules were observed to reorient upon disruption of the actin microfilament array, and it was therefore proposed that the actin cytoskeleton might contribute to cell elongation through the interaction between actin microfilaments and microtubules. The cross-talk between actin and microtubules has been demonstrated mostly by pharmacological studies where stabilising or disrupting one element affects the other (Collings, 2008). Recently, the dynamic association between actin microfilaments and microtubules was confirmed in interphase plant cells using an F-actin and microtubule dual-labelled line (Sampathkumar *et al.*, 2011).

Although the actin–microtubule interaction hypothesis remains to be tested, recent studies reveal that actin is required for global distribution of CSCs in primary cell walls (Crowell *et al.*, 2009; Gutierrez *et al.*, 2009). Perturbation of actin cytoskeleton resulted in aggregation and reduced motility of Golgi-localised CSCs and in an uneven distribution of plasma membrane-localised CSC. In xylem vessels, which are models for secondary wall synthesis, transverse actin bundles were positioned close to the sites of wall synthesis, and a disruption of actin by Latrunculin B resulted in a loss of transverse CSC bands (Wightman and Turner, 2008). These observations led to a hypothesis that transverse actin bands mark CSC delivery sites at the cell membrane in xylem vessels. If this hypothesis holds true, then the mechanism for CSC delivery in secondary walls may differ from primary walls, in which CSC delivery sites are marked by cortical microtubules.

References

Bashline L, DU J and Gu Y (2011) The trafficking and behavior of cellulose synthase and a glimpse of potential cellulose synthesis regulators. *Frontiers of Biology* **6**: 377–383.

Baskin TI (2001) On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. *Protoplasma* **215**: 150–171.

Baskin TI and Gu Y (2012) Making parallel lines meet: Transferring information from microtubules to extra-cellular matrix. *Cell Adhesion & Migration* **6**: 1–5.

Bessueille L, Sindt N, Guichardant M *et al.* (2009) Plasma membrane microdomains from hybrid aspen cells are involved in cell wall polysaccharide biosynthesis. *Biochemical Journal* **420**: 93–103.

Bringmann M, Li E, Sampathkumar A *et al.* (2012) POM-POM2/CELLULOSE SYNTHASE INTERACTING1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*. *Plant Cell* **24**: 163–177.

Cifuentes C, Bulone V and Emons AM (2010) Biosynthesis of callose and cellulose by detergent extracts of tobacco cell membranes and quantification of the polymers synthesized *in vitro*. *Journal of Integrative Plant Biology* **52**: 221–233.

Collings DA (2008) Crossed wires: Interactions and cross-talk between the microtubule and microfilament networks in plants In: Peter N (ed) *Plant Cell Monographs: Plant Microtubules, Development, and Flexibilty*, pp. 47–82. Berlin: Springer.

Crowell EF, Bischoff V, Desprez T *et al.* (2009) Pausing of golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell* **21**: 1141–1154.

Crowell EF, Gonneau M, Stierhof YD, Hofte H and Vernhettes S (2010) Regulated trafficking of cellulose synthases. *Current Opinion in Plant Biology* **13**: 700–705.

Doblin MS, Kurek I, Jacob-Wilk D and Delmer DP (2002) Cellulose biosynthesis in plants: From genes to rosettes. *Plant and Cell Physiology* **43**: 1407–1420.

Endler A and Persson S (2011) Cellulose synthases and synthesis in *Arabidopsis*. *Molecular Plant* **4**: 199–211.

Green PB (1962) Mechanism for plant cellular morphogenesis. *Science* **138**: 1404–1405.

Gu Y and Somerville C (2010) Cellulose synthase interacting protein: A new factor in cellulose synthesis. *Plant Signaling and Behavior* **5**: 1571–1574.

Gu Y, Kaplinsky N, Bringmann M *et al.* (2010) Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. *Proceedings of the National Academy of Sciences of the USA* **107**: 12866–12871.

Gutierrez R, Grossmann G, Frommer WB and Ehrhardt DW (2010) Opportunities to explore plant membrane organization with super-resolution microscopy. *Plant Physiology* **154**: 463–466.

Gutierrez R, Lindeboom JJ, Paredez AR, Emons AM and Ehrhardt DW (2009) *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nature Cell Biology* **11**: 797–806.

Haigler CH and Brown RM (1986) Transport of rosettes from the golgi-apparatus to the plasma-membrane in isolated mesophyll-cells of Zinnia-Elegans during differentiation to tracheary elements in suspension-culture. *Protoplasma* **134**: 111–120.

Harris D, Bulone V, Ding SY and DeBolt S (2010) Tools for cellulose analysis in plant cell walls. *Plant Physiology* **153**: 420–426.

Heath IB (1974) A unified hypothesis for the role of membrane bound enzyme complexes and microtubules in plant cell wall synthesis. *Journal of Theoretical Biology* **48**: 445–449.

Hepler PK and Newcomb EH (1964) Microtubules and fibrils in the cytoplasm of coleus cells undergoing secondary wall deposition. *Journal of Cell Biology* **20**: 529–532.

Herth W (1980) Calcofluor white and Congo red inhibit chitin microfibril assembly of Poterioochromonas: Evidence for a gap between polymerization and microfibril formation. *Journal of Cell Biology* **87**: 442–450.

Herth W (1985) Plasma-membrane rosettes involved in localized wall thickening during xylem vessel formation of *Lepidium sativum* L. *Planta* **164**: 12–21.

Kimura S, Laosinchai W, Itoh T *et al.* (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. *Plant Cell* **11**: 2075–2086.

Kudlicka K and Brown RM Jr. (1997) Cellulose and callose biosynthesis in higher plants (i. solubilization and separation of (1->3)- and (1->4)-[beta]-glucan synthase activities from mung Bean). *Plant Physiology* **115**: 643–656.

Lai-Kee-Him J, Chanzy H, Muller M *et al.* (2002) *In vitro* versus *in vivo* cellulose microfibrils from plant primary wall synthases: structural differences. *Journal of Biological Chemistry* **277**: 36931–36939.

Ledbetter MC and Porter KR (1964) Morphology of microtubules of plant cells. *Science* **144**: 872–874.

Lei L, Li S and Y G (2012) Cellulose synthase complexes: Composition and regulation. *Frontiers in Plant Science* **3**: 1–6.

Li S, Lei L, Somerville C and Gu Y (2012) Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. *Proceedings of the National Academy of Sciences of the USA* **109**: 185–190.

Lloyd C and Chan J (2008) The parallel lives of microtubules and cellulose microfibrils. *Current Opinion in Plant Biology* **11**: 641–646.

Mueller SC and Brown RM Jr. (1980) Evidence for an intra-membrane component associated with a cellulose microfibril-synthesizing complex in higher plants. *Journal of Cell Biology* **84**: 315–326.

Nicol F, His I, Jauneau A *et al.* (1998) A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. *EMBO Journal* **17**: 5563–5576.

Pagant S, Bichet A, Sugimoto K *et al.* (2002) KOBITO1 encodes a novel plasma membrane protein necessary for normal synthesis of cellulose during cell expansion in *Arabidopsis*. *Plant Cell* **14**: 2001–2013.

Paredez AR, Somerville CR and Ehrhardt DW (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**: 1491–1495.

Richmond TA and Somerville CR (2000) The cellulose synthase superfamily. *Plant Physiology* **124**: 495–498.

Sampathkumar A, Lindeboom JJ, Debolt S *et al.* (2011) Live cell imaging reveals structural associations between the actin and microtubule cytoskeleton in *Arabidopsis*. *Plant Cell* **23**: 2302–2313.

Saxena IM, Lin FC and Brown RM Jr. (1990) Cloning and sequencing of the cellulose synthase catalytic subunit gene of *Acetobacter xylinum*. *Plant Molecular Biology* **15**: 673–683.

Schindelman G, Morikami A, Jung J *et al.* (2001) COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in *Arabidopsis*. *Genes and Development* **15**: 1115–1127.

Seagull RW (1990) The effects of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibers. *Protoplasma* **159**: 44–59.

Somerville C (2006) Cellulose synthesis in higher plants. *Annual Review of Cell and Developmental Biology* **22**: 53–78.

Wightman R and Turner SR (2008) The roles of the cytoskeleton during cellulose deposition at the secondary cell wall. *Plant Journal* **54**: 794–805.

Wong HC, Fear AL, Calhoon RD *et al.* (1990) Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. *Proceedings of the National Academy of Sciences of the USA* **87**: 8130–8134.

Further Reading

Bischoff V, Desprez T, Mouille G *et al.* (2011) Phytochrome regulation of cellulose synthesis in *Arabidopsis*. *Current Biology* **21**: 1822–1827.

Brown RM and Saxena IM (2005) Cellulose biosynthesis: Current views and evolving concepts. *Annals of Botany* **96**: 9–21.

Crowell EF, Gonneau M, Vernhettes S and Hofte H (2010) Regulation of anisotropic cell expansion in higher plants. *Comptes Rendus Biologies* **333**: 320–324.

Desprez T, Juraniec M, Crowell EF *et al.* (2007) Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **104**: 15572–15577.

Guerriero G, Fugelstad J and Bulone V (2010) What do we really know about cellulose biosynthesis in higher plants? *Journal of Integrative Plant Biology* **52**: 161–175.

Mei Y, Gao HB, Yuan M and Xue HW (2012) The *Arabidopsis* ARCP protein, CSI1, which is required for microtubule stability, is necessary for root and anther development. *Plant Cell* **24**: 1066–1080.

Wasteneys GO (2004) Progress in understanding the role of microtubules in plant cells. *Current Opinion in Plant Biology* **7**: 651–660.