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REVIEW

The trafficking and behavior of cellulose synthase and a glimpse of potential cellulose synthesis regulators

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Abstract **Cellulose biosynthesis is a topic of intensive research not only due to the significance of cellulose in the integrity of plant cell walls, but also due to the potential of using cellulose, a natural carbon source, in the production of biofuels. Characterization of the composition, regulation, and trafficking of cellulose synthase complexes (CSCs) is critical to an understanding of cellulose biosynthesis as well as the characterization of additional proteins that contribute to the production of cellulose either through direct interactions with CSCs or through indirect mechanisms. In this review, a highlight of a few proteins that appear to affect cellulose biosynthesis, which includes: KORRIGAN (KOR), Cellulose Synthase-Interactive Protein 1 (CSI1), and the poplar microtubule-associated protein, PttMAP20, will accompany a description of cellulose synthase (CESA) behavior and a discussion of CESA trafficking compartments that might act in the regulation of cellulose biosynthesis.**

Keywords cellulose synthesis, cellulose synthase complex (CSC), dynamics, trafficking

Introduction

Cellulose, a polysaccharide comprised of β-(1→4)-glucan, is an attractive candidate for use as a biofuel feedstock due to its carbon-rich structure and its high abundance in plants. The elucidation of the mechanism by which plants synthesize cellulose might lead to methods to enhance cellulosic biofuel production. Decades ago in the study of cellulose biosynthesis, a membrane spanning six-lobed rosette structure that was observed in freeze-fractured tissue by electron microscopy was determined to be responsible for the biosynthesis of cellulose (Mueller and Brown, 1980). Within each lobe, it has been speculated that six cellulose synthase (CESA) proteins are arranged symmetrically such that each rosette is comprised of 36 CESA subunits, which corresponds to the production of a cellulose microfibril that is made up of 36 chains of β-(1→4)-glucan (Delmer, 1999). It has been confirmed that rosettes contain CESAs through the use of electron microscopy to visualize immunogold-labeled cotton cellulose synthase (Kimura et al., 1999). In *Arabidopsis thaliana*, ten CESA genes have been identified. Genetic and biochemical studies suggest that primary cellulose synthase complexes (CSCs), which are responsible for the production of cellulose during cell expansion, are comprised of CESA1, CESA3, and CESA6 where CESA6 is partially redundant with CESA2 and CESA5 (Scheible et al., 2001; Desprez et al., 2007; Doblin et al., 2002; Persson et al., 2007; Wang et al., 2008). Similarly, three different isoforms of CESA (CESA4, CESA7, and CESA8) are required to make up the secondary CSC that is responsible for cellulose synthesis after the completion of cell expansion (Taylor et al., 1999, 2000, 2003). Extensive sequence analysis revealed that divergence of primary and secondary CSC occurred prior to evolution of seed plants (Carroll and Specht, 2011).

The characterization of primary and secondary CESA mutants has improved our understanding of CSCs. A screen of temperature-sensitive mutants in which there was radial swelling of the root at elevated temperatures has revealed several cellulose deficient mutants. One radial swelling mutant, *rsw1*, which has been shown to be caused by a CESA1 point mutation, has a reduction in cellulose, decreased rosette density at the plasma membrane, and reduced primary CESA expression when grown at the

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restrictive temperature (Arioli et al., 1998). Irregular xylem mutants that are mapped to the secondary CESAs, CESA4 (IRX1), CESA7 (IRX3), and CESA8 (IRX5), also show cellulose deficiency phenotypes (Taylor et al., 1999, 2000, 2003).

The use of these CESA mutants along with various biochemical techniques has confirmed the identity of the three isoforms that comprise the primary and secondary CSCs. In support of the composition of the primary CSC, co-immunoprecipitation (co-IP) experiments show a physical interaction between CESA3 and CESA6 in detergent-solubilized protein extracts from dark-grown seedlings and *in vivo* bimolecular fluorescence complementation (BiFC) experiments suggest dimerization capability between each combination of CESA1, CESA3, and CESA6, which includes homodimerization (Desprez et al., 2007; Timmers et al., 2009). Also, the previously described RSW1 is able to pull down CESA3 and CESA6 in co-IP experiments at the permissive temperature but not at the restrictive temperature (Desprez et al., 2007; Wang et al., 2008). In the case of secondary CSCs, co-IP experiments suggest that CESA4 (IRX1), CESA7 (IRX3), and CESA8 (IRX5) can form a complex. In the *irx5* (*cesa8*) mutant background, co-IP is no longer observed when either CESA4 (IRX1) or CESA7 (IRX3) is used as the probe suggesting a crucial role for CESA8 in secondary CSC assembly (Taylor et al., 2003). Split-ubiquitin yeast two hybrid and BiFC experiments have shown the ability of heterodimerization between all combinations of secondary CESAs as well as the ability of CESA4 to form homodimers (Timmers et al., 2009).

Although several models of CESA arrangement and stoichiometry within the rosette have been proposed, indisputable experimental evidence has yet to be shown (Scheible et al., 2001; Doblin et al., 2002; Somerville, 2006; Timmers et al., 2009). In addition to CESA proteins, several other proteins have been shown to affect cellulose synthesis. Progress in the characterization of additional proteins involved in cellulose synthesis and in the trafficking and regulation of the CSC will provide valuable insight into the biosynthesis of cellulose.

Non-CESA proteins involved in cellulose synthesis

The mapping of cellulose deficient mutants has identified several non-CESA proteins (COBRA, KOBITO, FRAGILE FIBER1and2, CTL1/POM1, KORRIGAN) that have a direct or indirect effect on cellulose synthesis (Zuo et al., 2000; Schindelman et al., 2001; Pagant et al., 2002; Zhong et al., 2002; Zhong et al., 2004). Co-IP experiments in which CESA proteins have been successful in pulling down other CESA isoforms have been unsuccessful in identifying additional proteins associated with CSCs (Taylor et al., 2003; Desprez et al., 2007; Wang et al., 2008). Recently, the use of the central domain of CESA as bait in a yeast two-hybrid screen against an *Arabidopsis* cDNA library has given clues toward the discovery of proteins that are potential interacting partners with primary CESA proteins (Gu et al., 2010; Gu and Somerville, 2010). The characterization of proteins that either interact with the CSC or are components of the CSC will greatly improve our understanding of cellulose biosynthesis.

KORRIGAN (KOR)

KORROGAM (*KOR*) encodes an integral membrane endo-β-1,4-glucanase or cellulase that has been proposed to play several roles in primary and secondary cellulose synthesis. Several *kor* mutants have been isolated, but the plethora of phenotypes attributed to these mutants has caused the identification of the specific role of KOR to remain elusive. Two *korrigan* mutant alleles (*kor1-1* and *kor1-2*) contain T-DNA insertions in the promoter region of KOR that cause low expression levels. *kor1-1* confers a cell elongation defect while the more severe *kor1-2* mutant confers a cytokinesis defect in addition to its elongation defect (Nicol et al., 1998; Zuo et al., 2000). The polar distribution of KOR to the cell plate, which is critical for proper cell division, is mediated by two sorting signals, a dileucine motif and a tyrosine motif (Zuo et al., 2000). Although expression of a wild type *kor* cDNA construct complements the *kor1-2* mutant, expression of cDNA constructs in which either of the two sorting signals is modified by site-directed mutagenesis cannot complement the *kor1-2* mutant. In other *kor* mutants, point mutations cause several temperature sensitive alleles (*rsw2-1, 2, 3* and *acw1*) that have reduced cellulose content and increased pectin content (Lane et al., 2001; Sato et al., 2001). Two irregular xylem mutants (*irx2-1, 2*) identified to be *KOR* alleles have secondary cell wall specific phenotypes in which cellulose of the secondary walls is severely deficient while primary cell wall cellulose content is not changed significantly (Szyjanowicz et al., 2004).

In addition to phenotypic infrequencies in *KOR* mutants, the subcellular localization of KOR has also presented varying results. These discrepancies in the subcellular localization of KOR have been met with the proposal that KOR either cycles between intracellular compartments and the plasma membrane or that KOR confers its activity in intracellular compartments (Mølhøj et al., 2002). In xylem cells, KOR does not show significant co-localization with secondary cell wall thickenings (Szyjanowicz et al., 2004). Attempts to co-immunoprecipitate KOR with either primary or secondary CESAs have failed (Szyjanowicz et al., 2004; Desprez et al., 2007). Combined, these results cast doubt on the direct interaction between KOR and CSCs, however, cellulose defects in *kor* mutants and the co-regulation of KOR and CESA7 (IRX3) expression in cells actively synthesizing the secondary cell wall infer the importance of KOR in both primary and secondary cellulose synthesis (Nicol et al., 1998; Zuo et al., 2000; Lane et al., 2001; Sato et al., 2001; Mølhøj

et al., 2002; Szyjanowicz et al., 2004).

Cellulose synthase-interactive protein 1 (CSI1)

In an attempt to identify additional components of the CSC, the central domain of primary CESAs have been used as bait in a yeast two hybrid (Y2H) screen of the *Arabidopsis* cDNA library. Cellulose synthase interactive protein 1 (CSI1) was identified through this screen and further characterized to reveal its involvement in CESA dynamics (Gu et al., 2010). Through the use of spinning disc confocal microscopy, fluorescent protein-labeled CESAs have been shown to move bidirectionally in linear trajectories along microtubules (Paredez et al., 2006). Through a similar technique, red fluorescent protein (RFP)-labeled CSI1 particles were observed to co-localize with GFP-CESA3 on the plasma membrane of epidermal cells in etiolated *Arabidopsis* hypocotyls and to exhibit similar dynamics to plasma membrane localized YFP-CESA6 (Paredez et al., 2006).

The average velocity of CSCs in the plasma membrane that are actively synthesizing cellulose has been measured by several groups to be in the range of 275–365 nm/min (Paredez et al., 2006; DeBolt et al., 2007; Desprez et al., 2007; Gu et al., 2010). T-DNA insertion mutants of CSI1 show a reduction in CSC velocity in addition to showing a growth defect, a reduction in cellulose content, and a disruption in the linearity of the distribution of plasma membrane localized YFP-CESA6 (Gu et al., 2010). The co-localization and similar dynamics of CSI1 and CESAs in combination with the observations of altered CESA dynamics in *csi1* mutants support the Y2H results in suggesting that CSI1 is associated with primary CSCs. Since the linear tracks followed by CSCs align with microtubules (Paredez et al., 2006) and CSI1 mutants show a disruption in the linear distribution of CESAs, CSI1 might act as a link between CSCs and cortical microtubules. An analysis of the CSI1 protein sequence shows that CSI1 contains armadillo (ARM) repeats, which are often involved in protein–protein interactions (Tewari et al., 2010), and a C2 domain, which might bind with phospholipids or participate in protein–protein interactions (Davletov and Südhof, 1993; Benes et al., 2005). It is expected that ongoing studies will lead to the discovery of the roles of the different CSI1 domains and the mechanism by which CSI1 is involved in the movement and distribution of CSCs along microtubules.

PttMAP20

The use of the cellulose synthesis inhibitor, 2,6-dichlorobenzonitrile (DCB), has led to the identification of another potential protein involved in cellulose synthesis. DCB is a cellulose synthesis inhibitor that has been shown to inhibit the motility of CSCs in the plasma membrane and to cause an accumulation of CSCs (DeBolt et al., 2007; Wightman et al., 2009). One of the targets of DCB is PttMAP20, a microtubule-associated protein (MAP) in poplar (Rajangam et al., 2008). PttMAP20 is specifically expressed in developing xylem tissues and is co-regulated with secondary CESA genes. PttMAP20 binds to taxol-stabilized microtubules *in vitro* and transiently expressed YFP-PttMAP20 localizes to microtubules in tobacco leaves. The direct binding of DCB to PttMAP20 does not disrupt the binding of PttMAP20 to microtubules. Also, DCB does not affect the polymerization or dynamics of microtubules (DeBolt et al., 2007; Rajangam et al., 2008). Although these observations, taken together with the observation that CSCs travel along cortical microtubules (Paredez et al., 2006), create a plausible mode of action for DCB in cellulose synthesis inhibition through the disruption of PttMAP20 function, it is likely that DCB has additional targets.

The use of oryzalin, a microtubule-depolymerizing drug, can elucidate the importance of cortical microtubules in cellulose synthesis. In similar studies using oryzalin, the role of cortical microtubules appears to be more critical in secondary cell wall deposition in xylem cells (Gardiner et al., 2003; Wightman and Turner, 2008) than in primary CESA activity in hypocotyl cells (Paredez et al., 2006). Since PttMAP20 is specifically expressed in secondary xylem tissues, it is feasible that PttMAP20 may act as a mediator between microtubules and secondary CSCs and act as the target of DCB responsible for the cellulose synthesis defect in this cell type (Rajangam et al., 2008). However, DCB is also effective in disrupting the activity of primary CSCs where it likely acts on a different target. Since DCB does not interrupt PttMAP20 binding to microtubules, it has been suggested that DCB might disrupt an association between PttMAP20 and the CSC. No evidence for a direct association between PttMAP20 and CSCs has been shown as of yet. Further characterization of PttMAP20 might reveal the importance of microtubules as well as microtubule-associated proteins in the biosynthesis of cellulose.

CESA trafficking compartments, behavior, and dynamics

Since cellulose synthesis occurs at the plasma membrane in higher plants, the trafficking of the cellulose synthase complex (CSC) to and from the plasma membrane may act as a significant regulatory mechanism. An understanding of the mechanism and the pathway of CSC intracellular trafficking, delivery to the plasma membrane, internalization from the plasma membrane, and the potential of CSC recycling may bring insight into the regulation and organization of cellulose deposition in the cell wall.

Distinct CESA-containing compartments

The secretion pathway for transmembrane proteins typically begins at the endoplasmic reticulum (ER) where the protein is

incorporated into the membrane before being transported elsewhere. However, the Golgi apparatus represents the earliest stage of the trafficking pathway of CESA for which there is evidence (Haigler and Brown, 1986). Golgi localization of the rosette has been observed in freeze-fractured cells from *Zinnia elegans* suspension cultures through the use of scanning electron microscopy (SEM) (Haigler and Brown, 1986). Fluorescently labeled secondary wall CESA7 (YFP-IRX3) co-localizes with Golgi markers in xylem cells, where co-localization of YFP-IRX3 with ER markers is not observed (Wightman and Turner, 2008; Wightman et al., 2009; Wightman and Turner, 2010). Primary CESAs (YFP-CESA6 and GFP-CESA3) also have Golgi distribution (Paredez et al., 2006; Crowell et al., 2009; Gutierrez et al., 2009). To date, the pathway CESAs take to the Golgi remains elusive, though there is speculation that the ER is not involved (Wightman and Turner, 2010). Currently, it is unclear whether CESA delivery to the plasma membrane occurs directly from the Golgi to the plasma membrane during pausing events or if CESA passes through an intermediate compartment. A few CESA containing compartments have been identified (Table 1); yet the role of each compartment in CESA delivery, internalization, and/or recycling remains open for discussion (Crowell et al., 2009; Gutierrez et al., 2009).

Compartments that are co-labeled with VHA-a1 and GFP-CESA3 exhibit similar dynamics to the CESA-containing Golgi bodies and have been documented to associate and dissociate with the Golgi (Crowell et al., 2009). VHA-a1, a vacuolar H^+-ATPase, localizes to the trans-Golgi network (TGN) (Dettmer et al., 2006). Treatment with the endocytotic pathway marker, FM4-64, leads to rapid co-localization of FM4-64 and GFP-VHA-1a, suggesting that the TGN acts as an early endosome (EE) in endocytosis in addition to its role in the secretion system (Dettmer et al., 2006). This dual role of the TGN/EE suggests that the VHA-a1/GFP-CESA3 compartment could be involved in the secretion of CESA, the internalization of CESA, or both. In addition to the VHA-a1/GFP-CESA3 compartment, another primary CESA containing compartment, the microtubule-associated cellulose synthase compartment (MASC), was discovered (Crowell et al., 2009). Simultaneously, another research team discovered small CESA compartments (SmaCCs), which are believed to make up a population that includes MASCs and the VHA-a1/GFP-CESA3 compartments (Gutierrez et al., 2009; Crowell et al., 2010).

CESA localization to MASCs/SmaCCs can be observed in the lower hypocotyl or can be induced by treatment with mannitol (osmotic stress response), treatment with cellulose synthesis inhibitors such as isoxaben (Gutierrez et al., 2009) or CGA (Crowell et al., 2009), or treatment with the protein synthesis inhibitor, cycloheximide (Crowell et al., 2009). MASCs/SmaCCs co-localize with cortical microtubules and exhibit either linear motility or stationary behavior. MASC/SmaCC motility corresponds to the tracking of depolymerizing plus and minus ends of cortical microtubules and is compromised by taxol treatment (Gutierrez et al., 2009). MASCs/SmaCCs associate and dissociate with other MASCs/SmaCCs, with VHA-a1/GFP-CESA3 compartments, and with Golgi bodies (Crowell et al., 2009).

CESA internalization and recycling

MASCs/SmaCCs have been proposed to be involved in delivery (Gutierrez et al., 2009) and internalization (Crowell et al., 2009) of CESA. SmaCCs induced by osmotic stress conditions were observed delivering CESA to the plasma membrane upon mannitol washout. It is unclear whether the accumulation of SmaCCs during osmotic stress conditions represents novel CSCs in the secretion pathway that are awaiting incorporation into the plasma membrane or CSCs that were internalized into SmaCCs due to the osmotic stress to be subsequently recycled to the plasma membrane after mannitol removal (Gutierrez et al., 2009). Internalization, not secretion, has been suggested to be the significant role of MASCs based on the following observations: an increase in MASC distribution of CESA correlates with a decrease in plasma membrane CESA distribution, quick redistribution of

Table 1 A comparison of the observations and interpretations of MASCs/SmaCCs.

		Crowell et al., 2009	Guiterrez et al., 2009
Shared	CSC localization	Plasma membrane	Plasma membrane
		golgi bodies	Golgi bodies
		VHA-a1/GFP-CESA3	SmaCCs
		MASCs	
	Role of microtubules	MASCs are coincident with MTs	SmaCCs are coincident with MTs
		Oryzalin reduces MASC density	Oryzalin interferes with cortical tethering of SmaCCs
	Role of actin	Cell-wide distribution of CSC-labeled Golgi bodies	Cell-wide distribution of CSC-labeled Golgi bodies
Unique	Role of MASC/SmaCC under osmotic stress	MASC is an internalization/recycling compartment	SmaCC is a delivery compartment
	Insertion of CSC into plasma membrane	Pausing of Golgi bodies is coincident with CSC insertion	SmaCC may be involved in trafficking between Golgi and plasma membrane

CESAs from plasma membrane to MASCs occurs upon the introduction of osmotic stress, and MASC formation continues long after prolonged cycloheximide treatment in which a disruption in protein synthesis would cause the secretion system to halt (Crowell et al., 2009). Since SmaCCs and MASCs are thought to represent the same compartment, it is likely that MASCs/SmaCCs act in both the delivery and the internalization of CESA or in the recycling of CESA.

It is likely that other CESA internalization processes exist. Although the involvement of clathrin-mediated endocytosis (CME) in the internalization of CESA has been met with skepticism (Crowell et al., 2009; Crowell et al., 2010) due to the large size of the cytoplasmic portion of the CSC (Bowling and Brown, 2008), disruption of CME may have an effect on the composition of cell walls (Gu, unpublished). Malformed or disintegrating rosettes have been documented in the plasma membrane (Haigler and Brown, 1986) and CME may act in the internalization and recycling of CSC components that are no longer functional. Also, CME might play a role in altering the composition of the membrane to indirectly affect cellulose biosynthesis.

A mutant in rice, *brittle culm 3* (*bc3*), was mapped to a dynamin-related protein, OsDRP2A, which has been proposed to be involved in clathrin-mediated endocytosis. The brittle culm phenotype is attributed to cellulose deficiency and OsDRP2A has an effect on the abundance of OsCESA4 at the plasma membrane (Xiong et al., 2010).

Many radial swelling (*rsw*) mutants, such as the aforementioned *CESA1* and *kor* mutants, have been shown to have cellulose deficiency. The *rsw9* mutant, which confers a cell expansion phenotype that has been attributed to cellulose deficiency, has recently been shown to contain a mutation in a dynamin-like protein, DRP1A. DRP1A was revealed to play a significant role in endocytosis (Collings et al., 2008) and was reported to colocalize with clathrin light chain (Konopka and Bednarek, 2008). It is believed that an endocytosis defect is the cause of the cellulose deficiency and cell elongation defect in the *rsw9* mutant. Interestingly, osmotic stress through the addition of salt, sucrose, or mannitol was shown to rescue the endocytosis defect of *rsw9* (Collings et al., 2008).

Cytoskeleton and CSC dynamics

Cytoskeletal components have been the targets of several studies in the motility of CESA trafficking. While in the Golgi, CSCs are transported longitudinally throughout the cell in an actin-dependent manner. Treatment with Latrunculin B, an actin-depolymerizing drug, arrests the movement of Golgi bodies that contain CESAs (Wightman and Turner, 2008; Crowell et al., 2009; Gutierrez et al., 2009). In those cells that have been treated with Latrunculin B, plasma membrane localized CSCs are restricted to regions above stationary Golgi aggregates while the density of CSCs in other areas of the plasma membrane decreases (Crowell et al., 2009; Gutierrez et al., 2009). This indicates that the delivery event from the Golgi to the plasma membrane likely does not involve actin, but that actin is involved primarily in the cell-wide distribution of Golgi-localized CESAs.

Through the use of live cell imaging, pausing of primary CESA-containing Golgi bodies occurs in tandem with the deposition of CESAs in the plasma membrane in a linear arrangement at sites along cortical microtubules (Crowell et al., 2009). Treatment with oryzalin, a microtubule-depolymerizing drug, does not have a significant effect on the rate of GFP-CESA3 deposition in the plasma membrane (Gutierrez et al., 2009), but it does cause a uniform distribution of plasma membrane CESAs (Crowell et al., 2009). Taxol, a microtubule-stabilizing drug, causes an increase in the linearity of CESA arrangement (Crowell et al., 2009). In the case of the primary CESAs, microtubules seem to mark the site for preferential CESA deposition and may do so through an interaction with Golgi bodies, but microtubules do not appear to play an essential role in the activity of the deposition event (Crowell et al., 2009; Gutierrez et al., 2009).

There seems to be a different mechanism responsible for organization of secondary CESA deposition events in xylem cells (Wightman and Turner, 2008). Transverse bands of secondary cell wall deposition co-align with cortical microtubule bands. Thick actin cables involved in the motility of Golgi bodies run longitudinally throughout the cell, and thin actin filaments run transversely in alignment with the microtubule bands. Golgi pausing events, which are unaffected by oryzalin, occur in proximity to these bands and coincide with an increase in YFP-IRX3 (CESA7) signal. In xylem cells, it has been speculated that the transverse thin actin filaments hold the responsibility for marking sites for secondary CESA delivery while cortical microtubules act in keeping secondary CSCs in the plasma membrane after they have already been inserted and guiding the CSCs in the proper trajectory along microtubules (Wightman and Turner, 2008; Wightman and Turner, 2010).

Conclusion

An improved understanding of cellulose biosynthesis in plants requires a broadening of the identification and characterization of proteins that are involved in the activity, organization, regulation and trafficking of the CSC. With the identification of novel proteins that affect cellulose biosynthesis, a more complete image of the composition of the CSC, its behavior, its trafficking pathway, and its regulation mechanism may be established. Despite the recent progress in the field, the exact composition and stoichiometry of the CSC is still a mystery. The recently developed imaging of CSC has led to a remarkable increase in our understanding of the CSC in the primary wall. With the new tools on hand, it is expected that the field will expand fruitfully in the coming decade.

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References

Arioli T, Peng L, Betzner A S, Burn J, Wittke W, Herth W, Camilleri C, Höfte H, Plazinski J, Birch R, Cork A, Glover J, Redmond J, Williamson R E (1998). Molecular analysis of cellulose biosynthesis in *Arabidopsis.* Science, 279(5351): 717–720

Benes C H, Wu N, Elia A E H, Dharia T, Cantley L C, Soltoff S P (2005). The C2 domain of PKCδ is a phosphotyrosine binding domain. Cell, 121(2): 271–280

Bowling A J, Brown R M Jr (2008). The cytoplasmic domain of the cellulose-synthesizing complex in vascular plants. Protoplasma, 233 (1–2): 115–127

Carroll A, Specht C D (2011). Understanding plant cellulose synthases through a comprehensive investigation of the cellulose synthase family sequences. Front Plant Genet Genomics, 10.3389/fpls.2011.00005

Collings D A, Gebbie L K, Howles P A, Hurley U A, Birch R J, Cork A H, Hocart C H, Arioli T, Williamson R E (2008). Arabidopsis dynamin-like protein DRP1A: a null mutant with widespread defects in endocytosis, cellulose synthesis, cytokinesis, and cell expansion. J Exp Bot, 59(2): 361–376

Crowell E F, Bischoff V, Desprez T, Rolland A, Stierhof Y D, Schumacher K, Gonneau M, Höfte H, Vernhettes S (2009). Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis.* Plant Cell, 21(4): 1141–1154

Crowell E F, Gonneau M, Stierhof Y D, Höfte H, Vernhettes S (2010). Regulated trafficking of cellulose synthases. Curr Opin Plant Biol, 13 (6): 700–705

Davletov B A, Südhof T C (1993). A single C2 domain from synaptotagmin I is sufficient for high affinity Ca^{2+}/phospholipid binding. J Biol Chem, 268(35): 26386–26390

DeBolt S, Gutierrez R, Ehrhardt D W, Somerville C (2007). Nonmotile cellulose synthase subunits repeatedly accumulate within localized regions at the plasma membrane in *Arabidopsis* hypocotyl cells following 2,6-dichlorobenzonitrile treatment. Plant Physiol, 145(2): 334–338

Delmer D P (1999). Cellulose biosynthesis: Exciting times for a difficult field of study. Annu Rev Plant Physiol Plant Mol Biol, 50(1): 245–276

Desprez T, Juraniec M, Crowell E F, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S (2007). Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana.* Proc Natl Acad Sci USA, 104(39): 15572–15577

Dettmer J, Hong-Hermesdorf A, Stierhof Y D, Schumacher K (2006). Vacuolar H^+-ATPase activity is required for endocytic and secretory trafficking in *Arabidopsis.* Plant Cell, 18(3): 715–730

Doblin M S, Kurek I, Jacob-Wilk D, Delmer D P (2002). Cellulose biosynthesis in plants: from genes to rosettes. Plant Cell Physiol, 43 (12): 1407–1420

Gardiner J C, Taylor N G, Turner S R (2003). Control of cellulose synthase complex localization in developing xylem. Plant Cell, 15 (8): 1740–1748

Gu Y, Kaplinsky N, Bringmann M, Cobb A, Carroll A, Sampathkumar A, Baskin T I, Persson S, Somerville C R (2010). Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. Proc Natl Acad Sci USA, 107(29): 12866–12871

Gu Y, Somerville C (2010). Cellulose synthase interacting protein: a new factor in cellulose synthesis. Plant Signal Behav, 5(12): 1571–1574

Gutierrez R, Lindeboom J J, Paredez A R, Emons A M C, Ehrhardt D W (2009). *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. Nat Cell Biol, 11(7): 797–806

Haigler C H, Brown R M Jr (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(2–3): 111–120

Kimura S, Laosinchai W, Itoh T, Cui X, Linder C R, Brown R M Jr (1999). Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant vigna angularis. Plant Cell, 11 (11): 2075–2086

Konopka C A, Bednarek S Y (2008). Comparison of the dynamics and functional redundancy of the *Arabidopsis* dynamin-related isoforms DRP1A and DRP1C during plant development. Plant Physiol, 147 (4): 1590–1602

Lane D R, Wiedemeier A, Peng L, Höfte H, Vernhettes S, Desprez T, Hocart C H, Birch R J, Baskin T I, Burn J E, Arioli T, Betzner A S, Williamson R E (2001). Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-β-glucanase to cellulose synthesis and cytokinesis in *Arabidopsis.* Plant Physiol, 126(1): 278–288

Mølhøj M, Pagant S, Höfte H (2002). Towards understanding the role of membrane-bound endo-β-1,4-glucanases in cellulose biosynthesis. Plant Cell Physiol, 43(12): 1399–1406

Mueller S C, Brown R M Jr (1980). Evidence for an intramembrane component associated with a cellulose microfibril-synthesizing complex in higher plants. J Cell Biol, 84(2): 315–326

Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H (1998). A plasma membrane-bound putative endo-1,4-β-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis.* EMBO J, 17(19): 5563–5576

Pagant S, Bichet A, Sugimoto K, Lerouxel O, Desprez T, McCann M, Lerouge P, Vernhettes S, Höfte H (2002). KOBITO1 encodes a novel plasma membrane protein necessary for normal synthesis of cellulose during cell expansion in *Arabidopsis.* Plant Cell, 14(9): 2001–2013

Paredez A R, Somerville C R, Ehrhardt D W (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. Science, 312(5779): 1491–1495

Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P, Khitrov N, Auer M, Somerville C R (2007). Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in *Arabidopsis.* Proc Natl Acad Sci USA, 104(39): 15566–15571

Rajangam A S, Kumar M, Aspeborg H, Guerriero G, Arvestad L, Pansri P, Brown C J L, Hober S, Blomqvist K, Divne C, Ezcurra I,

Mellerowicz E, Sundberg B, Bulone V, Teeri T T (2008). MAP20, a microtubule-associated protein in the secondary cell walls of hybrid aspen, is a target of the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile. Plant Physiol, 148(3): 1283–1294

Sato S, Kato T, Kakegawa K, Ishii T, Liu Y G, Awano T, Takabe K, Nishiyama Y, Kuga S, Sato S, Nakamura Y, Tabata S, Shibata D (2001). Role of the putative membrane-bound endo-1,4-β-glucanase KORRIGAN in cell elongation and cellulose synthesis in *Arabidopsis thaliana.* Plant Cell Physiol, 42(3): 251–263

Scheible W R, Eshed R, Richmond T, Delmer D, Somerville C (2001). Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *ArabidopsisIxr1* mutants. Proc Natl Acad Sci USA, 98(18): 10079–10084

Schindelman G, Morikami A, Jung J, Baskin T I, Carpita N C, Derbyshire P, McCann M C, Benfey P N (2001). COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in *Arabidopsis.* Genes Dev, 15(9): 1115–1127

Somerville C (2006). Cellulose synthesis in higher plants. Annu Rev Cell Dev Biol, 22(1): 53–78

Szyjanowicz P M, McKinnon I, Taylor N G, Gardiner J, Jarvis M C, Turner S R (2004). The irregular *xylem 2* mutant is an allele of *korrigan* that affects the secondary cell wall of *Arabidopsis thaliana.* Plant J, 37(5): 730–740

Taylor N G, Howells R M, Huttly A K, Vickers K, Turner S R (2003). Interactions among three distinct CesA proteins essential for cellulose synthesis. Proc Natl Acad Sci USA, 100(3): 1450–1455

Taylor N G, Laurie S, Turner S R (2000). Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in *Arabidopsis.* Plant Cell, 12(12): 2529–2540

Taylor N G, Scheible W R, Cutler S, Somerville C R, Turner S R (1999). The irregular *xylem3* locus of *Arabidopsis* encodes a cellulose synthase required for secondary cell wall synthesis. Plant Cell, 11(5): 769–780

Tewari R, Bailes E, Bunting K A, Coates J C (2010). Armadillo-repeat protein functions: questions for little creatures. Trends Cell Biol, 20 (8): 470–481

Timmers J, Vernhettes S, Desprez T, Vincken J P, Visser R G F, Trindade L M (2009). Interactions between membrane-bound cellulose synthases involved in the synthesis of the secondary cell wall. FEBS Lett, 583(6): 978–982

Wang J, Elliott J E, Williamson R E (2008). Features of the primary wall CESA complex in wild type and cellulose-deficient mutants of *Arabidopsis thaliana.* J Exp Bot, 59(10): 2627–2637

Wightman R, Marshall R, Turner S R (2009). A cellulose synthase-containing compartment moves rapidly beneath sites of secondary wall synthesis. Plant Cell Physiol, 50(3): 584–594

Wightman R, Turner S (2010). Trafficking of the cellulose synthase complex in developing xylem vessels. Biochem Soc Trans, 38(3): 755–760

Wightman R, Turner S R (2008). The roles of the cytoskeleton during cellulose deposition at the secondary cell wall. Plant J, 54(5): 794–805

Xiong G, Li R, Qian Q, Song X, Liu X, Yu Y, Zeng D, Wan J, Li J, Zhou Y (2010). The rice dynamin-related protein DRP2B mediates membrane trafficking, and thereby plays a critical role in secondary cell wall cellulose biosynthesis. Plant J, 64(1): 56–70

Zhong R, Burk D H, Morrison W H 3rd, Ye Z H (2004). *FRAGILE FIBER3*, an *Arabidopsis* gene encoding a type II inositol polyphosphate 5-phosphatase, is required for secondary wall synthesis and actin organization in fiber cells. Plant Cell, 16(12): 3242–3259

Zhong R, Kays S J, Schroeder B P, Ye Z H (2002). Mutation of a chitinase-like gene causes ectopic deposition of lignin, aberrant cell shapes, and overproduction of ethylene. Plant Cell, 14(1): 165–179

Zuo J R, Niu Q W, Nishizawa N, Wu Y, Kost B, Chua N H (2000). KORRIGAN, an *Arabidopsis* endo-1,4-beta-glucanase, localizes to the cell plate by polarized targeting and is essential for cytokinesis. Plant Cell, 12(7): 1137–1152