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REVIEW

The Regulation of Cellulose Biosynthesis in Plants

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Cell walls define the shape of plant cells, controlling the extent and orientation of cell elongation, and hence organ growth. The main load-bearing component of plant cell walls is cellulose, and how plants regulate its biosynthesis during development and in response to various environmental perturbations is a central question in plant biology. Cellulose is synthesized by cellulose synthase (CESA) complexes (CSCs) that are assembled in the Golgi apparatus and then delivered to the plasma membrane (PM), where they actively synthesize cellulose. CSCs travel along cortical microtubule paths that define the orientation of synthesis of the cellulose microfibrils. CSCs recycle between the PM and various intracellular compartments, and this trafficking plays an important role in determining the level of cellulose synthesized. In this review, we summarize recent findings in CESA complex organization, CESA posttranslational modifications and trafficking, and other components that interact with CESAs. We also discuss cell wall integrity maintenance, with a focus on how this impacts cellulose biosynthesis.

INTRODUCTION

Plant cell walls are dynamic structures that define the shape and size of a plant cell, provide structural support to the plant body, protect cells from pathogens, and serve as nodes of communication between the symplast and apoplast. Plant cell walls are composed of several groups of polysaccharides including cellulose, hemicelluloses, and pectins, as well as structural proteins and phenolic compounds (Ivakov and Persson, 2012; Lampugnani et al., 2018). Cellulose, which consists of chains of β-1,4-linked Glc units, is synthesized at the plasma membrane (PM) and organized into microfibrils, which are the main load-bearing elements of cell walls. Native cellulose occurs primarily in paracrystalline structures that form as a result of inter- and intrachain hydrogen bonding as well as van der Waals forces (Saxena and Brown, 2005; Somerville, 2006; Nishiyama, 2009). The paracrystalline structure facilitates the high mechanical strength of a microfibril. Molecular simulations and freeze fracture electron microscopy imaging techniques demonstrate that, during extrusion, the microfibril undergoes a disordered amorphous phase before crystallization. It has been hypothesized that the initial disorganized zone of the forming microfibril allows for the parallel orientation of a forming protofibril and accommodates asynchronically synthesized glucan chains (Haigler et al., 2014).

Actively growing primary cell walls contain loosely connected microfibrils organized perpendicularly to the axis of expansion, which define the orientation of growth anisotropy (Green, 1962). Microfibrils in thin, flexible primary cell walls are dynamic in structure to allow cell expansion in growing tissues (Anderson et al., 2010). Their hydrophobic surfaces are bound to xyloglucan, the most common type of hemicellulose, whereas pectins bind to their hydrophilic surfaces and fill in spaces between microfibrils (Cosgrove, 2016). The surprisingly minor growth defects of an Arabidopsis mutant that lacks xyloglucans (Cavalier et al., 2008; Park and Cosgrove, 2012) is not fully consistent with classic "tether network" models of the plant primary cell wall in which the xyloglucan chains act as tethers that bind to cellulose microfibrils to form a load-bearing cellulose-xyloglucan network. Rather, xyloglucans likely play a role primarily in microfibril spacing and aggregation (Voxeur and Höfte, 2016; Xiao et al., 2016). Recent atomic force microscopy studies show that microfibrils form short lateral bundles and are arranged in a reticulate network, rather than being spaced and connected only through matrix polysaccharides (Zhang et al., 2016a). The interfibril spacing between cellulose microfibrils is ~20 nm, as determined by x-ray scattering analyses (Ye et al., 2018).

Cell growth is accomplished through cycles of wall relaxation, resulting in decreased water potential, leading to water uptake and increased turgor, which then again leads to wall relaxation. Cell wall loosening takes place at least in part as a result of the nonenzymatic activity of expansins, which mediate cell wall creep by targeting hydrogen bonds within cellulose and/or between microfibrils and matrix polysaccharides (McQueen-Mason and Cosgrove, 1994, 1995). Pectin methyl-esterases also facilitate cell wall loosening and subsequent growth if followed by the action of enzymes such as pectate lyase and polygalacturonase (Pelloux et al., 2007). Cell expansion and cellulose biosynthesis are generally tightly connected, though in some instances they can be transiently uncoupled, such as during diurnal variations in growth (Ivakov et al., 2017). Upon cessation of growth, some cell types, such as xylem vessels, fibers, or collenchyma, synthesize thick secondary cell walls that contain more cellulose than primary cell walls (Meents et al., 2018).

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In this review, we summarize recent findings in the field of cellulose biosynthesis with a focus on the structure of primary and secondary wall cellulose synthase (CESA) complexes (CSCs), their assembly, and the modulation of their activity and intracellular trafficking. We also discuss the role of cell-wall integrity sensing on the regulation of cellulose biosynthesis. Several excellent recent reviews provide a more in-depth discussion of cellulose structure and other aspects of cell wall synthesis, including the transcriptional control of CESAs (Somerville, 2006; Mutwil et al., 2008; Levesque-Tremblay et al., 2015; Park and Cosgrove, 2015; Anderson, 2016, 2018; Cosgrove, 2016, 2018a, 2018b; Kumar et al., 2016a; Chebli and Geitmann, 2017; Höfte and Voxeur, 2017; Lampugnani et al., 2018; Majda and Robert, 2018; Saffer, 2018).

STRUCTURE OF CSCs

Cellulose microfibrils are synthesized by large, motile PM-localized CSCs that are visualized as sixfold symmetrical rosettes. First reports noting "granules" associated with cell wall microfibrils in various plant species started emerging together with the development of electron microscopy (Brown, 1985). Freeze fracture analyses of the *Zea mays* terminal complexes (so-called because they were found at the ends of cellulose microfibrils) and intramembrane rosettes showed that they are associated with cellulose microfibrils and led to a model in which complexes of transmembrane proteins may be involved in cellulose biosynthesis (Mueller and Brown, 1980). Random sequencing of cotton cDNAs and comparison to bacterial genes involved in cellulose biosynthesis led to the identification of plant CESA genes (Pear et al., 1996). The substrate for CESAs is uridine diphosphate Glc (Verbančič et al., 2018), which is synthesized from the cytosolic invertase/uridine diphosphate Glc phosphorylase pathway (Barnes and Anderson, 2018). The CESA proteins are composed of a cytosolic N-terminal region involved in dimerization/oligomerization of CESA subunits (Kurek et al., 2002) followed by two transmembrane domains, a large cytoplasmic central loop that contains the substrate binding and catalytic regions, six additional transmembrane domains (Sethaphong et al., 2013; Slabaugh et al., 2014) and finally an intracellular C-terminal domain (Figure 1A).

In Arabidopsis, CESA1, CESA3, and CESA6-like proteins (CESA2, CESA5, CESA6, and CESA9) are involved in primary wall cellulose synthesis, whereas CESA4, CESA7, and CESA8 participate in secondary cell wall synthesis (Desprez et al., 2007; Persson et al., 2007). Null mutations in *CESA1* and *CESA3* are

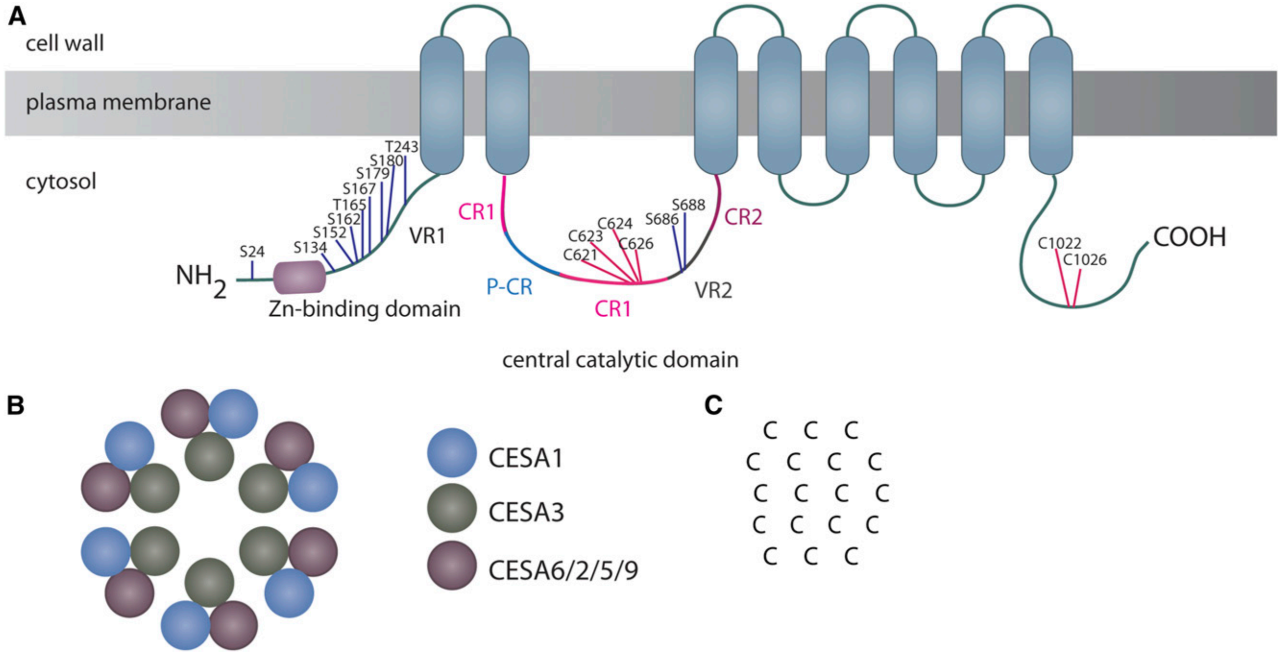

Figure 1. Schematic Representations of the Structure of a CESA Protein and a CSC.

(A) Domain structure of a CESA. The intracellular N-terminal domain contains a Zn binding domain and a variable region and is followed by two transmembrane domains. The large cytoplasmic central catalytic domain is divided into the conserved region, which flanks the plant-specific region on both sides, the variable region(s), which includes the class-specific region, and the conserved region(s). The six subsequent transmembrane domains are followed by the cytoplasmic C-terminal domain. CESA1 phosphorylations on various Ser and Thr residues are indicated (source: PhosPhAt 4.0, Zulawski et al., 2013; and references in the text). Several cysteines in the cytoplasmic loop and within the C-terminal domain that are S-acylated in CESA7 (Kumar 2016b) are depicted in pink. C, cellulose chain; CR1, conserved region 1; CR2, conserved region 2; P-CR, plant-specifc region; S, Ser; T, Thr.

(B) A schematic representation of a CSC consisting of 18 individual CESA proteins. The model is consistent with the rules outlined by Hill et al. (2014) and assumes that: a CSC is composed of six lobes that contain three CESA isoforms; the contacts between different isoforms is conserved; and the number of CESAs in each lobe is divisible by three.

(C) A model of a cellulose microfibril consisting of five layers of cellulose chains in a "34443" arrangement (C = cellulose chain).

lethal, indicating their indispensable role in the formation of a functional CESA complex. Single *cesa2*, *cesa5*, *cesa6*, or *cesa9* mutants are viable, but *cesa2 cesa5 cesa6* and *cesa2 ces6 ces9* triple mutants are not, indicating partial redundancy among these CESA genes (Desprez et al., 2007; Persson et al., 2007). Formation of a functional CSC in plants requires assembly of three different CESA isoforms drawn from distinct classes. For example, primary cell walls require CESA1, CESA3, and one additional isoform from the CESA6-like group. The regions defining these functional classes vary among CESA paralogs but are relatively conserved among their orthologs (Vergara and Carpita, 2001). The *CESA4*, *CESA7*, and *CESA8* genes are nonredundant and exhibit a varying degree of class specificity as shown by varying levels of complementation using chimeric domain swap constructs. In particular, CESA7 has the highest degree of class specificity as, for example, none of the N-terminal domain swaps with CESA4 or CESA8 complemented the *cesa7* mutant, whereas the N-terminal domain of CESA7 fused to CESA4 or CESA8 is able to partially complement the respective mutants (Kumar et al., 2017). A complementary study by Hill et al. (2018) that implemented larger domain swaps demonstrated that regions contributing to class specificity vary among CESA isoforms. For example, CESA8 specificity is defined by the central cytosolic loop, but the specificities of CESA4 and CESA7 were attributed to the residues within the C terminus. CESA functionalization occurs across distant plant clades and in some cases has arisen through convergent evolution (Norris et al., 2017).

The precise architecture of CSCs is becoming clearer. Initial models suggested that CSC rosettes consist of a hexamer of CESA hexamers (Herth, 1983; Doblin et al., 2002; Somerville, 2006). Though attractive, the concept of CSC containing 36 individual CESA subunits has been challenged on multiple occasions. Many approaches, including atomic force microscopy, wide angle x-ray scattering, small-angle neutron scattering analyses, and computational simulations of cellulose microfibrils, have coalesced on a model for a microfibril diameter of ~2.5 nm, consistent with microfibrils consisting of 18–24 cellulose chains (Fernandes et al., 2011; Newman et al., 2013; Thomas et al., 2013, 2015; Kuramae et al., 2014; Zhang et al., 2016a). In addition, a recent modeling study that examined three different modes of cellulose organization within an 18-chain microfibril demonstrated that the “34443” model is the most probable arrangement, in which the cellulose chains are stacked in the microfibril in an arrangement of three chains, followed by four chains, etc., as shown in Figure 1C (Kubicki et al., 2018). Analyses of primary cell wall (CESA1, CESA3, and CESA6) and secondary cell wall (CESA4, CESA7, CESA8) CESA stoichiometry using coimmunoprecipitation (Co-IP), mass spectrometry (MS), and quantitative immunoblotting demonstrated that each isoform occurs in equimolar amounts (Gonneau et al., 2014; Hill et al., 2014). This 1:1:1 stoichiometry, together with the trimeric nature of CESA proteins and analyses of microfibril width, suggests a model in which CSC rosettes contain 18 CESA subunits that synthesize 18 glucan chains because a 24-subunit rosette is not divisible by six (six lobes in a rosette) and then by three (number of CESA isoforms; Figure 1B; Hill et al., 2014). Consistent with this, improved transmission electron microscopy images of CSCs from the moss *Physcomitrella patens* combined with computational models support the notion that CSCs are composed of 18 CESA subunits (Nixon et al., 2016). However, CESA stoichiometry is not identical for all plant species. For example, in the developing xylem of aspen (*Populus tremuloides*), the stoichiometry of CESAs was 3:2:1 for PtCESA8a/b:PtCESA4:PtCESA7a/b (Zhang et al., 2018). To determine whether individual CESA subunits within a CSC play equivalent roles in cellulose synthesis, a complementation study was performed with catalytically inactive secondary *cesa* mutant proteins that did not affect CSC assembly (Kumar et al., 2018). Interestingly, not all CESA isoforms appeared to have equivalent activity, with CESA8 being the most and CESA4 the least active.

The distinction of CESAs being involved in primary or secondary wall synthesis is not as clear as once thought. During the transition from primary cell wall to secondary cell wall formation, CSCs are subject to remodeling and the primary cell wall CESA isoforms are replaced by those involved in secondary cell wall synthesis. This turnover was examined using transgenic Arabidopsis lines in which protoxylem identity was ectopically activated through inducible expression of *VASCULAR-RELATED NAC-DOMAIN7* (Watanabe et al., 2018). During the transition, secondary cell wall CESA7 was found to transiently coexist with primary cell wall CESA6 before its depletion from the PM and degradation in the lytic vacuole. Both classes of CESAs colocalize with the distinct cortical microtubule bands around the cell cortex (Watanabe et al., 2015, 2018).

POSTTRANSLATIONAL REGULATION OF CSCs

Various posttranslational modifications play important roles in regulating CESA function, and consequently affect cellulose biosynthesis. For example, rapid proteolytic degradation plays a role in controlling CESA abundance and activity (Rudolph et al., 1989). Early reports estimated CSC half-life to be <30 min (Jacob-Wilk et al., 2006). More recent direct measurements of the primary cell wall CESA1, CESA3, and CESA6 degradation rates in vivo indicate longer half-lives in etiolated Arabidopsis hypocotyls (Hill et al., 2018). In this tissue, 80% of the primary cell wall CESAs are still detectable 48 h after treatment with cycloheximide, which blocks de novo protein synthesis. CESAs are more rapidly degraded in light-grown seedlings, with only ~20% remaining 48 h after inhibition of protein synthesis. The longer half-lives of CESAs in etiolated hypocotyls could be advantageous because this type of morphogenesis requires higher rates of cell expansion and hence increased rates of cellulose synthesis. By contrast, elevated ambient temperatures, which also positively affect cell expansion, increased the turn-over of CESAs (Hill et al., 2018). Interestingly, destabilization of one CESA isoform leads to degradation of the entire complex. Calculations of CESA lifetime at the PM based on their density and insertion rates estimated their residence time to be ~7–8 min (Sampathkumar et al., 2013). The discrepancy between this rapid turnover at the PM and the much longer overall protein stability of CESAs indicates that upon the removal of CESA proteins from the PM, they do not undergo immediate proteolysis but rather are subject to compartmentalization and recycling.

Protein phosphorylation is the best-studied form of CESA posttranslational modification (Speicher et al., 2018). CESA phosphorylation occurs mostly at multiple residues within the hypervariable regions in the N terminus and in the central cytosolic loop (Figure 1A). A large number of these sites appear to be highly conserved in CESA proteins across plant species (Nühse et al., 2004; Speicher et al., 2018). Site-directed mutagenesis of a subset of Ser/Thr residues of CESAs designed to mimic (S/T→D/E) or block (S/T→A) their phosphorylation has revealed multiple roles for CESA phosphorylation (Chen et al., 2010). For example, phosphorylation of CESA7 has been linked to its degradation via a 26S proteasome-dependent pathway (Taylor, 2007). CESA phosphorylation plays a critical role in maintaining proper anisotropic cell expansion in hypocotyls and roots, at least partially through differential effects on the interaction of CESAs with microtubules. Interestingly, some phosphosite mutants of CESA1 or CESA3 resulted in an asymmetry of CSC particle velocities along cortical microtubules, in contrast with wild-type CESAs that travel bidirectionally with equal velocities (Chen et al., 2010). A study of primary cell wall CESAs in Arabidopsis suggests a link of phytochrome to CESA phosphorylation (Bischoff et al., 2011). This study found that the velocity of primary cell wall CSCs was dependent on which CESA occupied the third position of the primary cell wall CSCs in etiolated hypocotyls. Interestingly, CESA5-containing CSCs displayed a significant increase in particle velocity in response to red light treatment, and this difference was abolished in a mutant version of CESA5 in which four phosphorylation sites in the N-terminal domain were mutated to phosphomimics (Bischoff et al., 2011). This suggests that red light, acting through phytochrome, likely modulates cellulose synthesis at least in part by regulating phosphorylation of these residues. Further, many CSC-associated proteins (Table 1) are also phosphorylated, including KORRIGAN (KOR), POM-POM2/CELLULOSE SYNTHASE-INTERACTIVE1 (POM2/CSI1), and COMPANION OF CELLULOSE1 and 2 (CC1 and CC2) proteins (Speicher et al., 2018).

The kinases that phosphorylate CESAs at these various residues are largely unknown. One exception is the BRASSINOSTEROID INSENSITIVE (BIN2) protein kinase, which is involved in the response to brassinosteroids (BR; Sánchez-Rodríguez et al., 2017). Mutations blocking BR perception or synthesis, which result in constitutive activation of BIN2, displayed reduced levels of crystalline cellulose. In vitro kinase assays revealed that BIN2 phosphorylated a peptide derived from CESA1 at a residue corresponding to Thr-157 in the hypervariable domain. However, a second residue must already be phosphorylated on the peptide for efficient BIN2 phosphorylation. This priming is a common feature of other Glycogen synthase kinase3 kinases and implicates the involvement of partner kinase(s) in the regulation of CESA by BIN2. Thr-157 phosphorylation is likely functionally relevant, as a phosphonull mutation at this position in CESA1 abolished BIN2-dependent regulation of CESA activity. This suggests that this phosphorylation of CESA1 by BIN2, together with a second kinase, mediates the effects of BR on cellulose biosynthesis, contributing to the role of BR in regulating cell elongation.

Another type of reversible posttranslational modification involves S-acylation in which a fatty acid, usually palmitate, is attached to specific Cys residues via a thioester bond (Resh, 2006). The understanding of S-acylation in plants is limited but it has been shown to affect protein association with membranes and to affect protein stability (Li and Qi, 2017). Four cysteines in the variable region 2 (VR2) and C-terminal domain of CESA7 have been identified that undergo S-acylation (Kumar et al., 2016b). Disruption of these CESA7 acylation sites via mutation of the target Cys residues results in a reduction of crystalline cellulose, likely as a consequence of reduced trafficking of CESA from the Golgi to the PM (Kumar et al., 2016b).

CESA ASSEMBLY AND CELLULAR TRAFFICKING

Live-cell imaging using fluorescently tagged CESAs in Arabidopsis roots and hypocotyls reveal their presence in several compartments: Golgi, intracellular vesicles of possibly heterogenous nature, microtubule-associated vesicles in the cortical region of the cell, and at the PM (Paredez et al., 2006; Crowell et al., 2009; Gutierrez et al., 2009). The trafficking of CSCs among these locations within the cell plays a major role in the regulation of cellulose synthesis (summarized in the model in Figure 2). The first report of CSC rosettes in the Golgi apparatus in vascular plants came from the electron microscopy studies on mesophyll cells in *Zinnia elegans* in suspension cultures (Haigler and Brown, 1986). During their differentiation into tracheary elements, the *trans* face of the Golgi dictyosomesas well as the post-Golgi vesicles and protoplasmic fracture of the PM in freeze fracture contained the characteristic rosettes that were interpreted to be CSCs. The *trans*-Golgi network/early endosome (TGN/EE) compartment has been implicated in protein sorting and as a hub of plant exocytosis and endocytotic pathways (Viotti et al., 2010; Rosquete et al., 2018). The Golgi-localized STELLO1 and 2 (STL1/2) proteins regulate cellulose synthesis likely by controlling the assembly of CESA CSCs in the Golgi (Zhang et al., 2016b). The *stl1 stl2* mutants are hypersensitive to isoxaben (a cellulose biosynthesis inhibitor), have thinner secondary cell walls, and decreased levels of primary and secondary cell wall cellulose. Further, these mutants display a range of defects in the behavior of CESA3, including reduced velocity, reduced insertion into the PM, and a change in its distribution in the Golgi. STL proteins contain a domain with homology to glycosyltransferase family75 that faces the Golgi lumen and thus has the potential to interact with the Golgi-localized CESA3. These results are congruent with a role of STL proteins in accurate CSC assembly in the Golgi apparatus.

The delivery of CSCs to the PM plays an important role in the regulation of cellulose synthesis. Cortical microtubules (CMTs) play a role in defining the PM delivery sites for CESAs. Two laboratories identified CMT-associated vesicles that contained microtubule-associated CESA compartments (MASCs) or small CESA compartments (SmaCCs) and demonstrated that CESA insertion sites are not random, but rather colocalize with CMTs (Crowell et al., 2009; Gutierrez et al., 2009). Furthermore, CESA delivery to the PM coincided with the colocalization of both Golgi bodies and microtubules below the cell cortex (Crowell et al., 2009). The exocyst complex has been implicated in the delivery of CESA to the PM (Zhu et al., 2018). The exocyst is a multisubunit assembly that plays a role in a plethora of developmental events that depend on cellular processes

Table 1. CESA Interacting Proteins

Protein	Putative role	Partner CESA	Method[a]	Reference
KORRIGAN	Cellulose synthesis and CSC trafficking	CESA1 CESA3 CESA6 CESA4 CESA8	Gel filtration, Split-ubiquitin for membrane proteins, BiFC, GFP-TRAP/MS, Y2H	(Vain et al., 2014) (Zhu et al., 2018) (Mansoori et al., 2014)
CC1/2	Cellulose synthesis during salt stress	CESA1 CESA3 CESA6	Split-ubiquitin assay in yeast	(Endler et al., 2015)
POM2/CSI1	CSC-CMT interaction; formation of SmaCCs/MASCs; CSC trafficking	CESA1 CESA3 CESA6	Y2H, GST pull-down	(Gu et al., 2010) (Lei et al., 2015)
AP2M/μ2	CSC endocytosis	CESA1 CESA3 CESA6	Split-ubiquitin Y2H, GST pull-down	(Bashline et al., 2013)
Sec5B Sec10 Sec6 Sec15B Sec8 Sec3A Exo84B Exo70B1 Exo70A1 PTL1	CSC exocytosis	CESA6	Y2H, GFP-TRAP/MS	(Zhu et al., 2018)
TML TPLATE	CSC endocytosis	CESA6	Co-IP, BiFC	(Sanchez-Rodriguez et al., 2018)
BIN2	CESA phosphorylation; regulation of CESA activity	CESA1	in vitro protein kinase assay	(Sanchez-Rodriguez et al., 2017)
SHOU4	CSC exocytosis	CESA1 CESA3 CESA6	Y2H, Co-IP	(Polko et al., 2018)

[a]The methods shown are not necessarily for all the partner CESAs indicated. BIFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; GST, glutathione S-transferase; Y2H, yeast two-hybrid.

involving vesicular trafficking, cytokinesis, protein recycling, or cell polarity establishment (Zárský et al., 2013). POM2/CSI1 and CESA6 both interact with multiple subunits of the exocyst complex (Zhu et al., 2018). Moreover, POM2/CSI1 interacts with the PATROL1 (PTL1) protein, which was first identified by its role in the trafficking of AHA1, an H$^+$-ATPase, to the PM of guard cells (Hashimoto-Sugimoto et al., 2013). PTL1 was also found to interact with Sec-10 and colocalize with Sec5B, both subunits of the exocyst complex (Zhu et al., 2018). Disruption of *PTL1* resulted in impaired expansion of roots and hypocotyl cells, decreased cellulose levels, and slower CESA delivery rates to the PM. The *ptl1* and *pom2/csi1* mutations have an additive effect on several phenotypes, suggesting that they act in a nonlinear manner. A detailed sequential analysis of POM2/CSI1, CESA, and PTL cellular dynamics demonstrated that during CESA exocytosis, POM2/CSI1 appears first at the PM, followed by the tethering of CESAs accompanied by PTL1 and Sec5B. Based on these results, the authors proposed an elegant model in which POM2/CSI1 serves as a landmark for the CMT-marked insertion of CESA-containing exocytotic vesicles, with PTL1 priming the fusion of these vesicles (Zhu et al., 2018).

The paralogous *SHOU4* and *SHOU4L* genes also play an important role in regulating exocytosis of CESAs (Polko et al., 2018). SHOU4 was identified in a suppressor screen of the cellulose-deficient *fei1 fei2* mutant. The *shou4 shou4l* double mutants display a range of phenotypic defects including dwarfism, partial infertility, twisted growth of the roots, and impaired development of root hairs. The phenotypes are either absent or much milder in the respective single mutants, indicating functional redundancy. Interestingly, treatment of *shou4 shou4l* seedlings with low levels of isoxaben restored root hair growth and reverted root twisting, suggesting that these phenotypes result from excess cellulose in the mutant. Seed coat mucilage of *shou4 shou4l* mutants is characterized by increased levels of cellulose staining. Consistent with this, *shou4 shou4l* mutants display elevated CESA density at the PM due to enhanced exocytosis. This increased PM-localized CESA resulted in an increase in the level of amorphous cellulose, but wild-type levels of crystalline cellulose. SHOU4 and SHOU4L directly interact with CESAs. Further, *SHOU4* is haplo-insufficient, which indicates that the levels of SHOU4 are critical to its function. These results suggest a model in which SHOU4 acts as a "counter" of CESA levels at the PM, with the complex generating a negative feedback signal that regulates CESA exocytosis to maintain optimal levels of cellulose biosynthesis.

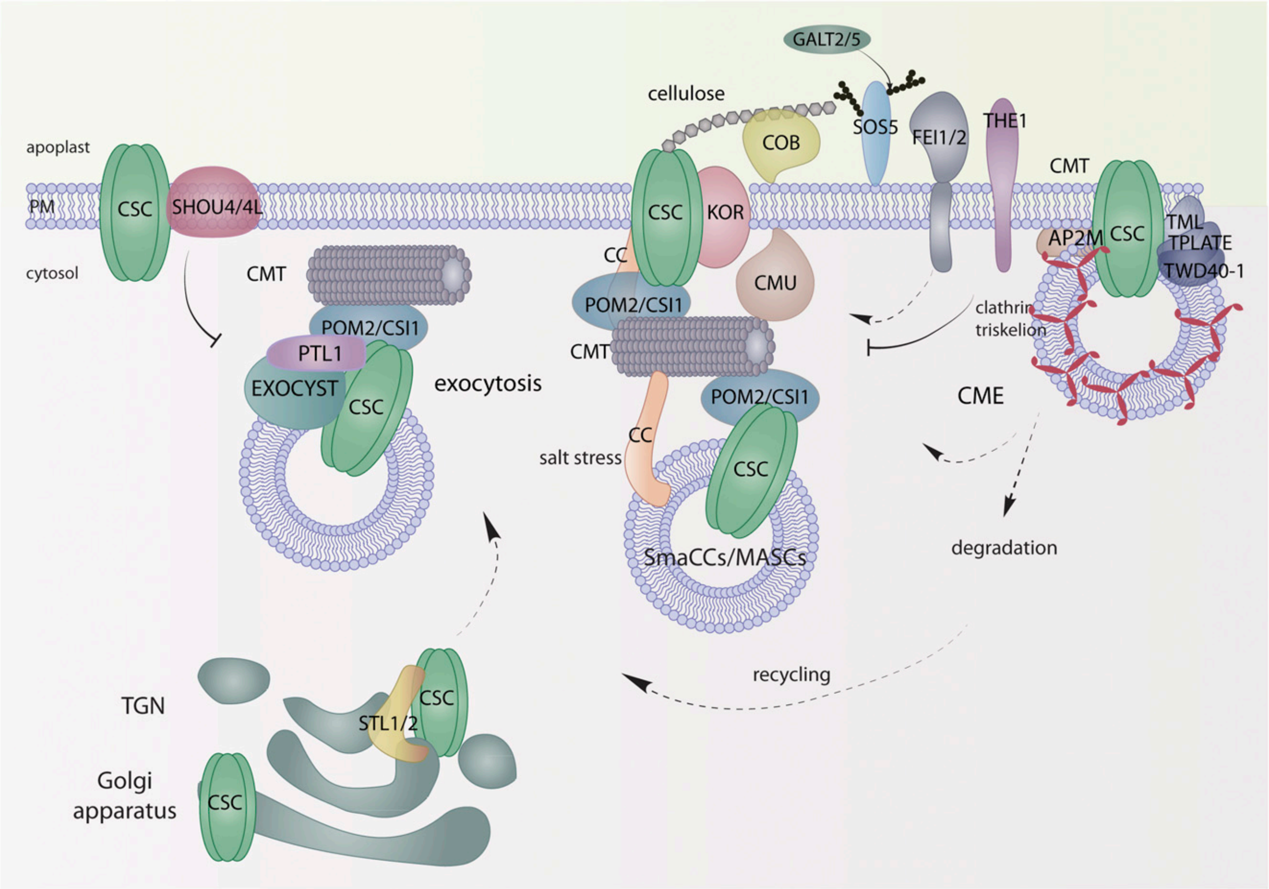

Figure 2. A Cartoon of CSC Trafficking and the Other Components of the CSC Machinery.

CSCs are assembled in the Golgi apparatus where they physically interact with STL proteins that assist their assembly and distribution in the Golgi. CSCs move through the TGN and follow the exocytosis route to the PM, where their insertion sites coincide with the pausing of the Golgi along the CMTs. The insertion of CSCs in the PM is preceded by POM2/CSI1, the exocyst complex, and PTL, which are required for CSC delivery. The CESA-interacting proteins SHOU4/4L negatively affect CSC exocytosis. POM2/CSI1 proteins also act as linkers between CSCs and CMTs and are necessary for the formation of SmaCCs/MASCs. The CC1 and CC2 belong to the CSCs, associate with SmaCCs/MASCs under salt stress and mediate the CMT and CSC dynamics, allowing for their recovery. CMU proteins regulate proper CMT spacing during cellulose biosynthesis. KOR is a component of CSCs required for optimal cellulose biosynthesis and COB is necessary for proper microfibril orientation. The SOS5 and FEI1/2 leucine-rich repeat-RLKs mediate cellulose biosynthesis and CrRLK1L THE1 inhibits cell expansion upon perturbation of cellulose biosynthesis. CSCs undergo CME that requires the AP2 complex and TPLATE complex members such as TWD40-1, TPLATE, and TML. The endocytosed CSCs may undergo recycling back to the PM or might be targeted for degradation.

The trafficking of CESAs from the TGN/EE may also be affected by pH. The BR-insensitive mutant *det3*, which encodes the C subunit of the V-ATPase (Schumacher et al., 1999), causes an increase in the pH of the TGN/EE, which reduces its motility and impairs the delivery of CESA3 to the PM (Luo et al., 2015). The exact mechanism through which pH affects CESA secretion remains to be identified but the authors hypothesize that the endomembrane pH may affect the secretion of CESA (and other cargos) from the Golgi via effects on cargo processing or vesicle tethering (Luo et al., 2015). A small Golgi-localized GTPase, RabH1B, also affects CESA exocytosis and cellulose biosynthesis (He et al., 2018). The *rab-h1b* mutant, which has increased CESA density at the PM, exhibits reduced levels of general endocytosis, though paradoxically decreased secretion of CESA from the Golgi. This is consistent with the notion that membrane trafficking in plants is a complex process and that disruption of one aspect likely has ripple effects on the entire system (Paez Valencia et al., 2016; Kanazawa and Ueda, 2017).

CSCs are internalized via clathrin-mediated endocytosis (CME), one of the best-studied endocytotic processes in plants (Paez Valencia et al., 2016). The Arabidopsis CESAs are cargo proteins of CME (Bashline et al., 2013). The medium subunit of the heterotetrameric CME adaptor protein2 (AP2) complex (AP2M, previously referred to as “μ2”) directly interacts with central domains of CESA3 and CESA6. The *ap2m-1* mutants show reduced endocytosis and, as a result, have increased levels of CESA density at the PM. Surprisingly, the higher CESA abundance does not lead to any changes in CSC particle velocity or cellulose content (Bashline et al., 2015). Further, *ap2m-1* mutants display increased elongation of primary roots and etiolated hypocotyls, but reduced sizes of rosettes, inflorescences, and siliques (Bashline et al., 2013). In line with these observations, TWD40-2, a member of the large adaptor TPLATE complex, has a critical role in CESA endocytosis (Bashline et al., 2015). The *twd40-2* mutants display additive phenotypes with *ap2m-1*, including increased CESA particles at the PM, decreased cellulose content, and further reductions in organ size as compared with single *twd40-2* and *ap2m-1* mutants. Surprisingly, the *twd40-2* mutant displays reduced elongation of etiolated hypocotyls, as opposed to the increased hypocotyl elongation of the *ap2m* mutant. The reduction in cellulose content in these mutants with reduced CESA endocytosis has been hypothesized to be the result of over-accumulation of nonfunctional CSCs at the PM. The increased elongation of hypocotyl and root cells in the *ap2m-1* mutant could be the result of increased anisotropic cell expansion from a modest increase in CESA density, but perhaps the *twd40-2* mutant has a more substantial effect on functional CESA levels such that it interferes with proper microfibril crystallization. In addition to TWD40-2, other members of the TPLATE complex, including TPLATE complex muniscin-like protein (TML) and TPLATE, interact with CESAs to mediate their endocytosis; these elements may also be involved in early CSC cargo recognition in plants (Sánchez-Rodríguez et al., 2018). The phosphatidylinositol kinases PI3K and PI4K mediate CESA trafficking, though they have distinct effects: PI3K is involved in the regulation of CME-mediated endocytosis of CESAs whereas PI3K plays a role in the exit of CESAs from the Golgi apparatus (Fujimoto et al., 2015).

Endocytosed CSCs are either degraded or recycled back to the cell surface. Isoxaben-induced disruption of cellulose biosynthesis or exposure to changes in osmoticum leads to the depletion of CESA particles from the PM and the formation of SmaCCs/MASCs (Crowell et al., 2009; Endler et al., 2015; Lei et al., 2015; Worden et al., 2015). In addition to interacting with microtubules, POM2/CSI1 mediates the formation of SmaCCs/MASCs (Lei et al., 2015), with the multifunctional C2 domain of POM2/CSI1 colocalizing with isoxaben-induced SmaCCs/MASCs that are formed likely as a result of endocytosis. Moreover, SmaCCs/MASCs are almost undetectable in *pom2/csi1* mutants. The recovery of PM CESA localization after isoxaben treatment requires functional POM2/CSI1 protein (Lei et al., 2015). This indicates that SmaCCs/MASCs could function as a reservoir of CESAs during stress responses, but it remains to be determined if the internalized CESAs need to first pass through the TGN/EE pathway or can be delivered directly to the cell surface.

CSC trafficking is also important during cytokinesis in plants, in which a new cell wall is formed de novo (Samuels et al., 1995). Live-cell imaging experiments of dividing cells in Arabidopsis roots show that CESAs are delivered in different stages during cell plate formation. First, primary cell wall CESAs localize to the phragmoplast as early as during the formation of the tubulo-vesicular network (Chen et al., 2018). This is accompanied by cellulose deposition as shown by Pontamine Fast Scarlet S4B staining and immunocytochemistry with CBM3a (a bacterially derived carbohydrate binding module that specifically recognizes crystalline cellulose; Blake et al., 2006) detection of crystalline microfibrils (Miart et al., 2014). Next, CESAs are delivered to the forming cell plate both from the neighboring Golgi bodies and directly from the PM of the mother cell. Finally, the CESAs are removed from the central zone of the cell plate by a clathrin-mediated process and from the membrane of the mother cell and then delivered to the periphery of the forming cell plate. This last stage has been hypothesized to contribute to the growth of the cell plate at the peripheral zone (Miart et al., 2014). Field-emission-scanning microscopy experiments showed that cellulose microfibrils in newly formed cell walls of diving cells in Arabidopsis inflorescence stems do not have a predominant orientation, but rather form meshwork-like structures (Fujita and Wasteneys, 2014).

ADDITIONAL CSC-INTERACTING COMPONENTS

One of the best-studied aspects of CSC biology is their interaction with CMTs. The relationship between cellulose microfibrils and CMTs was noted in a theoretical article that presented a model in which CESAs could move in the PM along the tracks defined by microtubules (Heath, 1974). The association of microfibrils with the CMTs has been found in multiple studies (Ledbetter and Porter, 1963; Fisher and Cyr, 1998; Gardiner et al., 2003) but remained controversial because some findings showed that they do not always coalign (Emons, 1982; Emons and Wolters-Arts, 1983) and that microfibrils could still be deposited correctly even after disruption of MTs (Himmelspach et al., 2003; Sugimoto et al., 2003). The demonstration that fluorescently labeled CESA particles on the PM moved precisely along the CMT tracks provided strong support for a role of CMT in the direction of CESA movement (Paredez et al., 2006).

The relationship between CMTs and CESAs was further strengthened by the identification of the *POM2/CSI1* gene. *POM2/CSI1* was initially discovered in a genetic screen for root elongation mutants (Hauser et al., 1995) and subsequently found to be coexpressed with the primary cell wall *CESA* genes (Persson et al., 2005). POM2/CSI1 interacts with the large central domain of primary cell wall CESAs and regulates their velocity at the PM (Gu et al., 2010; Bringmann et al., 2012; Li et al., 2012). POM2/CSI1 binds microtubules, moves along CMT trajectories, and regulates the CESA-CMT coalignment (Bringmann et al., 2012; Li et al., 2012). Similar to POM2/CSI1, its paralog, CSI3, also associates with CESAs and CMTs. CSI3 regulates cellulose biosynthesis in a *POM2/CSI1*-dependent manner as *pom2/csi1* loss-of function affects CSI3 velocity. These results indicate that while POM2/CSI1 is a key link between CESAs and CMTs, CSI3 may play an auxiliary scaffolding role in regulating CESA velocity (Lei et al., 2013).

A recent study employed a combination of near-total internal reflection fluorescence microscopy and automated CSC particle tracking to demonstrate that the velocity of CSCs is independent of their proximity to CMTs (Woodley et al., 2018). Further, disrupting microtubule polymerization could either decrease (oryzalin) or increase (*mor1* mutant) CSC speed and these effects of microtubule dynamics were likely independent of CESA catalytic activity. These results suggest that the role of CMTs in regulating CSC speed is complex and independent of a direct interaction.

Multiple additional proteins have been identified that directly interact with CESAs (Table 1) and/or play a role in CESA function. For example, KOR, an endo-β-1,4-endoglucanase, has been shown to play an important role in cellulose biosynthesis in multiple plant species (Nicol et al., 1998; Bhandari et al., 2006; Maloney et al., 2012; Xie et al., 2013). The *kor-1* mutants display reduced CESA velocity, suggesting that KOR is required for optimal cellulose biosynthesis (Vain et al., 2014). KOR directly interacts with CESA likely in a transient manner and may be required for untangling of the newly formed glucan chains (Vain et al., 2014). The *kor1-3* allele, which was identified based on hypersensitivity to the microtubule destabilizing drug oryzalin, affects cortical microtubule organization, as did treatment with isoxaben (Paredez et al., 2008). This further supports the long-standing hypothesis that there is feedback between CMTs and cellulose microfibrils/CESAs (Fisher and Cyr, 1998; Peng et al., 2013; Worden et al., 2015).

The CMT-interacting CELLULOSE SYNTHASE MICROTUBULE UNCOUPLING (CMU) proteins are necessary for proper CMT spacing during cellulose synthesis (Liu et al., 2016). The *cmu1 cmu2* mutants display lateral instability of microtubules and uncoupling of CESAs from the microtubules. This results in instability of CESA movement, which deviates from the mostly straight trajectories observed in wild-type hypocotyls and is restored by disruption of microtubule organization by oryzalin. The lateral displacement of microtubules was also abolished after treatment with isoxaben. Based on these findings, the authors proposed a model in which CMU proteins allow the CMTs to withstand the forces generated by CSC movement (Liu et al., 2016).

Glycosyl phosphatidylinositol (GPI)-anchored proteins such as COBRA (COB) or SALT-OVERLY SENSITIVE5 (SOS5)/FASCICLIN-LIKE PROTEIN4 also regulate cellulose biosynthesis. *COB* was initially identified in a genetic screen for root tip mutants with defects in anisotropic cell expansion (Benfey et al., 1993) and was later shown to regulate the orientation of cellulose microfibrils (Roudier et al., 2005). Phylogenetically, COB belongs to a large family whose members are expressed throughout development in both dicots and monocots and whose expression can be regulated by environmental stimuli (Roudier et al., 2002; Brady et al., 2007). COB localizes mostly to the longitudinal walls of elongating root cells and its distribution was altered upon inhibition of CMTs (Roudier et al., 2005). COB is detected in multiple cellular compartments, including the Golgi, possibly secretory vesicles, and the apoplast at different distances from the PM, the latter suggesting that COB is cleaved. Consistent with this, the GPI anchor was found to be cleaved from maize Brittle Culm1 (BC1), a COB-like protein, and the cleaved protein released into the cell wall (Liu et al., 2013). BC1 possesses a carbohydrate binding module that interacts specifically with crystalline cellulose, and modulation of BC1 function alters microfibril crystallinity. These results suggest that COB acts in the assembly of cellulose microfibrils at least in part by modulating cellulose crystallinity.

COBL2 has been shown to regulate crystalline cellulose deposition in Arabidopsis seed coat mucilage secretory cells (Ben-Tov et al., 2015). Though mucilage is primarily composed of pectin (Willats et al., 2001; Voiniciuc et al., 2015), cellulose also plays an important structural role in regulating mucilage adherence to the seed surface, with CESA3 and CESA5 being the key players in this process (Harpaz-Saad et al., 2011; Mendu et al., 2011; Sullivan et al., 2011; Griffiths et al., 2015; Ben-Tov et al., 2018). CESA3, CESA5, and CESA10 have been shown to localize around the columella of the seed coat epidermal cells in a unique coiled pattern. Upon mucilage extrusion, the cellulosic rays "unwind" in a counterclockwise manner (Griffiths et al., 2015). The biology of seed coat mucilage structure, function and formation has been discussed recently in several excellent reviews (Francoz et al., 2015; Voiniciuc et al., 2015; Griffiths and North, 2017; Golz et al., 2018).

CELLULOSE AND PLANT CELL WALL INTEGRITY MAINTENANCE

Similar to yeast, plant cells monitor changes in cell wall integrity (CWI) and in turn signal back to regulate cell wall synthesis, including cellulose synthesis (Humphrey et al., 2007; Steinwand and Kieber, 2010; Wolf et al., 2014; Höfte, 2015; Wolf, 2017; Polko et al., 2018). An early clue regarding plant CWI sensing mechanisms originated from the observation that tomato (*Solanum lycopersicum*) cell cultures adapted to the presence of the cellulose synthesis inhibitor, 2,6-dichlorbenzonitrile, and could grow despite the fact that they synthesize virtually no cellulose (Shedletzky et al., 1990). Ectopic lignification is commonly induced in response to reduced cellulose synthesis in multiple tissues, supporting the idea that plant cell wall remodeling is an active process (Caño-Delgado et al., 2003; Xu et al., 2008; Bischoff et al., 2009; Denness et al., 2011). The mechanism underlying CWI maintenance likely requires receptors that sense changes in the cell wall. Recent studies suggest that decreased cellulose biosynthesis results in an active inhibition of cell expansion, mediated in part through a wall sensing system. Receptor-like kinases (RLKs) from several distinct clades have been proposed to be involved in cell wall sensing (Steinwand and Kieber, 2010; Wolf and Höfte, 2014; Wolf, 2017). Loss-of-function mutations in *THESEUS* (*THE1*), which encodes a member of the *Catharanthus roseus* protein kinase-1-like (CrRLK1L) family, suppress growth defects and gene expression changes in cellulose-deficient mutants, such as *cesa6*^{prc1}, without restoring wild-type cellulose levels (Hématy et al., 2007). Several other CrRLK1L family members, including FERONIA (FER), CURVY1, HERCULES1/2, ANXUR1/2 (ANX1/2), and ERULUS have also been implicated in regulating cell expansion, cell wall sensing, and cell wall synthesis (Wolf, 2017).

CrRLK1L proteins are composed of an extracellular N-terminal domain with two tandem malectin-like domains, which may bind to cell wall carbohydrates (Schallus et al., 2008; Nissen et al., 2016; Du et al., 2018), a single transmembrane domain, and a cytosolic Ser/Thr kinase domain. The growth defects in *cesa6*^{prc1} have been proposed to reflect an active response of cells to decreased cellulose and the perception and/or signaling of the reduced cellulose to be mediated by THE1 (Hématy et al., 2007). A novel, hypermorphic allele of *THE1* has been recently characterized (Guo et al., 2009; Merz et al., 2017). This *the1-4* allele is a T-DNA insertion that results in the production of a truncated THE1 protein lacking the kinase domain. In contrast with the hypomorphic *the1-3* allele, *the1-4* enhanced the effects of reduced cellulose biosynthesis, suggesting that this mutant protein activates THE1 signaling. This suggests that, similar to other RLKs such as FEI1 and FER, kinase activity is not always essential for signal activation, but rather kinase activity actually attenuates THE1 signaling. THE1 also acts as a receptor for the rapid alkalinization factor (RALF)34 peptide (Gonneau et al., 2018), similar to FER, ANX1/2, and BUDDHA's PAPER SEAL1/2, which also bind RALF peptide ligands (Haruta et al., 2014; Ge et al., 2017; Stegmann et al., 2017). THE1/RALF34 signaling regulates lateral root initiation, suggesting that THE1 may integrate CWI with growth and development (Gonneau et al., 2018). FER is perhaps the best-studied member of the CrRLK1L family and is involved in multiple pathways, including pollen tube reception in the female gametophyte, root hair development, and BR and ethylene signaling (Huck et al., 2003; Escobar-Restrepo et al., 2007; Deslauriers and Larsen, 2010; Ngo et al., 2014; Yu et al., 2014). FER is required to maintain CWI during salt stress through binding to pectin and by inducing Ca^{2+} transients that are necessary for cell acclimation to salt stress (Feng et al., 2018). Related CrRLK1L family members, such as ANX1 and 2, have also been linked to cell wall synthesis, though they appear to primarily affect pectin synthesis (Nissen et al., 2016).

FEI1 and FEI2 are leucine-rich repeat RLKs that regulate cell wall synthesis in various contexts and have been implicated in cell wall-related signaling. Double *fei1 fei2* mutants display reduced growth anisotropy that is associated with reduced cellulose biosynthesis as determined by assays measuring incorporation of ^{14}C Glc into cellulose in roots (Xu et al., 2008; Basu et al., 2016). Root swelling in *fei1 fei2* mutants is suppressed by inhibitors of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid, but not by inhibitors of ethylene signaling or by genetic disruption of ethylene perception (Xu et al., 2008). The *fei1 fei2* phenotype is also reverted by mutations in auxin biosynthesis genes, which are also able to partially suppress other cellulose-deficient mutants such as *cesa6^{prc1}-1*, *cob*, and *sos5*, providing a link between auxin signaling and cell wall function (Steinwand et al., 2014). Genetic analyses indicate that the FEIs act in a linear pathway with SOS5 in roots (Xu et al., 2008; Basu et al., 2016), though the interaction may be more complex during cellulose synthesis in seed coat mucilage (Griffiths et al., 2014, 2015, 2016).

Analysis of mutations in the arabinogalactan-protein-specific galactosyltransferases *GALT2* and *GALT5* reveals that they also act in the SOS-FEI5 pathway and suggests that glycosylation of SOS5 contributes to its function. The *galt2*, *galt5*, and *galt2 galt5* mutants phenocopy *sos5* and *fei1 fei2* and display reduced levels of cellulose biosynthesis (Basu et al., 2015, 2016). A functional fluorescently tagged SOS5 is localized primarily to the PM in Arabidopsis roots and is retained in the apoplast after plasmolysis (Xue et al., 2017). The presence of the N-terminal fasciclin domain and the GPI anchor of SOS5, as well as its N- and O-glycosylations, stabilize its localization to the PM but are not essential for its function, which mostly requires the soluble C-terminal fasciclin domain.

High exogenous Suc exacerbates many cell-wall–related phenotypes, such as *cesa6^{prc1}*, *pom-pom*, *cobra*, *sabre*, *sos5*, and *fei1 fei2* (Hauser et al., 1995; Schindelman et al., 2001; Xu et al., 2008; Basu et al., 2016). A recent study suggests a mechanism that might underlie this phenomenon (Yeats et al., 2016). The *shaven3 shaven3-like1* (*shv3 svl1*) double mutant displays reduced hypocotyl elongation in darkness, specifically in the presence of exogenous Suc. This is associated with lower CESA velocities, reduction of cellulose content, and increased starch content. Similarly, *fer* mutants have increased starch (Yang et al., 2015). The Suc-dependent inhibition of hypocotyl elongation, reduced levels of cellulose, and increased starch accumulation in *shv3 svl1* were suppressed by a mutation in *SUC1*, which encodes a PM-localized Suc/H^+ symporter. This suggests that Suc accumulation could trigger cell expansion defects. In support of this hypothesis, both *shv3 svl1* and *fer* exhibit hyperpolarization of the PM and overaccumulation of intracellular Suc. The overaccumulation of Suc likely results in partitioning of carbon to starch rather than cellulose. It has been hypothesized that SHAVEN3 and SHAVEN3-LIKE3 and SVL1 are involved in signaling that coordinates proton pump activity with cellulose biosynthesis (Yeats et al., 2016). Interestingly, high exogenous Suc was shown to result in a depletion of CESA levels from the PM (Polko et al., 2018), perhaps linking the hyperpolarization of the PM to CESA trafficking.

CONCLUSIONS AND OUTSTANDING QUESTIONS

Tremendous progress has been made in our understanding of the mechanisms regulating cellulose biosynthesis and cell wall formation. The cellulose microfibril has more subtlety than perhaps was previously recognized. Details of its structure likely influence how matrix polysaccharides interact with its distinctive hydrophobic and hydrophilic surfaces to form the strong, yet extensible structure of primary cell walls. Wall extensibility may be controlled at limited regions ("biomechanical hotspots") where xyloglucans interact with microfibrils. The role of individual CESAs in primary-versus-secondary cell wall synthesis has been defined, as has the requirement of multiple CESAs for a functional CSC. Recent advances in this field include the first structures of bacterial and plant CESAs and revised estimates of microfibril composition from 36 to 18 chains. The previous model of a CSC being composed of a hexamer of hexamers has thus been replaced by a model with 18 to 24 subunits. The identification of new components of the CSC and novel regulators of CSC trafficking has improved our understanding of its dynamics during development and in response to exogenous cues, but undoubtedly additional components regulating these processes remain to be identified. In particular, the role of the posttranslational modifications of CESAs in regulating their interaction with the trafficking machinery needs to be determined as do the functions of most of the kinases involved in CESA phosphorylation. Additionally, how CESA density at the PM is monitored has not been elucidated, nor have mechanisms directing subcellular trafficking of the CSCs. Further, how these elements interact with each other, and their role in different cell types and in response to various environmental and developmental cues in the regulation of CESA function and cell wall synthesis, are important questions to be addressed. Additionally, the ligands for most of the RLKs involved in CWI need to be defined and the crosstalk among the various cell-wall sensing RLKs is key to understanding how the multiple inputs are integrated to bring about a coherent response. Multidisciplinary approaches that span areas of biochemistry, physics, genetics, physiology, and cell biology are needed to propel the field forward.

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AUTHOR CONTRIBUTIONS

Both authors contributed equally to this work.

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