Biosynthesis and properties of the plant cell wall

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The characterization of cell wall mutants of Arabidopsis thaliana, combined with biochemical approaches toward the purification and characterization of glycosyltransferases, has led to significant advances in understanding cell wall synthesis and the properties of cell walls. New insights have been gained into the formation of cellulose and the functions of the matrix polysaccharides rhamnogalacturonan-II and xyloglucan.

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Abbreviations

AtFUT1	Arabidopsis thaliana FUCOSYLTRANSFERASE1
AtXT1	Arabidopsis thaliana XYLOSYLTRANSFERASE1
CESA1	CELLULOSE SYNTHASE1
CSL	CELLULOSE SYNTHASE-LIKE
cyt1	cytokinesis1
IRX2	IRREGULAR XYLEM2
KOR	KORRIGAN
mur2	murus2
RG-I	rhamnogalacturonan-I
rsw1	root swelling1
SCD	sitosterol-cellodextrins
SG	sitosterol-β-glucoside

Introduction

The deposition and modification of cell wall material play essential roles during plant growth and development, the responses of plants to the environment, and the interactions of plants with symbionts and pathogens [1]. As cell migrations do not contribute to the development of the plant body, the planes of cell divisions and the ordered deposition of cell wall material ultimately determine the shapes of plant cells and organs. Most photosynthetically fixed carbon is incorporated into cell wall polymers, making plant cell walls the most abundant source of terrestrial biomass and renewable energy. Cell wall material is also of great practical importance for human and animal nutrition, and as a source of natural fibers for textiles and paper products. For these reasons, the study of cell wall synthesis is of considerable interest from both a basic and an applied point of view.

Two types of cell walls can be distinguished. Primary walls are deposited during cell growth, and need to be both mechanically stable and sufficiently extensible to permit cell expansion while avoiding the rupture of cells under their turgor pressure. Primary cell walls consist mainly of polysaccharides that can be broadly classified as cellulose, the cellulose-binding hemicelluloses, and pectins. The latter two classes of cell wall components are often referred to as matrix polysaccharides. These are synthesized within Golgi cisternae, whereas cellulose is generated at the plasma membrane in the form of paracrystalline microfibrils. Secondary cell walls are deposited after the cessation of cell growth and confer mechanical stability upon specialized cell types such as xylem elements and sclerenchyma cells. These walls represent composites of cellulose and hemicelluloses, and are often impregnated with lignins. In addition to polysaccharides, plant cell walls contain hundreds of different proteins. Many of these proteins are considered to be 'structural' proteins [2], whereas others participate in cell wall remodeling and turnover [3].

This review focuses on recent advances in understanding the biosynthesis and function of plant cell wall polysaccharides, with an emphasis on the model system *Arabidopsis thaliana*. As the genome sequence of this small crucifer has recently been determined [4], the coding regions of all glycosyltransferases and other enzymes that are involved in cell wall synthesis and modification are available in public databases. Now, the challenge is to identify candidate genes for glycosyltransferases and other cell-wall-related proteins, and to determine their function. Strategies to accomplish these goals have been outlined in recent review articles [5–7]. Because of space limitations, advances in the characterization of cell wall proteins and lignification pathways are not included in this contribution, and the reader is referred to recent reviews on these subjects [8,9].

The synthesis of cellulose in higher plants

In recent years, substantial progress has been made in understanding the synthesis of cellulose. It is a linear 1,4-β-D-glucan that assembles into paracrystalline microfibrils, each of which contains an estimated 36 parallel polysaccharide chains. Cellulose synthesis occurs at rosette-like structures that consist of six hexagonally arranged subunits that are embedded in the plasma membrane [10]. As each rosette is believed to synthesize one microfibril, some models propose that each of the six rosette subunits is composed of six 1,4-β-D-glucan synthases, each of which forms a single glucan molecule from cytoplasmic UDP-D-glucose [11**,12]. In this scenario, 36 1,4-β-D-glucan chains would emerge at the apoplastic side of the plasma membrane, and would assemble into cellulose microfibrils in a process that may be aided by additional proteins such as KORRIGAN (KOR; see below).

The catalytic subunit of cellulose synthase is believed to be encoded by members of a multi-gene family of transmembrane proteins that have sequence similarities to bacterial cellulose synthases, such as *acsA* from *Acetobacter xylinum* [13] and *celA* from *Agrobacterium tumefaciens* [14,15].

Table 1

Mutants in the *CESA* and *CSL* genes of *Arabidopsis*.

Gene name	Mutant	Phenotype(s)	References
CESA1	rsw1	Root swelling, stunted growth (rsw1-1), seedling lethality (rsw1-2)	[17,32••]
CESA3	ixr1	Resistance to isoxaben, stunted growth (antisense plants)	[11••,18]
CESA4	irx5	Irregular structure of xylem elements	[37],a
CESA6	ixr2	Resistance to isoxaben	[19•]
	prc1	Reduced length of roots and hypocotyls	[20]
CESA7	irx3	Irregular structure of xylem elements	[21]
CESA8	irx1	Irregular structure of xylem elements	[22]
CSLA9	rat4	Resistance to root transformation by Agrobacterium	[37]
CSLD3	kojak	Short and defective root hairs	[39•,40]

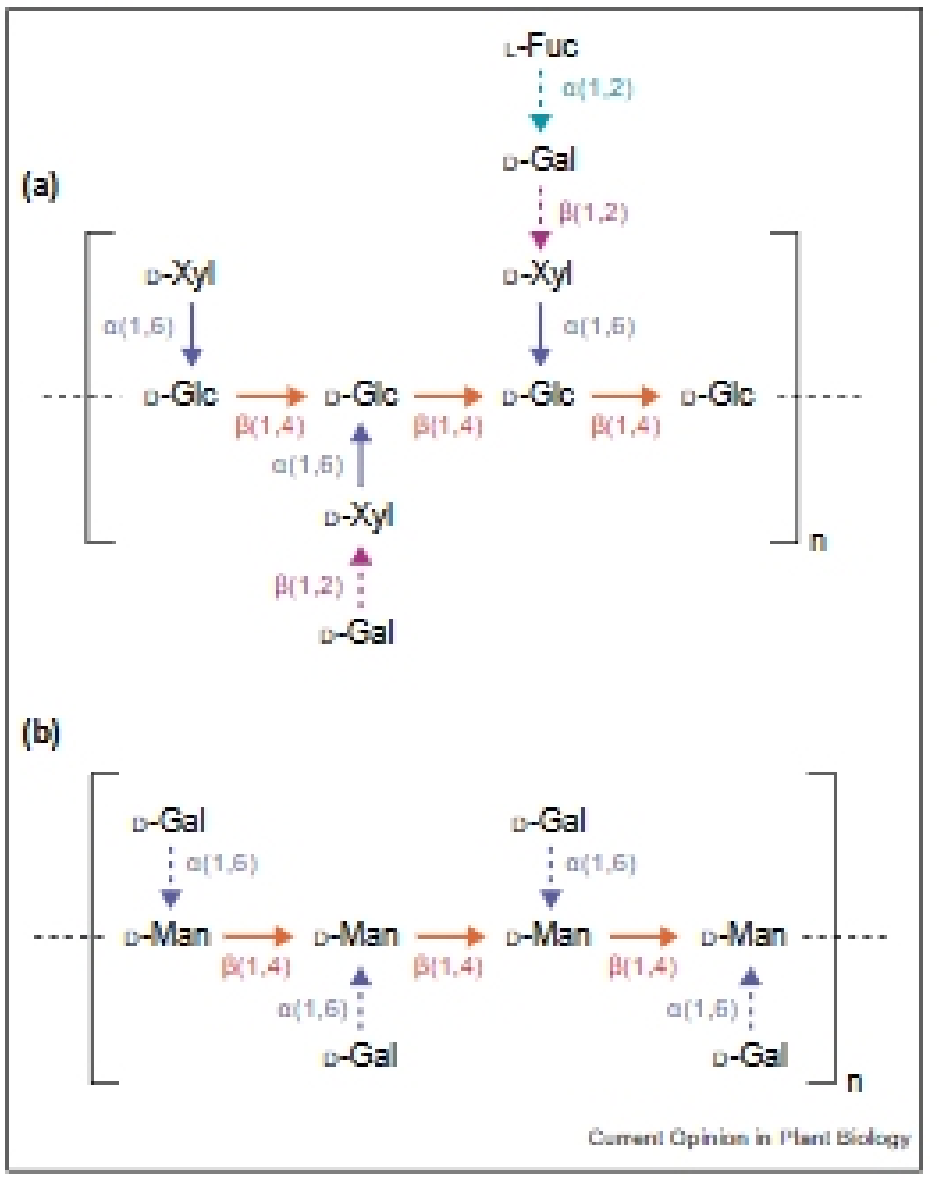
[a]S Turner, personal communication. Abbreviations: prc1, procuste1; rat4, resistant to transformation by Agrobacterium tumefaciens4.

The *Arabidopsis* genome harbors ten members of this gene family (*CELLULOSE SYNTHASE1* [*CESA1*] through *CESA10*), all of which contain eight transmembrane domains, a D,D,D,QxxRW motif that is believed to be part of the active site, and a putative zinc-binding domain that may mediate protein–protein interactions [16]. Soon after the cloning of the temperature-sensitive *root swelling1* (*rsw1*) allele of *CESA1* in 1998 [17], mutations in five additional CESA isoforms were identified by analyzing plants that had defects in elongation growth, collapsed xylem elements or resistance to isoxaben, a herbicide that interferes with cellulose synthesis during primary wall formation (Table 1). The phenotypes of these mutants and of *CESA3* antisense plants [18] indicate that the *CESA1*, *CESA3*, and *CESA6* genes are involved in the synthesis of cellulose in the primary cell wall [11••,17,18,19•,20], whereas the *CESA4*, *CESA7*, and *CESA8* genes appear to be primarily involved in cellulose synthesis during secondary wall formation ([21,22]; S Turner, personal communication). Although these observations offer some explanation for the large number of CESA isoforms in *Arabidopsis*, they do not fully explain why lesions in different catalytic subunits cause similar visible phenotypes, such as resistance to isoxaben or the collapse of xylem elements. One possible explanation is the need for at least two different catalytic subunits per rosette to produce a functional cellulose synthase complex [11••,12,20,22]. Taylor *et al.* [22] obtained biochemical evidence in favor of this scenario by demonstrating an association between CESA7 and CESA8 *in vitro*, an approach that may permit the identification of additional components of the cellulose synthase complex in the future.

CESA proteins in higher plants and their homologs in bacteria do not synthesize cellulose in the absence of additional gene products. In *Agrobacterium*, lipid-linked cellodextrins (i.e. short chains of 1,4-linked β-D-glucose) and an *endo*-β-D-glucanase participate in cellulose synthesis [14], raising the possibility that similar intermediates and enzymes play a role in plant cellulose synthesis. Screens for *Arabidopsis* mutants with defects in cell elongation, root-swelling phenotypes, abnormal cytokinesis, and irregular xylem structure led to the identification of several mutant alleles of the membrane anchored *endo*-1,4-β-D-glucanase KOR (the *KOR1* gene product, which is allelic to *ALTERED CELL WALL1* (*ACW1*), *RSW2*, and *IRREGULAR XYLEM2* (*IRX2*) ([23,24•,25], S Turner, personal communication). This protein is primarily localized to the cell plate [26] but has also been found in plasma-membrane fractions [23]. Mutations in the *KOR1* gene cause a decrease in cellulose formation [24•,25], which is partly compensated for by an increase in cell wall pectin [24•] and a change in pectin composition [27]. The strongest known *kor1* allele (i.e. *kor1-2*) causes defects in cell-plate formation and cytokinesis, and leads to extensive callus formation beyond the cotyledon stage of seedling development [26]. Interestingly, similar defects in cytokinesis have been observed in the *cytokinesis1* (*cyt1*) mutant of *Arabidopsis* [28], which appears to be deficient in cellulose synthesis because of a defect in protein glycosylation [29••]. As none of the published *kor1* mutants are known to be null alleles, it is not clear whether KOR1 function is absolutely required for cellulose synthesis in *Arabidopsis*. Furthermore, the *Arabidopsis* genome contains two transcribed *KOR1* homologs (*KOR2* and *KOR3* [30]) that may at least partly compensate for loss of KOR1 activity.

The postulated biochemical function of the *Arabidopsis* KOR1 protein has not been directly shown. However, recent work by Mølhøj *et al.* [31•] demonstrates that Cel16, the KOR1 ortholog from *Brassica napus*, acts as an *endo*-1,4-β-D-glucanase *in vitro*. Recombinant Cel16 protein that was expressed in *Pichia pastoris* hydrolyzed amorphous cellulose but did not act on crystalline cellulose, xyloglucan, or xylans [31•]. This suggests that KOR1 and related membrane-anchored endoglucanases may be involved in chain termination during cellulose biosynthesis or in the degradation of β-D-glucan chains that have not been properly incorporated into cellulose microfibrils. Alternatively, these enzymes may remove putative lipo-cellodextrin primers from the ends of growing β-D-glucan chains (see below) or act as cellodextrin ligases to join short β-D-glucan chains into longer molecules. The latter reaction is believed to be catalyzed by the celC endoglucanase during

Figure 1



Structures of (a) xyloglucan and (b) seed storage galactomannan. Solid arrows indicate linkages that are always present, whereas dashed arrows denote partial substitution patterns. Note that the xylose residues in xyloglucan and the galactose residues in galactomannan are both attached in α-1,6-linkage to the respective β-1,4-glycan backbones. Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose. Modified after [58].

cellulose synthesis in *Agrobacterium* [14]. Recombinant Cel16 protein loses its *endo*-1,4-β-D-glucanase activity upon enzymatic removal of *N*-linked glycans [31•], suggesting that this protein needs to be properly glycosylated to fulfill its function *in vivo*. This may explain why some mutants that have defects in the synthesis or processing of *N*-linked glycans are defective in cellulose synthesis, leading to embryo lethality. Examples of such mutants are the *cyt1* mutant, which has defective GDP-D-mannose pyrophosphorylase [29••], and the *knopf* and *gcs1* mutants, which have defective α-glucosidase I [32••,33,34]. CESA proteins do not appear to be glycosylated *in vivo* [32••], suggesting that some other components of the cellulose-synthesizing machinery are sensitive to structural changes in *N*-glycans.

As there is considerable evidence for the presence of lipid-bound cellodextrins during cellulose synthesis in *Agrobacterium*, lipo-glucosides have been suspected to serve as primers during cellulose synthesis in higher plants. Sitosterol-β-glucoside is an attractive candidate for a lipid-linked primer as it is produced at the plasma membrane where cellulose synthesis occurs [35]. Peng *et al.* [36••] recently demonstrated that cotton fiber membranes can convert sitosterol-β-glucoside (SG) molecules into sitosterol-cellodextrins (SCD) with up to four glucose residues, using UDP-glucose as the monosaccharide donor. Furthermore, they found that radiolabeled SCDs were incorporated into a labeled glucan product, supporting the notion that SG can act as a primer for cellulose synthesis *in vitro*. These authors also demonstrated that, when expressed in yeast, the cellulose synthase GhCESA1 from cotton catalyzes the conversion of SG into sitosterol-cellotriose (SG3), although other SCDs were not formed. This result establishes that GhCESA1 can act as a SG glucosyltransferase but clearly shows that additional components are needed to produce polymeric cellulose. These components may include additional isoforms of CESA [22] and the KOR *endo*-1,4-β-glucanase. Interestingly, Peng *et al.* [36••] found that cellulose synthesis in cotton fiber membranes is strongly inhibited by the Ca^{2+} chelator EGTA (i.e. ethylene glycol-bis-(2-aminoethyl ether)-N,N,N',N'-tetraacetate), which blocks the action of the KOR1 protein. The addition of a Ca^{2+}-independent *endo*-1,4-β-glucanase restored cellulose synthesis, suggesting that this enzyme activity is required for cellulose formation in higher plants. Although SG appears to serve as a primer for cellulose synthesis in cotton fibers, it is not clear how far this observation can be generalized. Lipid-linked 1,4-β-glucans have been reported to accumulate in the *kor1* allele *acw1* [24•] but the identity of the lipid moiety remains to be determined.

The *CESA* gene products belong to a much larger family of putative glycosyltransferases that have been termed CSL proteins for cellulose synthase-like proteins [16]. On the basis of similarities between the predicted amino-acid sequences, the *CSL* gene family in *Arabidopsis* has been subdivided into six groups, *CSLA* through *CSLF* plus *CSLG* [16]. A mutation in the *CSLA9* gene (at the *rat4* locus; see [37,38]) leads to resistance to root transformation by *Agrobacterium*, but the biochemical function of the encoded protein remains to be determined. Loss of function of the CSLD3 protein causes severe defects in the tip growth of root hairs [39•,40], which led to short and distorted hairs that frequently burst at their tips. Although the *CSLD3* gene is expressed throughout the plant, the mutation appears to affect root hair growth specifically. It has no effect on the elongation of pollen tubes [40], the only other plant cells to elongate by a tip-growth mechanism. Favery *et al.* [39•] found that a CSLD3::green fluorescent protein (GFP) fusion protein localized to the endoplasmic reticulum of tobacco epidermal cells. However, they could not exclude its partial localization to the Golgi or plasma membrane, where the glycosyltransferases of cell wall synthesis are expected to reside. The use of immunolocalization procedures on root hair cells from *Arabidopsis* plants should provide a better understanding of the role of CSLD3 in cell wall synthesis.

Somerville and co-workers [37] used mid-infrared microspectroscopy to characterize insertion mutants in