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# Glycosyltransferases and cell wall biosynthesis: novel players and insights

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Plants need an enormous biosynthetic machinery to synthesize the complex polysaccharides that are present in the plant cell wall. The isolation, characterization and mapping of wall mutants, together with biochemical approaches, have led to significant advances in our understanding of both wall polysaccharide synthesis at a molecular level and the function of polysaccharides in plant growth and development. Moreover, potential regulation mechanisms and associated protein factors are emerging from recent data.

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## Abbreviations

<b>AGP</b>	ADP-glucose pyrophosphorylase
<b>AtFUT1</b>	<i>A. thaliana</i> FUCOSYLTRANSFERASE1
<b>AtXT1</b>	<i>A. thaliana</i> XYLOSYLTRANSFERASE1
<b>CALS</b>	callose synthase gene
<b>CESA</b>	gene encoding the catalytic subunit of cellulose synthase
<b>CFL1</b>	<i>cotton FKS1-like1</i>
<b>CSC</b>	cellulose synthase complex
<b>CSL</b>	cellulose-synthase-like gene
<b>FTIR</b>	Fourier-transformed infrared spectroscopy
<b>GSL</b>	GLUCAN SYNTHASE-LIKE
<b>GT</b>	glycosyltransferase
<b>GUS</b>	$\beta$ -glucuronidase
<b>GUT1</b>	GLUCURONOSYLTRANSFERASE1
<b>irx</b>	<i>irregular xylem</i>
<b>KOR</b>	KORRIGAN
<b>ManS</b>	mannan synthase gene
<b>nonac-H18</b>	<i>non-organogenic callus with loosely attached cells-H18</i>
<b>pmr4</b>	<i>powdery mildew resistant4</i>
<b>qua</b>	<i>quasimodo</i>
<b>RGII</b>	rhamnogalacturonan II
<b>SG</b>	UDP-glucose:sterol glycosyltransferase

## Introduction

All plant cells are encapsulated in a cell wall, whose most prominent components are polysaccharides. This is not surprising as plants are able to generate an excess of sugars through photosynthesis. By contrast, nitrogen is limited during plant growth and development, and

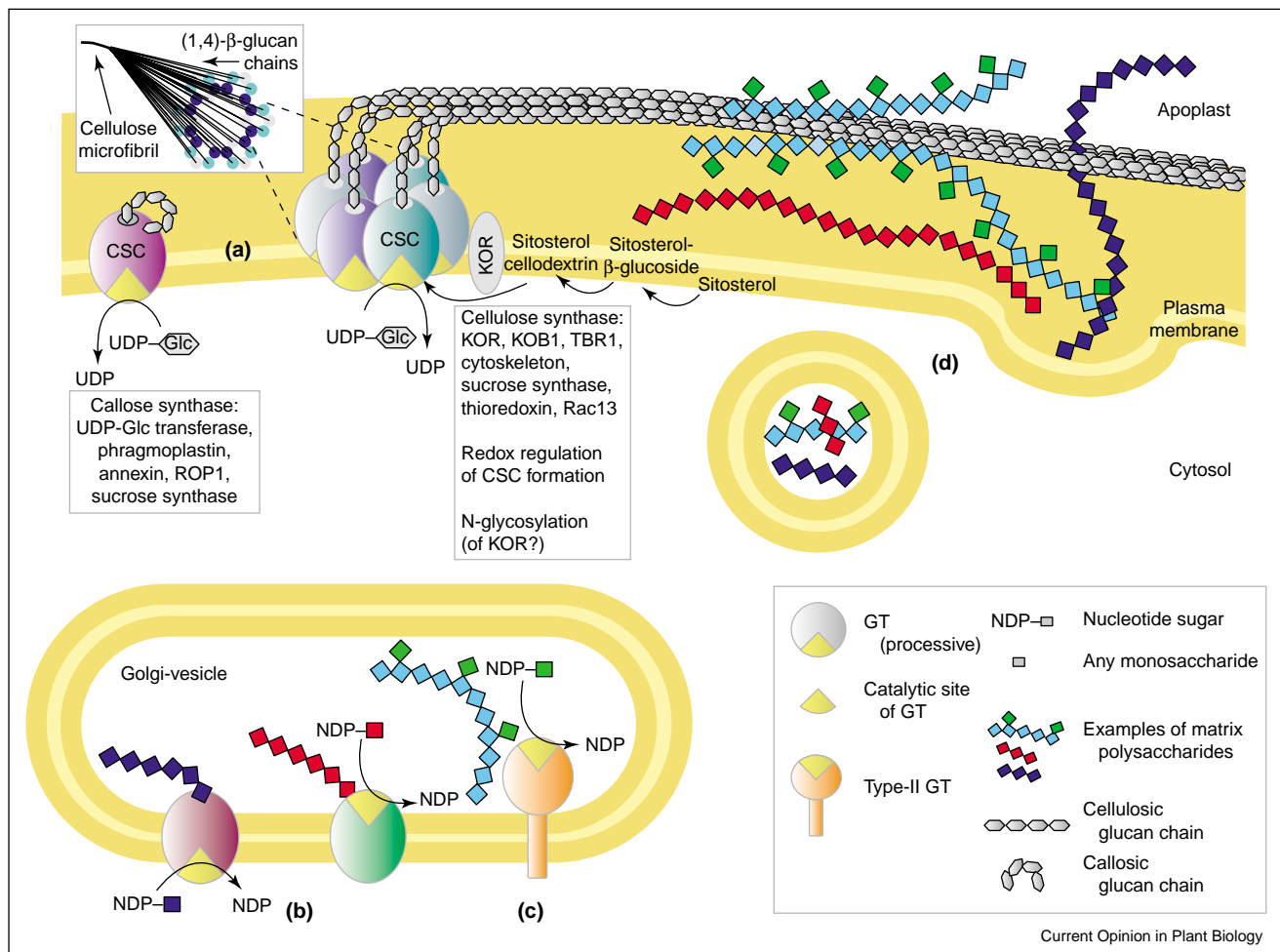
thus does not occur frequently in the wall, being present only in small amounts in structural glycoproteins and wall-modifying enzymes. The wall polysaccharides form coextensive networks and have a tremendous structural complexity. They consist of at least 14 different monosaccharides, which are connected by a variety of glycosidic linkages in the different polysaccharides. To assemble these polysaccharides, the plant cell needs a vast biosynthetic machinery. For instance, it has been estimated that the biosynthesis of the pectic polysaccharides alone would require the action of at least 53 different enzymatic activities [1]. A recent survey of genes in *Arabidopsis thaliana* has indeed indicated that the proportion of plant genes that encode carbohydrate-acting enzymes, such as glycosylhydrolases and glycosyltransferases (GTs), is greater than that in humans or other fully sequenced organisms [2].

The central process of polysaccharide synthesis is the action of GTs or glycosylsynthases [3]. These enzymes form glycosidic bonds by attaching a sugar moiety of an appropriate donor substrate, mainly a nucleotide sugar (G Seifert, this issue), to a specific acceptor substrate. In *Arabidopsis* alone, 415 putative GT genes have been identified to date on the basis of their gene sequence (<http://afmb.cnrs-mrs.fr/CAZY/>) and it seems likely that even more are present. For example, the activities of two closely related  $\alpha$ -xylosyltransferases that are not in CAZY have been confirmed (N Geshi, B Petersen, J Egelund, pers. comm.). Furthermore, a bioinformatic survey of GTs that are not in CAZY disclosed more than 30 accessions that have GT characteristics that are not weaker than those of the GTs already in CAZY (M Skjøt, P Ulvskov, pers. comm.). Most GTs have been classified into various gene families according to their topological protein-fold and the stereochemical outcome of the reaction that they catalyze [4]. The biological activity of only a few of these putative GTs has been demonstrated. Even fewer have been shown to be involved in wall polysaccharide synthesis or to affect wall structure. We summarize recent results in these areas in this review.

## Biosynthesis of cellulose and callose at the plasma membrane

Cellulose is the major load-bearing and omnipresent polysaccharide in the primary and secondary cell walls of plants. It consists of long parallel linear  $\beta$ -1,4-D-glucan chains that are assembled into crystalline microfibrils by hydrogen bonding (Figure 1). The amorphous  $\beta$ -1,3-D-glucan callose is less abundant than cellulose during normal plant growth, but this polysaccharide is present

Figure 1



Synthesis of cell wall polysaccharides. Glycosyltransferases (GTs) utilize nucleotide sugars as donor substrates to generate polysaccharides. The metabolism of the nucleotide sugars themselves is described in detail elsewhere (G Seifert, this issue). **(a)** Cellulose and callose are synthesized at the plasma membrane. Different CESA isoforms aggregate into higher-order rosettes (box in top left corner) to produce (1,4)- $\beta$ -glucan chains that coalesce into cellulose microfibrils. Proteins that are known or suspected to interact [10] with the CSC, and mechanisms that are known to be important for cellulose synthesis and CSC activity, are shown in the central text box. The proposed pathway of primer synthesis for cellulose synthesis [24], which starts with sitosterol and requires KOR endoglucanase activity, is also depicted. CALS and proteins reported to interact with it (text box; [10]) are schematized on the left. **(b)** By contrast, matrix polysaccharides are synthesized within the Golgi apparatus. **(c)** Type-II GTs, which consist of a transmembrane stem and a catalytic domain, play a major role in decorating polysaccharides with side-chains. **(d)** The synthesized matrix polysaccharides are secreted by exocytosis into the apoplast, where they form highly ordered networks with cellulose microfibrils.

in the cell plate of dividing cells, in pollen mother cell walls and tubes, and in the sieve plates of phloem elements. In addition, callose is deposited after plants are exposed to a range of abiotic and biotic stresses, including wounding or pathogen attack. The proteins that are thought to catalyze glucan-chain elongation in cellulose and callose synthesis are processive GTs that belong to the GT2 family (Table 1). In *Arabidopsis*, as many as ten or twelve GT2 family members form the cellulose synthase catalytic subunit (*CESA*) and callose synthase (*CALS/GLUCAN SYNTHASE-LIKE* [*GSL*]) gene families (<http://cellwall.stanford.edu>).

Substantial progress has been made in the recent past in understanding the *in vivo* synthesis of cellulose in plants, and lately, it has also become possible to synthesize sizable amounts of plant cellulose *in vitro* [5\*\*]. Most of the new concepts, such as the existence of at least two different catalytic subunits in a cellulose synthase complex (CSC), or the requirements for N-glycosylation and for sitosterol- $\beta$ -glucoside as a primer molecule for cellulose biosynthesis, have been summarized recently by Reiter [6]. In this review, we focus on additional concepts and new results. Continuing on from the model of at least two different catalytic subunits in a CSC [7–10], it has

Table 1

Glycosyltransferase and other genes involved in plant cell wall biosynthesis from *Arabidopsis thaliana* and other plants.

(a) Glycosyltransferases						
Species and gene <sup>a</sup>	GT family <sup>b</sup>	Activity	Mutant (mutagen <sup>c</sup> )	Mutant wall	Major phenotype(s)	Reference(s)
<i>AtCESA1</i>	2	n.d.	<i>rsw1</i> (EMS)	Reduced cellulose.	Swollen root, abnormal dark morphology, reduced growth of hypocotyls and roots ( <i>rsw1-10</i> ).	[6,15*]
			Antisense	Slightly reduced cellulose in leaves.	Stunted growth.	[14]
<i>AtCESA2</i>	2	n.d.	Antisense	n.d.	Reduced stem elongation at elevated temperature.	[14]
<i>AtCESA3</i>	2	n.d.	<i>ixr1</i> (sd, EMS)	Unaltered cellulose.	Semidominant resistance to isoxaben.	[8,34*]
			<i>cev1</i> (EMS)	Reduced cellulose.	Stunted root growth, induction of defense responses.	[33*]
			<i>eli1</i> (EMS)	Reduced cellulose, ectopic lignin.	Stunted root growth, induction of defense responses.	[34*]
			Antisense	Slightly reduced cellulose in leaves.	Stunted growth.	[14]
<i>AtCESA4</i>	2	n.d.	<i>ixr5</i> (Ds, EMS)	Reduction of secondary cellulose.	Irregular xylem vessels.	[11*]
<i>AtCESA6</i>	2	n.d.	<i>prc1</i> (T-DNA)	Reduced cellulose, incomplete cell walls.	Reduced growth of dark-grown hypocotyls and roots.	[6,13*]
<i>AtCESA7</i>	2	n.d.	<i>ixr2</i> (sd, EMS)	Unaltered cellulose.	Semidominant resistance to isoxaben.	[59]
			<i>ixr3</i> (EMS)	Reduction of secondary cellulose.	Collapsed xylem, weak stem.	[6,13*]
<i>AtCESA8</i>	2	n.d.	<i>fra5</i> (sd, EMS), <i>fra5</i> (OX)	Reduction of secondary cellulose.	Fragile fibers, weak stem, extremely thin fiber walls, collapsed xylem vessels.	[60*]
			<i>ixr1</i> (EMS)	Reduction of secondary cellulose.	Weak stem, collapsed xylem vessels.	[6,13*]
			<i>fra6</i> (EMS)	Reduction of secondary cellulose.	Reduced fiber wall thickness.	[60*]
<i>AtGSL5</i>	2	n.d.	<i>pmr4</i> (EMS, RNAi)	Lack of wound-induced callose.	Powdery mildew resistance, induction of defense responses.	[31**,32*]
<i>AtGSL6</i>	2	n.d.	n.d.	n.d.	Localizes to cell plates.	[29]
<i>AtCSLA7</i>	2	n.d.	(Ds)	n.d.	Embryo lethal, affects pollen growth.	[61]
<i>AtCSLA9</i>	2	n.d.	<i>rat4</i> (T-DNA)	Increase of Gal in older tissues.	Resistant to root transformation by <i>Agrobacterium</i> .	[62,63]
<i>AtCSLB6</i>	2	n.d.	(T-DNA)	Altered FTIR spectrum (cellulose region).	n.d.	[39]
<i>AtCSLD3</i>	2	n.d.	<i>kojak</i>	n.d.	Growth of root hairs is defective.	[64,65]
<i>TfManS</i>	2	Mannan mannosylIT (putative mannan synthase)	OX	Two-fold increase in mannan	No visible phenotype	[38**]
<i>AtFUT1</i>	37	Xyloglucan $\alpha$ -1,2 fucosylIT	<i>mur2</i> (EMS)	98% reduction of xyloglucan fucosylation,	Altered trichome papillae.	[40]
			<i>atfut1</i> (T-DNA)	OX	increase in xyloglucan O-acetylation.	No visible phenotype.
<i>PsFT1</i>	37	Xyloglucan $\alpha$ -1,2 fucosylIT	n.d.		No visible phenotype.	[43*]
<i>AtFUT3</i>	37	n.d. (not active on xyloglucan, AGPs)	OX	n.d.	Stunted growth, curly leaves, not able to set seed.	[41]
<i>AtFUT4</i>	37	n.d. (not active on xyloglucan, AGPs)	OX	33% increase in fucose, change in other MS.	No visible phenotype.	[41]
<i>AtFUT5</i>	37	n.d. (not active on xyloglucan, AGPs)	OX	33% increase in fucose, change in other MS.	No visible phenotype.	[41]
<i>AtMUR3</i>	47	Xyloglucan $\beta$ -1,2 galactosylIT	<i>mur3</i> (EMS)	Absence of fucogalactosyl-side-chain in xyloglucan.	Altered trichome papillae, reduced wall strength in hypocotyls.	[46**,47]

Table 1 Continued

<b>(a) Glycosyltransferases</b>						
Species and gene <sup>a</sup>	GT family <sup>b</sup>	Activity	Mutant (mutagen <sup>c</sup> )	Mutant wall	Major phenotype(s)	Reference(s)
<i>AtXT1</i>	34	Xyloglucan $\alpha$ -1,2 xylosylT	n.a.			[48**]
<i>TfGMGT</i>	34	Galactomannan $\alpha$ -1,6 galactosylT	OX	Higher substitution level of Gal for mannan.	n.d.	[49–51]
<i>AtQUA1</i>	8	n.d.	<i>qua</i> (T-DNA)	25% reduction in GalA.	Dwarfed growth, reduced cell adhesion.	[52*]
<i>NpGUT1</i>	47	n.d.	<i>nolac-H18</i> (T-DNA)	Absence of GlucoseA in RGII, altered RGII dimerization antisense.	Reduced cell adhesion, crumbled shoot behavior.	[53*]
<b>(b) Other proteins</b>						
<i>AtKOR</i>		1,4- $\beta$ -D-glucanase	<i>kor1</i> (T-DNA)	Increased pectin, reduced cellulose.	Cell elongation and cytokinesis defects, localizes to growing cell plate and plasmamembrane.	[6]
			<i>rsw2</i> (EMS) <i>irx2</i> (EMS) <i>acw1</i> (EMS)			
<i>AtKOB1</i>	n.d.		<i>kob1</i> (T-DNA, EMS)	Reduced cellulose, increased pectin, ectopic callose and lignin.	Dwarfism, sterility, short etiolated hypocotyls, incomplete cell walls.	[20*]
<i>AtCYT1</i>		Mannose-1-phosphate guanylyl-T	<i>cyt1</i>	Strongly reduced cellulose, accumulation of callose.	Cytokinesis defect, isotropic embryo growth.	[6]
<i>AtKNF</i>		$\alpha$ -glucosidase I	<i>knf</i> (EMS, FN), <i>gcs1</i> (T-DNA)	Strongly reduced cellulose.	Isotropic embryo growth, thin cell walls, cytokinesis defect.	[6]
<i>AtRSW3</i>		$\alpha$ -subunit of glucosidase II	<i>rsw3</i> (EMS), SGT5691 (Ds)	Reduced cellulose.	Swollen root, no mucilage, various morphological abnormalities.	[67]
<i>AtTBR1</i>		n.d.	<i>tbr1</i> (EMS)	Strongly reduced cellulose in trichomes.	No trichome birefringence, slightly reduced trichome density.	[21]

<sup>a</sup>Only genes that have been biochemically characterized or for which mutants have been identified are included. <sup>b</sup>Families classified according to <http://afmb.cnrs-mrs.fr/CAZY/>. <sup>c</sup>Alleles are derived from mutagenesis using ethylmethanesulfonate (EMS), fast neutron radiation (FN), insertional T-DNA (T-DNA), RNA interference (RNAi), an insertional transposable element (Ds) or constitutive overexpression (OX), or are semidominant alleles (sd). Abbreviations: *acw1*, altered cell wall1; *At*, *Arabidopsis thaliana*; *cev1*, constitutive expression of *VSP1*; *cyt1*, cytokinesis defective1; *eli1*, ectopic lignin1; *fra5*, fragile fiber5; *FT1*, FUCOSYLTRANSFERASE1; *Fuc*, fucose; *Gal*, galactose; *GalA*, galacturonic acid; *gcs1*, glucosidase1; *GMGT*, galactomannan galactosyltransferase; *irx1*, isoxaben-resistant1; *knf*, *knopf*; *Man*, mannose; *MS*, monosaccharides; n.d., not demonstrated; *Np*, *Nicotiana plumbaginifolia*; *prc1*, *procuste1*; *Ps*, *Pisum sativum*; *rat4*, resistant to *Agrobacterium transformation4*; *rsw*, radially swollen; *T*, transferase; *Tf*, *Trigonella foenum-graecum*.

now been shown that a third catalytic subunit is required in the secondary CSC of *Arabidopsis* and rice [11\*,12]. In a screen for additional cellulose-deficient mutants, Taylor *et al.* [11\*] identified a novel complementation group, *irregular xylem5* (*irx5*). *IRX5* was cloned and shown to encode *AtCESA4*. The phenotype of *irx5* plants is identical to that caused by mutations in two other members of the *CESA* gene family (*IRX1* [i.e. *AtCESA8*] and *IRX3* [i.e. *AtCESA7*]). *IRX5*, *IRX3* and *IRX1* are coexpressed in exactly the same cells, and all three proteins interact in detergent-solubilized extracts. The association of *IRX1* and *IRX3* is reduced to undetectable levels in the absence of *IRX5*. The data suggest that *IRX5*, *IRX3* and *IRX1* are all essential components of the CSC, and that the presence of all three subunits is required for correct complex assembly.

Although the biological functions of six *AtCESA* genes (i.e. *AtCESA1*, *AtCESA3*, *AtCESA4*, *AtCESA6*, *AtCESA7* and *AtCESA8*) have been elucidated to some extent

during recent years using classical genetic approaches [6,13\*], the functions of the remaining four *Arabidopsis CESA* genes (i.e. *AtCESA2*, *AtCESA5*, *AtCESA9* and *AtCESA10*) are less clear. It seems that loss of the function of one of these four genes, in contrast to the other six, yields minimal or no phenotypes, implying functional redundancy. For example, none of the T-DNA insertion mutants for *CESA2*, *CESA5*, *CESA9* or *CESA10* has a convincingly altered visible phenotype in ambient conditions. Neither do these mutants have reduced growth of etiolated hypocotyls nor a cellulose reduction or changed cell wall sugar composition in major plant tissues (A Münster, M Pauly, W-R Scheible, unpublished). A minor phenotype reported for strong *CESA2-antisense* lines was a small reduction in stem elongation rate, but not final plant height, at elevated temperature [14]. Expression studies of the *CESA* genes facilitate functional studies using loss-of-function mutants because only genes that are expressed at the same stage or in the same tissue need to be examined for potentially redundant functions.

Using *AtCESA*-promoter:: $\beta$ -glucuronidase (GUS) fusions, *CESA2* and *CESA5*, like *CESA1*, *CESA3* and *CESA6*, were shown to be expressed at the sites of primary wall synthesis. When *CESA9* and *CESA10* promoters were used in GUS fusions, GUS staining was observed in the petioles of rosette leaves ([13<sup>\*</sup>]; A Kochevenko, W-R Scheible, unpublished). *CESA9* is also expressed in a region proximal to the shoot apical meristem during vegetative growth and in flowers (A Kochevenko, W-R Scheible, unpublished). Beeckman *et al.* [15<sup>\*</sup>] used semi-quantitative reverse transcription (RT)-PCR to investigate the expression of all ten *AtCESA* genes in young seedlings, in the stems and flowers of three-week old plants, and in developing embryos. All ten genes were expressed in the three post-embryonic tissues. Only *CESA1*, *CESA2*, *CESA3* and *CESA9* are significantly expressed in developing *Arabidopsis* embryos at the heart and torpedo stages, however, and the expression of these genes declines towards the bent-cotyledon stage [15<sup>\*</sup>]. As the *CESA2* and *CESA9* proteins share extensive sequence similarity, it will be interesting to investigate the effects of a *CESA2 CESA9* double knockout on early embryo development, as well as on stem elongation rate and plant development in general.

Plant CESA proteins possess a conserved region near their amino-terminus that contains two putative zinc fingers that have high homology to the RING-finger motif. Kurek *et al.* [16<sup>\*\*</sup>] showed that the cotton protein GhCESA1 can bind two zinc atoms, and yeast two-hybrid analysis indicates that GhCESA1 and GhCESA2 interact via this domain to form homo- and heterodimers. Biochemical analyses show that this dimerization occurs under oxidative conditions via intermolecular disulfide bonds in the zinc-finger domain. In the same yeast two-hybrid screen, a thioredoxin clone and a metallothionein clone were also found to interact with the zinc-binding domain of GhCESA1 [10], suggesting that these proteins might be involved in the redox modulation of cellulose synthase. Kurek *et al.* [16<sup>\*\*</sup>] also showed that a herbicide that inhibits the synthesis of crystalline cellulose and leads to a disruption of rosette architecture affects the oxidative state of the zinc-finger domain, which is necessary for rosette stability. Taken together, the results support a model in which at least part of the processes of rosette assembly and function may involve oxidative dimerization between CESA subunits. An oxidative environment should therefore favor cellulose synthesis. This concept correlates with the observed occurrence of a strong increase in hydrogen peroxide production during the formation of secondary cell wall in cotton fibers [17]. Starch synthesis, another bulk-carbon metabolic pathway in plants, is also regulated by redox-modulated activation/inactivation of the catalytic subunit (AGPB) of ADP-glucose pyrophosphorylase (AGP) [18<sup>\*</sup>,19<sup>\*</sup>]. In the case of AGPase, however, oxidative conditions and the dimerization of AGPB lead to an inactivation of the enzyme,

suggesting that carbon partitioning between growth (cellulose production) and storage (starch formation) can be mediated by redox regulation.

Besides the catalytic subunits of cellulose synthase, other proteins are required for cellulose synthesis in plants. The  $\beta$ -1,4-endocellulase KORRIGAN (KOR; see [6]) has been clearly linked with cellulose synthesis. Various other proteins — including sucrose synthase, cytoskeletal components such as microtubules and actin, redox proteins (see above), Rac13, and a lipid-transfer protein — have also been implicated in cellulose synthesis or its regulation (see [10]; Figure 1). Recently, Höfte and co-workers [20<sup>\*</sup>] identified a novel protein named KOBITO1 (KOB1). They suggested that this protein might be part of the cellulose synthesis machinery because it localizes to the plasma membrane and is necessary for the normal synthesis and deposition of cellulose microfibrils during rapid cell expansion in *Arabidopsis*. KOB1 has two homologs in the *Arabidopsis* genome and is plant specific. The *TRICHOME BIREFRINGENCE1 (TBR1)* gene, which is essential for secondary cellulose production in *Arabidopsis* trichomes [21], encodes another novel plant-specific protein with predicted plasma-membrane localization (A-S Nita, R Eshet, D Delmer, W-R Scheible; unpublished). *TBR1* has several close homologs of unknown function in the *Arabidopsis* genome. Ongoing and future research will show whether, when and where these proteins are required for cell wall synthesis and which proteins they interact with.

Lipid-linked cellodextrins participate in cellulose synthesis in *Agrobacterium* [22], and sitosterol- $\beta$ -glucosides were recently proposed to act as primer molecules for cellulose synthesis in plants [23<sup>\*\*</sup>,24]. In *Arabidopsis*, however, the results of some recent experiments question this concept. Sitosterol- $\beta$ -glucoside is produced from UDP-glucose and sitosterol by the enzyme UDP-glucose:sterol glycosyltransferase (SG), which is encoded by two genes in *Arabidopsis*. *Sg1 sg2* T-DNA-insertion double mutants exhibit a greater than 30-fold reduction in sitosterol- $\beta$ -glucoside levels (W-R Scheible, H Schaller, C Somerville, unpublished) but are not noticeably affected in plant growth and stature, cellulose content or cell wall composition (A Münster, W-R Scheible, unpublished). In *Arabidopsis* sterol synthesis mutants, such as *fackel*, *hydra1* or *dwarf1*, sitosterol levels are reduced to extremely low or undetectable levels, but cellulose levels are reduced by only 30–40% (in *fackel* and *hydra1*) or not at all (in *dwarf1*) [25<sup>\*</sup>]. These results suggest that sitosterol- $\beta$ -glucosides do not limit or even are dispensable for cellulose synthesis in *Arabidopsis*. It is possible that alternative primer molecules are sufficiently abundant to initiate cellulose synthesis in *Arabidopsis* when sitosterol and sitosterol- $\beta$ -glucosides are strongly decreased or absent. Nevertheless, the presence of embryonic growth defects, cell wall gaps, reduced cellulose contents and ectopic callose and

lignin deposition in sterol biosynthesis mutants such as *fackel* and *hydra1* [25<sup>•</sup>] indicate that sterols and/or sterol-glucosides may well contribute to cellulose and cell wall synthesis. Their contribution might, however, occur via another mechanism, such as the provision of a special plasma-membrane-lipid environment to the CSC. It is also conceivable that some sterols act as signalling molecules to control the expression of cell wall synthesis genes. In this context, it is intriguing that the HOME-ODOMAIN-ZIPPER (HD-ZIP) transcription factor gene *INTERFASCICULAR FIBERLESS1/REVOLUTA(FL1/REV)*, which governs lateral meristem initiation and interfascicular fiber differentiation in *Arabidopsis*, contains a sterol/lipid-binding START domain [26,27].

Important progress has also been made in the identification and functional characterization of genes that encode callose synthases. A novel plant gene, *cotton FKS1-like1 (CFL1)*, whose product has homology to the putative catalytic subunit of the yeast  $\beta$ -1,3-glucan synthase (FKS1), has been isolated from cotton [28]. The CFL1 protein localizes to *in-vitro*-synthesized callose pellets, suggesting that CFL1 is involved in callose synthesis. Hong *et al.* [29] characterized one of the *Arabidopsis* *CALS/GSL* genes, demonstrating the localization of its protein product to the growing cell plate, the interaction of this protein with phragmoplastin, and enhanced callose synthase activity in transgenic tobacco cells in which this *CALS/GSL* was expressed. Doblin *et al.* [30] identified two putative *CALS/GSL* full-length cDNAs in *Nicotiana glauca* pollen tubes, and proposed that they encode the catalytic subunits of  $\beta$ -glucan synthases that are involved in wall synthesis in pollen tubes. More recently, it was shown that the *Arabidopsis* *CALS12/GSL5* gene is indeed responsible for the production of papillary and wound-induced callose in response to biotic and abiotic stresses, and that the other eleven *Arabidopsis* callose synthases cannot compensate for the loss of this gene function [31<sup>••</sup>,32<sup>•</sup>]. The deposition of callose is thought to reinforce the cell wall after pathogen attack and is regarded as a defense response. It is surprising, therefore, that *Arabidopsis powdery mildew resistant4 (pmr4)* mutants [31<sup>••</sup>], which lack pathogen-induced callose, are more resistant than wildtype plants to pathogens rather than more susceptible. This resistance can be explained, however, by the activation of the salicylic acid defense pathway in *pmr4* mutants. It is interesting that mutations in the cellulose synthase gene *AtCESA3* also lead to the activation of stress defense pathways [33<sup>•</sup>,34<sup>•</sup>]. The cell wall and its components apparently give feedback to the plant by triggering biotic/abiotic stress responses [35].

### Biosynthesis of the matrix polysaccharides in the secretory pathway

Production of the matrix polysaccharides, such as the crosslinking hemicelluloses and pectic polysaccharides,

as well as the glycosylation of structural proteins, such as extensins or AGPs, is thought to occur in the secretory pathway of the cell, that is, the endoplasmic reticulum and the Golgi apparatus (Figure 1). Some of the putative processive GTs (i.e. glycan synthases) that are localized in these organelles are encoded by the cellulose-synthase-like (*CSL*) genes, which were named according to their sequence homology to *CESA* genes [36,37]. The *CSL* genes found in *Arabidopsis* and rice have been subdivided into eight groups (<http://cellwall.stanford.edu>) on the basis of their gene sequence similarity. Depending on the number of amino-terminal transmembrane domains, the proposed catalytic site of some CSLs might face the cytosol, whereas that of others might face the lumen of the Golgi apparatus (Figure 1; [13<sup>•</sup>]). A *CSL*-gene termed mannan synthase (*ManS*) was identified in an endosperm expressed sequence tag (EST) collection from guar [38<sup>••</sup>]. Upon expression of *ManS* in soybean somatic embryos, microsomal fractions were able to transfer mannose from GDP-mannose to a high molecular weight beta-mannanase degradable product (i.e. ManS) [38<sup>••</sup>]. These data demonstrate that *CSL* genes might indeed be involved in the synthesis of  $\beta$ -glycans other than cellulose in the Golgi apparatus, as hypothesized earlier [39]. In addition, plants with a loss-of-function mutation in some of the *CSL* genes have a developmental phenotype (Table 1).

Numerous advances have been made in understanding the molecular components necessary for the synthesis of xyloglucan, the major hemicellulose present in the primary walls of non-graminaceous plants. Xyloglucans consist of a  $\beta$ -1,4-linked glucose backbone, which is substituted between 50–75% by  $\alpha$ -1,2-linked xylosyl residues. In most plant species, these xylosyl residues are further substituted by  $\beta$ -1,2-linked D-galactosyl residues at specific positions, which in turn can be further substituted by  $\alpha$ -1,2-linked fucosyl-residues. The first enzyme identified as being involved in the synthesis of xyloglucan was a fucosyltransferase ([40]; see recent review by Reiter [6]). This enzyme is predicted to be a Golgi-localized type-II membrane protein that consists of a hydrophobic amino-terminal domain, which is thought to be involved in anchoring the protein in the membrane, and a globular carboxy-terminal domain, which represents the catalytic domain (Figure 1). *A. thaliana* *FUCOSYL-TRANSFERASE1 (AtFUT1)* is a member of a gene family that contains another nine members in *Arabidopsis* (Table 1). To date, none of these enzymes has shown an ability to fucosylate xyloglucan when expressed heterologously, suggesting that these enzymes are involved in the fucosylation of other polysaccharides or are responsible for a different GT activity ([41]; Table 1). The *mur2* lesion in the *AtFUT1* gene results in a drastic decrease (by 98%) in the fucosylated xyloglucan present in walls [42<sup>••</sup>]. A T-DNA-insertional mutant of *AtFUT1* also has essentially no fucosylated xyloglucan in several tissues, but

confirmed the lack of a visible *mur2* phenotype [43<sup>•</sup>]. Interestingly, the overexpression of *AtFUT1* did not increase the fucosylation of xyloglucan, but increased the xyloglucan *O*-acetylation of the galactosyl residue by approximately 50% [43<sup>•</sup>]. This is consistent with the virtual absence of *O*-acetylation when fucosylation but not galactosylation is reduced, as in the *mur1*, *atfut1* and *mur2* mutants [43<sup>•</sup>,44], or when both fucosylation and galactosylation are reduced, as in the *mur3* mutant ([45]; see below). In turn, this suggests an interdependence between the fucosylation and *O*-acetylation of galactosyl residues, which is mediated by their respective transferases. Together, the results described above suggest that xyloglucan fucosylation is carried out by a single gene in *Arabidopsis*. Furthermore, it seems that the action of this fucosyltransferase is not rate limiting because several tissues that contain nearly wildtype levels of fucosylated xyloglucan express *AtFUT1* at a very low level [43<sup>•</sup>]. Despite the drastic change in the xyloglucan structures of *atfut1* or *mur2* mutants, these mutants have no observable changes in plant growth and development or in wall strength (Table 1). Structural changes such as xyloglucan fucosylation could, therefore, be a remnant of evolution or could confer a selective advantage under growth conditions that have not yet been identified.

A galactosyltransferase that is involved in the synthesis of xyloglucan has been identified through the map-based cloning of the *mur3* locus [46<sup>••</sup>]. Heterologous expression of MUR3 revealed that this enzyme only transfers a galactosyl residue to a specific xylosyl residue, illustrating the narrow acceptor substrate specificity of such GT activities. As a result, the xyloglucans of *mur3* plants lack the entire fucosylgalactosyl side-chain, which is compensated for by an increased abundance of galactosyl residues at an alternative xylosyl residue. Testing of the tensile parameters of tissues indicated a significant reduction in wall strength in etiolated *mur3* hypocotyls, suggesting that the galactosylated but not the fucosylated side-chains of xyloglucan have a mechanical role [47]. An *Arabidopsis* xylosyltransferase (*AtXT1*) that adds a xylosyl residue to cello-oligosaccharides has also been identified in an acceptor-depending assay ([48<sup>••</sup>]; Table 1). MUR3 activity only adds the xylosyl residue to a specific position, indicating that at least one if not two more xylosyltransferases are necessary to generate the xyloglucan structure present in *Arabidopsis*. Seven additional *AtXT1* homologs have been identified in the *Arabidopsis* genome, but heterologous expression of six of these revealed that none acts as a xylosyltransferase on cello-oligosaccharides [48<sup>••</sup>] and thus their function still remains unknown. The glucan synthases that are necessary to assemble the xyloglucan backbone have not yet been identified.

Another biosynthetic enzyme that has been identified is a GT from fenugreek that is specific to galactomannan

biosynthesis [49]. Analysis of the *in vitro* enzyme products revealed that this GT recognizes six units of the mannan backbone and transfers a single galactosyl residue to a specific position on the mannan backbone [50]. Constitutive expression of the enzyme in tobacco resulted in an endosperm mannan with a higher degree of galactosylation [51]. Experiments like these highlight the potential biotechnological applications of wall-biosynthetic genes.

Recently, GTs that could be involved in the synthesis of the pectic polysaccharide(s) were identified for the first time. One *Arabidopsis* mutant, *quasimodo* (*qua*), that had a T-DNA insertion in a putative GT had a dwarfed growth phenotype and a cell-adhesion defect ([52<sup>•</sup>]; Table 1). Analysis of the cell wall material of this mutant revealed only a reduction in galacturonic acid (of 25%); thus, it has been suggested that *QUA* encodes a galacturonosyltransferase. However, biochemical proof of such an activity is lacking. Another gene that is involved in pectin biosynthesis (termed *nolac-H18* for non-organogenic callus with loosely attached cells) was discovered in a T-DNA-insertion mutant from a haploid line of *Nicotiana plumbaginifolia* [53<sup>•</sup>]. The callus of this mutant exhibited decreased cellular adhesion. The cell walls of *nolac-H18* showed a drastic decrease in arabinose and glucuronic acid. The purification and analysis of rhamnogalacturonan II (RGII), one of the pectic polysaccharides, revealed that the *nolac-H18* mutant lacked a terminal galactose-glucuronic acid side-chain. As a result of the altered RG II structure, the borate dimerization of RGII was significantly reduced, which could be responsible for the reduced-cell-adhesion phenotype. The specific structural change in RGII suggests that the affected gene, *NpGUT1*, might encode a  $\beta$ -glucuronosyltransferase that is involved in RGII synthesis. However, this specific transferase activity remains to be proven.

## Conclusions and future perspectives

The fully sequenced genomes of *Arabidopsis* and rice and the availability of numerous bioinformatics tools have inundated scientists with possible gene candidates that encode putative GTs and other important components of the biosynthetic machinery for wall polysaccharides. Unfortunately, even the clustering or classification of GTs into families does not enable us to predict the precise donor, acceptor and product specificity of each putative enzyme [4]. As a result, the activities of just six GTs (Table 1) from the hundreds of gene candidates have been demonstrated unambiguously by biochemical assays. In addition, another 18 genes that are likely to play a direct role in the biosynthesis of wall polysaccharides have been identified (Table 1), mainly through the availability of mutants.

At present, the major bottleneck in confirming that candidate genes are indeed involved in wall biosynthesis is

the ability to demonstrate the biochemical function of the gene products. Removing this bottleneck will require the availability of assays that include the appropriate donor- and acceptor substrate, and in which no loss of enzymatic activity occurs upon protein extraction or expression. Another instructive approach examines the wall structures of mutants obtained by reverse-genetic tools (see [54] for review). The change in wall polysaccharide structure produced by the mutation of a candidate GT gene might, however, be minor due to polysaccharide abundance or tissue specificity. Hence, this approach necessitates rather detailed analyses of the wall, which are usually labor intensive and time consuming.

Several technical advances to accelerate the analysis of wall structure have been made recently (see [35] for review). One promising technique includes the use of Fourier-transformed infrared spectroscopy (FTIR) of whole plant tissues [55]. Using this technique, it has been possible to cluster mutants into groups that exhibit, for example, cellulose defects or to rank mutants according to the strength of their wall defects. The use of FTIR on wall preparations also seems to be a promising technique for the rapid screening of potential wall mutants [56]. A second way to assess the structure of a wall rapidly is to use oligosaccharide profiling. Here, wall material is treated with enzymes or chemicals that solubilize a specific polysaccharide and generate characteristic oligosaccharide fragments. Analysis of the fragments using mass spectrometry [45], or in a more quantitative but more labor-intensive fashion using derivatization with fluorescent tags followed by gel electrophoresis [57], gives detailed clues as to the structural alteration. Miniaturizing the profiling method to facilitate the use of laser-catapulting dissection permits the oligosaccharide profiling of walls from as few as 500 cells by mass spectrometry (N Obel, M Pauly, unpublished). Another potentially powerful approach to assess small amounts of wall polysaccharides is the use of the recently introduced glycan arrays [58]. Up to 10 000 glycan samples can be rapidly and reproducibly assayed, for example, with polysaccharide-specific antibodies with a detection limit of an approximately 80 fg/sample. Methods such as these will not only be vital in our quest to accelerate progress in revealing the enzymes that are involved in wall biosynthesis, but will also provide important insights into the functions of various wall polysaccharides and their substructures in plant growth and development.

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