

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	<i>Arabidopsis thaliana</i> FUCOSYLTRANSFERASE1
AtXT1	<i>Arabidopsis thaliana</i> XYLOSYLTRANSFERASE1
CESA1	CELLULOSE SYNTHASE1
CSL	CELLULOSE SYNTHASE-LIKE
<i>cyt1</i>	<i>cytokinesis1</i>
<i>IRX2</i>	<i>IRREGULAR XYLEM2</i>
KOR	KORRIGAN
<i>mur2</i>	<i>murus2</i>
RG-I	rhamnogalacturonan-I
<i>rsw1</i>	<i>root swelling1</i>
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
<i>CESA1</i>	<i>rsw1</i>	Root swelling, stunted growth (<i>rsw1-1</i>), seedling lethality (<i>rsw1-2</i>)	[17,32**]
<i>CESA3</i>	<i>ixr1</i>	Resistance to isoxaben, stunted growth (antisense plants)	[11**,18]
<i>CESA4</i>	<i>irx5</i>	Irregular structure of xylem elements	[37],a
<i>CESA6</i>	<i>ixr2</i>	Resistance to isoxaben	[19*]
	<i>prc1</i>	Reduced length of roots and hypocotyls	[20]
<i>CESA7</i>	<i>irx3</i>	Irregular structure of xylem elements	[21]
<i>CESA8</i>	<i>irx1</i>	Irregular structure of xylem elements	[22]
<i>CSLA9</i>	<i>rat4</i>	Resistance to root transformation by <i>Agrobacterium</i>	[37]
<i>CSLD3</i>	<i>kojak</i>	Short and defective root hairs	[39*,40]

^aS Turner, personal communication. Abbreviations: *prc1*, *procuste1*; *rat4*, resistant to transformation by *Agrobacterium tumefaciens* 4.

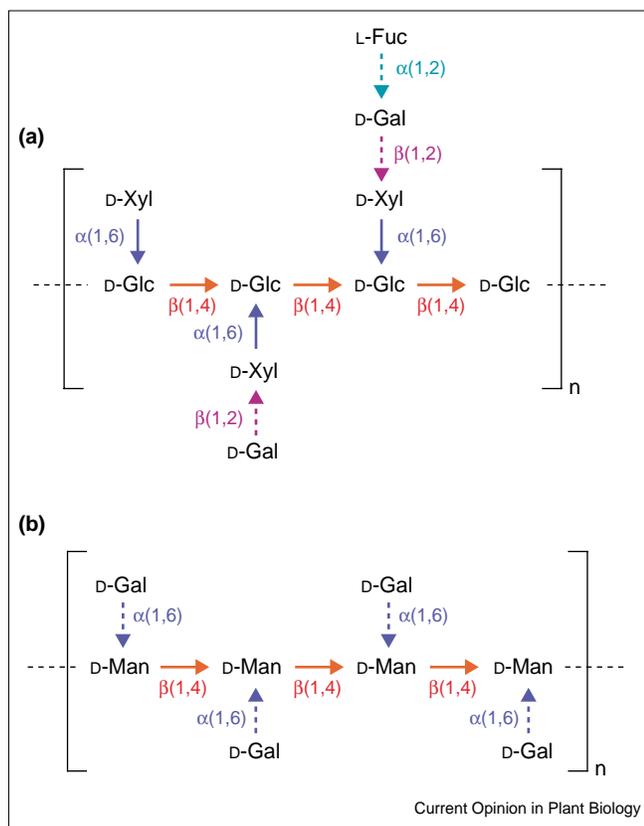
The *Arabidopsis* genome harbors ten members of this gene family (*CELLULOSE SYNTHASE1 [CESA1]* through *CESA10*), all of which contain eight transmembrane domains, a D,D,D,QxxRW motif that is believed to be part of the active site, and a putative zinc-binding domain that may mediate protein–protein 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 (cyl1)* 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 \bullet], 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 $\bullet\bullet$], and the *knopf* and *gcs1* mutants, which have defective α -glucosidase I [32 $\bullet\bullet$,33,34]). CESA proteins do not appear to be glycosylated *in vivo* [32 $\bullet\bullet$], 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 $\bullet\bullet$] 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-celotriose (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 $\bullet\bullet$] 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 \bullet] 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 CSLE 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 \bullet ,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 \bullet] 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

most of the *CSL* genes in *Arabidopsis*. This procedure detects changes in the composition and structure of cell wall polysaccharides, and can be applied to the walls of single cells. Although the spectra are usually difficult to interpret, they provide useful information on which cell types are affected by specific mutations, and offer some guidance as to which polysaccharides should be studied in detail using standard methods of carbohydrate chemistry. As an example of the promise of this method, Bonetta *et al.* [37] found a significant change in the cellulosic region of the infrared spectrum in a mutant that had an insertion in *CSLB6*.

The biosynthesis and function of xyloglucan

Xyloglucan is the major hemicellulose in the primary walls of most higher plants, and typically consists of a 1,4- β -D-glucan backbone carrying 1,6- α -D-xylose moieties on three consecutive glucose residues (Figure 1a). The second and third xylose residues within each 'XXXG' building block may carry D-galactose in β -1,2-linkage, and the second of these galactose residues is usually substituted by L-fucose in α -1,2-linkage. The gene that encodes xyloglucan fucosyltransferase from *Arabidopsis* (AtFUT1) was cloned in 1999 [41] and found to be a member of a multi-gene family that has ten members [42]. AtFUT1 is expressed at similar levels in all plant organs, whereas the other putative fucosyltransferases (AtFUT2 through AtFUT10) displayed more complex expression patterns. Most of them showed a low abundance of mRNA in reverse transcriptase polymerase chain reaction (RT-PCR) experiments [42]. These results suggest that the putative proteins encode organ-specific xyloglucan fucosyltransferases or glycosyltransferases that are involved in the fucosylation of other polysaccharides.

Arabidopsis contains fucose residues in xyloglucan, rhamnogalacturonan-I (RG-I), RG-II, arabinogalactan proteins, and N-linked glycans [43,44]. RG-II contains fucose in two different positions, and has recently been found to harbor an L-galactosyl residue that may be attached by a fucosyltransferase-like protein because the two monosaccharides are structurally related (L-fucose is 6-deoxy-L-galactose). As the N-linked glycan fucosyltransferase is not a member of the *AtFUT* gene family [45], at least five fucosyltransferases are unaccounted for and may be encoded by specific *AtFUT* genes.

Recent work by Vanzin *et al.* [46••] has shown that the *murus2* (*mur2*) mutation [47] is a single nucleotide change within the *AtFUT1* gene. This change causes loss of enzymatic activity because it leads to an amino-acid substitution close to the carboxy-terminus of the protein. *mur2* plants lack fucosylated xyloglucan in all major organs, which makes it unlikely that any of the other *AtFUT* genes encodes a xyloglucan fucosyltransferase. The mutant plants do not show any changes in their growth habit or physiology, raising the question of why xyloglucan fucosylation has persisted during evolution. Fucosylated xyloglucan is believed to serve as the source of signal molecules that control auxin-mediated expansion growth [48],

which is difficult to reconcile with the normal growth habit of *mur2* plants. On the basis of computer simulations and *in vitro* binding studies, xyloglucan fucosylation has been proposed to facilitate xyloglucan-cellulose interactions by favoring a planar conformation of the xyloglucan backbone [49]. Nonetheless, *mur2* plants do not show any obvious weakening of their primary cell wall as determined by break-force measurements, suggesting that the mutant plants can assemble a reasonably stable xyloglucan-cellulose network.

Although the genetics and biochemistry of xyloglucan fucosylation are reasonably well understood, the complexities of β -D-glucan synthesis, xylosylation and galactosylation remain to be determined. A xyloglucan galactosyltransferase from *Arabidopsis* has recently been cloned and shown to be a member of a multi-gene family (N Carpita, W-D Reiter, unpublished data). In an effort to identify xyloglucan xylosyltransferase genes in *Arabidopsis*, Faik *et al.* [50••] exploited the observation that the *Arabidopsis* genome contains seven coding regions with a high degree of sequence similarity to galactomannan galactosyltransferase from fenugreek [51], but does not contain the seed storage galactomannans found in this legume and related species. The fenugreek enzyme attaches D-galactose residues in α -1,6-linkage to a β -1,4-D-mannan backbone, whereas xyloglucan xylosyltransferase attaches D-xylose residues in α -1,6-linkage to a β -1,4-D-glucan backbone (Figure 1). These similarities in donor and acceptor substrates, together with the identical α -1,6-linkage patterns, prompted Faik *et al.* [50••] to express six of the *Arabidopsis* coding regions in the methylotropic yeast *Pichia pastoris*, and to analyze the recombinant proteins for xyloglucan xylosyltransferase activities. One of the six proteins catalyzed the conversion of cellopentaose to the xylosylated derivative GXGGG, with the xylose residue attached in α -1,6-linkage. This protein was termed *Arabidopsis thaliana* XYLOSYLTRANSFERASE1 (AtXT1). A similar enzymatic activity was identified in solubilized microsomal fractions from rapidly growing pea seedlings, which are a rich source of xyloglucan biosynthetic enzymes. On the basis of these results Faik *et al.* [50••] concluded that AtXT1 represents a *bona fide* xylosyltransferase that acts on the β -1,4-D-glucan backbone of xyloglucan. The function of the AtXT1 homologs within the *Arabidopsis* genome remains to be determined but it appears likely that at least some of these proteins play a role in xyloglucan xylosylation.

The roles of cross-linking glycans in wall strength and expansion growth

Plant cell walls contain a large number of cross-links between polysaccharides that provide mechanical strength while permitting rearrangements during expansion growth. The cellulose-xyloglucan network is generally considered the major load-bearing element within the primary wall, with additional contributions from Ca^{2+} cross-links between homogalacturonans [1]. It has recently been shown that the extremely complex pectic component rhamnogalacturonan-II is cross-linked via borate, which

forms a tetra-ester between apiose residues of two different RG-II molecules [52]. The recent characterization of two cell wall mutants of *Arabidopsis* (i.e. *mur1* and *mur2*) has provided interesting insights into the significance of this cross-link.

The *mur1* mutation leads to the absence of L-fucose from all shoot organs because of a defect in the *de novo* synthesis of GDP-L-fucose [53,54]. The mutant plants are slightly dwarfed and show a two-fold decrease in the strength of elongating inflorescence stems [53], which was previously thought to be caused by an absence of xyloglucan fucosylation. Characterization of the xyloglucan fucosyltransferase mutant *mur2* [46••] indicated that the structural alterations in *mur1* xyloglucan could not be responsible for the phenotypes observed in *Arabidopsis mur1* because *mur2* plants lack fucosylated xyloglucan but have a normal growth habit and wall strength. In a parallel development, O'Neill *et al.* [55••] demonstrated that borate cross-linking between RG-II molecules was significantly reduced in *mur1* plants, presumably because of the replacement of two L-fucose residues by the structurally similar monosaccharide L-galactose. These structural alterations are relatively minor and do not directly affect the apiose residues that are involved in the formation of cross-links. This suggests that RG-II must adopt a highly specific conformation for efficient borate cross-linking to occur. Growth of *mur1* plants in the presence of millimolar concentrations of borate rescues their visible phenotype [55••], presumably by restoring RG-II cross-linking via a mass-action mechanism. It remains to be seen whether the wall strength phenotype of *mur1* plants can similarly be rescued by high concentrations of borate in the growth medium.

Conclusions and future directions

Substantial progress has been made during the past two years in the genetic dissection of cellulose synthesis using *Arabidopsis thaliana* as a model system. Mutants in six out of the ten *Arabidopsis* cellulose synthase isoforms have been isolated via forward genetic approaches, suggesting that there is little functional redundancy within the *CESA* gene family. This can be explained at least partly by the presence of more than one *CESA* isoform within cellulose synthase complexes, and the involvement of distinct *CESA* gene products in the synthesis of primary and secondary cell walls. A role for the KOR endoglucanase in cellulose synthesis has been established by several groups, suggesting that cellulose formation in higher plants is mechanistically similar to that in *Agrobacterium*. Biochemical data suggest a role for lipid-linked primers in cellulose synthesis but direct genetic evidence for this is not yet available. Considerable progress has also been made in identifying glycosyltransferases that are involved in the synthesis of xyloglucan. The major challenge now is to identify enzymes that are involved in the formation of the 1,4- β -D-glucan backbone of this polysaccharide.

The progress in identifying cell wall biosynthetic enzymes has been driven primarily by biochemical and forward

genetic approaches. This suggests that the purification of glycosyltransferases and the characterization of cell wall mutants will continue to make significant contributions toward an understanding of cell wall synthesis. Many mutants with changes in cell shapes and sizes are likely to show defects in cell wall formation, providing an opportunity to identify cell-wall-related genes. Where mutations in cell wall biosynthetic enzymes do not lead to obvious visible phenotypes, biophysical procedures such as Fourier-transform infrared spectroscopy [37,56] can be used to identify potential mutants that are altered in cell wall composition and/or ultrastructure.

Current sequence information indicates that virtually all of the coding regions that are involved in substrate generation [57] and glycosyl transfer reactions [6] appear to be members of multi-gene families. This does not necessarily reflect genetic redundancy but may be a consequence of the unusual diversity and complexity of plant cell wall polysaccharides. It may not be possible to identify mutations in many of these genes in forward genetic screens for plants with easily recognizable changes in morphology. However, it is now possible to identify insertion mutants in virtually any *Arabidopsis* gene by accessing databases that list lines with known gene disruptions (for example, see <http://signal.salk.edu/cgi-bin/tdnaexpress>). These lines can then be subjected to detailed phenotypic analyses, including the structural analysis of carbohydrates. Despite recent progress in predicting the likely functions of many gene products, bioinformatics approaches alone will not be sufficient to identify all of the coding regions that are involved in cell wall biogenesis. Accordingly, a combination of biochemical and forward and reverse genetic approaches is probably the best strategy to unravel the complexities of plant cell wall formation.

Acknowledgements

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