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Biosynthesis of plant cell walls

Michael Handford[1]
Departamento de Biología, Facultad de Ciencias, Universidad de Chile,
Las Palmeras 3425, Ñuñoa, Santiago, Chile.

Abstract

M. Handford. 2006. Biosynthesis of plant cell walls. Cien. Inv. Agr. 33(3):179-196. The cell wall plays a major role in determining the expansion, growth and shape of plant cells, and thus of the plant itself. Additionally, cell wall breakdown products are used as developmental signals. Moreover, the cell wall is in the front line of defence, forming a significant barrier to penetration by pathogens and pathogen vectors. There is great diversity in the polysaccharides of the cell wall which is composed of cellulose microfibrils, with the matrix polysaccharides, pectins and hemicelluloses binding non-covalently to the cellulose molecules, crosslinking them in an extensive network. Agronomically, a variety of industries are reliant on cell wall polysaccharides, particularly in the processing of foods. However, surprisingly little is known about their precise role(s) *in planta*. This is even more apparent considering that the polysaccharide composition of different cell types and organs differs, and changes over time, suggesting that the polysaccharides have a variety of specific roles. With the completion of genome sequencing projects in model and commercially-important plant species, significant progress has been made in elucidating the proteins responsible for cell wall synthesis, leading to potential biotechnological applications.

Key words: *Arabidopsis thaliana*, cell wall, cellulose, Golgi apparatus, nucleotide-sugar, transporter.

Introduction

All plant cells are surrounded by an extracellular matrix termed the cell wall. The principal component of the cell wall is cellulose, although frequently a variety of other polysaccharides (hemicelluloses and pectins), proteins and phenolic compounds are also present. During the growth and development of the plant, the cell wall is deposited as a series of layers. Firstly, the middle lamella between adjacent cells is laid down, followed by the deposition of the primary cell wall. In some cell types, a secondary cell wall is subsequently deposited, and in certain instances, the cell dies, as is the case of xylem vessels and tracheids. The synthesis of the cell wall is heavily compartmentalised. Whereas the proteins are synthesised at the endoplasmic reticulum, and subsequently modified in the Golgi apparatus, before being carried in vesicles to the cell surface, the phenolic compounds are made in the cytosol. Of the polysaccharides, both cellulose and callose are synthesised at the cell membrane, and are extruded directly into the cell wall. On the other hand, hemicelluloses and pectins are made in the Golgi apparatus and exocytosed to the plasma membrane for deposition extracellularly. Cell wall proteins modify the nature and interaction of the polysaccharides, in accordance with the stage of development of the cell.

The plant cell wall performs a number of functions. Its primary role is to contain and resist the osmotic pressure exerted by the cytoplasm/cell membrane inside. Additionally, by cementing adjacent cells, the wall contributes to the strength and rigidity of the organism as a whole. The gel-like properties of the extracellular matrix also serve as a

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[1] Corresponding author: mhandfor@uchile.cl

water reserve in certain succulent plants, such as *Aloe barbadensis* Miller (Aloe vera). Finally the cell wall is a formidable barrier to be overcome by potential pathogens, and is a source of signalling molecules in the defence process. Agronomically, the polysaccharides which make up the cell wall constitute dietary fibre and are useful in such diverse industries as food processing and paper manufacture. For example, galactomannan is used when making ice creams, and during the manufacture of paper, arabinoxylan, a major component of pine cell walls must be removed. As a consequence of the biological and commercial importance of these polysaccharides, and therefore of the proteins responsible for cell wall synthesis, modelling and degradation, research in this area has thrived, particularly in the last five years.

This review will concentrate on our current understanding of cell wall synthesis and will focus on the polysaccharide component of the extracellular matrix. The reader is referred to excellent reviews on general cell wall architecture and metabolism (Carpita and McCann, 2000), structure (Somerville *et al*., 2004), growth (Cosgrove, 2005), its role in the pathogen response (Vorwerk *et al*., 2004) and a recent compilation concerning the interest surrounding cell wall proteins and proteomics (Jamet *et al*., 2006).

Cell wall deposition and composition

The cell wall is born during cell division. At the plane of cell division a new cell wall is laid down, physically dividing the two daughter cells, with short narrow intercellular cytosolic connections, or plasmodesmata integrated into the structure. Such cell walls must resist pressures up to 3.0 MPa, and adjacent cells are held in place by the middle lamella, rich in pectic polysaccharides. Therefore, as the cell and plant grows, often in a polarised fashion, coordinated expansion of multiple cells is required. Whereas all cells possess a primary cell wall, certain cells, such as wood fibre cells and xylary elements, deposit a secondary cell wall between the primary wall and the plasma membrane, performing specific functions (Figure 1).

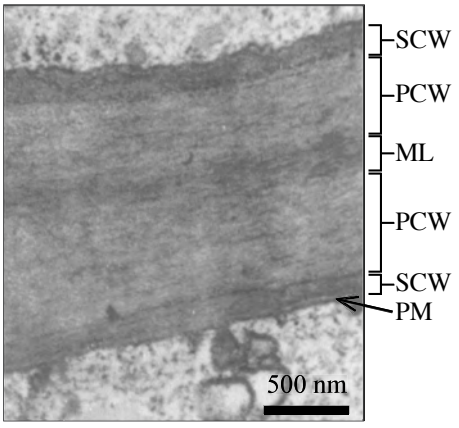

Figure 1. The plant cell wall is a layered extracellular matrix. Transmission electron micrograph of an *in vitro* cultivated megagametophyte cell of *Araucaria araucana* (Mol.) Koch. PCW, primary cell wall; SCW, secondary cell wall; ML, middle lamella; PM, plasma membrane. Courtesy of Liliana Cardemil and Miguel Jordan, Universidad de Chile, Santiago, Chile.

Figura 1. *La pared celular de la planta es una matriz extracelular depositada en capas. Microfotografía electrónica de una célula de megagametofito cultivado* in vitro *de* Araucaria araucana *(Mol.) Koch. PCW, pared celular primaria; SCW, pared celular secundaria; ML, lámina media; PM, membrana plasmática. Cortesía de Liliana Cardemil y Miguel Jordan, Universidad de Chile, Santiago, Chile.*

Cell walls contain a rich diversity of polysaccharides. This diversity stems from the dozen or so constituent sugars in the polymers, some of which may belong to either the D- or L- series (e.g. galactose), and the fact that the monosaccharides can be linked in linear and branched polymers by both α- and β-linkages. In addition, modifications, such as acetylation and methyl-esterification add to the variety of structures encountered in the cell wall.

The principal component of the cell wall is cellulose. Cellulose is a highly insoluble unbranched polymer composed of (1→4)β-linked D-glucose units. Each cellulose chain is held by hydrogen bonds and van der Waals forces in crystalline microfibrils, composed of 30-36 adjacent, parallel strands. Such microfibrils are readily visible by electron microscopy, and encircle the cell, perpendicular to the plane of cell expansion. On the other hand, the other polysaccharide components, hemicelluloses and pectins are made in a soluble form and

generally do not form microfibrils due to lateral modifications such as branching and/or esterification. Interconnecting the cellulose microfibrils is a crosslinking network of neutral hemicelluloses, so-named as they possess (1→4)β-linked sugar backbones as found in cellulose. In dicotyledonous species, the main crosslinking polysaccharide is xyloglucan, although xylans and their derivatives perform a similar function in monocotyledonous cell walls. Xyloglucan shares the same backbone as cellulose, although 75% of the glucose residues are appended with D-xylose. To the xylose residues, D-galactose, and in turn L-fucose units are sometimes added. Other hemicelluloses include the mannans containing (1→4)β-linked D-mannose, which are usually found in low abundance except in certain specialised cell types such as seed endosperm.

Another network is formed by the acidic pectins, creating a gel-like matrix. The diversity of pectins include homogalacturonan, with a (1→4)α-linked D-galacturonic acid backbone and rhamnogalacturonan I (RG-I), composed of alternating (1→2)α-L-rhamnose-(1→4)α-D-galacturonic acid units. Unlike homogalacturonan, RG-I is a branched polymer with side chains of (1→5)α-L-arabinan and (1→4)β-D-galactan, the latter of which may be arabinosylated. The length of the arabinan, galactan and arabinogalactan sidechains is a critical factor in determining porosity of the cell wall. A third pectin polysaccharide, rhamnogalacturonan II (RG-II) has a backbone similar to homogalacturonan, although RG-II is highly branched, with four sidechains (A-D) containing a dozen different sugars held together by around 20 different linkages (O'Neill *et al*., 2004). This complex molecule is well conserved throughout evolution, with similar structures in angiosperms, gymnosperms, bryophytes and pteridophytes implying that it performs a fundamental function in the cell wall. It is believed that the three pectin classes are covalently linked to each other, and that RG-I forms the main structure of the pectin network *in muro*, with homogalacturonan and RG-II forming sidechains (Vincken *et al*., 2003). RG-I aligns with the cellulose microfibrils and non-covalent interactions assist in stabilising the pectin network. Such interactions take the form of intermolecular calcium bridges, spanning carboxyl residues of the galacturonan backbone. This ionic interaction only forms in the extracellular space because on synthesis, the carboxyl residues are heavily masked by methyl-esterification, and pectin methyl-esterases present in the wall cleave the methyl esters, exposing the carboxyl residues to calcium ions. The coordination of calcium ions also has the effect of trapping water, resulting in a highly-hydrated gel in the wall. Reflecting the ionic bridges formed between the pectin molecules is the observation that this polysaccharide is extracted from the cell wall in the presence of calcium chelators such as EDTA and EGTA. A second form of non-covalent interaction involves RG-II. *In vivo* studies in *Arabidopsis thaliana* L. demonstrated that 95% of RG-II molecules exist as dimers in the cell wall, and that this interaction is mediated by borate ions (O'Neill *et al*., 2001). Borate forms a diester bridge between D-apiose residues present in sidechain A of RG-II, thus interconnecting two chains. The boron-mediated spontaneous dimerisation of RG-II molecules, themselves linked to RG-I, would form a mechanism of self-assembly of the pectin network in the cell wall. The hemicelluloses and pectins are relatively insoluble during the extraction of these polysaccharides from the cell wall. However, they are synthesised in soluble form and the highly-hydrated, porous nature of the cell wall permits the diffusion of the newly-synthesised polysaccharides in a matter of minutes (Baron-Epel *et al*., 1988; Vorwerk *et al*., 2004). On reaching their destination, modifications such as the formation of intermolecular non-covalent crosslinkages, and the formation of larger polymers significantly reduces the solubility of hemicelluloses and pectins in the cell wall.

Several genes encoding proteins involved in cell wall synthesis have been identified via a forward genetic strategy, employing high-throughput chemical methods to detect changes in the cell wall composition of mutagenised plants (Reiter *et al*., 1993). In addition, several sensitive techniques, including mass spectroscopy have been developed to assist in the identification and subsequent analysis of mutants with defects in specific polysaccharides (Lerouxel *et al*., 2002). These studies have shed some light on the role of

individual sugar residues in the polysaccharide component of the cell wall. For example, the *mur3* mutant of *Arabidopsis* is deficient in galactose residues in xyloglucan, diminishing the interaction between the xyloglucan and cellulose thus affecting the tensile strength of the cell wall (Reiter *et al*., 1997; Ryden *et al*., 2003). In addition, the tensile strength of cell walls is reduced in *mur3*, which have a lower capacity to form borate dimers between RG-II molecules. This defect is rescued if the plants are sprayed with borate, implying that this polysaccharide plays a key role in cell-cell adhesion (Ryden *et al*., 2003).

It should be noted that whereas xyloglucan is the main crosslinking hemicellulose in dicots, and in the non-commelinoid monocots, its function is replaced by glucuronarabinoxylan, (1→4)β-D-xylan decorated by (1→2)α-linked L-arabinose and D-glucuronic acid residues, in the commelinoid monocots which include grasses, cereals, and bromeliads. In addition, a mixed (1→3),(1→4)β-D-glucan is also present in the order Poales (grasses and cereals). Although unbranched, this β-glucan develops a corkscrew structure, a result of the (1→3)β-linkages which join short chains of linear (1→4)β-D-glucan units. Whereas in general in dicots, cellulose, hemicellulose and pectin are present in roughly equal amounts, in monocots, hemicelluloses are much more abundant than cellulose, with pectins making up just 10% of the structure (Cosgrove, 1997).

The composition of the cell wall is highly regulated during the processes of cell growth, division and differentiation. This is highlighted by the fact that the composition of the 35-40 cell types in the same plant differs substantially. Such variations unfortunately complicate cell wall polysaccharides analysis, as during an extraction, the investigator is typically merging the components of many different cell types, leading to the averaging of cell-specific variations. Therefore, the use of relatively homogeneous samples holds certain advantages. Such systems include callus, *Zinnia elegans* L. cells, which can be induced to differentiate synchronously into vascular tissue (McCann *et al*., 2001), and the large and abundant cambial cells of *Populus* species (Mellerowicz *et al*., 2001). On a more microscopic level, adjacent cells may present differences in composition, and antibodies raised against specific cell wall epitopes have greatly increased our understanding of the temporal and spatial dynamics of cell wall metabolism. For example, the dead xylary elements of *Arabidopsis* have scarce deposition of mannans, in contrast to the relative abundance of this hemicellulose in the adjacent xylem parenchyma cells (Handford *et al*., 2003). However, a monoclonal antibody raised against the terminal (1→2)α-L-fucose residues present in xyloglucan (CCRC-M1; Puhlmann *et al*., 1994), revealed an even more subtle difference; the composition of the cell wall of a single cell differs depending on the adjacent cell type. In lateral root cap cells, the CCRC-M1 epitope is abundant in the periclinal wall facing the exterior, yet is virtually absent from the walls facing adjacent lateral root cap cells (anticlinal walls) or the periclinal wall between the lateral root cap cell and the underlying epidermal cell (Freshour *et al*., 1996). Similar temporal and spatial variations in pectin epitopes have been documented for the (1→4)β-D-galactan sidechains of RG-I in peas (McCartney *et al*., 2001) and for the pattern of methyl-esterification of the homogalacturonan backbone (Willats *et al*., 2001).

Whereas all cells possess a primary cell wall, only certain cell types contain a secondary cell wall, which frequently has specialised functions, reflected in its distinct composition. Deposition of the secondary cell wall only begins once expansion of the primary cell wall has ceased. Hemicelluloses are typically more abundant in the secondary cell wall than in the primary wall, as shown by the prevalence of mannans in the former only (Meier and Reid 1982; Bacic *et al*., 1988; Handford *et al*., 2003). Mannans also fulfil certain specialised roles in plants. For example, an abundance of *O*-acetylated mannan (acemannan or acetomannan) results in the formation of a highly-hydrated gel in the extracellular space, which is employed by succulent plants such as *A. barbadensis* (Aloe vera) as an efficient means of retaining water (Femenia *et al*., 2003). Other macromolecules are also found in the secondary cell wall. Lignins serve to impermeabilise the cell wall, as found for instance in xylary elements and form

a latent defence mechanism against pathogens. The cytosolically-synthesised monolignols are glycosylated before being secreted into the extracellular matrix. Once there, peroxidases, laccases and monolignol radical binding proteins catalyse their polymerisation into an extensive highly-crosslinked network which associates via covalent interactions with the polysaccharide and protein components of the secondary cell wall (Davin and Lewis, 2005). Cutins and suberins may also be present in certain cell types to increase the impermeability of the cell and to provide a protective barrier (Kolattukudy, 2001).

Biosynthesis of cell wall polysaccharides

Given the metabolic load and complexity of the extracellular matrix, it is estimated that up to 15% of genes encoded by plants may be involved in aspects of cell wall synthesis, assembly, modelling and breakdown (Carpita *et al*., 2001). Here, our current understanding of polysaccharide biosynthesis is examined.

The enzymes responsible for elongating glycan chains and forming branches are called glycosyltransferases. They require a specific acceptor molecule and, as substrate, sugars activated by the presence of a nucleotide (nucleotide-sugars) mainly in the form of UDP- and GDP-sugars (Feingold and Avigad, 1980; Scheible and Pauly, 2004). These enzymes take two forms, multimembrane-spanning (type III) processive glycosyltransferases, and type II glycosyltransferases, the latter possessing a hydrophobic N-terminus, believed to anchor the protein in the membrane, and a C-terminal catalytic domain.

Cellulose and callose synthesis

Both cellulose and callose, a (1→3)β-D-glucan manufactured in the wounding response, cell plate formation and in pollen tip growth, are synthesised at the plasma membrane. Significant strides have been made in determining the enzymes and mechanisms responsible for the synthesis of cellulose, the most abundant polymer on Earth (Saxena and Brown, 2005). The discovery and cloning of the genes which encode cellulose synthase was carried out after searching for plant orthologues of the corresponding bacterial cellulose synthase genes (*celA*) from *Acetobacter xilinum* and *Agrobacterium tumefaciens*. *Gossypium hirsutum* (cotton) fibres were chosen as a model system because they contain up to 98% cellulose. A cDNA library corresponding to the commencement of cellulose synthesis in cotton fibres, when the expression of cellulose synthases would be expected to be greatest, was constructed (Pear *et al*., 1996). The cellulose synthase genes (*CESA*) obtained encode proteins of about 110 kDa and consistent with a membrane localisation, possess eight transmembrane domains. Such *CESA* genes were subsequently characterised in *Arabidopsis* (Arioli *et al*., 1998), which possesses a family of ten related proteins.

Physically, the cellulose synthases form rosettes embedded in the plasma membrane which are readily visible by electron microscopy after freeze-fracture and rotary-shadowing of the membrane. In higher plants, each symmetrical rosette is composed of six protein subunits, and each subunit contains five or six CESA proteins. Each protein is capable of synthesising a single linear (1→4)β-D-glucan chain, giving rise to the 30-36 parallel cellulose polymers that constitute the microfibril. Evidence from functional and immunoprecipitation studies suggests that each subunit is not a homomeric complex; rather three different CESA proteins are required to produce a functional complex (Taylor *et al*., 2003). Our current knowledge in *Arabidopsis* is that CESA1, 3 and 6 are required for primary cell wall biosynthesis whereas subunits CESA4, 7 and 8 manufacture the cellulosic component of secondary cell walls, supported by coexpression of the latter in developing stems (Taylor *et al*., 2003). Different CESA proteins are also required for primary and secondary wall formation in aspen (*Populus tremuloides*; Kalluri and Joshi, 2004). It appears that CESA proteins in each rosette subunit are stabilised as dimers by a coordinated zinc ion between the zinc finger domains present towards the N-termini of the proteins (Kurek *et al*., 2002). This interaction could therefore be an initial step in organising each pentameric/hexameric subunit, and ultimately the rosette supercomplex. Interestingly, the topology of

the cellulose synthase complexes in the plasma membrane appears to play a key role in the shape of the cellulose molecules synthesised. Whereas in higher plants the rosettes favour the formation of interwined microfibrils, the flat ribbons produced in *Erythrocladia subintegra* are synthesised by rectangular complexes of cellulose synthase enzymes (Tsekos *et al*., 1996).

It is clear however, that other factors play important roles in cellulose biosynthesis in plants. The *cytokinesis defective1* (*cyt1*) mutant of *Arabidopsis* is severely deficient in cellulose synthesis, consistent with the development of swollen root tips and weakened cell walls (Lukowitz *et al*., 2001). The *cyt1* mutant is profoundly limited in *N*-glycosylation, and can be phenocopied in wild-type plants by the addition of tunicamycin, an inhibitor of the *N*-glycosylation process. These results suggest that *N*-glycosylation of cellulose synthase (CESA1 contains four potential *N*-glycosylation sites; Arioli *et al*., 1998), plays a part in protein folding, stabilisation of the rosette complexes or in regulating their enzymatic activity.

Apart from decorations of cellulose synthase, other proteins are also required for efficient cellulose synthesis (Scheible and Pauly, 2004). Analysis of an *Arabidopsis* mutant with dwarfed hypocotyls identified *korrigan*, a membrane bound endo-1,4-β-glucanase, with a reduced amount of crystalline cellulose in the cell wall (Nicol *et al*., 1998; His *et al*., 2001). Although the protein is not directly linked to the CESA rosettes (Zuo *et al*., 2000; Szyjanowicz *et al*., 2004), it has been postulated that the glucanase helps to reorientate misplaced cellulose chains, thus helping in the assembly of the thicker cellulose microfibrils. An alternative role for KORRIGAN is that it cleaves a nascent glucan from a primer molecule required for cellulose synthesis. A candidate for such a primer is sterol-β-glucoside (Peng *et al*., 2002), which CESA may use in conjunction with UDP-D-glucose to form an oligo(1→4)β-D-glucan. The sterol component could be subsequently removed by KORRIGAN once a critical glucan length has been reached. However, the assays demonstrating that sterol-β-glucoside serves as a potential primer were carried out *in vitro* (Peng *et al*., 2002), and the *in vivo* synthesis of cellulose is still a matter of debate and a definitive role for such a primer has yet to be established. Another potential candidate to facilitate the synthesis of cellulose is sucrose synthase, which could efficiently funnel the UDP-D-glucose obtained from the metabolism of sucrose into the CESA complexes (Salnikov *et al*., 2001). This process of substrate-channelling is explored further in the analysis of non-cellulosic polysaccharide synthesis (see Nucleotide-sugar transporters and multi-protein complexes). Due to conservation throughout evolution, similar patterns of expression of genes encoding cellulose synthases, endo-1,4-β-glucanase and sucrose synthase is being actively explored in commercially important woody species (Joshi *et al*., 2004; Bhandari *et al*., 2006).

There is increasing evidence for a direct interplay of signals between the deposition of the extracellular cellulose and the internal dynamics of the cell (Smith and Oppenheimer, 2005). Electron microscopy imaging frequently reveals that the microfibrils are laid down parallel to the cortical microtubular network, suggesting that there could be a direct or indirect interaction between the cellulose synthase rosettes and this component of the cytoskeleton (Ledbetter and Porter, 1963). In addition, application of microtubule disrupting drugs, such as colchicine dramatically disrupts the typically-ordered pattern of cellulose synthesis. At a molecular level and consistent with the inhibitor studies, is the observation that *FRAGILE FIBER2* (*FRA2*) encodes a katanin-like microtubule-severing protein, and *fra2* mutants possess both aberrant deposition of cortical microtubules and disrupted cellulose deposition (Burk and Ye, 2002). As outlined below (see Nucleotide-sugar transporters and multi-protein complexes), a type II glycosyltransferase has also recently been shown to interact with cytoskeletal components. In conclusion, a variety of factors, including *N*-glycosylation, lipids, and structural and enzymatic proteins interact directly or indirectly with the CESA rosettes to influence the synthesis and orderly deposition of cellulose.

Significant progress has also been made in elucidating the mechanisms involved in callose biosynthesis. In *Arabidopsis*, twelve callose synthases (CALS) are encoded in the genome, and one of these, CALS1, is specifically-localised at the plasma membrane during cell plate formation (Hong *et al*., 2001). Functional *in vivo* evidence, via the over-expression of the *Arabidopsis* protein in tobacco, demonstrated that the enzyme was a callose synthase and that there was more callose deposited at the cell plate in transgenic plants compared to wild-type controls (Hong *et al*., 2001). The importance of callose in other cellular processes has also been examined. It was generally believed that the wound-induced callose plug that forms close to a fungal infection site was a plant defence mechanism mounted to resist pathogen attack. Paradoxically however, the *pmr4*/*CALS12* mutant of *Arabidopsis*, deficient in callose synthase is more *resistant* to *Blumeria graminis* (powdery mildew fungus), a consequence of a stimulation of the salicylic acid pathway (Jacobs *et al*., 2003; Nishimura *et al*., 2003). Apart from indicating that the callose synthase homologues are not able to supplement the loss of activity of CALS12, this result suggests that the fungus is, in some way, dependant on the deposition of callose in order to pathogenise the plant efficiently.

Non-cellulosic polysaccharides synthesis

The linear, homopolymeric nature of cellulose means that relatively few proteins are required for its synthesis. However, as outlined above, the same cannot be said for the hemicelluloses and pectins. These polysaccharides contain a much greater diversity of sugars and linkages, and are found in both branched and linear forms. Bioinformatic analysis indicates that there are 415 genes encoding for putative glycosyltransferases in the *Arabidopsis* genome (Henrissat *et al*., 2001), although this number is almost certainly higher if different parameters are used in the data mining (Scheible and Pauly, 2004). Although such an estimate is higher than the 350 different linkages present in the various cell wall polysaccharides, of which 53 are found in pectic polysaccharides alone (Mohnen, 1999), the high number may reflect preferences for selected substrates, or the glycosylation of other non-polysaccharide molecules (Bowles, 2002).

For the synthesis of hemicelluloses with a backbone similar to that of cellulose, such as xyloglucan, galactan, mannan and xylan it has been proposed that cellulose synthase-like (CSL) glycosyltransferases are responsible. There are an estimated 29 CSL enzymes in the *Arabidopsis* genome and 37 in *Oryzae sativa* (rice) forming eight families (CSLA – CSLH) which could function similar to cellulose synthase (Richmond and Somerville, 2000; Somerville *et al*., 2004). Thus, a single multi-membrane spanning protein would take the nucleotide-sugar from the cytosol, transfer it across the Golgi membrane and extrude the nascent (1→4)β-D-glycan chain into the lumen. Alternatively, as the reaction topology of CSL action has yet to be established, the nucleotide-sugar substrate may enter the Golgi lumen by a different mechanism prior to use by a lumenal catalytic domain of these enzymes (Figure 2A). Interestingly, the CSL proteins lack the N-terminal zinc binding motif, so unlike the CESA proteins, they may not form dimers or other oligomeric structures in the Golgi membrane.

Using a variety of approaches, mutants have been identified in several CSL genes in *Arabidopsis*, providing intriguing insights into their roles *in planta*. These mutants have dramatic phenotypes, namely absent root hairs (AtCSLD3/Kojak; Favery *et al*., 2001), resistance to Agrobacterial root transformation (AtCSLA9/rat4; Zhu *et al*., 2003) and embryo lethality (AtCSLA7; Goubet *et al*., 2003). However, whereas it is clear that CSLs play crucial roles in plant development, two critical features have proved difficult to determine; a demonstration of the subcellular localisation of these proteins and secondly an indication of their polysaccharide-producing capabilities *in vivo*. The first feature remains outstanding, although the biochemical evidence strongly suggests that these polysaccharides, such as mannans are synthesised in the Golgi apparatus, as shown in *Pisum sativum* (pea), *Pinus radiata* (pine) and *Arabidopsis* (Dalessandro *et al*., 1988; Piro *et al*., 1993; Handford *et al*., 2003). However, experimental evidence which should lead to

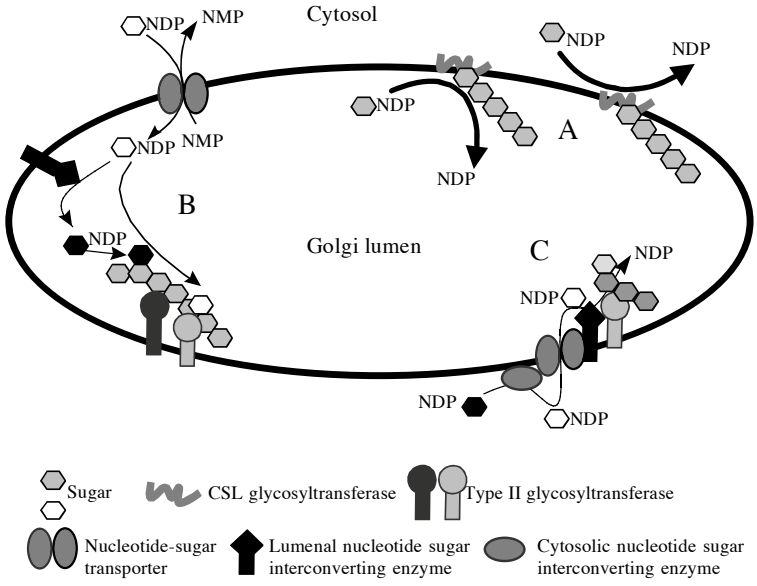

Figure 2. Current models for the synthesis of non-cellulosic polysaccharides in the Golgi apparatus. A. Multi-membrane spanning cellulose synthase-like (CSL) glycosyltransferases catalyse the lumenal synthesis of polysaccharides. It is envisioned that (1→4)β-D-glycan backbones are produced this way although it is unknown whether such enzymes require cytosolic or lumenal nucleotide-sugars. B. Type II glycosyltransferases anchored in the membrane are required for the decoration of the glycan backbone with lateral sidechains. Such enzymes require the activity of transporters to import the nucleotide-sugar substrate, the identity of which may be altered by a lumenal nucleotide-sugar interconverting enzyme. The nucleoside diphosphatase and the putative inorganic phosphate transporters required to complete the cycle are omitted for clarity. C. A hypothetical model of a multi-protein complex which would efficiently channel the cytosolically-synthesised nucleotide-sugars into non-cellulose polysaccharides in the Golgi lumen. The changing identity of the sugar moiety is highlighted.

Figura 2. *Modelos para la síntesis de polisacáridos no celulósicos en el aparato de Golgi. A. Una glicosiltransferasa con múltiples pasos transmembrana tipo celulosa sintetasa (CSL) cataliza la síntesis de polisacáridos en el lumen del Golgi. Se ha propuesto que el esqueleto de los (1→4)β-D-glicanos son producidos de esta manera, aunque se desconoce si tales enzimas requieren de nucleótidos-azúcar citosólicos o lumenales. B. Se requieren de glicosiltransferasas tipo II ancladas en la membrana para la decoración del esqueleto del glicano con cadenas laterales. Tales enzimas requieren de la actividad de transportadores para importar los substratos de nucleótidos-azúcar cuya identidad puede ser alterada por una enzima de interconversión. Para una mayor claridad y comprensión del modelo, se han omitidos la nucleósido difosfatasa y transportadores putativos de fosfato inorgánico que se requieren para completar el ciclo. C: Modelo hipotético de un complejo multiproteico que podría canalizar eficientemente, desde el citosol al lumen, un nucleótido-azúcar al polisacárido no celulósico sintetizado en el Golgi. La identidad cambiante del azúcar es resaltada.*

addressing the second feature has recently been obtained. *Cyamopsis tetragonoloba* (guar) seeds accumulate massive quantities of the storage carbohydrate galactomannan which amounts to 90% of this tissue (Reid *et al*., 1992). The enzyme responsible for the synthesis of the galactomannan backbone is (1→4)β-D-mannan synthase and Dhugga *et al*. (2004) created an expressed sequence tag (EST) library from guar at the developmental stage with maximal activity of this enzyme. In this way, it was elegantly shown that the mannan synthase of guar was indeed a CSL type protein.

A second heterologous expression system, that of *Drosophila melanogaster* S2 cells which lack endogenous glycan synthases, and therefore provides a system with lower interference, was developed to analyse the activity of *Arabidopsis* and rice CSLs (Liepman *et al*., 2005). Of the three CSLs which were detected in microsomal fractions isolated in transformed S2 cells, AtCSLA9, AtCSLE1 and OsCslH1, AtCSLA9 showed substantial incorporation of

GDP-D-mannose into a mannanase-sensitive product. Interestingly, when supplied with a mixture of GDP-D-glucose and GDP-D-mannose, AtCSLA9 formed a (1→4)β-linked glucomannan. On incubation with either UDP-D-xylose or UDP-D-galactose, the substrates required for xylan and galactan synthesis, respectively, neither polysaccharide was generated, indicating a certain specificity by this CSL for the synthesis of (1→4)β-D-glycans related to mannan. Other members of the CSLA family were also shown to possess mannan synthase and/or glucomannan synthase activity, confirming the biological activity of the proteins (Liepman *et al*., 2005). It is tempting, but premature to speculate that all nine members of the CSLA family are involved in the synthesis of mannans. Moreover, it has not been shown that members of other CSL groups are incapable of synthesising mannans, but the development of this heterologous system paves the way for further analyses of additional genes. Unfortunately, the embryo lethality of the *atcsla7* mutant, a CSL shown to possess mannan synthase activity by Liepman *et al.* (2005), prevented a detailed analysis of the cell wall in the mutant (Goubet *et al*., 2003). The discovery of leaky alleles may help to determine the role of AtCSLA7 *in vivo*, and it will be very interesting to see whether mannans are affected. On the other hand, functional redundancy among members of the same family may neutralise any differences in the cell wall, as indicated in the case of *atcslA9* (Zhu *et al*., 2003).

Whilst it is possible to envision that CSL type enzymes could be responsible for the *in vivo* synthesis of (1→4)β-D-glycan backbones, it is hard to reconcile how the same enzymes could catalyse the addition of lateral sidechains found on many of the non-cellulosic polysaccharides. This function is carried out by so-called type II glycosyltransferases (Figure 2B). Experiments have demonstrated that the catalytic domains of these enzymes face the lumen of the Golgi apparatus (Wulff *et al*., 2000; Sterling *et al*., 2001). The abundance of galactomannan in seed endosperm, as in the case of isolating a mannan synthase from guar (see above, Dhugga *et al*., 2004), again proved useful in the cloning and characterisation of the first type II glycosyltransferase. A galactosyltransferase, which adds galactose in an (1→6)α-D-linkage to the mannan backbone was purified from *Trigonella foenum-graecum* (fenugreek) and the corresponding cDNA subsequently obtained (Edwards *et al*., 1999). The 51-kDa protein possessed the structural characteristics of type II glycosyltransferases.

Significant progress has subsequently been made in identifying the glycosyltransferases needed for xyloglucan synthesis. The α1,2-fucosyltransferase responsible for the addition of the terminal L-fucose units, AtFUT1, was the first glycosyltransferase cloned in *Arabidopsis* (Perrin *et al*., 1999) and an orthologue was subsequently identified in pea (Faik *et al*., 2000). Despite being a member of a family of nine-related members, AtFUT1 appears to be the only xyloglucan-specific fucosyltransferase in this species (Sarria *et al*., 2001). Therefore, it has been proposed that one, or more, of the AtFUT1 homologues catalyses the addition of α1,2-linked L-fucose to the heavily-glycosylated arabinogalactan proteins (van Hengel and Roberts, 2002). In a systematic screen for cell wall composition mutants, *mur2* was identified as one with a significant reduction in the L-fucose content of the cell wall (Reiter *et al*., 1997), and a reduction to just 2% of wild-type levels of fucosylation of xyloglucan, due to a point mutation in *AtFUT1* (Vanzin *et al*., 2002). The strength of the *mur2* cell wall was unchanged compared to wild-type cell walls, the mutant grew normally (Vanzin *et al*., 2002) and transgenic plants constitutively over-expressing AtFUT1 had no increase in L-fucose decoration of xyloglucan (Perrin *et al*., 2003), presumably because of limiting xyloglucan oligosaccharide acceptor substrate. Xyloglucan crosslinks cellulose microfibrils (Pauly *et al*., 1999) and from *in vitro* and computer modelling analyses, L-fucose residues were believed to play a significant role in this interaction (Levy *et al*., 1991; 1997). However, the results obtained *in vivo* from under- and over-expressing AtFUT1 lines cast doubt on these assertions.

The mutant *mur3,* with reduced L-fucose content, was identified but is present in a different complementation group than *mur2*, indicating that a different locus was affected (Reiter *et*

al., 1997). A map-based cloning approach pinpointed the mutation to a xyloglucan-specific galactosyltransferase that acts only on specific D-xylose residues in the xyloglucan oligosaccharide, suggesting that the enzyme has a very tight preferred specificity (Madson *et al*., 2003). As a consequence of the mutation, the terminal L-fucose attached to the D-galactose are also absent in the mutant, providing an explanation for the original *mur3* phenotype, which could be rescued by expression of a wild-type copy of the gene. The specificity of the xyloglucan galactosyltransferase is mirrored in the specificity of a xylosyltransferase which only transfers D-xylose residues to certain residues of the (1→4)β-D-glucan backbone (Faik *et al*., 2002).

More recently, glycosyltransferases involved in arabinan and glucuronoxylan synthesis have been identified. The *arabinan deficient1* mutant (*arad1*) of *Arabidopsis* has a >50% reduction in L-arabinose residues (Harholt *et al*., 2006), and reduced labelling by antisera raised against the (1→5)α-L-arabinan chains of RG-I (Willats *et al*., 1998). Although the *in vitro* activity of this enzyme has yet to be presented, the ARAD1 protein possesses the structural characteristics of a type II glycosyltransferase, so is likely to be involved directly as a arabinan α-1,5-arabinosyltransferase (Harholt *et al*., 2006). Regarding glucuronoxylan, the *fragile fiber8* (*fra8*) mutant of *Arabidopsis* suffers a specific reduction of (1→2)α-linked D-glucuronic acid residues on the xylan backbone of this polysaccharide in secondary cell walls (Zhong *et al*., 2005). This causes a reduction in the thickness of interfascicular fibres and xylem vessels of the mutant, leading to partially collapsed vasculature and a significant reduction in the mechanical strength of the stems. The D-glucuronic acid sidechains of xylan could therefore play an important role in crosslinking in the secondary cell wall. Interestingly, the D-xylose content of the cell wall of *fra8* mutants is also diminished suggesting that there may be coordinated synthesis of (1→4)β-D-xylan and the addition of D-glucuronic acid sidechains, possibly by an enzymatic complex. Again however, although localised to the Golgi (Zhong *et al*., 2005), biochemical proof of the enzymatic activity of FRA8 has still to be shown.

Given the abundance and agronomic importance of pectins, it is perhaps surprising that so few proteins involved in its synthesis have been cloned and characterised. However, there are two exciting clues and both link pectins to roles in cell-cell adhesion. The *quasimodo1* mutant of *Arabidopsis* has an adhesion defect and a significantly reduced D-galacturonic acid and homogalacturonan content, as shown by immunocytochemistry using monoclonal antibodies specific for this polysaccharide (Bouton *et al*., 2002; Leboeuf *et al*., 2005). Homogalacturonan α-1,4-galacturonosyltransferase activity is reduced in the mutant, but the activity of (1→4)β-D-xylan synthase is also limited, suggesting that QUASIMODO1 plays a role in both pectin and hemicellulose biosynthesis (Orfila *et al*., 2005). Although the T-DNA tagged gene has been cloned, and is a type II glycosyltransferase (Bouton *et al*., 2002), positive evidence for its enzymatic activity is lacking. In a separate development, the *nolac-H18* mutant of *Nicotiana plumbaginifolia* has loosely-attached cells when grown in suspension culture and lacks a galactoglucuronic acid disaccharide moiety on sidechain A of RG-II. Despite these residues not being directly involved in borate diester formation (O'Neill *et al*., 2004), only about half the RG-II extracted from the mutant is found as a dimer, compared to >95% in wild-type tobacco (Iwai *et al*., 2002). From the analysis of the RG-II in the mutant, it was proposed that *nolac-H18* is deficient in a glucuronyltransferase, and although the gene affected is a putative glycosyltransferase, its specificity has yet to be tested.

Nucleotide-sugar metabolism

The substrates used by all glycosyltransferases are nucleotide-sugars. Apart from 'activating' the sugars, the identity of the nucleotide moiety plays a fundamental role in determining their final destination. For example, the D-glucose found in the xyloglucan backbone is supplied as UDP-D-glucose, yet GDP-D-glucose is the substrate required for the synthesis of glucomannan. The majority of nucleotide-sugars, including all GDP-sugars such as GDP-D-mannose, -L-fucose and -D-galactose are manufactured in the cytosol (Seifert, 2004).

However, there is increasing evidence that some, but not all UDP-sugars are synthesised in the lumen of the Golgi apparatus (Figure 2B). UDP-D-glucuronic acid decarboxylase, UDP-D-xylose epimerase and UDP-D-galacturonic acid epimerase, which synthesise UDP-D-xylose, UDP-L-arabinose and UDP-D-galacturonic acid, respectively, have been cloned and shown to be associated with the Golgi membrane and indeed code for membrane-anchored proteins (Harper *et al*., 2002; Burget *et al*., 2003; Molhoj *et al*., 2004; Usadel *et al*., 2004). However, the precursor for these three UDP-sugars, UDP-D-glucuronic acid is made in the cytosol from UDP-D-glucose, by UDP-D-glucose dehydrogenase, a non-membrane bound enzyme (e.g. Johansson *et al*., 2002). Thus there is a need for cytosolically-synthesised nucleotide-sugars to reach the Golgi lumen for use by type II glycosyltransferases either directly or via lumenal interconversion. Furthermore, as the reaction topology of CSL enzymes has not been demonstrated, it is plausible that the catalytic domains of such processive glycosyltransferases also face the Golgi lumen. Therefore, given the highly unfavourable route of directly crossing the lipid bilayer, nucleotide-sugar transporter proteins were proposed to exist to import the substrate enzymes.

Nucleotide-sugar transporters and multi-protein complexes

Much work has been done in animal and yeast systems on such transporters (Gerardy-Schahn *et al*., 2001; Ishida and Kawakita, 2004). Transport of nucleotide-sugars is saturatable, temperature-dependent and susceptible to the addition of detergents (Berninsone and Hirschberg, 2000). On a biochemical level, nucleotide-sugar transporters function as antiporters, coupling the import of specific nucleotide-sugars with the exit of the corresponding nucleoside-monophosphate (Figure 2B). The nucleoside-monophosphates are formed by the action of a nucleoside-diphosphatase, which hydrolyses the byproduct of the reaction catalysed by the lumenal glycosyltransferases (Orellana *et al*., 1997; Wulff *et al*., 2000), although the fate of the inorganic phosphate also produced is not known. Biochemical evidence for the transport of UDP-D-glucose and GDP-L-fucose across the Golgi membrane of peas has been obtained (Muñoz *et al*., 1996; Wulff *et al*., 2000), the import of which was shown to be mediated by distinct proteins (Wulff *et al*., 2000). Interest in plant nucleotide-sugar transporters is substantial, as they are a potential control point for glycan synthesis via substrate level-control. In the *Arabidopsis* genome there could be at least 20 nucleotide-sugar transporters (Norambuena *et al*., 2002; Handford *et al*., 2004), although few have been characterised in detail. The *Arabidopsis* nucleotide-sugar transporters characterised to date are capable of transporting GDP-D-mannose, UDP-D-galactose and UDP-D-glucose (Norambuena *et al*., 2002, 2005; Baldwin *et al*., 2001; Bakker *et al*., 2005), but it is predicted that many other nucleotide-sugars should be transported into the Golgi. However, their role *in vivo* is the subject of ongoing experimentation: unlike the phenotypes exhibited by glycosyltransferase and nucleotide-sugar synthesis mutants, to date investigations into the importance of nucleotide-sugar transporters *in planta* are lacking, although mutations in these genes in other organisms can produce severe growth and developmental defects (Handford *et al*., 2006). Furthermore, nucleotide-sugar transporters can regulate synthesis of specific subsets of macromolecules. For example, in human cells with a defective GDP-D-fucose transporter there is a loss of fucosylation of *N*-linked glycans whereas *O*-linked glycans are less affected (Sturla *et al*., 2003). The differential effect could be explained by substrate-channelling and it has recently been proposed (Seifert, 2004; Usadel *et al*., 2005) that plant nucleotide-sugar transporters, type II glycosyltransferases and nucleotide-sugar interconverting enzymes form complexes in the Golgi membrane (Figure 2C). For example, an *Arabidopsis* mutant deficient in an enzyme required for the cytosolic synthesis of UDP-D-galactose (UDP-D-glucose epimerase, UGE4) has defects in glycosylation of selected polysaccharides in a cell-specific manner (Seifert *et al*., 2002). These results indicate that UGE4 is specifically complexed with a UDP-galactose transporter and specific galactosyltransferase(s) whereas other isoforms of the enzyme may be coupled in different complexes. The composition of such

complexes has taken a new turn recently. The *katamari1* mutant (Tamura *et al*., 2005) was identified as one with defects in cell elongation, a disrupted actin cytoskeletal network and a disorganised endomembrane system, of which the Golgi apparatus plays a central role. It is well-established that the Golgi network and vesicles traffic along actin microfilaments (and not the microtubular network) for the secretion of hemicelluloses, pectins and glycoproteins at the plasma membrane (Boevink *et al*., 1998; Baldwin *et al*., 2001) and an intact actin network is required for this process to proceed efficiently (Hu *et al*., 2003). Intriguingly, the locus disrupted in the *katamari1* mutant is allelic to *MUR3*, the gene that encodes a xyloglucan-specific galactosyltransferase (see above, Madson *et al*., 2003). The N-terminal region of KATAMARI1/MUR3 spans the Golgi membrane and is exposed to the cytosol, and immunoprecipation experiments demonstrated that KATAMARI1/MUR3 interacts directly or indirectly with actin (Tamura *et al*., 2005). Therefore, this protein carries out a dual role in cell wall synthesis and cytoskeletal organisation and the identity of other proteins associated with KATAMARI1/MUR3 could shed light on the interplay between the Golgi and the cytosol.

Concluding remarks

With the completion of genome and EST sequencing projects of a variety of dicot and monocot plant species, cell wall research, like all areas of plant science has received a wealth of information, permitting the identification of hundreds of genes potentially involved in diverse aspects of cell wall metabolism. It is becoming increasingly clear that many of these sequences are members of gene families, and analysing the roles of such a vast quantity of proteins poses difficulties. In an attempt to discover genes which had not been identified in previous forward genetic screens, Brown *et al*. (2005) and Persson *et al*. (2005) analysed the profiles of genes co-expressed with those known to be involved in secondary cell wall cellulose biosynthesis. Using this innovative approach, several novel genes were identified, genes which would otherwise have been overlooked by more conventional methods. Mutations in some of these genes did indeed result in secondary cell wall phenotypes (Brown *et al*., 2005; Persson *et al*., 2005).

Whilst occasionally mutations in genes involved in cell wall metabolism result in discernible phenotypes, as in the case of *pmr4*/*CALS12*, in other instances, highly sensitive, and at times expensive techniques such as Fourier transform infrared spectoscropy or mass spectroscopy are needed to analyse subtle, tissue-specific changes (Pilling and Höfte, 2003). In this regard, two more readily-accessible procedures are proving to be powerful tools for cell wall research. Firstly, PACE (Polysaccharide Analysis using Carbohydrate gel Electrophoresis) is a relatively new arrival in glycan analysis, yet is reliable, simple, quantitative and economical compared to more traditional methods. PACE works on the principle that a fluorophore can be added to the non-reducing end of an oligosaccharide for subsequent separation in polyacrylamide gels. Many samples can be run in parallel (up to 30 in one gel) and the oligosaccharides, which are liberated by enzymes of known specificity, appear as distinct bands when viewed under ultraviolet light, the identity of which can be determined if standards are included in the gel. It has been extensively optimised (Goubet *et al*., 2002) and used to analysis cell wall polysaccharides, such as mannans (Handford *et al*., 2003) and pectin (Goubet *et al*., 2003; Barton *et al*., 2005). A second method is the use of polyclonal and monoclonal antibodies raised against specific polysaccharides. As well as providing essential data on the spatial distribution of individual hemicelluloses and pectins (e.g. Freshour *et al*., 1996; McCartney *et al*., 2001; Willats *et al*., 2001; Clausen *et al*., 2004; Willats *et al*., 2004), new insights are being gained into the temporal distribution of polysaccharide deposition. For example, a glucomannan antisera revealed that the deposition of this hemicellulose in developing conifer xylem follows a diurnal rhythm yet in mature tracheids, the glucomannan is distributed uniformly (Maeda *et al*., 2000; Hosoo *et al*., 2002).

With the huge agronomical importance of the cell wall, considerable interest surrounds biotechnological means to alter the

polysaccharide and/or lignin composition to increase yield or to facilitate the processing of the plant material. In this regard, an exciting development concerns the manipulation of galactomannan, a gum used by the food industry as a thickener and stabiliser, but also employed in paper processing and mining. Whereas the (1→4)β-D-mannan backbone of fenugreek galactomannan is highly substituted with (1→6) α-D-galactose residues (mannose:galactose 1:1), that of tobacco is sparsely galactosylated (1:30). However, in tobacco seeds expressing the fenugreek enzyme galactomannan galactosyltransferase (Edwards *et al*., 1999), the transgenic line contains around 4 times more D-galactose substitutions, demonstrating that the polysaccharide composition of plant polysaccharides can be altered *in vivo* (Reid *et al*., 2003). However, for industrial purposes, relatively sparsely galactosylated galactomannan is preferred. Towards this end, Edwards *et al*. (2004) succeeded in altering the level of substitution in the model legume *Lotus japonicus* from a mannose:galactose of 1:1.2, to a ratio of 6:1, by suppressing the activity of the galactosyltransferase. The potential applications of such an approach are clearly tremendous.

Research into cell wall metabolism, at both pure and applied levels, is rapidly evolving and a deeper understanding of the complexities of the highly dynamic extracellular matrix is being attained. Fortunately, public funding has been sought to maintain web-based resources, such as WallBioNet (xyloglucan.prl.msu.edu) and the Cell Wall Navigator (bioweb.ucr.edu/Cellwall) to help maintain the global cell wall community abreast of current developments.

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Resumen

La pared celular juega un importante rol en la determinación de la expansión, crecimiento y forma celular, como también de la planta misma. Adicionalmente, los productos de la degradación de la pared celular son usados como señales de desarrollo. Por otra parte, la pared celular está en la línea frontal de defensa, constituyendo una importante barrera a la penetración de patógenos y vectores patógenos. Hay una gran diversidad en los polisacáridos de la pared celular, la que está compuesta por microfibrillas de celulosa, embebidas en una matriz de pectinas y hemicelulosas unidas en forma no covalente a las moléculas de celulosa, formando una extensa red. Agronómicamente, varias industrias dependen de los polisacáridos de la pared celular, particularmente para el procesamiento de alimentos. Sin embargo, asombrosamente, poco se sabe sobre su rol exacto en la pared celular de la planta. Esto se hace incluso más evidente si se considera que la composición de los polisacáridos de diferentes tipos de células y órganos difieren, y cambian en el tiempo, sugiriendo que los polisacáridos tienen una variedad de funciones específicas. Al completarse los proyectos de secuenciación genómica de plantas modelo y de importancia económica, se han obtenido progresos significativos en determinar las proteínas responsables de la síntesis de la pared celular, todo lo cual conduce a potenciales aplicaciones biotecnológicas.

Palabras clave: *Arabidopsis thaliana*, azúcar-nucleótido, pared celular, celulosa, aparato de Golgi, transportador.

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