The structure, function, and biosynthesis of plant cell wall pectic polysaccharides

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Abstract

Plant cell walls consist of carbohydrate, protein, and aromatic compounds and are essential to the proper growth and development of plants. The carbohydrate components make up ~90% of the primary wall, and are critical to wall function. There is a diversity of polysaccharides that make up the wall and that are classified as one of three types: cellulose, hemicellulose, or pectin. The pectins, which are most abundant in the plant primary cell walls and the middle lamellae, are a class of molecules defined by the presence of galacturonic acid. The pectic polysaccharides include the galacturonans (homogalacturonan, substituted galacturonans, and RG-II) and rhamnogalacturonan-I. GALacturonans have a backbone that consists of α-1,4-linked galacturonic acid. The identification of glycosyltransferases involved in pectin synthesis is essential to the study of cell wall function in plant growth and development and for maximizing the value and use of plant polysaccharides in industry and human health. A detailed synopsis of the existing literature on pectin structure, function, and biosynthesis is presented.

1. Introduction

The plant cell wall is a complex macromolecular structure that surrounds and protects the cell, and is a distinguishing characteristic of plants essential to their survival. As a consequence of limited mobility, plants are plastic in their ability to withstand a variety of harsh environmental conditions and to survive attack by pathogens and herbivores. The structure formed by the polysaccharides, proteins, aromatic, and aliphatic compounds of the cell wall enables plants to flourish in diverse environmental niches.

Cell wall structure is continually modified to accommodate the developmental stage and the environmental condition. The plant cell lays down the middle lamella and the primary wall during initial growth and expansion of the cell. In many cells, the wall is thickened and further strengthened by the addition of a secondary wall (Fig. 1). The primary wall is characterized by less relative cellulose and greater pectin compared to secondary walls. The primary wall is thought to contribute to wall structural integrity, cell adhesion, and signal transduction. The major fraction of primary wall non-cellulosic polysaccharides in the Type-I walls of dicot and non-graminaceous species are the pectic polysaccharides. It is the focus of this literature review to bring together the available knowledge of the fine structure, function, and biosynthesis of the pectic polysaccharides of the plant cell wall, with respect to plant growth and development.

2. Pectin structure

The pectic polysaccharides comprise a class of GalA-containing polysaccharides that are abundant in the plant cell wall; comprising as much as 30% of dicot, gymnosperm, and non-Poales monocot walls.1,2 The walls synthesized by the order Poales (formerly the Gramineae) and related orders contain considerably less pectin; approximately 10% by weight.3 It has been estimated that ~90% of the uronic acids in the wall derive from the GalA residues of pectic polysaccharides.4 The structural classes of the pectic polysaccharides include homogalacturonan (HG), xylogalacturonan (XGA), apioagalacturonan (AGA), rhamnogalacturonan II (RG-II), and rhamnogalacturonan I (RG-I).5 The fine structure of the pectic polysaccharides governs the biological role(s) of these molecules within the cell wall. Expanding our knowledge of how pectin structure is modified during growth and in response to environmental stimuli is essential to understanding the role of these biological molecules in plant biology.

2.1. Homogalacturonan

HG is a polymer of α-1,4-linked D-galacturonic acid (Fig. 2) that can account for greater than 60% of pectins in the plant cell wall.1 HG is abundant in potato (Solanum tuberosum) primary walls and,
accordihg to immunohistochemical analysis, is particularly dense
in the middle lamellae of this species.\textsuperscript{5} HG comprises at least
23\% of Arabidopsis (\textit{Arabidopsis thaliana}) leaf walls\textsuperscript{6} and
~10\% of sycamore suspension culture cell walls (\textit{Acer pseudoplatanus}).\textsuperscript{7}
The walls of fruits, such as tomato and mango, have up to 35\%\textsuperscript{8}
and ~52\% uronic acid,\textsuperscript{9} respectively. HG GalA residues may be
methyl-esterified at the C-6 carboxyl or acetylated at the O-2 or
O-3 (Fig. 2).\textsuperscript{1} The pattern and degree of methylesterification and
acetylation varies from source to source. Methylesterification is
hypothesized to be tightly regulated by the plant in a developmen-
tal and tissue-specific manner.\textsuperscript{10} For example, suspension-cultured
cotton HG was ~50\% methylated with non-random distribution.\textsuperscript{11}

The unmethylated C-6 of HG GalA residues is negatively
charged and may ionically interact with Ca\textsuperscript{2+} to form a stable gel
with other pectic molecules if >10 consecutive unmethyl-esterified
GalA residues are coordinated.\textsuperscript{12} The hypothesized in vivo struc-
ture of the HG–calcium complex is sometimes referred to as the
egg-box model (Fig. 3).\textsuperscript{12} The egg-box model describes the close
packing of HG that occurs upon Ca\textsuperscript{2+}-induced gelling, which ac-
counts for ~70\% of the pectic gel in the cell walls of plants.\textsuperscript{13} In vi-
tro, citrus peel pectin was used to demonstrate that a pectin gel
can be formed by addition of salts to pectin de-methylesterified
by orange peel pectinmethylesterase (PME). It was postulated that
the gel formation was mediated by cations in solution, hydrogen
bonding, and hydrophobic interactions.\textsuperscript{14} NMR spectroscopy of a
calcium pectate gel prepared from orange peel pectin established
that the HG backbone has a twofold helical structure (2\textsuperscript{1}), consis-
tent with the egg-box model; however, a small amount of the 3\textsuperscript{1}
helical structure also occurs naturally.\textsuperscript{13}

2.2. HG is covalently crosslinked to RG-I, RG-II, and possibly
other wall polymers

The backbone of HG is covalently linked to RG-I and RG-II, and is
also hypothesized to be covalently crosslinked to xylglucan (XG)
hemicellulose polysaccharides in muro. It has long been observed that pectic polymers are released from wall preparations by endopolygalacturonase (EPG) treatment that hydrolyzes the glycosidic bonds of the HG backbone to produce monomeric, dimeric, or oligomeric fragments. HG, RG-I, and RG-II polysaccharides fail to resolve independently by size exclusion chromatography prior to fragmentation by EPGase digestion. In soybean soluble polysaccharides, stretches of α-(1,4)-linked galacturonic acid were found flanked by RG-I fragments, providing evidence that HG and RG-I are directly and covalently connected through backbone residues. It has also been suggested that HG polysaccharides are linked to xyloglucan based on fragments of XG which were not readily solubilized from walls unless treated with EPG. Further support is provided by discovery of an XG diagnostic fragment, isopimeraose, that was released from the acidic or pectic fractions of driselase-digested, alkali-extracted walls of Arabidopsis, rose, sycamore, tomato, spinach, maize, and barley, suggesting a covalent-crosslink between pectin and a neutral polysaccharide, such as XG.

In vitro synthesis of HG with endogenous acceptors yields large polymers with a degree of polymerization (DP) of up to 150 residues that may reflect the size of polysaccharides present in muro. The endogenous acceptors in this study, however, were not exhaustively characterized, thus the results reported may represent longer chains than might be found in muro. In agreement with the hypothesis of long chain HG in pectin, HG isolated from apple, commercial beet pectin, and commercial citrus pectin were 21,000, 19,000, and 24,000 Da in size, which translates to approximately 72–100 GalA residues in length. Comparable HG domains isolated from dried citrus peel were between 17,000 and 20,600 Da in size, demonstrating that long chain HGs are found in the walls of these species. Reliable sources of EPG of high purity have made digestion of walls with EPG a popular method of cell wall solubilization, which prevents further characterization of HG domain chain length. The HG intra-RG-I linkers identified in soybean were found to be 4–10 residues in length, fragments much shorter than the previously characterized HG from citrus and beet walls. Because the fragments were isolated from soybean cotyledons, it is unknown if the structure extends to other species or other tissues. The detailed characterization of HG polysaccharide domains and linker structure will aid in the understanding of HG function in plant growth and development.

2.3. Substituted galacturonans: apiogalacturonan and xylogalacturonan

The α-apiose-substituted apiogalacturonan (AGA) is found in the walls of aquatic plants such as the duckweeds (Lemnaceae) and the marine seagrasses (Zosteraceae). Apiose residues are beta-2-linked, 3-linked, as well as 2- and 3-linked to single GalA residues of HG (Fig. 4A). The characterization of AGA by mild extraction of Lemna walls showed that the substitution of HG can also occur as apiose, a disaccharide of apiose (Api-f-1,3′-Api-f-1). The level of apiosylation of HG, assessed by the GalA to Api ratio, was observed to be 4 to 1 in Zosteraceae, to 4 to 5 in Lemnaceae. The content of AGA in plant walls appears to fluctuate widely from 0.2% to 20% of non-cellulosic polysaccharides in the dormant buds and the green fronds of giant duckweed, respectively. The abundance of AGA suggests a specifically important structural role in the wall framework of these water-born plants.

Xylogalacturonan (XGA) is HG substituted by α-xylene residues at the C-3 of GalA backbone residues (Fig. 4B). The characterized XGA in pectic extracts of the Zosteraceae marine seagrass consisted of HG substituted by a xylose disaccharide.

Figure 4. The substituted galacturonans xylogalacturonan and apiogalacturonan. Apiogalacturonan (A) is characterized by apiose and 3′-linked apiose at the 2 position of galacturonan backbone residues. Xylogalacturonan (B) is characterized by xylose and 2-linked xylobiose (not shown) at the 3 position of galacturonan backbone residues.
that is a ubiquitous component of plant walls making up 2.4. Substituted galacturonan: rhamnogalacturonan II

Xyl and by Xyl branched at the O-2 by another Xyl residue. species, is the galacturonan backbone substituted at the O-3 by polysaccharide structure, which has been observed in multiple soybean walls and closely related species. The most abundant XGA relatively minor component of the wall or to be a structure specific to observed previously in plant cell walls, it is likely to make up a relatively minor component of the wall or to be a structure specific to soybean walls and closely related species. The most abundant XGA polysaccharide structure, which has been observed in multiple species, is the galacturonan backbone substituted at the O-3 by Xyl and by Xyl branched at the O-2 by another Xyl residue.

2.4. Substituted galacturonan: rhamnogalacturonan II

Rhamnogalacturonan II (RG-II) is a substituted galacturonan that is a ubiquitous component of plant walls making up ~4% of suspension-cultured sycamore walls and ~8% of Arabidopsis leaf walls. An RG-II molecule is recognized as a stretch of HG backbone approximately seven to nine residues long with four well-defined side chains, designated A through D (Fig. 5). The structure of RG-II is highly complex with 12 different types of glycosyl residues, including the rare sugar species 2-aceric acid, 2-keto-3-deoxy-D-lyxo heptulosaric acid (Dha), and 2-keto-3-deoxy-D-manno octulosonic acid (Kdo). Despite its complexity, the conservation of RG-II structure across species, modifications shown in red text are those that are present in the RG-II of Pteridophyte and Lycophyte species. Adapted from O'Neill et al. (2004).

An RG-II molecule is recognized as a stretch of HG backbone to be a structure specific to soybean walls and closely related species. The most abundant XGA polysaccharide structure, which has been observed in multiple species, is the galacturonan backbone substituted at the O-3 by Xyl and by Xyl branched at the O-2 by another Xyl residue.

(Fig. 6). The three-dimensional conformation of RG-II sidechain A was found to be mostly stationary in solution, while sidechain B was dynamic. The stationary behavior of sidechain A may provide a binding surface for borate and also contribute directly to the mechanical stability of the RG-II dimer. The backbone of the structure of rhamnogalacturonan I (RG-I) has repeating units of \(-\alpha-D-GalpA-1,2-\alpha-D-Rhap-1,4-\beta-D-GalpA\) as characterized from suspension-cultured sycamore walls (A. pseudoplat-
The RG-I backbone and representative sidechains. The structure of RG-I with sidechains of α-(1,5)-L-arabinan, β-(1,4)-galactan, and Type-I arabinogalactan. The α-(1,5)-L-arabinan chains that originate from the RG-I backbone may be branched with long chains of mono- or di-meric α-arabinan or mono-, di-, or oligomeric branches of β-(1,3)-linked Gal. Type-II arabinogalactan may have branches of 6-linked or 3,6-linked galactose residues. Adapted from O’Neill et al. (2003).

Figure 7. The structure of RG-I backbone and representative sidechains. The structure of RG-I with sidechains of α-(1,5)-L-arabinan, β-(1,4)-galactan, and Type-I arabinogalactan. The α-(1,5)-L-arabinan and galactan side chains from 4-linked to approximately half of the rhamnose residues of the galactan chain that has branches of one or more Ara residues decorate RG-I galactan chains. Thus far, GlcA residues have not been found linked directly to the RG-I backbone and nor have they been found linked to RG-I arabinan.

The complexity of the pectic polysaccharides, and their conservation, to a greater or lesser degree, throughout the plant kingdom, infers specific and important biological functions in the plant cell wall.

3. A structural model of the primary cell wall

Current models of the primary plant cell wall structure are based on the hydrogen, covalent and ionic bonding between two or more structural components of the wall. To determine how the many described components of the wall come together as a complete functional wall in vivo is an objective of current cell wall research: how to discern how the matrix polysaccharides of the primary wall function within the frame-work of the cellulose-xylloglucan structural network? The structure and role of cellulose and hemicellulose in primary walls and the integration of known cellulose, hemicellulose, and pectin structure into a practical three-dimensional model of the primary wall are discussed in the following section.

3.1. Cellulose in primary walls

Cellulose is the foremost load bearing network of the primary and secondary wall. The percentage dry weight of cellulose in a dicot such as Arabidopsis ranges from 15% of leaf to 33% of stem walls. The walls of monocot grass species have approximately 6–10% cellulose in leaves and 20–40% in stems. Cellulose is a polymer of β-(1,4)-D-Glc residues that associate with other cellulose chains by hydrogen bonding and Van der Waals forces.

The cellulose chains of plant walls are synthesized at the plasma membrane by cellulose synthase complexes that contain multiple cellulose synthase (CesA) subunits which form a rosette structure. The rosettes consist of 6 globular CesA-containing complexes each of which synthesizes growing cellulose chains of 6–10 cellulose molecules, which are referred to as 2 nm fibers. Six of the 2-nm fibers then may associate to form microfibrils of approximately 36 glucan chains. The microfibrils average 30 nm in width, a size that may be visualized by spectroscopic methods. The cellulose chains of the primary wall were of low molecular weight compared...
to cellulose chains of the secondary wall.68,69 Cellulose chains may align in parallel (Type I) or antiparallel (Type II)70 orientation to each other. Only the Type I conformation is known to naturally occur in plants; however, concentrated alkaline treatments may cause Type II cellulose to form during harsh extraction procedures. The cellulose chains may form the Type Iα or Type Iβ conformation depending on the extent of staggering of the chains in relation to each other. Type-Iα and Type-Iβ are recognized by the triclinic or monoclinic unit cell, respectively, of the crystalline cellulose.70 The inter-conversion of Type Iα and Type Iβ allomers may be induced by mild alkali70 or by the bending of the cellulose chains,71 not unlike the reorienting that cellulose microfibrils undergo to run parallel with the surface of the plasma membrane after synthesis. It is also thought that the interaction of cellulose microfibrils with hemicelluloses may affect the ratio of Type Iα to Type Iβ cellulose.71 For example, the developing tracheid of the Japanese hinoki cypress (Caryota obtusa) formed greater amounts of the metastable Iα in the primary wall, while greater amounts of the stable Iβ were formed in the secondary wall.72 Primary wall cellulose microfibrils are highly crystalline and oriented parallel to the direction of elongation, contrary to the orientation found in secondary walls.73 The differences in the size, conformation, crystalline form, degree of crystallinity, and orientation of primary wall cellulose microfibrils are attributed to the stresses that cellulose microfibrils undergo during rapid cell expansion in the pectinaceous primary wall environment.69,72

3.2. Hemicellulose

The hemicelluloses are often described as those wall polymers that (1) are solubilized from the wall by alkaline solvents and (2) are β-(1,4)-linked pyranosyl residues that have the O-4 in the equatorial position.2 These are characteristics that result in a cellulose-like conformation and cause a tendency to hydrogen-bond to cellulose chains. Xylans, mannans, and xyloglucan fit this technical definition, but arabinogalactan is also considered a hemicellulose. The hemicelluloses are more abundant in secondary walls than in the primary walls of both dicots and monocot species. Monocot species have significantly more hemicellulose and less pectin than dicots, and also have mixed linkage glucans that make up a major proportion of monocot hemicellulose polysaccharides.73 Xylan polysaccharides comprise linear chains of β-(1,4)-o-Xylp residues and may be found as arabinoxylan (AX), glucuronoarabinoxylan (GAX), glucuronoxylan (GX), or the unsubstituted homoxylan (Fig. 8). Xylans also are decorated by acetyl groups at the O-2 or O-3 position.74 The arabinose residues of AX are primarily terminal residues linked to the 2-position of the xylose backbone in dicots and non-graminaceous species.3,75 Alternate forms of AX have

![Figure 8](Image)

**Figure 8.** The basic structure of xylan and xyloglucan. Glucuronoarabinoxylan (A) is β-(1,4)-o-xylan substituted by glucuronic acid at the O-2 and by arabinose at the O-2 and O-3. Xyloglucan (B) is β-(1,4)-o-glucan, like cellulose, but is substituted at the O-6 by xylose that may be further modified (for known identities of R, see Table 1). The most common pattern of XG backbone substitution is a regular pattern of 3-substituted glucose residues followed by a single free glucose.
been identified in rye wholemeal having arabinose O-2 and O-3 doubly substituted xyloses, substituted arabinoses, terminal xylose, and terminal galactose substitutions.76 These structures have not, thus far, been confirmed in the primary walls of dicots, suggesting that they are specialized features of cereal walls. AX and GAX are the most abundant xylans in the primary walls of dicots not, thus far, been confirmed in the primary walls of dicots, sug-

sperm suspension-cultured cell walls (4-O-methylglucuronosyl residues.77 Incorporation of 4-O-methylglucuronic acid to form GX occurs in dicot secondary walls, but is not generally found in the walls of monocots or the primary walls of dicots.77

The mannans include the galactomannans (GMs) and the galac-
toglucuronans (GGMs) that are structurally important compo-

nents of the cell wall as well as an important source of storage polysaccharides. Mannans have a similar three-dimensional struc-
ture to cellulose.78 The specific functions of mannans in the walls of monocots or the primary walls of dicots.77

The walls of the graminaceous monocots, or grasses, are more

doubly substituted xyloses, substituted arabinoses, terminal xy-

osyl residues.77 Incorporation of 4-

Table 1

<table>
<thead>
<tr>
<th>Sourcea</th>
<th>Structure of xyloglucan sidechain</th>
<th>Designationb</th>
<th>Reference</th>
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<tr>
<td>Acer pseudoplatanus</td>
<td>Glc82</td>
<td>G</td>
<td>82</td>
</tr>
<tr>
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<td>X</td>
<td>82</td>
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<td>82</td>
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</tr>
<tr>
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<tr>
<td>Lycopersicon esculentum</td>
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<td>T</td>
<td>93</td>
</tr>
<tr>
<td>Argenia spinosa</td>
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<td>94</td>
</tr>
<tr>
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<tr>
<td>Acer pseudoplatanus</td>
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<tr>
<td>Acer pseudoplatanus</td>
<td>T-β-D-Xylp-1,6-Glc82</td>
<td>C</td>
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</table>

Xyloglucan sidechains have variable structure depending on the source of the walls from which the xyloglucan is isolated.

- The species from which the xyloglucan was isolated.
- A single letter abbreviation used to designate specific XG structures.
- The contribution of the cellulose–xyloglucan network to the structural integrity of the plant cell wall has been studied for many years and the subtleties of the interaction of XG, in all of its forms, with cellulose in the primary wall continue to unravel. Strong binding of XG to cellulose has been observed. The strength of the interaction derives from the strong non-covalent and additive interaction of hydrogen bonds between XG molecules and cellulose microfibrils.18,96 XG is likely to interact with cellulose microfibrils as they are synthesized into the primary wall matrix, causing microfibrils of smaller diameter (less chains per fiber) than those found in secondary walls.97 The binding of XG to cellulose is also known to weaken cellulose networks,98 but increases the expansibility of such networks;99 mechanical properties suited to the expansion and stresses characteristic of conditions during primary wall synthesis.

The XG is bound to cellulose microfibrils in three distinct domains; (1) XG that is endogluccanase accessible, (2) XG that is sol-
ubilized by concentrated alkali, and (3) XG that is neither enzyme accessible nor alkali soluble.100 Molecules of XG have been microscopically visualized to coat and tether the cellulose microfi-

birs101,102 and by virtue of the repeating unit structure of XG polysaccharides, bring order to the cellulose network of the plant cell wall, and also has a role in supplying energy stores in the seeds of plants53,90 and as a signal molecule.15,88,91 Of relevance to this review is the reported evi-
dence for a linkage between XG and pectins.

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birs101,102 and by virtue of the repeating unit structure of XG polysaccharides, bring order to the cellulose network.103 It has also been observed that XG from different sources (i.e., with distinct populations of the different side chains) binds differently to the cellulose microfibrils.98 The sidechains of XG modulate the binding
of XG to cellulose and thus are important in regulating the mechanical properties of the cellulose–XG network.

3.3. The primary cell wall pectic network

The covalent crosslinking of the pectic polysaccharides HG, RG-I, and RG-II has been demonstrated repeatedly in the literature by the EPGase-dependent release of pectic polysaccharides from the wall. The available data suggest that the RG-I and RG-II backbones are continuous with the HG backbone, not that of RG-I sidechains, as suggested by Vincken et al. (2003). If the backbones of the pectins are continuous, the pectic network may be thought of as a macromolecular structure having specific domains of HG, RG-I, and RG-II, however, the arrangement of these domains in vivo is not known. The linkage of HG, RG-I, and RG-II through backbone glycosidic linkages is just one possible way in which the pectins are crosslinked. The pectic network is based on multiple levels of crosslinking that include, but are not limited to, backbone glycosidic linkages, calcium crosslinking, borate ester crosslinking, and covalent linkages to phenolic and possibly other compounds.

The HG domains of pectin may self-associate depending on the degree of methylesterification and thus the affinity of HG for calcium ions. RG-I has a unique backbone of alternating 2-linked Rha backbones of the pectins are continuous, the pectic network may make up >2% of the wall. The most abundant phenolic species found in the walls of Arabidopsis are para-coumaryl and feruloyl acids, which present the opportunity for crosslinking, though in most instances this has not been proven. Complexes of feruloylated-xylloglucan and a-p-coumaroylated-arabinoxylan have been isolated from bamboo shoot walls. If feruloylated α-(1,5)-linked arabinan and β-(1,4)-linked galactan were also isolated from spinach walls. The pulp of spruce and pine wood yielded lignin-carbohydrate complexes. Interestingly, a small relative amount of arabinose was also found in conjunction with the lignin–carbohydrate complexes, not associated with arabinoxylan based on carbohydrate linkage analysis. As such, the data implicate crosslinking via ferulic and/or p-coumaric esters to arabinogalactan, α-(1,5)-linked arabinan and β-(1,4)-linked galactan in these complexes, which is consistent with the structure of RG-I sidechains.

The structural proteins of the wall make up 2% of wall dry weight and comprise a variety of wall-associated proteins. The fraction remaining after endopolygalacturonase, endoglucanase, and alkali extraction of sycamore cell walls produced a residue from which further pectic polysaccharides were released only by protease treatment. The release of pectins by protease treatment is likely due to a linkage with the structural protein of the cell wall.

The arabinogalactan proteins (AGPs), proline-rich proteins (PRPs), glycine-rich proteins (GRPs), and wall-associated kinases (WAKs) are wall-associated proteins and are hypothesized to aid in the wall structural reinforcement and regulatory pathways. AGPs are highly glycosylated, similar to animal proteoglycan glycoproteins, and are localized to the cell surface by a glycoprophosphatidylinositol-lipid anchor at the plasma membrane. AGPs are typically glycosylated by arabinogalactan sidechains that are 3-linked-O-galactan branched at the C-6 by terminal galactose or arabinose residues (Type I arabinogalactan). Potential signaling and/or structural roles have yet to be determined for each specific AGP. The PRPs are wall-associated proteins that are secreted into the wall matrix wherein they ultimately become crosslinked, conferring strength to the wall. The expression and incorporation of PRPs into the wall can be induced by oxidative bursts that occur during responses to stress, suggesting that these proteins play a role in the defense responses of the plant. The expression of GRPs is also induced by stress. GRPs are hypothesized to interact with components of signaling pathways, and thus, may be regulators of wall structure.

WAKs have been implicated in cell elongation, morphogenesis, and defense against pathogens. Undoubtedly, wall-associated proteins serve complex and biological roles with regard to wall structure.
3.5. An ultrastructural model of the plant cell wall

Ultrastructural models of the plant cell wall have been formulated based on known cell wall structures in an attempt to integrate available knowledge into a functional structural wall model. The model presented in recent reviews of wall structure argues for two independent networks within the primary cell wall; the pectin–pectin and xyloglucan–cellulose network. In that model, the polysaccharides of the pectic-network, proteins, and phenolic compounds are organized independently around the framework of the cellulose–xyloglucan network. Such a model utilizes the well-established models of the pectin–pectin network and XG–cellulose network. However, there is now well-established evidence to show that a covalent pectin–pectin network exists through the linear backbones of the pectic polysaccharides and that the XG polysaccharides have a strong affinity for cellulose and that XG functions, in part, to coat and tether cellulose microfibrils to form the XG–cellulose networks. Furthermore, there is increasing evidence that pectin interacts, perhaps covalently with hemicellulose such as XG or xylan. Realistic wall models, therefore, must integrate the pectic network, the cellulose xyloglucan network and the available knowledge of other wall structural components that have been characterized. A revised wall model that better takes the current structure data into account, would demonstrate the highly crosslinked wall wherein pectin–pectin, pectin–XG, pectin–phenolics, pectin–protein, and XG–cellulose networks provide a cohesive wall network.

4. Function of pectic polysaccharides

The plant cell wall has a functional role in plant growth and development, by contributing to structural integrity, cell adhesion, and mediation of defense responses. The specific roles of pectic polysaccharides in these processes are being elucidated. The plant cell modulates wall structural character in response to growth, differentiation, and environmental stimuli. HG, RG-I, and RG-II are structurally diverse polysaccharides that contribute to primary wall function with regard to cell strength, cell adhesion, stomatal function, and defense response.

4.1. HG–calcium complexes contribute to wall strength

Calcium crosslinking of HG contributes to wall strength by bringing blocks of unmethylated HG chains into a tightly packed conformation that is dependent on three characteristics: the intramolecular conformation of HG, the charge separation between two GaLA molecules in a HG chain, and the efficiency with which HG chains pack together. The extent and pattern of methyl-esterification of HG directly affects the affinity of HG for calcium cations involved in the gelation of HG chains. A decline in wall expansibility and an increase in wall stiffening have been correlated with a decrease in arabinian and galactan RG-I sidechains and an increase in HG–calcium complexes. In bean pods (Phaseolus vulgaris), RG-I neutral sugar sidechains and HG steadily increased during exponential growth and cell expansion. At maturation, the arabanian and galactan were degraded, while the HG continued to accumulate forming HG–calcium complexes. The loss of RG-I sidechains coincided with de-methylation of the pectic component, facilitating HG–calcium complexation. In this study, in addition to tracking the wall polymers at five stages in pea pod development, the enzyme activities of α-arabinase and β-galactanase, pectinmethylesterase (PME), polygalacturonase (PG), and peroxidase (POD) were assayed. Dramatic changes in enzyme activity were observed in fully mature (24–55 days after flowering) and senescing (>55 days after flowering) pea pods. The activities of α-arabinase and β-galactanase and PME gradually increased up to the fully mature stage; thereafter β-galactanase and PME dramatically increased in senescing pods. The data suggest that the loss of RG-I sidechains in combination with the de-methylation of HG, but not the degradation of HG, contributes to the locking of wall components. In soybean, glycerinated hollow cylinders (GHCs) isolated from hypocotyls were used as a tool to study the effect of Ca2+ on wall tension and wall expansibility. Addition of a calcium chelator to the system dramatically increased wall expansibility, a response that ceased with addition of calcium. The calcium-induced wall stiffening may play a role in decreased wall expansibility and increased strength.

The transgenic expression of EPG in apple, tobacco, and Arabidopsis indicate that HG–calcium complexes likely play a role in wall strengthening and affect wall expansibility. Transgenic plant lines expressing polygalacturonase (PG) have been produced in apple (Mus domesticus), tobacco, and Arabidopsis, in order to study the changes in wall structure and the developmental abnormalities caused by in vivo pectin degradation. The HG extracted from PG-expressing apple leaf walls was reduced in content and molecular weight. In addition, wall weakening contributed to epidermal tearing of the stomatal guard cells in the apple leaves. Tobacco plants expressing the Aspergillus niger endopolygalacturonase-II (AnPG-II) had a dwarfed phenotype and a general weakening of walls that were unable to maintain cell shape and size against the force of turgor pressure.

4.2. RG-II borate complexes contribute to wall strength

Boron is hypothesized to function specifically in membrane proteins, plant reproduction, nitrogen fixation, and plant cell wall strengthening. Boron is directly involved in the reversible dimerization of two RG-II molecules that play a critical role in the expansive strength of the plant cell wall and has a specialized role in meristematic and reproductive systems of the plant. Symptoms of boron deficiency in plants such as slowed root growth, degeneration of new growth, and degeneration of meristematic regions and reproductive organs illustrate the importance of wall borate crosslinking. The Arabidopsis bor1-1 (high boron requiring) mutant is perpetually boron deficient, and bor1-1 plants are dwarfed with stems that fail to elongate and have a loss of apical dominance. The Arabidopsis mur1-1 mutant is morphologically similar to the bor1-1 mutant, but is deficient in the production of l-fucose, an essential component of RG-II structure caused by a lesion in GDP-manannose-4,6-dehydratase, an enzyme that synthesizes the substrate for addition of fucose into wall polysaccharides; GDP-l-fucose. Normal plants have ~95% of the RG-II molecules in the dimer form, whereas mur1-1 has only ~50% of RG-II molecules in the dimer form. The l-fucose in sidechains A and B of mur1-1 RG-II is replaced by l-galactose, but this intriguing in vivo substituion of wall borate crosslinking. The transgenic expression of EPG in apple, tobacco, and Arabidopsis has a strong affinity for cellulose and that XG to show that a covalent pectin–pectin network exists through the highly crosslinked wall wherein pectin–pectin, pectin–XG, pectin–phenolics, pectin–protein, and XG–cellulose networks provide a cohesive wall network.

The first definitive proof of a boron requirement in plants was in 1923. A number of reviews have documented the progression of boron research in plant biology through the years. Boron directly involved in the reversible dimerization of two RG-II molecules that play a critical role in the expansive strength of the plant cell wall and has a specialized role in meristematic and reproductive systems of the plant. Symptoms of boron deficiency in plants such as slowed root growth, degeneration of new growth, and degeneration of meristematic regions and reproductive organs illustrate the importance of wall borate crosslinking. The Arabidopsis bor1-1 (high boron requiring) mutant is perpetually boron deficient, and bor1-1 plants are dwarfed with stems that fail to elongate and have a loss of apical dominance. The Arabidopsis mur1-1 mutant is morphologically similar to the bor1-1 mutant, but is deficient in the production of l-fucose, an essential component of RG-II structure caused by a lesion in GDP-manannose-4,6-dehydratase, an enzyme that synthesizes the substrate for addition of fucose into wall polysaccharides; GDP-l-fucose. Normal plants have ~95% of the RG-II molecules in the dimer form, whereas mur1-1 has only ~50% of RG-II molecules in the dimer form. The l-fucose in sidechains A and B of mur1-1 RG-II is replaced by l-galactose, but this intriguing in vivo substitution only partially rescues RG-II dimerization in the mutant. The nolac-H18 mutant (non-organogenic callus with loosely attached cells) has a T-DNA insertion in the NgpGUTI (N. plumbaginifolia glucuronosyltransferase) gene in tobacco. The NgpGUTI mutant is deficient in a putative glucuronosyltransferase that has homology to animal exostosin glucuronosyltransferases, and fails to incorporate a GlcA residue, and the corresponding Gal branch [α-1-Galp-(1,2)-β-D-GlcP-(1,4)] into RG-II sidechain A. The coordination of boron by RG-II is dependent on the specific conformation of RG-II sidechain A, which is compromised in the mur1-1 and nolac mutants. The consequence of RG-II–boron complex disruption is a lack of wall expansibility that results in plants that have dwarfed stature, compromised cell adhesion, and defects in
reproductive tissue function.\textsuperscript{138,140} The disruption of meristematic regions in \textit{nolac} shoots was similar to that found in boron-deficient pumpkin plants (\textit{Cucurbita moschata})\textsuperscript{138,141} and is hypothesized to be a factor in \textit{nolac} and boron-deficient pumpkin meristematic regions.

4.3. HG–calcium complexes and RG-I sidechains contribute to cell adhesion

Cellular adhesion in plant tissues is mediated by the extracellular matrix or pectic polysaccharides of the plant cell wall. Cell adhesion is reduced in mutants that have insufficient: HG–calcium complexes, branched RG-I polysaccharides, or RG-II dimerization. The colorless non-ripening mutant (\textit{Cnr}), isolated from plantings of commercial tomato (\textit{Lycopersicon esculentum}), grows similar to wild-type fruits up to the mature-green stage, but does not ripen. When wild-type fruits are ripe-red, \textit{Cnr} tomatoes are yellow with white flesh. The mealy texture of \textit{cnr} tomato flesh\textsuperscript{142} and the large intercellular spaces in \textit{Cnr} pericarp compared to WT\textsuperscript{143} were an indication of altered cell adhesion.\textsuperscript{142} Decreased calcium crosslinking of pectins in the \textit{Cnr} tomato was suggested by increased solubility in water and an overall decrease in chelator soluble pectins, which was confirmed by EELs spectroscopy.\textsuperscript{143} The glycosyl residue composition of \textit{Cnr} mature-green walls showed decreases in Rha, Xyl, and uronic acids with increases in galactose compared to WT. Antibody that recognizes a region of unesterified homogalacturonan showed dramatically reduced binding to \textit{Cnr} middle lamellae compared to WT.\textsuperscript{143} Interestingly, antibody that binds to \(\alpha\)-(1,5)-arabinan was bound to cytosolic vesicles but not in the walls of \textit{Cnr}, suggesting that arabinan is not being incorporated into the walls of these plants. The changes in cell walls of the \textit{Cnr} tomato mutant correlate with reduced calcium-complexed HG and a lack of wall arabinan incorporation, which implicates these pectic polysaccharides in cell adhesion.

4.4. HG–calcium complexes and RG-I arabinan affect stomatal function

Guard cells were used as a model for cell wall architecture based on the turgor-driven cycle of expansion (opening) and contraction (closing) in stomata that necessitate wall strength as well as expansibility. Epidermal strips (\textit{Commelina communis}) were used to study the changes in stomatal opening in response to wall manipulation by purified wall degrading enzymes.\textsuperscript{144} The tremendous turgor pressure that builds up during stomatal opening (up to 5 MPa) causes a volume expansion of each guard cell of up to 70%.\textsuperscript{145} Guard cells were induced to open in epidermal peels by fusitocin and induced to close by ABA, such that normal opening of the stomata was able to be measured and tested after treatment with an assortment of wall degrading enzymes.\textsuperscript{144} Surprisingly, only pectinolytic enzymes and a feruloyl esterase had an appreciable affect on pore opening. Degradation of cellulose and hemicellulose by cellulase and xylanase enzymes had no affect on fusitocin-induced stomatal opening. Endoarabinase, that specifically hydrolyzes \(\alpha\)-(1,5)-l-arabinan, completely blocked pore opening, while feruloyl esterase, Faea, inhibited but did not stop guard cell pore opening. Open stomata treated with arabinase and induced to close with either ABA or mannitol failed to close, suggesting that the walls were ‘locked’ into place. Interestingly, a combination of endopolygalacturonase (EPG) and pectinmethylesterase (PME) produced much more widely open pores than either treatment alone. The addition of EPG/PME treatment after arabinase-induced wall locking then allowed the stomata to ‘unlock’ and close. The locking of guard cell walls is also reversed by treatment with strong calcium chelators. Based on these experiments, it is hypothesized that in vivo, the feruloylated RG-I arabinans form ester linkages either to other feruloylated RG-I arabinans, or to other wall molecules, providing a mechanism for spatial buffering of HG polymers, and thus, by not allowing the HG chains to come into close proximity the HG is inhibited from locking into place by calcium crosslinking (Fig. 9).\textsuperscript{144}

4.5. Pectic polysaccharides mediate defense; a barrier and signaling mechanism

The wall provides a physical barrier that pathogens must break down in order to gain entry into the cell to establish infection. Both bacteria and fungal phytopathogenic organisms produce wall hydrolytic enzymes as essential virulence factors that allow entry into plant cells. Wall fragments produced by hydrolytic enzymes may subsequently become signaling molecules to the plant of an impending infection. An early physiological response by the plant may minimize, or end, an attack by phytopathogenic organisms. The action of oligosaccharides as signaling molecules, or oligosacharins, in plant defense is well documented.\textsuperscript{146–148} Chitin, chitosan, \(\beta\)-glucan and oligogalacturonides are known to be oligosaccharide elicitors of defense responses.\textsuperscript{148} Oligogalacturonides or OGAs, derived from pectic HG, have specialized functions beyond those of structural components in the elicitation of phytoalexins (antibiotic) and reactive oxygen species (ROS).\textsuperscript{149,150} Treatment of sodium polypectate or cowpea (\textit{Vigna unguiculata}) walls with PC produced OGAs that were elicitors.\textsuperscript{150,152} The elicitor activity was not affected by the specific PG or if the resulting OGA was terminally 4,5-unsaturated, but did depend on an OGA DP of 9–16.\textsuperscript{153,154} Phytoalexin elicitor-active OGAs cause changes in gene expression including the induction of genes in a pathway for coumarin phytoalexin biosynthesis, demonstrating that OGA elicitor activity works to induce defense response pathways.\textsuperscript{155,156} PG-expressing tobacco plants were also constitutively upregulated in defense responses as a result of wall architectural changes brought about by the fragmentation of HG by PG enzymes.\textsuperscript{157}

The action of OGAs is thought to function primarily in defense, but has also been shown to stimulate morphogenesis in specific systems where studied. For example, calcium-dependent induction of flowering shoot growth occurred in tobacco thin-cell layer explants when sycamore-derived OGAs of 12–14 residues in length were applied.\textsuperscript{158,159} In addition, specific Arabidopsis apoplastic resident proteins were identified that may be affectors of the biological responses elicited by OGAs.\textsuperscript{160} These proteins included a polygalacturonase-inhibiting protein, two lectins, an alpha-glucosidase, an alpha-xilosidase, and a leucine-rich repeat protein. The functions of the specific responses elicited by OGAs, thus, include but are not limited to, defense.

5. Biosynthesis of pectic wall polymers

Pectin biosynthetic glycosyltransferase (GT) enzymes require specific nucleotide-sugar substrates and acceptors for activity. The current model of pectin biosynthesis predicts a Golgi luminal GT active site and nucleotide-sugar substrate, which is thought to be imported into the Golgi lumen by membrane spanning protein transporters or alternatively synthesized within the Golgi lumen.\textsuperscript{161–164} Here the pertinent historical and progressive research in the synthesis of nucleotide sugars, glycosyltransferases, pectimethyltransferases, and O-acetyltransferases contributing to the construction of plant cell wall pectic polysaccharides is summarized.

5.1. Subcellular localization of pectin biosynthetic glycosyltransferases

The pectic polysaccharides are synthesized in the Golgi apparatus of the plant cell, sorted to vesicular compartments, and
secreted to the apoplastic space. The Golgi apparatus is a complex organelle made up of stacks of flattened vesicles containing proteins geared to the sorting and processing of cargo in specific Golgi vesicles. A Golgi stack has four defined regions: the cis, medial, trans Golgi, and the trans-Golgi network (Fig. 10). Cargo intended for secretion is transported through the endoplasmic reticulum (ER) to the cis face of the Golgi body. Wall polysaccharides are continually synthesized and moved through Golgi stacks from the cis face to the trans face of the Golgi where they are sorted and packaged into the vesicles of the trans-Golgi network (TGN) for transport to the plasma membrane, the cell plate of dividing cells or the vacuole.

In plants, transport from the ER to the Golgi may occur via clathrin coated COPII vesicles or by direct connections that physically link ER and Golgi membranes. Movement through the Golgi is thought to occur via two complementary mechanisms as described by the vesicle shuttle model and the cisternae maturation model. The vesicle shuttle model proposes that each cisterna is stable and functionally specific, while the cargo is transported stack to stack by vesicular shuttles. The cisternae maturation model proposes that the resident processing enzymes may be regulated by retrograde transport of enzymes in COPI vesicles while cargo is moved in bulk within the cisternae; from cis to trans Golgi. Transport in the secretory system is known to involve a host of proteins that regulate and orchestrate the initiation, formation, and direction of secretory vesicle movement associated with the Golgi. Vesicle trafficking in the Golgi is a complex process for which there are many valuable reviews.

The synthesis of polysaccharides in the Golgi and their movement to the plasma membrane have been tracked by histochemical analysis of plant cells. Antibody probes that recognize epitopes of carrot extensin-1, sycamore deesterified RG-I/HG, and purified sycamore XG had differential binding to specific Golgi stacks. RG-I epitopes appeared in the cis and medial Golgi and the XG epitopes were present in the medial, trans, and TGN, while the extensin

![Figure 9. The model of arabinan and HG–calcium complexes based on the behavior of stomatal pore openings in response to wall degrading enzymes. The α-(1,5)-α-arabinan of RG-I may be esterified to ferulic acids that are thought to dimerize providing a linkage between RG-I molecules (A). According to Jones et al. (2003), the arabinan provides a mechanism for the inhibition of HG calcium complexes (yellow/white diamonds (GalA/HG); light yellow circles (calcium), which contribute to wall stiffening (B). Removal of ferulic acid crosslinks by an α-(1,5)-arabinan-specific endoarabinase or ferulic acid esterase promotes the formation of HG–calcium complexes and wall 'locking’.]

![Figure 10. The plant Golgi apparatus. Each Golgi apparatus is composed of flattened vesicles within which glycoproteins and wall polysaccharides (cargo-colored squares) are synthesized largely by membrane bound glycosyltransferases (colored sticks). The direction of cargo flow is depicted by the large black arrows. The Golgi cargo is received from the endoplasmic reticulum (ER) at the cis-face of the Golgi and traverses to the medial, trans, and the trans-Golgi network (TGN) for further processing and export.](http://example.com/golgi_diagram.png)
epitopes were observed throughout the Golgi. The spatially
distinct localization of these Golgi products demonstrated that gly-
coproteins and wall polysaccharides are both simultaneously syn-
thesized in the Golgi, but appear to have different sorting and
export programs. The differential localization of RG-I, XG, and
extension epitopes also suggest the separation of glycoprotein, as
well as acidic and neutral polysaccharide biosynthetic machinery,
within the Golgi apparatus.

The localization of pectic polysaccharide epitopes within the
cisternae of the Golgi sets the stage for biochemical localization of
the activities necessary for pectin biosynthesis. Multiple activi-
ties are necessary for the synthesis of HG, RG-I, and RG-II (Table
2) and many glycosyltransferases are required for the complete
synthesis of pectic polysaccharides. The subcellular local-
ization of pectin biosynthetic HG:α1,4-GalAT, RG-I:β1,4-GalT, and
α1,5-AraT has been investigated. HG:GalAT activity was de-
tected exclusively in Golgi-enriched fractions based on the correla-
tion with latent UDPase activity, a Golgi-resident activity. Pea
homogenates were subjected to discontinuous sucrose density
gradient, which separates membrane fractions derived from ER, Golgi, and the plasma membrane according to relative
density. Proteinase K treatment of GalAT activity-positive Golgi
fractions, with and without dissolution of membranes by Triton
X-100 detergent, demonstrated that GalAT activity is preserved
from ER, Golgi, and the plasma membrane according to relative
density. Proteinase K treatment of GalAT activity-positive Golgi
fractions, with and without dissolution of membranes by Triton
X-100 detergent, demonstrated that GalAT activity is preserved
in the presence of Proteinase K without Triton X-100 but is lost
after addition of detergent, a situation that can exist only if the ac-
tive site of the HG:GalAT is located in the lumen of the Golgi, and
thus is protected from Proteinase K degradation. Pectin biosyn-
thetic β1,4-GalAT activity in potato stems (S. tuberosum) and arar-
binosyltransferase (AraT) activity in mung bean (Vigna radiata) were
also localized specifically to the Golgi, suggesting that the pectin
biosynthetic machinery is likely located within the Golgi
apparatus.

5.2. Nucleotide-sugar interconverting enzymes in pectin
biosynthesis

Diverse biosynthetic pathways lead to the synthesis of the spe-
cific nucleotide-sugars required for plant pectin biosynthesis
(Fig. 11). Progress has been rapid in the elucidation of the A. thali-
ona nucleotide-sugar interconverting pathways due to the comple-
tion of the Arabidopsis genome sequence. Nucleotide-sugars may be
formed via salvage pathways from sugars recycled from the wall
polysaccharides or from sugars supplied to cultured cells. Such
nucleotide-sugars, or primary sugar phosphates derived directly from photosynthesis metabolism, are converted into a diverse array of sugar donor molecules by the nucleotide-sugar
interconverting enzymes (NIEs) (Table 3). For this discussion, the advances in Arabidopsis NIE discovery will be covered as a
means to condense the material currently existing in the literature
in reference to plant and bacterial NIEs. Nucleotide-sugars are sub-
strates for the enzyme-catalyzed transfer of a sugar group to an
acceptor molecule in polysaccharide biosynthesis. If the appropri-
ate nucleotide-sugar is not synthesized in the plant, synthesis is
hindered. Nucleotide-sugars are supplied to wall biosynthetic gly-
cosyltransferases by NIEs that regulate wall polysaccharide biosyn-
thesis and are themselves frequently regulated by elements of the
NIE pathway. Evidence of NIE regulation of nucleotide-sugar
availability is observed in the biological shift from primary wall to
secondary wall synthesis: the abundance of nucleotide-sugars and
their precursors is coordinately shifted to reflect an up-regulation
in hemicellulose and cellulose nucleotide-sugar substrates and a
down-regulation in pectic polysaccharide nucleotide-sugar
substrates. Arabidopsis mutants have been isolated that demon-
strate the role of NIEs in pectin biosynthesis. The nucleotide-sugar
interconverting pathway mutant, murr1, showed disrupted RG-II
synthesis, stemming from a lesion in a GDP-α-mannose-4,6-dehydro-
genase gene (GMD1). GMD1 is a key component in the synthesis of
gDP-fucose that is necessary for the correct synthesis of cell wall
RG-II and of fucosylated xyloglucans. UDP-α-4-glucose epimerase
(UGE) catalyzes the epimerization of UDP-α-Glc to UDP-α-Gal,
which has an effect on the specific incorporation of Gal onto XG
sidechains, Type-II arabino-galactan (β-1,6-galactan) and to a lesser
extent RG-I. The Arabidopsis root hair deficient mutants (rh1 1,
reb1-1/rh1d1-2, reb1-2/rh1d1-3, and rh1d4-1) are deficient in UGE4,
lack root hairs, and show weakened swollen walls of root tricho-
blast cells. Monoclonal antibodies specific to fucosylated XG (CCRC M1) and fucosylated AGPs (CCRC M7) showed an
absence of CCRC M1 label in rhd root sections and a clear reduction in CCRC-M7 label. In addition, binding of the AGP-specific
monoclonal antibodies, JIM14 and LM2, was reduced in trichoblast
cells, indicating that the specific and dramatic alterations were
brought about by a mutation in the NIE UGE4.

Evidence for NIE involvement in, and regulation of, wall biosyn-
thesis is provided by biochemical and kinetic data on the activities
of specific NIEs that are themselves regulated by components of
the nucleotidic-sugar interconversion pathway. UDP-α-Xyl is syn-
thesized by the decarboxylation of UDP-α-GlcA by UDP-α-Xyl
synthase (UXS), and UDP-α-Xyl is in turn utilized for the synthesis of UDP-α-Ara. Representatives of the UXS gene family were
expressed in Escherichia coli. The product of UXS (UDP-Xyl)
down-regulates upstream components of the nucleotide-sugar
interconverting pathway by negative feedback: UDP-Glc dehydro-
genase and UDP-Glc pyrophosphorylase are strongly inhibited by
UDP-Xyl. The activity of UXS is itself down-regulated by UDP-
Xyl, UMP, UDP, and UTP. The activity of an additional NIE is also
regulated by other products of the nucleotide-sugar interconver-
ting pathway. The UDP-Api/UDP-Xyl synthase (AXS) synthesizes
UDP-Api or UDP-Xyl from UDP-GlcA, depending on reaction condi-
tions. AXS activity is inhibited by UDP-GalA by as much as 69%. It
is proposed that UDP-GalA is a regulator of AXS in vivo. Rham-
nose biosynthesis (RHM) is responsible for the synthesis of UDP-α-Rha from UDP-α-Glc by three distinct activities; UDP-α-gluc-
ose-4,6-dehydratase, UDP-4-keto-6-deoxy-α-glucose-3,5-epimerase,
and UDP-4-keto-α-rhamnose 4-keto-reductase. The activity of RHM is inhibited by UDP-Rha, UDP-Xyl, and UMP, and thus, the levels of UDP-Glc and UDP-Rha in the cell are regulated by the concentrations of these nucleotide-sugars. Regulation of
NIEs in plant cells by negative feedback inhibition provides a
mechanism for control of wall polysaccharide biosynthesis.

5.3. HG glycosyltransferases

The HG backbone is a polymer of α1,4-linked GalA residues and is
proposed to require several α1,4-GalATs (HG:GalATs) to synthesize
the entire complement of HG required throughout plant develop-
ment (Table 2). HG:GalATs specifically catalyze the transfer of
α-GalA from UDP-α-GalA onto a growing stretch of HG (E.C.
2.4.1.43) via a lumenally facing HG:GalAT catalytic domain. GAUT1, the only functionally proven HG:GalAT, has been shown to
be Golgi localized, and the other GAUT1-related gene family
members are also predicted to be Golgi-localized Type-II membrane
proteins. The activity of plant HG:GalAT was first critically evalu-
ated in pea. Unmethylated UDP-α-GalA was the preferred nucleo-
tide-sugar substrate for elongation of endogenous acceptors in
particulate membrane fractions of mung bean (V. radiata). HG:
GalAT activity has also been characterized in tomato (L. esculentum),
tobacco (Nicotiana tabacum), azuki bean (Vigna angularis), pea (P. sativum), petunia (Petunia axillaris), pumpkin (C. moschata), and Arabidopsis (A. thaliana).
The soluble HG:GalAT activity from tobacco microsomal
membrane particulate preparations catalyzed the transfer of GaIA
### Table 2
Predicted glycosyltransferases required for pectin biosynthesis\(^{a,b}\)

<table>
<thead>
<tr>
<th>Glycosyltransferase</th>
<th>Parent polymer</th>
<th>Enzyme acceptor</th>
<th>Structure reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG glycosyltransferases</td>
<td>HG/RG-II</td>
<td>(\alpha)-Galp(-\alpha(1\rightarrow4)\alpha)-Galp(-\alpha(1\rightarrow))</td>
<td>180</td>
</tr>
<tr>
<td>RG-I glycosyltransferases</td>
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<td>(\alpha)-Rhap(-\alpha(1\rightarrow4)\alpha)-Galp(-\alpha(1\rightarrow))</td>
<td>180,190</td>
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<tr>
<td>RG-I glycosyltransferases</td>
<td>RG-III</td>
<td>(\alpha)-Galp(-\alpha(1\rightarrow2)\cdot\alpha)-Rhap(-\alpha(1\rightarrow))</td>
<td>19</td>
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<tr>
<td>RG-I glycosyltransferases</td>
<td>RG-I</td>
<td>(\alpha)-Rhap(-\alpha(1\rightarrow4)\alpha)-Galp(-\alpha(1\rightarrow))</td>
<td>48,44,180</td>
</tr>
<tr>
<td>RG-I glycosyltransferases</td>
<td>RG-II</td>
<td>(\alpha)-Galp(-\alpha(1\rightarrow2)\cdot\alpha)-Rhap(-\alpha(1\rightarrow))</td>
<td>19</td>
</tr>
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<td>(\alpha)-Rhap(-\alpha(1\rightarrow4)\alpha)-Galp(-\alpha(1\rightarrow))</td>
<td>18,180,30</td>
</tr>
<tr>
<td>RG-I glycosyltransferases</td>
<td>RG-II</td>
<td>(\alpha)-Galp(-\alpha(1\rightarrow4)\cdot\alpha)-Rhap(-\alpha(1\rightarrow))</td>
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<td>185,53</td>
</tr>
</tbody>
</table>

a: unknown anomeric configuration or attached glycolyl residue.

b: The glycosyltransferases necessary for the synthesis of homogalacturonan (HG), rhamnogalacturonan-I (RG-I), xylogalacturonan (XGA), apioagalacturonan (AGA), and rhamnogalacturonan-II (RG-II) are listed based on the current understanding of pectic polysaccharide structure according to the corresponding structure references.

c: Multiple GalATs for synthesis of HG, XGA, AGA, and RG-II.

from UDP-\(\alpha\)-GalA preferentially to the non-reducing end of OGA acceptors of DP 10 or greater.\(^21,23\) The products formed were of an \(\alpha-(1,4)\)-configuration as demonstrated by the endo- or exopolygalacturonase degradation of the reaction products to yield GalA.\(^21\) The tobacco-solubilized enzyme, under the low relative UDP-GalA conditions used, added only single GalA residues in a non-processive manner.\(^21\) However, the pumpkin detergent-permeabilized GalAT activity, under higher relative UDP-GalA concentrations, catalyzed the addition of up to 5 GalA residues,\(^23\) while petunia-solubilized GalAT activity added up to 27 GalA residues,\(^23\) with the number of residues added being dependent on the concentration of UDP-GalA used. The bulk of the in vitro HG:GalAT data from multiple sources strongly suggests that the characterized HG:GalATs are not processive.

The first gene encoding an HG:GalAT was isolated from Arabidopsis suspension culture cells.\(^22\) LC–MS/MS of trypsin-digested partially purified solubilized Arabidopsis GalAT active fractions identified two proteins in a NCBI BLAST (http://www.ncbi.nlm.nih.gov/) of protein sequences corresponding to GAUT1 (At1g61130) and GAUT7 (At2g38650), members of the ga\textit{lactu}ronosyl\textit{t}ransferase1 (GAUT1)-related gene family. Anti-GAUT1 polyclonal antibodies were able to immunoadsorb GalAT activity from the partially purified Arabidopsis solubilized GalAT enzyme preparations, providing evidence that the GAUT1 gene encodes a pectin biosynthetic HG:GalAT.\(^22\)

The open reading frames of GAUT1 and GAUT7 were amplified from Arabidopsis RNA and truncated to remove the transmembrane domain for expression in human embryonic kidney (HEK293) cells. A \textit{Trypanosoma cruzi} mannosidase signal sequence and C-terminal HA epitope tag were engineered onto GAUT1 and GAUT7 allowing the recombinant protein to be secreted directly to the culture medium. The truncated GAUT1 and GAUT7 proteins were recovered from the HEK cell medium by immunoprecipitation with anti-HA antibodies from the cell medium. GAUT1, but not GAUT7, immunoprecipitates yielded incorporation of \([\text{\textsuperscript{14}C}]\)GalA into discreetly sized OGA acceptors when incubated with UDP-[\text{\textsuperscript{14}C}]GalA. GAUT1 catalyzed elongation of OGA acceptors in an \(\alpha-(1,4)\)-configuration; an activity consistent with a role of GAUT1 as an HG:GalAT in pectin biosynthesis.

The GAUT1 and GAUT7 amino acid sequences are part of a distinct subfamily, which includes 15 GAUT genes and 10 GATL genes, of the CAZy database (http://www.cazy.org/)\(^{24}\) GT8 family. The GAUT1-related family is defined by a C-terminal amino acid motif common to both the GAUT and GATL proteins, but that is not a characteristic of other GT8 member proteins: \([\text{\textsuperscript{14}C}]\)GalA into discreetly sized OGA acceptors when incubated with UDP-[\text{\textsuperscript{14}C}]GalA. GAUT1 catalyzed elongation of OGA acceptors in an \(\alpha-(1,4)\)-configuration; an activity consistent with a role of GAUT1 as an HG:GalAT in pectin biosynthesis.

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Further study of this gene family may lead to the discovery of many unique GalAT activities. In addition, many new structures may be identified in the process of defining the expression patterns.

**Figure 11.** The plant nucleotide-sugar interconverting pathways. Species of nucleotide-sugars created from UDP-\(\alpha\)-Glc (A) and myo-inositol (B) in an alternate pathway. The proposed mechanism of plant CMP-\(\alpha\)-Kdo (C) and GDP-\(\alpha\)-Fuc synthesis (D).
and the specific linkages catalyzed by the genes of the GAUT1-related gene family.

A subfraction of HG is composed of XGA; HG decorated with xylose residues at the O-3 of backbone GalA residues.29 A xylosyltransferase in Arabidopsis was identified that is reported to be an XGA xylosyltransferase.241 This would be the first glycosyltransferase identified in the synthesis specifically of XGA.

5.4. Pectin methyltransferase

The modification of pectic polysaccharides by addition of methyl groups at the C-6 carboxyl group, or acetyl groups at the O-2 or O-3 of GalA residues is regulated in a developmental manner.242 Pectin methyltransferases (PMTs) act specifically on pectic polysaccharides during synthesis in the Golgi apparatus. Pectins are secreted in a highly methylesterified form.243,244 After the deposition of pectins in the apoplast, pectic methylesterases (PMEs) selectively remove the methyl groups. The extent and pattern of methylation of pectic polysaccharides affect the functional characteristics of the pectic polysaccharides,245 for example, by altering the affinity of pectins for calcium ions and altering the accessibility of pectins to wall hydrolases.16 The pectic polysaccharides are methylated by PMTs that catalyze the transfer of a methyl group from the donor S-adenosyl-methionine (SAM) to the target pectic polysaccharide.229,246,247 The import of SAM into the Golgi lumen is necessary for the methylation of HG248 where PMT activities have been localized.249–251 The catalytic activity of PMTs has been detected and partially characterized in the extracts of

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>NDP-sugarb</th>
<th>Enzymatic reactionc</th>
<th>Isoform</th>
<th>Locusd</th>
<th>Reference</th>
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<tr>
<td>UDP-glucose epimerase (UGE)</td>
<td>UDP-Glc</td>
<td>E.C.5.1.3.2</td>
<td>UGE1</td>
<td>At1g12780</td>
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<td>UDP-xylene epimerase (UXE)</td>
<td>UDP-Ara</td>
<td>E.C.5.1.3.5</td>
<td>UXE1/MUR4</td>
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<td>UDP-glucuronic acid epimerase (GAE)</td>
<td>UDP-GalA</td>
<td>E.C.5.1.3.6</td>
<td>GA1/UGl/Ae3</td>
<td>At4g30440</td>
<td>206</td>
</tr>
<tr>
<td>UDP-glucose dehydrogenase (UDG)</td>
<td>UDP-Gal</td>
<td>E.C.1.1.1.22</td>
<td>UGD1</td>
<td>At5g39320</td>
<td>207,208</td>
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<tr>
<td>UDP-xylene synthase (UXS)</td>
<td>UDP-Xyl</td>
<td>E.C.4.1.1.35</td>
<td>UX51/AUD3</td>
<td>At3g53520</td>
<td>209</td>
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<tr>
<td>UDP-rhamnose synthase (RHM)</td>
<td>UDP-α-Rha</td>
<td>E.C.4.2.1.76d</td>
<td>RH1M/UR51</td>
<td>At1g78570</td>
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<td>UDP-xylose synthase (UXS)</td>
<td>UDP-Api/UDP-Xyl</td>
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<td>AX51</td>
<td>At2g27860</td>
<td>212</td>
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<tr>
<td>GDP-mannose dehydrogenase (GMD)</td>
<td>GDP-4-keto-6-deoxy-Man</td>
<td>E.C.4.2.1.47</td>
<td>GDP1/MUR1</td>
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<td>GER1</td>
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<td>GME1</td>
<td>At5g28840</td>
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<td>Kdo synthase (KDS)</td>
<td>CMP-Kdo</td>
<td>E.C.2.5.1.55</td>
<td>KDS1A/Kdop5f</td>
<td>At1g79500</td>
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<tr>
<td>CMP-Kdo synthase (CKS)</td>
<td>CMP-Kdo</td>
<td>E.C.2.7.7.38</td>
<td>MIOX4</td>
<td>At4g26260</td>
<td>223</td>
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</tbody>
</table>

a Adapted from Seifert et al. (2004).

b The nucleotide-sugar synthesized.

c The Enzyme Commission number (E.C.x.x.x.x) of each enzyme activity is listed based on the chemical reaction carried out.

d The locus of the Arabidopsis gene is given as the AGI code that refers to the position of the gene on Arabidopsis chromosomes.

e The RHM has catalytic activities equivalent to the combined bacterial E.C. 5.1.3.13, E.C. 1.1.1.33 and E.C. 4.2.1.76.

f E.C. numbers were not assigned for these activities.

g CMP-Kdo synthase enzymes have not been identified in Arabidopsis; however, the protein and corresponding gene have been identified in maize (ZmCKS) and a putative protein accession number has been identified in Arabidopsis (AC007202).
Although the precise functions of PMTs are not yet fully characterized, their expression patterns suggest that these enzymes are involved in various biological processes, including cell growth, organ development, and pathogen defense. The diversity of PMT isoforms with distinct substrate specificities and pH optima indicates the existence of multiple PMT enzymes required for the synthesis of complex pectin structures.

**Table 4**

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Fraction</th>
<th>pH optimum</th>
<th>Apparent $K_{m}$ for UDP-Gal (μM)</th>
<th>$V_{max}$ (pmol min$^{-1}$ mg$^{-1}$)</th>
<th>Acceptor</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Vigna radiata</td>
<td>Particulate</td>
<td>6.3–7.0</td>
<td>1.7</td>
<td>4700</td>
<td>Endogenous</td>
<td>229</td>
</tr>
<tr>
<td>Vigna radiata</td>
<td>Particulate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Endogenous</td>
<td>230</td>
</tr>
<tr>
<td>Brassica rapa</td>
<td>Particulate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Endogenous</td>
<td>230</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Endogenous</td>
<td>230</td>
</tr>
<tr>
<td>Acer pseudoplatanus</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Endogenous</td>
<td>231</td>
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<td>Particulate</td>
<td>7.8</td>
<td>8.9</td>
<td>150</td>
<td>Endogenous</td>
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<tr>
<td>Nicotiana tabacum</td>
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<td>6.3–7.8</td>
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<td>290</td>
<td>Exogenous/endogenous</td>
<td>21,162</td>
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<td>Vigna angularis</td>
<td>Permeabilized</td>
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<tr>
<td>Pisum sativum</td>
<td>Permeabilized</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Exogenous/endogenous</td>
<td>177</td>
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<td>Petunia axillaris</td>
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<td>7.0</td>
<td>170</td>
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<td>Exogenous</td>
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<tr>
<td>Cucurbia moschata</td>
<td>Particulate</td>
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<td>1700</td>
<td>15,000</td>
<td>Exogenous</td>
<td>236</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Solubilized</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Exogenous</td>
<td>228</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>Particulate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Exogenous</td>
<td>237</td>
</tr>
</tbody>
</table>

ND: Not determined.

a The activity was detected from membrane particulate preparations (particulate), detergent-permeabilized membranes (permeabilized) or detergent-solubilized membranes (solubilized).

b The acceptor may be endogenous (that existing in the preparation) or exogenous (previously extracted pectins of variable purity).

Multiple PMT enzymes are required for pectin synthesis as evidenced by the discovery of PMT isoforms in flax Golgi membranes separated in sucrose density gradients. The PMT isoforms were identified as PMT activities that had distinct pH optima and also separated in sucrose density gradients. The PMT isoforms were evidenced by the discovery of PMT isoforms in flax Golgi membranes (110 kDa), and PMT18 (18 kDa). The PMT18 protein appeared to harbor PMT activity and was found in partially purified preparations of both PMT5 and PMT7, suggesting that PMT18 may be the catalytic subunit of PMT5 and PMT7. Confirmation that these proteins are PMTs has not yet been presented. Mutagenesis of the Arabidopsis genome has led to the identification of a putative PMT mutant at the At1g78240 gene locus that is predicted to encode a ~43 kDa protein with sequence similarity to known methyltransferases. The mutant is referred to as the *qua1*/*qua2* mutant.
gesting a functional linkage between QUA1 and QUA2. The transcriptions of additional QA2 isoforms were co-expressed with GAUT9 and GAUT1. The cooperativity of PMT activity with exog-enously added HG acceptors in mung bean and the co-expression of GAUT and PMT transcripts, may reflect a physical dependence of HG:GalATs on PMTs.

### 5.5. Pectin acetyltransferase

Pectin O-acetyltransferase (PAT) activity catalyzes the transfer of an acetyl group from acetylCoA to a pectic polysaccharide acceptor. Acetyl groups decorate the GalA residues of pectic polysaccharides at the O-2 or O-3 positions. Acetyl groups may decorate the GalpA residues of HG and RG-I; however, acetylation was not detected on the RG-II backbone. O-acetylation was detected, however, on the 4-O-methyl-fucose residue and aceric acid residue of RG-II sidechain. The functional consequences of acetylation are not clear but may play a role in preventing pectin breakdown by microbial hydrolases. PAT activity has been detected in micro-somal membrane preparations of potato. The PAT activity at 30 °C was found to have a pH optimum of 7.0, an apparent Km of 35 μM for acetyl-CoA and a Vmax of 0.9 pmol min⁻¹ mg⁻¹ protein.

### 5.6. RG-I glycosyltransferases

The biosynthesis of RG-I requires multiple glycosyltransferase activities to synthesize a backbone of [1-2]-α-L-Rhap-(1-4)-α-L-rhamnopyranosyltransferase (α1,2-GalAT) responsible for synthesis of the RG-I backbone have not been identified. Pectin enzymes are useful as potential models with which to query plant genomes and may be of use in identification of the RG-I:RhaT. Recently, an α1,3-RhaT (WapR) and an α1,6-RhaT (MigA) were identified in *Pseudomonas aruginosa* that are responsible for the transfer of rhamnosyl residues to the core glycan structure of lipopolysaccharide (LPS). Bacterial nucleotide interconverting genes have successfully been used to identify the nucleotide interconverting enzymes UGE1 in *Escherichia coli* and UGE2 in *Streptococcus pneumoniae* Cap1 J bacterial UGE. The RHM2 in *Arabidopsis thaliana* was identified from bacterial protein family hidden markov models (HMM) of NDP-rhamnose synthase (PFAM: PF01370). In addition, hydrophobic cluster analysis in conjunction with the identified conserved glycosyltransferase domains of *Acetobacter xylinum* cellulose synthase (CesA) was used to identify CesA homologs in plants. Hydrophobic cluster analysis aids in the identification of homologs across species that may not retain great conservation of the primary amin-o acid sequence but retain function based on the secondary protein structure predicted by hydrophobic amino acid clustering. These methods provide hope for identification of additional pectin GTs in the future.

### 5.7. RG-I galactosyltransferases

The galactan side chains of RG-I are composed primarily of β-1,4-D-galactan with some branches of β-1,6- and β-1,3-Gal. The galactosyltransferases (GalTAs) catalyze transfer of β-Galp residues from UDP-β-Galp to an acceptor molecule. Pectin GaTs are hypothesized to catalyze the initiation of galactan side chains directly onto the backbone Rha residues of RG-I, elongate galactan chains, initiate branch points onto galactan chains, and elongate galactan side chains. Synthesis of these structures may require up to 10 or more different pectin-specific GalTAs (see Table 2).

The characterization of GaT activities necessary for RG-I galactan biosynthesis has been carried out in *flax*, *mung bean*, *poto-ta*, *radish*, and *soybean* (Table 6). The β1,4-GaT activity of *flax* has been detected, and peA homogenates was localized to the Golgi apparatus by sucrose density gradient centrifugation. Fractionation of Golgi membranes into high-, medium-, and low-density microsomes showed that the galactan β1,4-GaT activity had a differential distribution in density gradients from glycoprotein GaT activity. The products of the above pectin GaT activity were not characterized, and thus, may represent both elongating and branching galactan GaT activities.

Pectin RG-I:GaT activity that specifically elongates existing Gal branches on the RG-I backbone was recovered from microsomal membrane preparations of mung bean. The GaTAs catalyzed the transfer of a Galp onto β-1,4-galactan acceptor molecules. Cleavage of the reaction products specifically by endo-β-1,4-galactanase verified the linkage catalyzed. Similar experiments were carried out in *potato*, *soybean*, and *pea* homogenates. The GaT activity in pea microsomal membranes elongated a β-1,4-galactan acceptor that was associated with a high molecular weight PG- and RG-A lyase-digestible pectin. The partially purified pectin biosynthetic GaT activity isolated from soybean was able to transfer more than 25 galactosyl units onto an oligogalactan of DP 7, which is approaching the length of native pectin galactan branches isolated from soybean RG-I. The GaT activity that elongates a growing galactan chain is discerned from GaT activity that initiates a Gal linkage onto the rhamnosyl C-4 of the RG-I backbone or onto the C-4 of an existing Gal linked to the RG-I backbone. GaT activity that utilizes a β-1,4-Galp-α-L-Rhap-α-L-Rhap acceptor was identified in potato microsomal membranes. This GaT activity was suggestive of a chain initiation GaT that adds a single Gal onto the firstly added Gal on the RG-I backbone. Such an activity would catalyze the second initiating step in RG-I galactan.

---

**Table 5**

Pectin biosynthetic pectin methyltransferase (PMT) activity

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Fraction</th>
<th>pH optimum</th>
<th>Apparent Kᵐ</th>
<th>Vmax (pmol min⁻¹ mg⁻¹)</th>
<th>Acceptor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vigna radiata</em></td>
<td>Particulate</td>
<td>6.8</td>
<td>59</td>
<td>2.7</td>
<td>HG</td>
<td>252,247,257</td>
</tr>
<tr>
<td><em>Linus usitatissimum</em></td>
<td>Particulate</td>
<td>6.8</td>
<td>10–30</td>
<td>ND</td>
<td>HG</td>
<td>253</td>
</tr>
<tr>
<td><em>Linus usitatissimum</em></td>
<td>Solubilized</td>
<td>7.1</td>
<td>30</td>
<td>ND</td>
<td>HG</td>
<td>246</td>
</tr>
<tr>
<td><em>Linus usitatissimum</em></td>
<td>Particulate</td>
<td>5.5</td>
<td>20</td>
<td>ND</td>
<td>Low MeOxy-HG</td>
<td>256,256,259</td>
</tr>
<tr>
<td><em>Linus usitatissimum</em></td>
<td>Particulate</td>
<td>7.0</td>
<td>20</td>
<td>ND</td>
<td>High MeOxy-HG</td>
<td>256,256,259</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>Particulate</td>
<td>7.8</td>
<td>38</td>
<td>49</td>
<td>HG</td>
<td>254,250</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>Solubilized</td>
<td>7.8</td>
<td>18</td>
<td>7.3</td>
<td>HG</td>
<td>260</td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>Permeabilized</td>
<td>6.8</td>
<td>230</td>
<td>600–1300</td>
<td>HG</td>
<td>255</td>
</tr>
<tr>
<td><em>Linus usitatissimum</em></td>
<td>Solubilized</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>HG</td>
<td>259</td>
</tr>
</tbody>
</table>

ND: Not determined.

a Particulate (particulate), detergent-permeabilized (permeabilized) or detergent-solubilized (solubilized) membranes were used.

b The HG acceptor, with variable levels of further characterization except where noted (high or low Methyl-esterified HG (MeOxy-HG)).

c The PMT activity was further purified from a solubilized membrane preparation to yield PMT5, PMT7, and PMT18.
synthesis. This activity catalyzed the addition of Gal residues from UDP-[1-14C]galactose onto small RG-I backbone fragments with a low degree of galactosylation, but not onto RG-I with a high degree of galactosylation, RG-I without galactosylation, galactan, or galactooligosaccharides. In addition, the activity was separable from a different β1,4-GalT activity that did incorporate [1-14C]Gal onto highly galactosylated RG-I.279 Similarly, a GalT activity isolated from flax elongated existing single Gal residues of RG-I; a potential GalT involved in galactan chain initiation.278

The β-1,3-galactan and β-1,6-galactan structures are primarily associated with wall AGPs; however, some have been isolated in association with RG-I sidechains. A GalT activity isolated from 6-day old radish root microsomal membrane preparations showed elongation of β-1,3- and β-1,6-galactooligosaccharides.273 The elongation of de-arabinosylated AGs from AGP polysaccharides was also elongated by the GalT in these fractions.273

The Arabidopsis MUR3 encodes a glycosyltransferase in GT47 that is predicted to be an XG-GalT. GT47 has 9 subfamilies (A, B, C1, C2, C3, D1, D2, E, and F) that show varying similarity to animal exostosins. MUR3 is a putative GalT based on wall glycolyl residue composition phenotype that has a distinct reduction in wall Gal content. Further characterization of the mur3 mutant plants that had a wild-type-like phenotype revealed via wall analyses that mur3 mutant plants had reduced galactosylation. The enzymatic activity of heterologously expressed MUR3 showed activity of a galactosyltransferase that is specific for transfer of a galactose to the third xylose substituted Glc in a xyloglucan XXXG.280 Whether any of the other GT47 family members are GalTs involved in RG-I synthesis, remains to be determined.

5.8. RG-I arabinosyltransferase

The synthesis of α-1,5-linked arabinan by α1,5-AraT involves the transfer of Ara residues from UDP-L-Ara to acceptor molecules in an α-(1,5) configuration. As many as 18 AraTs may be required for the synthesis of the complex branched arabinans of pectic polysaccharides.265 AraT activity has been characterized in mung bean179 and French bean283 (Table 7). Pectin biosynthetic AraT activity is derived from membrane fractions and requires Mn2+ ions.

Arabianin AraT activity was first observed in mung bean permeabilized microsomal membranes.284 The product of addition of [14C]arabinose to mung bean permeabilized membranes was an arabianin, as characterized by paper chromatography of the sugars released by acid hydrolysis.284 Similar experiments carried out in French bean produced products that were degraded by pectinase;283 however, structural characterization of the acceptor was not carried out. In 1992, a partially purified 70 kDa putative AraT was recovered from active protein fractions using a monoclonal antibody found to inhibit AraT activity in microsomal membranes.285 Despite the repeated solubilization of AraT activity and the partial purification of a putative AraT protein, the AraT was not identified and the enzyme products of the putative AraT were not rigorously characterized. Recently, however, an authentic α1,5-AraT activity was observed by the incubation of UDP-L-Ara with mung bean hypocotyl Golgi membranes and exogenous α-1,5-L-arabinooligosaccharides. The products were determined to be α-1,5-linked by α-1-arabinofuranosidase cleavage and NMR structure determination.288

The complex branched arabinans of pectic RG-I polysaccharides also require the activities of a β1,3-AraT protein that was identified in mung bean membrane preparations.179,186,287 The mung bean Golgi-enriched membranes incorporated L-[14C]Ara onto endogenous 1,5-linked arabinofuranooligos, but very inefficiently. The reaction products were not released with an α1,5-arabinofuranosidase and only approximately 25% of the radio-labeled products were released by an arabinofuranosidase that cleaved 1,2-, 1,3-, or 1,5-linkages.179 Similar activity assays were carried out with mung

Table 6

Pectin biosynthetic galactosyltransferase (GalT) activity

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Fractiona</th>
<th>pH optimum</th>
<th>Apparent Km for UDP-Galp (µM)</th>
<th>Vmax (pmol min⁻¹ mg⁻¹)</th>
<th>Linkage</th>
<th>Acceptorb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linum usitatissimum</td>
<td>Particulate</td>
<td>6.5</td>
<td>ND</td>
<td>ND</td>
<td>β-1.4</td>
<td>Endogenous</td>
<td>270</td>
</tr>
<tr>
<td>Linum usitatissimum</td>
<td>Particulate</td>
<td>6.5</td>
<td>ND</td>
<td>ND</td>
<td>β-1.4</td>
<td>Endogenous</td>
<td>270</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Particulate</td>
<td>6.5</td>
<td>1200</td>
<td>2000–3000</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>279</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Solubilized</td>
<td>7.5</td>
<td>ND</td>
<td>ND</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>279</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Solubilized</td>
<td>3.6</td>
<td>ND</td>
<td>ND</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>279</td>
</tr>
<tr>
<td>Pistum sativum</td>
<td>Particulate</td>
<td>7.0–8.0</td>
<td>ND</td>
<td>ND</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>280</td>
</tr>
<tr>
<td>Raphanus sativus</td>
<td>Particulate</td>
<td>5.9–6.3</td>
<td>410</td>
<td>1000</td>
<td>β-1.6</td>
<td>Exogenous</td>
<td>273</td>
</tr>
<tr>
<td>Glycine max</td>
<td>Permeabilized</td>
<td>6.5</td>
<td>32</td>
<td>200</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>277</td>
</tr>
<tr>
<td>Glycine max</td>
<td>Solubilized</td>
<td>6.5</td>
<td>1200</td>
<td>2000–3000</td>
<td>β-1.4</td>
<td>Exogenous</td>
<td>274,281</td>
</tr>
</tbody>
</table>

ND: Not detected, ?: unknown linkage.

α: Particulate (particulate), detergent-permeabilized (permeabilized) or detergent-solubilized (solubilized) membranes were used.

b The acceptor molecules used in the references listed were both endogenous and exogenous and have been characterized to a greater or lesser degree; additional information is noted as available.

Table 7

Pectin biosynthetic arabinosyltransferases (AraT) activity

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Fractiona</th>
<th>pH optimum</th>
<th>Apparent Km for UDP-Ara (µM)</th>
<th>Vmax (pmol min⁻¹ mg⁻¹)</th>
<th>Linkage</th>
<th>Acceptorb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigna radiata</td>
<td>Permeabilized</td>
<td>6.0–6.5</td>
<td>ND</td>
<td>ND</td>
<td>? Ara</td>
<td>Endogenous</td>
<td>284</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Permeabilized</td>
<td>6.0–6.5</td>
<td>ND</td>
<td>ND</td>
<td>? Ara</td>
<td>Endogenous</td>
<td>286,283</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Solubilized</td>
<td>6.5</td>
<td>33,000</td>
<td>200</td>
<td>β-1.3-Ara</td>
<td>Exogenous</td>
<td>179</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Solubilized</td>
<td>6.5</td>
<td>330</td>
<td>200</td>
<td>β-1,4-Ara</td>
<td>Exogenous</td>
<td>287</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Solubilized</td>
<td>6.5</td>
<td>ND</td>
<td>ND</td>
<td>α-1,5-Ara</td>
<td>Exogenous</td>
<td>288</td>
</tr>
</tbody>
</table>

ND: Not detected, ?: unknown linkage.

a Particulate (particulate), detergent-permeabilized (permeabilized) or detergent-solubilized (solubilized) membranes were used.

b The acceptors utilized in the referenced studies were endogenous or exogenous with variable degrees of characterization; additional information is listed where available.
bean Golgi membranes incubated with UDP-β-L-Ara and 2-amino-
benzamide (2-AB) labeled 1,5-linked arabinofuranosidicacchar-
dies. The reaction products were found to be β-L,1,3-Ara residues
linked to the terminal Ara residues of the 2-AB labeled accep-
tors. 186 The linkage and ring conformation of the terminal β-linked
residue were confirmed by partially methylated alditol acetates
and NMR spectroscopy. 186

A β1,4-AraT activity was identified which catalyzes the trans-
fer of a terminal β-Ara from UDP-β-L-Ara onto the terminal non-
reducing residue of a β-1,4-galactooligosaccharide labeled at the
reducing end with 2-AB. 287 The conformation of the reaction pro-
ucts was determined by ESI-MS/MS, linkage analysis, and NMR.
The linkage described naturally occurs in RG-I galactan side-
chains. 56 The Ara1Gal7–2-AB molecule was not further utilized as
an acceptor for either AraT or GaT activity, suggesting that the ter-

5.9. RG-II glycosyltransferase

The backbone on RG-II is α-1,4,α-GaLa-linked HG. GAUT1 has
recently been discovered, which catalyzes the addition of GaLa residues
onto HG oligomers, 228 and could, conceivably, synthe-
size the backbone of RG-II. There are 15 GAUT genes in the
Arabidopsis T-DNA insertion mutant arad1 has reduced cell
wall arabinin with little change in the composition of other glyco-
syl residues and no apparent physical phenotype and is hypothe-
sized to be a putative cell wall AraT. 289 The mutant wall
compositional phenotype was complemented by the ARAD1 trans-
gene under the 35S promoter. ARAD1 (AT2g35100) is a GT47 glyco-

Areas of pectin research which are lagging include the identifi-
cation of GTs responsible for the biosynthesis of pectin. Progress
has been made in recent years, however, GT activities for less than
a handful of linkages have been characterized and fewer genes
have been identified. Generally speaking, the substrates and accep-
tors must frequently be generated by each researcher in order to
carry out the analyses. Undoubtedly, the lack of discoveries in pectin
biosynthesis is in part due to the difficulty with which the activities
may be assayed.

Acknowledgments

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Science Center is a collaborative effort between Oak Ridge National
Laboratory and other research sites, for more information please go
to www.bioenergycenter.org.

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