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Current Opinion in Plant Biology

Pectin structure and biosynthesis

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Pectin is structurally and functionally the most complex polysaccharide in plant cell walls. Pectin has functions in plant growth, morphology, development, and plant defense and also serves as a gelling and stabilizing polymer in diverse food and specialty products and has positive effects on human health and multiple biomedical uses. Pectin is a family of galacturonic acid-rich polysaccharides including homogalacturonan, rhamnogalacturonan I, and the substituted galacturonans rhamnogalacturonan II (RG-II), and xylogalacturonan (XGA). Pectin biosynthesis is estimated to require at least 67 transferases including glycosyl-, methyl-, and acetyltransferases. New developments in understanding pectin structure, function, and biosynthesis indicate that these polysaccharides have roles in both primary and secondary cell walls. Manipulation of pectin synthesis is expected to impact diverse plant agronomical properties including plant biomass characteristics important for biofuel production.

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Current Opinion in Plant Biology 2008, **11**:266–277

This review comes from a themed issue on
Physiology and metabolism
Edited by Ken Keegstra and Markus Pauly

1369-5266/$ – see front matter
Published by Elsevier Ltd.

DOI 10.1016/j.pbi.2008.03.006

Introduction

Pectin is the most structurally complex family of polysaccharides in nature, making up ~35% of primary walls in dicots and non-graminaceous monocots, 2–10% of grass and other commelinoid primary walls, and up to 5% of walls in woody tissue [1,2] (Department of Energy, Energy Efficiency and Renewable Energy, Biomass Program: http://www1.eere.energy.gov/biomass/feedstock_databases.html). Pectin is abundant in walls that surround growing and dividing cells, walls of cells in the soft parts of the plant, and in the middle lamella and cell corners. Pectin is also present in the junction zone between cells with secondary walls including xylem and fiber cells in woody tissue. Pectin is a component of all higher plant walls and of the walls of gymnosperms, pteridophytes, bryophytes and Chara, a charophycean algae believed to be the closest extant relative of land plants [3]. The correlation of increased amounts of the pectin RG-II in vascular plants, and its appearance as plants adapted to upright growth on land and developed lignified secondary walls [4], suggests that pectin has fundamental roles in both primary and secondary wall structure and function. An understanding of pectin structure and synthesis is crucial to understanding, and potentially modifying, wall structure so as to promote efficient production of biofuel from recalcitrant plant lignocellulosic biomass [5,6].

Several reviews on pectin biosynthesis synthesis [2,7,8], plant wall biosynthesis [9–12], and regulation of cell wall synthesis [13,14] have recently been published. The goal of this paper, after a brief review of pectin function, is to summarize recent developments in our understanding of pectin structure and biosynthesis, with special attention to genes that encode pectin biosynthetic transferases. For a more detailed summary of the enzymology of pectin synthesis, readers are directed to four comprehensive reviews [6–8,15].

Overview of pectin function

Multiple lines of evidence indicate a role for pectin in plant growth, development, morphogenesis, defense, cell–cell adhesion, wall structure, signaling, cell expansion, wall porosity, binding of ions, growth factors and enzymes, pollen tube growth, seed hydration, leaf abscission, and fruit development [2,16]. Pectin is also used as a gelling and stabilizing agent in the food and cosmetic industries and has multiple positive effects on human health including lowering cholesterol and serum glucose levels, reducing cancer [17], and stimulating the immune response [18]. Pectin is also used in the production of a variety of specialty products including edible and biodegradable films, adhesives, paper substitutes, foams and plasticizers, surface modifiers for medical devices, materials for biomedical implantation, and for drug delivery. The complexity of pectin structure provides a multiplicity of structural epitopes that impart unique functions. The identification of genes encoding proteins that catalyze or regulate pectin synthesis is instrumental to understand pectin structure/function relationships.

Pectin structure

Pectins are a family of covalently linked galacturonic acid-rich plant cell wall polysaccharides [19]. Galacturonic acid comprises approximately 70% of pectin, and all the pectic polysaccharides contain galacturonic acid linked at the *O*-1 and the *O*-4 position.

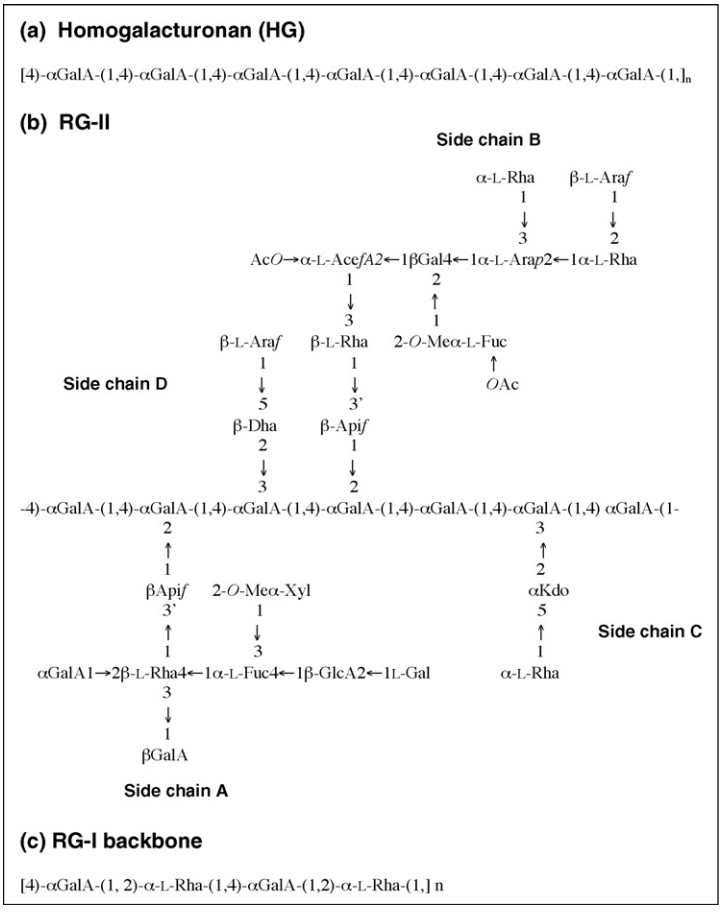
Homogalacturonan (HG)

The most abundant pectic polysaccharide is homogalacturonan (HG), a linear homopolymer of α-1,4-linked galacturonic acid that comprises ~65% of pectin (Figure 1a). HG is partially methylesterified at the C-6 carboxyl, may be *O*-acetylated at *O*-2 or *O*-3 [1], and may contain other potentially crosslinking esters of uncertain structure [17,20]. HG has been shown to be present in stretches of approximately 100 GalA residues in length [21], although shorter regions of HG have been detected interspersed between other pectic polysaccharides [22••]. The other pectic polysaccharides are considerably more complex in structure than HG and include the substituted HGs rhamnogalacturonan II (RG-II) (Figure 1b), xylogalacturonan (XGA), and apiogalacturonan (AP), along with the structurally more variable pectic polysaccharide rhamnogalacturonan I (RG-I) (Figure 1c and d).

Substituted HG: rhamnogalacturonan II (RG-II)

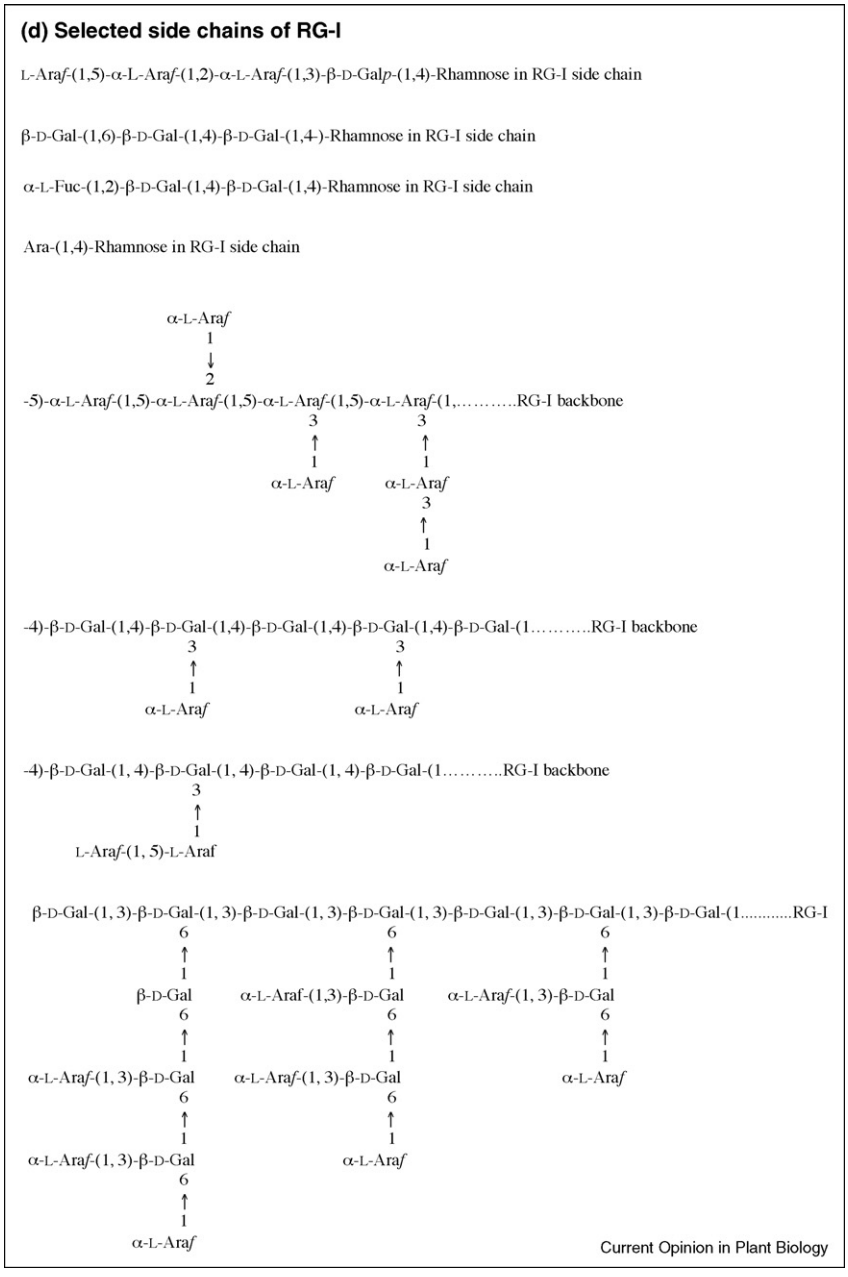
The most structurally complex pectin, RG-II, makes up ~10% of pectin [3] (Figure 1b). Its structure is largely conserved across plant species and consists of an HG backbone of at least 8 (and most probably more) 1,4-linked α-D-GalA residues decorated with side branches (a–d) consisting of 12 different types of sugars in over 20 different linkages. RG-II usually exists in plant walls as RG-II dimers crosslinked by a 1:2 borate diol ester between the apiosyl residues in side chain A of two RG-II monomers [3]. RG-II dimerization crosslinks HG domains and yields a macromolecular pectin network [4]. The fact that mutations that cause even minor modifications to RG-II structure lead to reduced RG-II dimer formation and severe growth defects such as dwarfism suggests that the dimerization of RG-II in the wall is crucial for normal plant growth and development.

Figure 1



Selected representative structures of specific regions of the pectic polysaccharides. **(a)** Homogalacturonan, HG, unsubstituted backbone, degrees of polymerization of up to 100 have been described. **(b)** Rhamnogalacturonan II, RG-II, structure is almost invariant across species. **(c)** Rhamnogalacturonan I, RG-I, region of disaccharide repeat backbone. **(d)** Selected RG-I backbone side chains. The bottom structure is a so-called type II arabinogalactan since similar structures are also found in arabinogalactan proteins. All sugars are D isomers and in the pyranose ring form unless otherwise designated. See Table 1 and references [3,15] for references for structures and reference [3] for relatively minor species-specific modifications of RG-II structure.

Figure 1.



(*Continued*).

Other substituted HGs

Two other substituted galacturonans, xylogalacturonan (XGA) and apiogalacturonan (AP) are more restricted in their expression. XGA is an HG substituted at *O*-3 with a β-linked xylose. The 3-linked xylose has occasionally been found to be further substituted at *O*-4 with an additional β-linked xylose [23]. XGA is most prevalent in reproductive tissues, although XGA has also been detected in Arabidopsis stems and leaves [24•]. The observation that a recently identified XGA biosynthetic gene is upregulated in response to pathogen attack suggests that at least some of the substituted HGs, such as XGA, may function to make HG more resistant to degradation by endopolygalacturonases produced during pathogen attack [25••]. Apiogalacturonan (AP), HG substituted at *O*-2 or *O*-3 with D-apiofuraose, is present in aquatic monocots such as *Lemna*.

Rhamnogalacturonan I (RG-I)

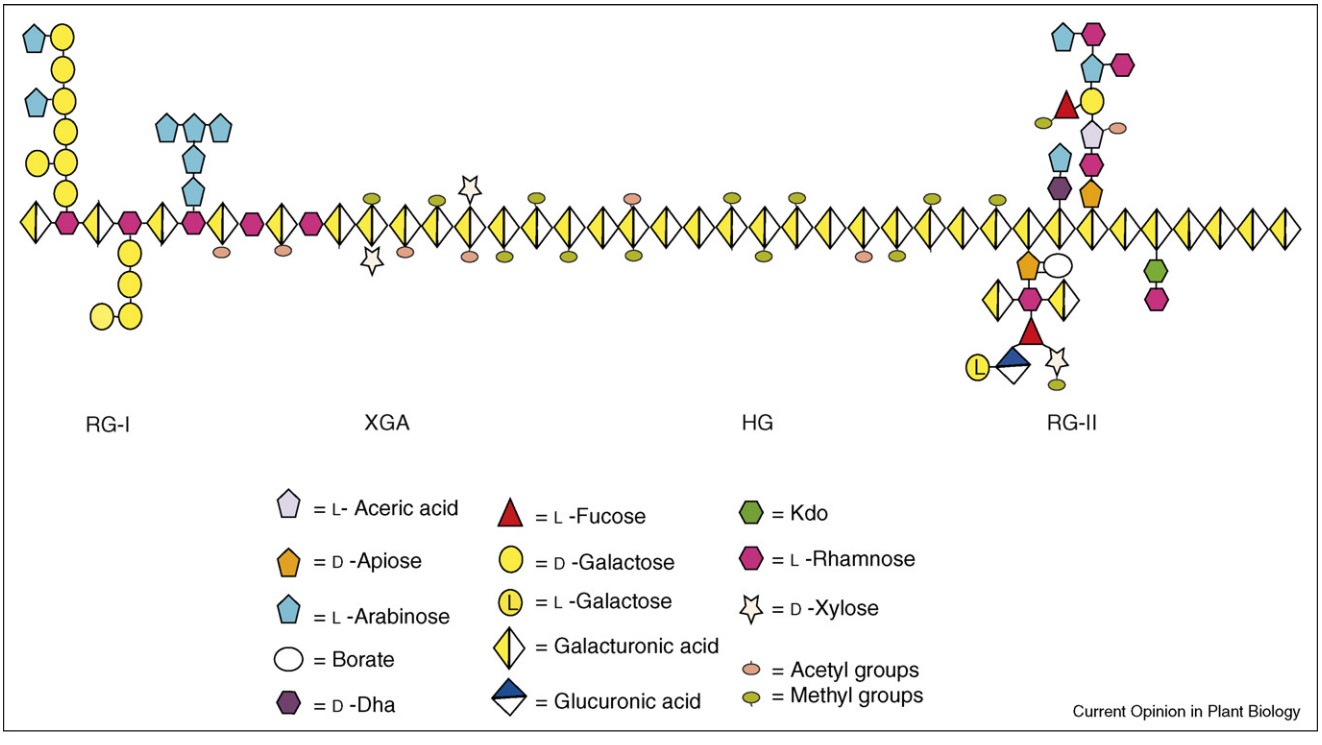
RG-I represents 20–35% of pectin. It contains a backbone of the disaccharide repeat [-α-D-GalA-1,2-α-L-Rha-1-4-]$_n$

(Figure 1c) and exhibits a high degree of cell type and develop-dependent expression in the type and number of sugars, oligosaccharides, and branched oligosaccharides attached to its backbone (Figure 1d) [2,16,26]. The reason for this level of variation in RG-I structure is not understood but suggests diverse functional specialization. Between 20 and 80% of the rhamnosyl residues in the RG-I backbone have side chains containing individual, linear, or branched α-L-Ara*f* and β-D-Gal*p* residues. The side chains include α-1,5-linked L-arabinan with 2- and 3-linked arabinose or arabinan branching, β-1,4-linked D-galactans with degrees of polymerization (DP) of up to 47 [22••], β-1,4-linked D-galactans with 3-linked L-arabinose or arabinan branching, and β-1,3-linked D-galactan with β-6-linked galactan or arabinogalactan branching (reviewed in references [7,27]. The side chains may also contain α-L-Fuc*p*, β-D-Glc*p*A, and 4-*O*-Me β-D-Glc*p*A residues [27].

Model of how the pectic polysaccharides are linked

It is generally believed that the pectic polysaccharides are covalently crosslinked since harsh chemical treatments or digestion by pectin-degrading enzymes are required to isolate HG, RG-I, and RG-II from each other and from walls. There is currently no consensus as to how the pectic polysaccharides are linked to each other, or to other polymers, in the wall; however, the available data [22••,28,74] support a model with HG, RG-I, and RG-II linked via their backbones (Figure 2). There are also indications based on co-elution of pectins with other wall polymers and mutant phenotype studies that pectins may be covalently linked to, or tightly associated with, other types of wall polysaccharides including xyloglucans [29•] and xylans [30]. Analyses of soluble soybean polysaccharides indicate that, at least in soybean, β-1,4-linked xylose of degrees of polymerization of up to 7 may be attached to *O-3* of HG [30]. Other studies suggest that at least a dimer of β-1,4-linked xylose may be attached to RG-I in *Diospyros kaki* leaves [31]. Although it remains to be determined how general such structures are in diverse plant species, these results open up the possibility that pectins may serve to hold at least some hemicelluloses in the wall. The structural data suggesting a link between pectins and xylans, a class of hemicelluloses abundant in secondary walls, are intriguing in light of the mutant phenotypes of several GAUT1-related gene family members [32••], a family that includes proven and putative pectin biosynthetic glycosyltransferases. For example, the mutant phenotypes of *qua1/GAUT8* [33,34•], *irx8/GAUT12* [35•,36••] and *parvus/glz1/GATL1* [37••,38] include effects on both

Figure 2



Schematic structure of pectin showing the four pectic polysaccharides homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) linked to each other. The representative pectin structure shown is not quantitatively accurate, HG should be increased ~12.5-fold and RG-I increased ~2.5-fold to approximate the amounts of these polysaccharides in walls. Figure is modified from reference [8]. Monosaccharide symbols used are taken partly from the Symbol and Text Nomenclature for Representation of Glycan Structure from the Consortium for Functional Glycomics (http://www.functionalglycomics.org/glycomics/molecule/jsp/carbohydrate/carbMoleculeHome.jsp).

xylan and HG content and/or synthesis. Clarification of the specific enzyme activity affected in these mutants will probably contribute a clearer understanding of how pectins and hemicelluloses, or their synthesis [39•,40] are associated.

Pectin biosynthesis

Location of pectin synthesis

Autoradiographic pulse chase experiments, immunocytochemical localization studies with anti-pectin-specific antibodies, and pectin biosynthetic enzyme subcellullar fractionation and topology studies demonstrate that pectin is synthesized in the Golgi and transported to the wall in membrane vesicles. Pectin synthesis occurs simultaneously in numerous Golgi stacks in the cell in a process that appears to include a compartmentalization of specific biosynthetic enzymes to drive the construction of increasingly complex pectin polysaccharides through the *cis*, medial and *trans* Golgi cisternae [41]. The polymers are targeted to the wall by the movement of Golgi vesicles, presumably along actin filaments that have myosin motors [42].

Mechanism of pectin synthesis

All evidence to date suggests that pectin is synthesized in the Golgi lumen by membrane bound, or associated, Golgi-localized glycosyltransferases (GTs) that transfer glycosyl residues from nucleotide-sugars onto oligosaccharide or polysaccharide acceptors. It is not clear, however, how synthesis of any of the pectic polysaccharides is initiated or whether lipid or protein donors are involved [40,43]. During synthesis some pectic glycosyl residues are modified by methyltransferase-catalyzed esterification or *O*-methylation, by acetyltransferase-catalyzed acetylation, and at least in *Chenopodiaceae* (e.g. spinach), by feruloylation driven by feruloyltransferases. The substrates for these modifications appear to be *S*-adenosylmethionine (SAM), acetylCoA, and feruloylCoA, respectively. The recent demonstration that SAM is transported into pea Golgi lumen via a Golgi membrane-localized transporter with an apparent Km of 30 μM [44•] agrees with previously reported Kms for HG methyltransferase activity in plant microsomal membrane preparations and is consistent with pectin synthesis models depicting transport of cytosolic substrates into the Golgi via Golgi membrane-bound transporters.

HG appears to be inserted into the wall as a highly methylesterified polymer that is deesterified to varying degrees by wall-localized pectin methylesterases [45]. The conversion of HG from a methylesterified form to the negatively charged form has been associated with the cessation of growth [46], with the binding of positively charged ions and proteins to the wall, and with the association of HG molecules to each other via Ca^{++} binding. The resulting HG:HG salt bridges contribute to cell–cell adhesion [47]. The spatial partitioning of HG esterification and deesterification during synthesis, or in the wall, is not understood, although it has been shown that the HG in the middle lamella is relatively unesterified and that localized regions of esterified HG exist in some cell walls.

Types of pectin biosynthetic enzymes

Many of the genes encoding nucleotide-sugar biosynthetic enzymes required for pectin synthesis have been identified [48–51]. Most of these genes exist in gene families, and it has been proposed that unique gene family members function in the synthesis of specific pectic polysaccharides. Definitive evidence for this hypothesis remains to be presented. Approximately 67 glycosyltransferase, methyltransferase and acetyltransferase activities are predicted to be required for pectin synthesis [6]. Table 1 lists those transferases for which enzyme activity has been demonstrated *in vitro* and/or for which the encoding gene has been conclusively identified. Conclusive identification of pectin biosynthetic genes requires proof of enzyme activity of the encoded protein. To date, this has only been accomplished for four glycosyltransferases (Table 1).

Enzymatically proven pectin biosynthetic transferases

Homogalacturonan α1,4-Galacturonosyltransferase (HG:α1,4GalAT): GAUT1 [32••]

A membrane-bound α1,4-galacturonoslytransferase that transfers galacturonic acid (GalA) from UDP-α-D-GalA onto the non-reducing end of homogalacturonan polysaccharide and oligosaccharide acceptors, but with a preference for HG of a degree of polymerizatyion (DP) >9, has been identified in every higher plant species tested. A gene encoding an HG:α1,4-GalAT highly expressed in Arabidopsis cell suspension cultures was identified by partial purification of the enzyme from detergent-solubilized protein preparations. Out of 20 proteins identified by tandem MS sequencing, only two contained sequence domains indicative of glycosyltransferases, and only one, Galacturonosyltransferase 1 (GAUT1; At3g61130) yielded HG:GalAT activity when transiently expressed in human HEK293 cells [32••]. Anti-GAUT1 antibodies also immunoabsorbed HG:GalAT activity from partially purified Arabidopsis protein preparations, confirming that GAUT1 encodes HG:GalAT and indicating that *in vitro* GAUT1 catalyzes the elongation of HG oligosaccharide acceptors resulting in the synthesis of polymeric HG. The other predicted glycosyltransferase in the protein preparation used to identify GAUT1 had 36% sequence identify (59% similarity) to GAUT1 and was named GAUT7 (At2g38650) (see below). GAUT7 did not have GalAT activity when transiently expressed in HEK293 cells [32••], but recent results suggest that GAUT7 may be part of a GalAT complex (MA Atmodjo *et al.*, unpublished). GAUT1 and GAUT7 both encode proteins with characteristics consistent with the biochemical properties of HG:GalAT: a basic PI and an apparent type II

Table 1

List of enzymatically confirmed pectin biosynthetic transferases and identified genes[a]

Type of transferase[b]	Type of GT or transferase activity	Parent polymer[c] (side chain)	Enzyme[d] acceptor substrate *Enzyme activity* (unless noted: enzyme adds to the glycosyl residue on the left*)	Reference[e] for structure for GTs and for enzyme activity for MTs and ATs	Gene identified [reference][f]
GT	D-GalAT	HG/RG-II	*GalAα1,4-GalA *α1,4-GalAT*	[2]	GAUT1 At3g61130 HG:GalAT [32••]
GT	D-GalT	RG-I	L-Rhaα1,4-GalA *β1,4-GalT*	[2]	
GT	D-GalT	RG-I	Galβ1,4-Rha *β1,4-GalT*	[2,72]	–
GT	D-GalT	RG-I	Galβ1,4-Gal *β1,4-GalT*	[2]	–
GT	D-GalT	RG-I/AGP[g]	Galβ1,3-Gal *β1,3-GalT*	[2]	–
GT	D-GalT	RG-I/AGP[g]	Galβ1,3-Gal *β1,6-GalT*	[2]	–
GT	D-GalT	RG-I/AGP[g]	Galβ1,6-Galβ1,3-Gal *β1,6-GalT*	[2]	–
GT	L-AraT	RG-I	L-Arafα1,2-Araf *1,5-L-ArafT*	[2]	–
GT	L-AraT	RG-I	L-Arafα1,5-Araf *α1,5-L-ArafT*	[2]	–
GT	L-AraT	RG-I	L-Arafα1,3-Araf *α1,3-L-ArafT*	[2]	–
GT	L-Ara*p*T	RG-I/AGP[g]	L-Arafα1,5-Araf *β1,3-L-ArapT*	[67]	–
GT	L-Ara*p*T	RG-I	Galβ1,4-Gal *α14-L-ArapT*	[68]	–
GT	D-Api*f*T	HG/RG-II (A, B)	GalAα1,4-GalA β1,2-Api*f*T	[2]	–
GT	D-XylT	RG-II (A)	L-Fucα1,4-L-Rha *α1,3XylT*	[2]	RGXT1 RG-II:XylT At4g01770, RGXT2 At4g01750, [52••]
GT	D-XylT	HG/XGA	GalAα1,4-GalA *β1,3 XylT*	[2]	XGD1 At5g33290) XGA:XylT-[25••] Confirmation of linkage and anomeric configuration needed
GT	D-GlcAT	RG-II (A)	L-Fucα1,4-L-Rha *β1,4GlcAT*	[2]	–
MT	HG:GalA-6-*O*-MT	HG	GalAα1,4-GalA$_{(n)}$	[2,6,60•]	
AT	HG: GalA-3-*O*-AT	HG	GalAα1,4-GalA$_{(n)}$	[2,6]	
AT	RG-I: GalA-3-*O*/2-*O*-AT	RG-I	GalAα1,2-L-Rhaα1,4$_{(n)}$	[2,6]	
MT	RG-I: GlcA-4-*O*-MT	RG-I	GlcAβ1,6-Gal	[2,6]	
MT	RG-II: xylose-2-*O*–MT	RG-II	D-Xylα1,3-L-Fuc	[2,6]	
MT	RG-II: fucose-2-*O*-MT	RG-II	L-Fucα1,2-D-Gal	[2,6]	
AT	RG-II: fucose-AT	RG-II	L-Fucα1,2-D-Gal	[2,6]	
AT	RG-II: aceric acid 3-*O*-AT	RG-II	L-Ace*f*Aβ1,3-L-Rha	[2,6]	

[a] Glycosyltransferases for which enzyme activity has been measured or for which a putative gene has been identified, and list of pectin acetyl- and methyl-transferases. Activity of glycosyltransferases based on the transfer of a sugar from a nucleotide-sugar onto the indicated disaccharide acceptor region at the non-reducing end of the indicated acceptor.

[b] Type of transferase: GT, glycosyltransferase; MT, methyltransferase; AT, acetyltransferase.

[c] HG: homogalacturonan; RG-I: rhamnogalacturonan I; RG-II: rhamnogalacturonan II; XGA: xylogalacturonan.

[d] All sugars are D sugars and have pyranose rings unless otherwise indicated. *indicates non-reducing end of the acceptor onto which the indicated glycosyltransferase transfers the glycosyl residue.

[e] References for the structure. *Note*: Owing to limitations of the number of references that could be cited, only those structure references not listed in Ridley *et al.* [2] are cited here. See reference [2] for other original references on pectin structure.

[f] References for gene encoding the GT.

[g] Enzyme activity would also be required to synthesize arabinogalactan proteins (AGPs) (see reference [73]).

membrane protein topology predicting a short N-terminal region, a single membrane spanning region, and a larger C-terminal domain. GAUT1 belongs to a superfamily of 25 genes in Arabidopsis named the GAUT1-related gene family, which includes 15 GAUT genes with 37–100% identity (56–100% similarity) to GAUT1, and 10 GAUT1-like genes (GATLs) with 39–44% identifying (43–53% similarity) with GAUT1. The GAUT1-related genes are a subclass of the Arabidopsis CAZy (Carbohydrate Active Enzymes database; http://www.cazy.org) GT-8 family [32••,53]. The GAUTs fall into three evolutionary-related clades: Clade A: (GAUTS 1–7); Clade B (GAUTs 8–11) and Clade C (GAUTs 12–15). GAUT proteins are predicted to encode proteins of 61–78 kDa with a predicted signal anchor (GAUTs 1, 6–15) or signal peptide (GAUTs 3-5), consistent with a type II membrane topology or an intra-microsomal membrane location, respectively. The only exception is GAUT2 whose sequence does not predict passage in, or through, the ER/Golgi system. GAUTs 1, 3, 7, 8 and 9 have been identified as Golgi-resident proteins [54,55], consistent with the location of the catalytic domain of HG:α1,4-GalAT in the Golgi lumen [56]. The GATL genes encode proteins with predicted signal peptides and masses of 33–44 kDa.

GAUT1 is an HG:α1,4-GalAT; however, its precise function in pectin synthesis is not known. For example, it is not known whether GAUT1 functions only during the

elongation stage of pectin synthesis or also in HG initiation. It is also not clear whether GAUT1 synthesizes HG as well as the backbone for RG-II, or whether the HG synthesized by GAUT1 is the same HG that is covalently attached to the RG-I backbone. Furthermore, the extent to which GAUT1 functions alone, or in a complex or complexes with other proteins is not known. Current results suggest that, at least *in vitro*, GAUT1 can function in a complex (MA Atmodjo *et al.*, unpublished). The recovery of high molecular weight fractions with HG:GalAT activity [57] supports a role of complexes in pectin synthesis. The size, number of subunits, stoichiometry, and the function of pectin biosynthetic complexes, however, remains to be determined.

RG-II: α1,3xylosyltransferase (RG-II: α1,3XylT): RGXT1 and RGXT2 [52••]

Two *Arabidopsis thaliana* α-D-1,3-xylosyltransferases *RGXT1* (At4g01770) and *RGXT2* (At4g01750) have been identified, which when expressed as truncated soluble forms in baculovirus-transfected insect cells, transfer xylose from UDP-α-D-Xyl onto fucose in an α1,3-linkage. This is the same linkage present in sidechain A of RG-II that contains *2-O*-methyl D-Xyl attached in an α1,3-linkage to α-L-Fuc [52••]. The demonstration that the enzymes transfer xylose onto RG-II isolated from *RGXT1* and *RGXT2* mutant walls, but not onto RG-II from wild-type Arabidopsis walls [52••], provides strong enzyme function data that RGXT1 and RGXT2 encode RG-II:α1 and 3XylTs. RGXT1 and RGXT2 share 90% sequence identity, encode proteins of 361 and 367 amino acids, respectively, and are members of CAZy GT-family 77, a novel family of 27 putative *Arabidopsis thaliana* glycosyltransferases [58]. Although the biochemical data supporting the identity of RGXT1 and RGXT2 as RG-II:α1, 3XylTs is strong, it is noteworthy that no clear difference in the structure of RG-II isolated from walls of *RGXT1* and *RGXT2* mutants compared with wild-type walls was obtained [52••]. This result suggests that there may be gene redundancy and that a double gene knockout mutant (or more) may be required to recover an RG-II phenotype in the mutant. In this regard, it is noteworthy that two additional Arabidopsis genes, At4g01220 and At1g56550 are 68–75% identical to *RGXT1* and *RGXT2*, although their enzyme activity has not yet been determined [52••].

Xylogalacturonan: xylosyltransferase (XGA:XylT): XGD1 [25••]

Recently, xylogalacturonan xylosyltransferase, XGD1 (xylogalacturonan deficient 1, At5g33290) was identified in Arabidopsis [25••]. The gene encodes a Golgi-localized type II membrane protein from CAZy family 47, which when expressed in *Nicotiana benthamiana*, catalyzes the transfer of xylose from UDP-α-D-xylose onto HG oligosaccharides (the so-called oligogalacturonides). Since the *xgd1* mutant produces HG with reduced levels of β-1,3-xylose compared with wild-type XGA, it is likely that XGD1 catalyzes the transfer of xylose onto HG in a β-1,3 linkage, although the anomeric configuration of the product synthesized by XGD1 was not directly determined. In agreement with this conclusion is the fact that XGD1 mutants have 15–30% reduced xylose content in adult leaves, the location of the highest expression of XGD1 in wild-type plants, and more importantly, have a significant reduction in xylose in the wall fraction enriched for xylogalacturonan. Taken together the results indicate that XGD1 is a XGA:XylT and, most probably, a XGA:β-1,3XylT, although direct proof of the anomeric configuration and linkage of the xylose to GalA in HG remains to be determined.

Putative pectin biosynthetic transferases

An increasing number of putative pectin biosynthetic glycosyltransferases and methyltransferases have been identified, largely as a result of characterizing walls of mutant or transgenic plants, sequence similarity to known genes from other organisms and/or via trancriptome co-expression studies with tissues that produce large amounts of specific wall polysaccharides. Such putative pectin biosynthetic genes require demonstration of enzyme activity before their definitive role in pectin synthesis is confirmed. Nonetheless, their identification is an important first step to identify genes to target for enzyme function studies.

Putative HG biosynthetic enzymes

Putative HG–methyltransferase (HG–MT)

The GalA in HG of most cell walls is highly (often >50%) methylesterified at the *C*-6 carboxyl group [46]. The enzyme that catalyzes this reaction is called HG–methyltransferase (HG–MT) to distinguish it from methyltransferases that methylate RG-I or RG-II. *In vitro* HG–MT activity uses *S*-adenosylmethionine as a methyl donor, is membrane bound, and has a catalytic site located on the luminal side of the Golgi [2]. The ability of UDP-GalA to stimulate HG–MT activity in intact membrane vesicles, and of HG and pectin to serve as exogenous acceptors for HG–MT in detergent-permeabilized membranes support a model that a region of HG is synthesized before its methylation by HG–MT [2,6]. Since some HG–MT have higher activities with partially esterified pectin acceptors, compared with polygalacturonic acid, it is likely that plants contain multiple HG–MTs that distinguish the degrees of methylation of HG acceptors. The methylesterification of pectin could be regulated at the level of the methyltransferase level or by substrate availability. The report that overexpression of Arabidopsis *S*-adenosylmethionine synthetase in flax yields increased SAM synthetase activity and pectin methylesterification in the wall, with no concomitant increase in HG–MT activity [59], indicates that the degree of methylesterification of HG may be regulated at least partly, at the level of substrate (i.e. SAM) concentration.

No gene encoding HG–MT has been definitively identified. Recently, however, it has been suggested that Quasimodo2 (Qua2) (At1g78240), a Golgi-localized Arabidopsis protein with a putative methyltransferase domain may be an HG–MT [60•]. Arabidopsis *Qua2-1* mutant plants have a 50% reduction in purifiable homogalacturonan and are dwarfed, a common phenotype of pectin mutants. While such results are consistent with a putative function of QUA2 as an HG–MT, demonstration of the enzyme activity of QUA2, and of the 29 related QUA2 homologs in Arabidopsis, is required to determine which, if any, of these genes encode methyltransferases required for pectin synthesis. It is interesting to note, however, that QUA2 and a homolog are transcriptionally co-expressed with GAUT8/QUA1, a putative pectin biosynthetic gene (see below). Furthermore, two additional sets of QUA2 homologs are co-expressed with GAUT1 (an HG–GalAT, see above) and with GAUT9 (a putative pectin biosynthetic gene [60•].

Putative HG:GalTAs or Xylan:XylTs

Three different GAUT mutants have characteristics consistent with GAUT gene function in HG and/or xylan synthesis. Arabidopsis *qua1/gaut8* (At3g25140) mutants are reduced in both GalA content (~25% in rosette leaves or total plant walls [33], ~30% in stem walls [34•], somewhat in suspension cell walls [61]) and to a lesser extent in xylose content. Protein extracts from *qua1* stems have reduced HG:α-1,4-GalAT and β-1,4-xylosyltransferase activities [34•]. These results are difficult to understand since HG and XGA are generally thought to be separate polysaccharides. The characteristics of the QUA1 (and IRX8, see below) mutants could indicate that pectin and xylan syntheses are connected, for example, via a covalent linkage of the polymers, via synthesis in biosynthetic complexes, or via compensation upon loss of one of the polysaccharides. The pleiotropic affects of the QUA1 mutant, combined with the inability to recover enzyme activity from the recombinantly expressed or purified GAUT8 protein, make the enzyme function of GAUT8/QUA1 unclear at present.

GAUT12/IRX8 (At5g54690) mutants are dwarfed with collapsed xylem, reduced secondary wall thickness, reduced amounts of glucuronoxylan and a subfraction of HG [35•], and contain glucuronoxylan that is largely devoid of a reducing end sequence 4-β-Xyl-1,4-β-Xyl-1,3-α-L Rha-1,2-α-GalA-1,4-Xyl [36••]. These results suggest that GAUT12/IRX8 may be a HG:GalAT involved in the synthesis of a subfraction of HG to which β-1,4-xylan is attached [35•] or a α1,4-GalAT that adds GalA into the above-mentioned xylan reducing end sequence [36••]. Proof of the function of GAUT12 will require determination of its enzyme activity.

A third GAUT1-related gene family member that has characteristics consistent with a defect in pectin synthesis and in secondary wall xylan synthesis is one of the GATL genes, PARVUS/GLZ1/GATL1 (At1g19300) [37••,38,62,63]. Mutants in this gene, when grown under low humidity, are semi-sterile dwarfs with reduced anther dehiscence, slightly elevated mole percent Rha, Ara, and Gal, slightly reduced GalA, and larger reductions in xylose compared with the wild type. The mutant also has reduced branching of the RG-I backbone, with accompanying overall increased amounts of wall pectin and decreased xylan [37••,62]. Xylan side branches in *parvus/glz1/gatl1* have comparable amounts of methylGlcA but lack non-methylated GlcA side branches [37••] and also lack the reducing end sequence 4-β-Xyl-1,4-β-Xyl-1,3-α-L-Rha-1,2-α-GalA-1,4-Xyl, the latter modification being similar to that in the irx8/gaut12 mutants described above ([36••], see also article by WS York and MA O'Neill in this issue of *Curr Opin Plant Biol*). The described glycosyl residue changes, and the structural modifications are consistent with a possible role of the PARVUS/GLZ1/GATL1 gene (like IRX8/GAUT12) in the synthesis of the xylan, possibly the xylan-primer, or in the synthesis of the structure to which the reducing end xylan oligosaccharide is attached, possibly a fraction of pectin.

Putative RG-II biosynthetic enzymes

Putative RG-II:GlcAT

Nicotiana plumbaginifolia T-DNA nolac-H18 callus mutant *NpGUT1* is non-organogenic, has reduced intercellular attachment, and 86% reduced levels of glucuronic acid. The gene mutated in the callus is NpGUT1, which has 60% sequence homology to animal heparin sulfate biosynthetic glucuronosyltransferases. Since RG-II isolated from cell walls of the nolac-H18 mutant are devoid of glucuronic acid and exhibit 82% reduced RG-II dimer formation, it has been proposed that *NpGUT1* encodes RG-II β-1,2GlcAT that transfers GlcA onto the L-fucose in RG-II side chain A [64]. Demonstration of the enzyme activity of *NpGUT1* will be required to confirm the proposed function of the putative RG-II:β-1,2GlcAT.

Putative RG-I biosynthetic enzymes

Putatutive RG-I:α1,5AraT)

RG-I contains L-arabinose in multiple linkages with the majority of the arabinose in the furanose ring form, although there is a small amount of terminal arabinopyranose in some RG-I side chains [65]. Most enzymatic studies of pectin AraT activity have been carried out using the donor UDP-β-L-arabinopyranose and two types of enzyme activity that use the arabinopyranose form of the sugar have been described. A Golgi-localized α1,5-arabinan:β-(1→3) arabinopyranose AraT activity that transfers a single arabinopyranose residue onto the non-reducing end of α1,5-arabinooligosaccharide acceptors has been identified [66,67]. Other studies have identified an arabinopyranosetransferase activity that transfers individual arabinopyranosyl residues onto

1,4-linked β-D-galactooligosaccharides [68] in an α configuration. In neither case has a gene been identified. It has been proposed that the β-1,4-galactan:α1,4AraT-catalyzed addition of a non-reducing end terminal α-L-arabinopyranosyl residue onto β-1,4-galactan oligosaccharide prevents further galactosylation of the galactooligosaccharides [68,69].

An Arabidopsis gene that encodes a putative arabinan:α-1,5-arabinosyltransferase (*ARAD1*; At2g35100, CAZy GT47) was identified through biochemical and immunochemical analyses of the Arabidopsis T-DNA insert mutant *ARABINAN DEFICIENT 1* [70•]. *ARAD1* encodes a predicted 52.8 kDa protein with a single N-terminal transmembrane domain region followed by a proposed catalytic domain. Walls from *ARAD1* homozygous knockout mutant leaves and stems have 25 and 54%, respectively, reduced levels of Ara compared with wild-type walls [70•]. Transformation of *arad1* plants with the *ARAD1* gene restores Ara in the wall to wild-type levels, providing evidence that *ARAD1* affects wall arabinose levels. Immunocytochemical analyses of leaf, inflorescence stem, and the stem using the anti-α-1,5-arabinan antibody LM6 indicate the reduced immunolabeling in walls that were not associated with proteins, suggesting that the mutant was affected in the synthesis of α-1,5-arabinans, but not in glycoprotein synthesis [70•]. Furthermore, comparison of RG-I from wild type and *ARAD1* walls showed that *arad1* RG-I had a 68% reduction in Ara content, predominantly due to a reduction in 5-linked Ara*f* along with 2,5*f*-linked Ara and 2,3,5-linked Ara*f*. The results provide strong evidence that *ARAD1* is a putative RG-I arabinan:α-1,5-AraT. Confirmation of the proposed α1,5arabinosyltransferase activity of ARAD1, however, will require expression of an enzymatically active protein.

The recent identification of a UDP-arabinopyranose mutase (UAM) from rice that catalyzes the reversible formation of UDP-Ara*f* from UDP-Ara*p* [71••] should facilitate the identification of AraTs that catalyze the transfer of arabinofuranose, rather than arabinopyranose.

Conclusions: challenges and future directions in studying pectin synthesis

The first step in understanding how the 67 or more pectin biosynthetic transferases work together to synthesize the pectic polysaccharides is to identify the genes encoding the biosynthetic enzymes. The identification of the four glycosyltransferases involved in the synthesis of HG, XGA, and RG-II is a start, but rapid progress in identifying the other pectin biosynthetic enzymes will require overcoming the following challenges: (1) remaining uncertainty regarding pectin structure and how and where in the cell the pectic polysaccharides are crosslinked; (2) the growing body of evidence that species-, cell type-, and developmental state-specific differences in pectin structure exist, making it likely that the number and types of enzymes required to synthesize pectin are plant, tissue, cell type and developmental state specific; (3) the limitation that knowledge of the structure of 'mature' pectic polysaccharides from the wall does not necessarily reflect the structures as they are synthesized, but rather structures after insertion into the wall and after any subsequent modification *in muro* by chemical and/or enzyme-catalyzed reactions; (4) the lack of availability, and in some cases identity, of the biosynthetic substrates and acceptors; and (5) the great difficulty in recovering enzyme activity in heterologously expressed genes. Despite these challenges, however, progress is being made and putative pectin biosynthetic genes are being identified via mutant phenotype characterization and by identification of genes co-transcribed with *de facto* pectin biosynthetic genes. The hold up, however, remains the definitive identification of enzyme function. To date, success in proving enzyme activity has required a focused, and often time consuming, gene-by-gene effort. Whether broader, more global methods can be developed to obtain the definitive enzyme activity data needed to prove gene function is not clear. However, the increasing evidence that wall synthesis occurs, at least partly, via protein complexes makes efforts to purify and characterize protein complexes with a dual emphasis on enzyme activity and subunit structure determination, particularly important. Likewise, the development of methods to either isolate single cell types in plants, so as to study cell-type-specific synthesis, or potentially more powerful, the development of methods to study synthesis in single cells, would greatly catapult the field forward. Finally, there is a continued need to know the identity of, and have available, the diverse sugar, and acceptor substrates used by the pectin biosynthetic transferases.

Acknowledgements

The following funding is gratefully acknowledged for supporting the writing of this review and the research that drives the author's work in this area: NRI, CSREES, USDA Award 2006-35318-17301; NSF MCB awards 0313509 and 0646109; and the BioEnergy Science Center, the U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.

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