Evolving Views of Pectin Biosynthesis

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Annu. Rev. Plant Biol. 2013. 64:747–79

First published online as a Review in Advance on March 1, 2013

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-042811-105534

Keywords

plant cell wall biosynthesis, homogalacturonan, rhamnogalacturonan, glycosyltransferase, protein complex, biomass

Abstract

Recent progress in the identification and characterization of pectin biosynthetic proteins and the discovery of pectin domain–containing proteoglycans are changing our view of how pectin, the most complex family of plant cell wall polysaccharides, is synthesized. The functional confirmation of four types of pectin biosynthetic glycosyltransferases, the identification of multiple putative pectin glycosyl- and methyltransferases, and the characteristics of the GAUT1:GAUT7 homogalacturonan biosynthetic complex with its novel mechanism for retaining catalytic subunits in the Golgi apparatus and its 12 putative interacting proteins are beginning to provide a framework for the pectin biosynthetic process. We propose two partially overlapping hypothetical and testable models for pectin synthesis: the consecutive glycosyltransferase model and the domain synthesis model.

Contents

HG: homogalacturonan

RG-I: rhamnogalacturonan I

RG-II: rhamnogalacturonan II

INTRODUCTION

Building a complete picture of the pectin biosynthetic process requires a detailed understanding of pectin structure. However, pectin is not a single structural cell wall polymer; rather, it is a family of plant cell wall polysaccharides and/or glycan domains that contain galacturonic acid (α-D-GalA) linked at both the 1 and 4 positions. The pectic polysaccharides are generally grouped into three major types: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and the substituted galacturonan rhamnogalacturonan II (RG-II). Some plant cell walls also contain additional substituted galacturonans, known as apiogalacturonan (AGA) and xylogalacturonan (XGA) (see structural details below).

Glycosidic linkage data indicate that these different pectin types/domains are covalently linked to one another in the wall, although in no case has the full structure for a complete pectin macromolecule been determined. When considering pectin structure as presently understood, it is important to realize that it is virtually impossible to extract intact pectin from plant cell walls for structural studies. The reported pectin structures were determined using procedures that likely broke covalent bonds during the extraction process. This makes biosynthetic studies challenging because our picture of pectin as it exists in the plant may be incomplete.

Pectic polysaccharides have numerous and distinct functions in plants. These include promoting cell-cell adhesion (112) via, for example, RG-II dimerization (9) and HG cross-linking (19); providing structural support in soft tissues (i.e., in primary walls); influencing secondary wall formation in fibers and woody tissues (61, 139, 155, 156); providing a reservoir of oligosaccharide signaling molecules important for plant growth, development, and defense responses (138, 145); and acting as a hydration polymer to, for example, affect wall rheology and support seed and root growth and function (58, 97, 178). One goal of pectin synthesis research is to use the directed modification of pectin structure to develop a structure-function-level understanding of how pectin performs its diverse functions in the plant.

Plant cell wall matrix polysaccharides, including pectin, are synthesized in the Golgi apparatus [recently reviewed by Driouich et al. (30)]. Numerous biochemical and cell biology data support the model that pectin is synthesized and modified as it moves through the

different Golgi cisternae and the *trans*-Golgi network and is transported via vesicles to the cell wall. However, one cannot exclude the possibility that the initiation of pectin synthesis occurs earlier in the secretory pathway—for example, in the endoplasmic reticulum. Assuming that unique enzymes are required to catalyze the formation of each unique pectic glycosidic linkage and modification, pectin synthesis requires at least 67 different transferases, including glycosyltransferases (GTs), methyltransferases (MTs), and acetyltransferases (ATs) (103).

Our original view of pectin synthesis, referred to as the consecutive GT model, was that these transferases work independently and sequentially to initiate, elongate, and branch the pectic polysaccharides (**Figure 1*a***). However, based on recent progress described in this review, we now propose an additional hypothetical but data-supported model for pectin synthesis: the domain synthesis model (**Figure 1*b***). We present here a brief description of these models to provide readers with two perspectives as they progress through the review.

In the consecutive GT model (**Figure 1*a***), an increasingly complex pectin structure, exemplified by HG and RG-I, is produced by a series of GTs that consecutively add sugars from nucleotide-sugar substrates onto growing polymer chains as they move through the *cis*-, medial-, and *trans*-Golgi apparatus, a process akin to an assembly line. In the domain synthesis model (**Figure 1*b***), an oligo- or polysaccharide primer is synthesized by an initiation enzyme using nucleotide sugars or possibly lipid-linked sugars, and the primers are elongated by elongation enzymes to make pectic glycan domains such as HG and RG-I. The domains are then transferred en bloc onto a growing polysaccharide. This mechanism would enable the synthesis of polysaccharides and proteoglycans with diverse pectin domains. The evidence for and against these models is summarized at the end of this review.

Pectin structure and synthesis have been reviewed multiple times over the past decade, including in several recent reviews (19, 30, 58, 103). In this review, following a brief overview of pectin structure, we summarize our current understanding of how pectin is synthesized and indicate which aspects of the process are biochemically proven and which are putative or hypothesized. We also present updates in the field, including the identification of pectin biosynthetic complexes (6, 56), data that lead us to propose the domain synthesis model for pectin synthesis.

AGA: apiogalacturonan

XGA: xylogalacturonan

Pectin domain: a region of pectin (such as HG, RG-I, or RG-II) within a polysaccharide or wall proteoglycan

Glycosyltransferases (GTs): enzymes that catalyze the transfer of a glycosyl residue from an activated-sugar donor (e.g., nucleotide sugar) onto an acceptor, forming a new glycosidic bond in the product

PECTIN STRUCTURE

Extensive chemical analyses of cell walls and wall fractions from many plant species and tissues have identified primary plant cell walls as composed of the polysaccharides cellulose, hemicellulose, and pectin, with a small amount (~10%) of diverse structural and enzymatic proteins (5). Pectin is particularly abundant in primary walls of dicots and nongraminaceous (nongrass) monocots and is enriched in some fruits, such as citrus and apple. Pectin is also present, albeit in much lower amounts, in secondary walls and in the primary walls of grasses. Our current knowledge of the sugar composition and linkages in pectin structure is derived mostly from primary walls of leaves and fruit tissues or from suspension-culture cells. These studies have led to the view that there are three major pectic polysaccharides: HG, RG-I, and RG-II (**Figure 2**).

Approximately 65% of pectin is HG, which is a homopolymer of D-GalA linked in an α-1,4 configuration (**Figure 2*a***). HG is partially methylesterified at the *O*-6 position and to a lesser extent acetylated at *O*-2 and *O*-3. There is evidence that contiguous regions of unesterified HG interact via Ca^{2+} salt bridges in the wall (19, 122, 172). In some species or tissues, HG may be β-xylosylated at *O*-3 or apiosylated at *O*-2 and/or *O*-3, giving rise to XGA and AGA, respectively. RG-II constitutes ~10% of pectin and is the most complex, but also the most structurally conserved, pectic polysaccharide (**Figure 2*b***). RG-II has an HG backbone substituted with side chains A–D. Side chains A (an

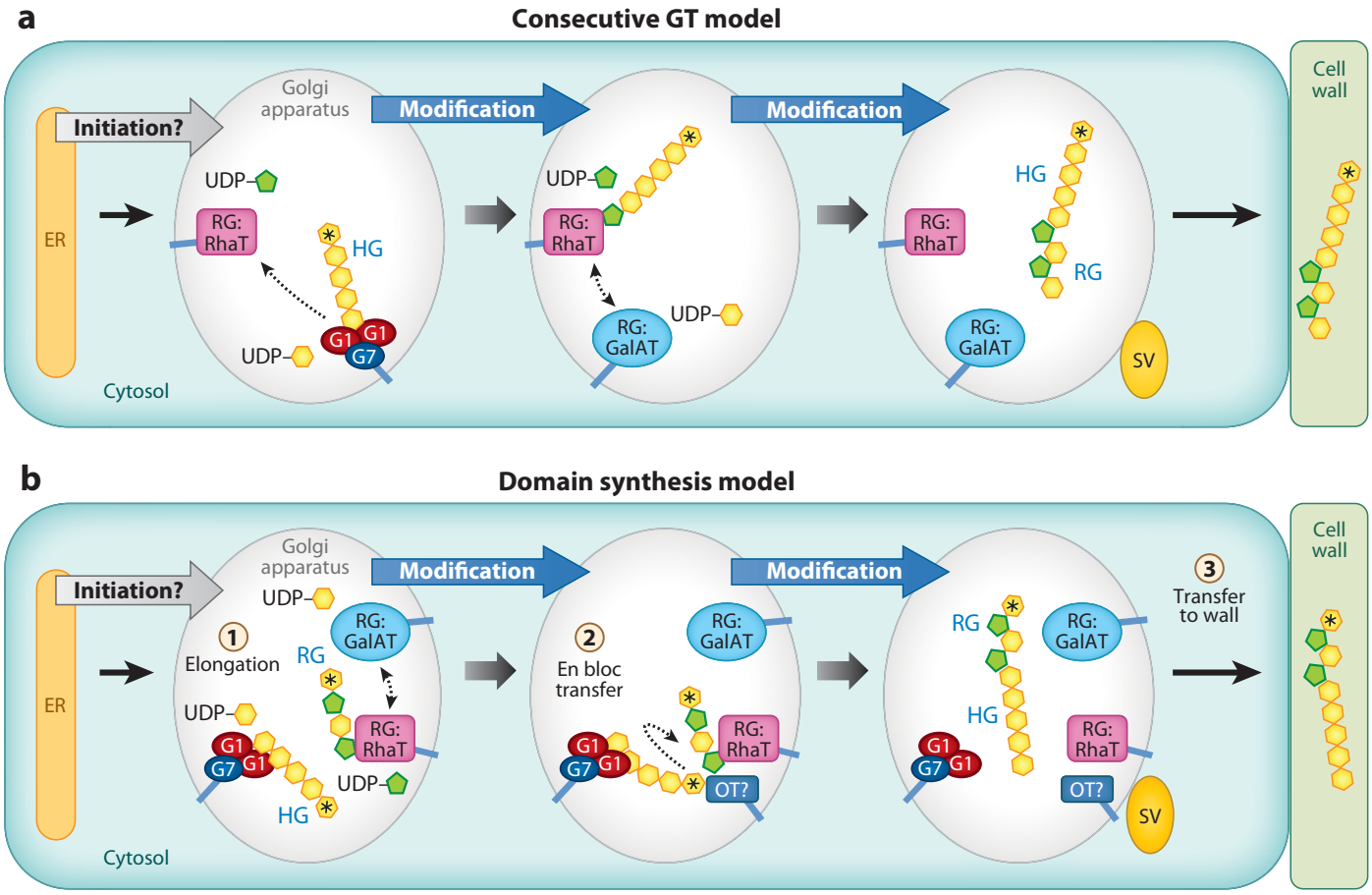


Figure 1

Two hypothetical models for pectin biosynthesis. (*a*) The consecutive glycosyltransferase (GT) model. Individual GTs consecutively add sugar residues to the nonreducing end of growing oligo- or polysaccharides to synthesize the backbone and side branches of pectin. Whether HG or RG-I is synthesized first is not yet known. For clarity, only the GTs involved in each step are shown, but this is not meant to imply that the different GTs do not exist in the same compartment. (*b*) The domain synthesis model. ① Elongation of oligosaccharide domains—e.g., the homogalacturonan (HG) domain by the heterotrimeric GAUT1:GAUT7 HG:α-1,4-D-galacturonosyltransferase (GalAT) complex (G1- and G7-containing complex), and the rhamnogalacturonan I (RG-I) domain presumably by a yet-to-be-identified RG:rhamnosyltransferase (RG:RhaT) and RG:galacturonosyltransferase (RG:GalAT). ② En bloc transfer of a pectin domain [e.g., via oligosaccharyltransferase (OT)] onto another domain (which could also be a growing polysaccharide or a proteoglycan), resulting in a growing pectin structure. This step might occur repeatedly until fully mature polymeric structures are produced. ③ Transfer of the mature polysaccharide (or glycoconjugate polymer) to the wall. In each step, modification of the sugar residues of the growing oligo- or polysaccharides may occur (e.g., methylesterification and/or acetylation). Note that it is not yet known how or where pectin synthesis is initiated or whether the growing oligo- or polysaccharides are free or tethered at the reducing ends (denoted by *asterisks*) to possible lipid or protein moieties. Yellow hexagons and green pentagons represent GalA and Rha residues, respectively. Additional abbreviations: ER, endoplasmic reticulum; SV, secretory vesicle.

octasaccharide) and B (a nonasaccharide in most species) are *O*-2 linked to the HG backbone via β-D-apiosyl (Api*f*) residues. Side chains C and D (both disaccharides) are linked to the HG backbone at *O*-3. The complex and conserved RG-II, with its 12 different sugars in more than 20 linkages, has a critical function in plant growth and development. Abundant evidence shows that RG-II generally exists as an RG-II borate diester dimer, ostensibly linking HG-connected pectin in the wall (9). Even minor modifications of the RG-II structure have deleterious effects on plant growth, including dwarfism (111).

RG-I constitutes 20–35% of pectin. Unlike HG and RG-II, it has a disaccharide

repeat backbone of [4)-α-D-GalA-(1,2)-α-L-Rha-(1,]$_n$ in which the GalA residues are highly acetylated at *O*-2 or *O*-3 (**Figure 2*c***). However, other than its backbone, it is not possible to describe a conserved RG-I structure owing to the cell-type- and developmental-stage-dependent branching, as shown by immunohistochemical studies using many different antibodies reactive against cell wall polymers (37, 54, 170). Specifically, 25–80% of the Rha residues in the RG-I backbone may be substituted at *O*-4 with linear or branched oligosaccharides or polysaccharides. RG-I side chains include α-1,5-linked L-arabinan, which may be branched with arabinose or arabinan at *O*-2 and *O*-3; β-1,4-linked D-galactan, which may be branched at *O*-3 with L-Ara or arabinan; and β-1,3-linked D-galactan branched at *O*-6 with galactan or arabinogalactan (**Figure 2*d***). Some RG-I side chains may contain α-L-Fuc, β-D-GlcA, or 4-*O*-methyl-β-D-GlcA.

Increasing amounts of structural data indicate that HG, RG-I, and RG-II are connected by covalent linkages via their backbones (**Figure 2*e***), forming an interconnected pectin structure in the wall. However, it is not known whether HG, RG-I, and RG-II are arranged in a specific order or what the representative lengths are for each domain within the larger pectin structure. As an example, various lengths (degrees of polymerization) of HG in the wall have been reported, ranging from short stretches of 4–10 residues found interspersed between RG-I domains (108, 162) to isolated HG homopolymers with a degree of polymerization of up to 300 GalA residues (142).

It is not currently possible to isolate and determine the complete structure of "native" pectin from the primary wall, because isolation requires chemical and/or enzymatic treatments that release pectic fragments with concomitant loss of the original structure. There are many reports of the characterization of pectin-enriched fractions isolated from cell walls using salt or weak base extraction. However, considerable evidence (118, 150) indicates that pectin is also present in most if not all fractions isolated from the originally insoluble cell walls, including those often referred to as largely hemicellulose fractions, e.g., from 1M and 4M potassium hydroxide (KOH) fractions. Furthermore, by using relatively mild extraction conditions and extensively analyzing parenchymatous and immature tissues from both monocots (150) and dicots (47, 144, 153, 160), Selvendran and colleagues found that all these cell walls contain an insoluble pectic fraction that constitutes 10–50% of wall pectin. Surprisingly, this insoluble pectin is found in close association with other wall polymers and is abundantly present in the final cellulose-enriched fraction. The presence of such insoluble pectin is not well appreciated across the plant cell wall research field. However, it provides additional evidence that at least a portion of pectin is strongly associated, perhaps covalently, with other polymers/glycoconjugates in the wall, consistent with a pectin-containing glycoconjugate-based wall infrastructure, as suggested by the recent identification of the pectin domain–containing proteoglycan structure arabinoxylan-pectin-arabinogalactan protein 1 (APAP1) (162) (structure *iii* in **Figure 2*e***) (see also sidebar, Proteoglycan with Pectin Domains).

APAP1: arabinoxylan-pectin-arabinogalactan protein 1

Finally, it is difficult to obtain fractions of plant cell walls highly enriched for a single cell type at a single developmental stage. Thus, most cell wall structural studies provide an average structure based on many cell types, leaving open the question of what the specific wall structure is for each cell type. Immunolabeling studies of plant tissue sections using hundreds of antibodies against distinct wall carbohydrate epitopes have indeed identified diverse expression patterns of the respective epitopes in plant cell walls from different species, cell types, and developmental stages (13, 37, 54, 170). Such results clearly show that the precise structure of the plant cell wall is not static but dynamic. Accordingly, the pectin synthesis mechanism must be able to accommodate the diverse structures synthesized in diverse cell types. Many cell wall studies are conducted on suspension-culture cells because they are a source of actively growing cells, which are generally considered

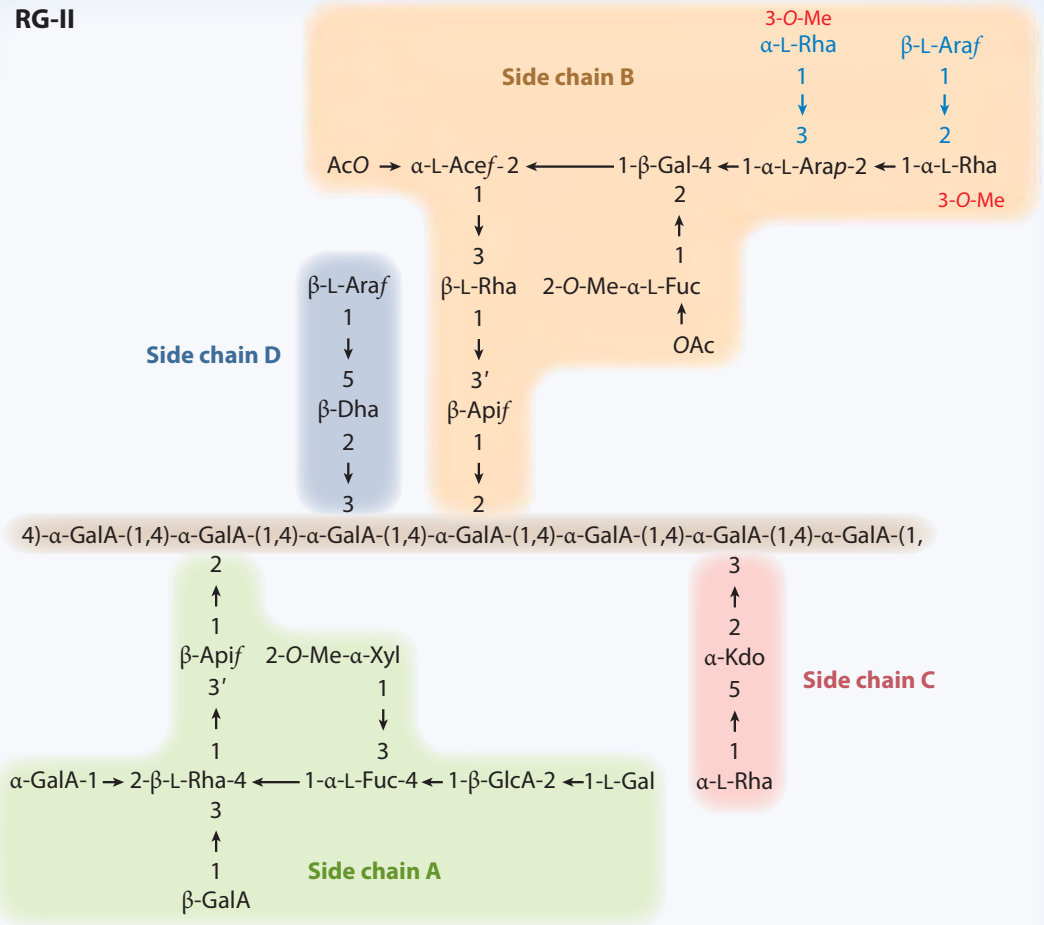
a HG

[4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,]$_n$

Modifications:

- Up to 80% of the carboxyl groups may be methylesterified.
- GalA may be *O*-acetylated at *O*-2 or *O*-3.
- Some GalA may be substituted at *O*-3 with β-D-Xyl, forming xylogalacturonan (XGA). The Xyl may be further elongated at the *O*-2 position by another β-D-Xyl; additional extension with Xyl residues has been observed in soybean.
- GalA in some plants, especially aquatic, may be β-substituted with D-Api*f* at *O*-2 and/or *O*-3, forming apiogalacturonan (AGA).
- Possible *trans*-esters have been proposed between the carboxyl group and *O*-2 or *O*-3 hydroxyls.

b RG-II



Modifications:

- The order of side chains A–D on the backbone has not been definitively shown.
- Red denotes structures present in RG-II of some nonflowering plants (e.g., pteridophyte and lycophyte species).
- Blue denotes structures not present in RG-II of *Arabidopsis* or closely related species.

c RG-I backbone

[4)-α-GalA-(1,2)-α-L-Rha-(1,4)-α-GalA-(1,2)-α-L-Rha-(1,]$_n$

Figure 2

(*Continued*)

d Selected side chains or portions of side chains for which linkage to the Rha in the RG-I backbone has been demonstrated

L-Ara*f*-(1,5)-α-L-Ara*f*-(1,2)-α-L-Ara*f*-(1,3)-β-Gal-(1,4)-Rha in RG-I backbone

α-L-Ara*f*-(1,3)-β-Gal-(1,4)-Rha in RG-I backbone

4
↑
1
α-L-Ara*f*

β-Gal-(1,6)-β-Gal-(1,4)-Rha in RG-I backbone

β-Gal-(1,6)-β-Gal-(1,4)-β-Gal-(1,4)-Rha in RG-I backbone

α-L-Fuc-(1,2)-β-Gal-(1,4)-β-Gal-(1,4)-Rha in RG-I backbone

Ara-Ara-Rha in RG-I backbone

Fuc-Ara-Rha in RG-I backbone

Gal-Gal-Gal-Gal-Rha in RG-I backbone

Modifications:

- Most or all of the GalA in the RG-I backbone is *O*-acetylated at *O*-2 or *O*-3; the acetylation position is species-specific. Acetylation of the backbone Rha residues has also been reported. There is no evidence for methylesterification of GalA in the RG-I backbone (in contrast to HG).
- 25–80% of the Rha in the RG-I backbone is substituted at *O*-4 with linear or branched oligo- or polysaccharides. The specific locations of the side chains along the RG-I backbone are not known.
- ~40 different structures have so far been identified as side chains at *O*-4 of the backbone Rha. The length of the side chain ranges from 1 to ~30 residues.
- For arabinan side chains, α-L-Ara*f* is the residue linked at *O*-4 of the backbone Rha. Most arabinan in RG-I consists of a 5-linked backbone of α-L-Ara*f*, which may be branched at *O*-2 and *O*-3.
- For galactan and arabinogalactan side chains, Gal is the residue linked at *O*-4 of the backbone Rha.
- For galactan side chains, both linear 4-linked β-D-galactans and branched 3- and 6-linked β-D-galactans may be present.
- Galactan side chains may be further branched with Ara-containing side chains, thus forming arabinogalactan.
- Galactan side chains may be terminated by single α-L-Fuc, β-D-GlcA, or 4-*O*-methyl-β-D-GlcA residues.
- Some RG-Is (e.g., from Chenopodiaceae species such as spinach and sugar beet) are esterified with phenolics, including ferulic and/or coumaric acid—e.g., feruloylation at *O*-2, *O*-3, and *O*-5 of Ara in α-L-arabinans and on *O*-6 of Gal in β-D-galactans.

e Interconnections between glycan domains in pectin and in the pectin-containing proteoglycan APAP1

Figure 2

Overview of the structures of the main pectic domains in plant cell wall pectic polysaccharides and in pectin-containing cell wall proteoglycans: (*a*) homogalacturonan (HG), (*b*) rhamnogalacturonan II (RG-II), (*c*) rhamnogalacturonan I (RG-I) backbone, (*d*) selected side chains or portions of side chains for which linkage to the Rha in the RG-I backbone has been demonstrated, and (*e*) interconnections between glycan domains in pectin (*i* and *ii*) and in the pectin-containing proteoglycan arabinoxylan-pectin-arabinogalactan protein 1 (APAP1) (*iii*). Structural information for HG is from References 5 and 19; for RG-II is from Reference 9; for RG-I is from References 5, 38, 58, 89, 103, 134, 151, and 182; for the interconnections between pectin domains is from References 24, 65, and 108; and for APAP1 is from Reference 162. Unless otherwise noted, all sugars are D-sugars in the pyranose ring form. Abbreviations for sugar residues: Ace*f*, aceric acid; Api*f*, apiofuranose; Ara*f*, arabinofuranose; Ara*p*, arabinopyranose; Dha, 3-deoxy-D-lyxo-2-heptulosaric acid; Fuc, fucose; Gal, galactose; GalA, galacturonic acid; GlcA, glucuronic acid; Kdo, 3-deoxy-D-manno-octulosonic acid; Rha, rhamnose; Xyl, xylose. Abbreviations for nonpectin portions of APAP1: AG, arabinogalactan; AGP, arabinogalactan protein; Xylan1 and Xylan2, hemicellulosic glycan domains.

PROTEOGLYCAN WITH PECTIN DOMAINS

Pectins are generally considered cell wall polysaccharides. Although there have been reports over the past 40 years that they may be covalently linked to proteins or other wall polymers, in the absence of definitive chemical linkage data to support such claims, the view of pectins as polysaccharide polymers has persisted. However, the recent identification and structural confirmation of a plant cell wall proteoglycan that contains pectic HG and RG-I domains covalently attached to a cell wall protein indicates that pectin domains are also present in cell wall proteoglycans. Tan et al. (162) identified arabinoxylan-pectin-arabinogalactan protein 1 (APAP1) from the media of *Arabidopsis* suspension-culture cells. APAP1 consists of pectin and hemicellulose domains covalently linked to arabinogalactan protein. The pectin domain–containing proteoglycan was also identified in *Arabidopsis* cell walls. Although the mechanism for APAP1 synthesis is not yet known, the possibilities include intracellular synthesis in the endomembrane system and/or transglycosylation in the wall. The existence of APAP1 makes it necessary to consider a pectin biosynthetic process that could accommodate the synthesis of both pectic polysaccharides and pectin-containing cell wall proteoglycans.

to entail only a few so-called dedifferentiated cell types. However, it is important to note that cells grown in vitro may retain some characteristics of the original tissue from which they were derived, but may also express an in vitro–induced growth state that may not reproduce the original wall structure as present in planta.

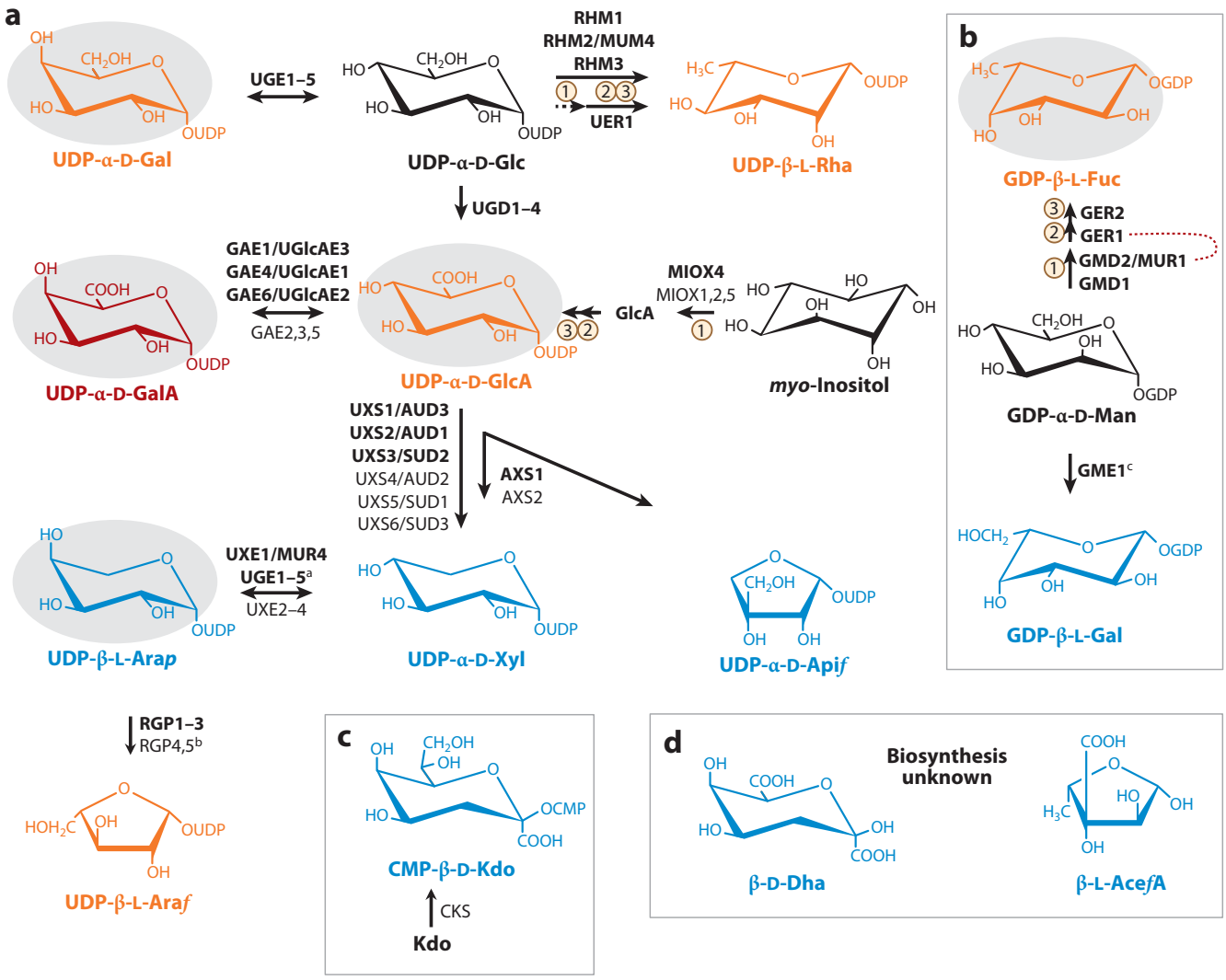
SUBSTRATES FOR PECTIN BIOSYNTHESIS

Pectin biosynthesis requires multiple nucleotide sugars as activated-sugar donor substrates. The nucleotide sugars are generated from a few nucleotide-sugar precursors (UDP-Glc, GDP-Man) by the action of interconversion enzymes and/or through the so-called salvage pathway via the conversion of free sugars to nucleotide sugars in two sequential reactions catalyzed by sugar kinases and UDP-sugar pyrophosphorylase (**Figure 3**, **Supplemental Table 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). The majority of enzymes involved in these pathways have been identified and characterized (8, 9). Because the availability of the diverse nucleotide sugars is a key factor for the synthesis of pectin and other wall polysaccharides, tight regulation of nucleotide-sugar biosynthesis is critical.

Multiple isoforms exist for most of the nucleotide-sugar biosynthetic enzymes, especially those that function in the interconversion pathway (**Figure 3**). Some of the isoforms may have overlapping expression and/or function. For instance, whereas single mutants of *Arabidopsis* UDP-Glc dehydrogenase isoforms 2 and 3 (*ugd2* and *ugd3*, respectively) have no visible growth phenotype, *ugd2 ugd3* double mutants show abnormal seedling development, dwarfed plants, and lower seed set (136). Similarly, *Arabidopsis rgp1* and *rgp2* single mutants, which are defective in two UDP-Ara mutase isoforms, grow normally despite reduced wall Ara content, yet *rgp1 rgp2* double mutants are male gametophytic lethal owing to pollen defects (135). Individual nucleotide-sugar isoforms, however, may serve a unique function with regard to spatial (e.g., at the tissue, cellular, or subcellular levels) and/or developmental expression, formation of enzyme complexes, and enzymatic properties (8, 9). For example, analyses of the expression patterns and phenotypes of double, triple, and quadruple mutants of the five distinct *Arabidopsis* UDP-Glc 4-epimerase isoforms (UGE1–5) suggest that UGE2 and -4 function in overall plant growth and development, UGE3 is specific for pollen development, and UGE1 and -5 may function during stress (141). The UGE isoforms also have different catalytic efficiencies, exogenous NAD^+ cofactor requirements, and subcellular localizations (7).

Subcellular compartmentalization of the nucleotide-sugar biosynthetic enzymes may function to direct the sugar substrates to the proper biosynthetic machinery that produces specific polysaccharides (8, 9). Most of the interconversion enzymes and all the identified

Supplemental Material



[a]UGE1 and -3 also have UDP-Xyl 4-epimerase activity, whereas UGE2, -4, and -5 have only weak UDP-Xyl 4-epimerase activity.
[b]*Escherichia coli*–expressed recombinant RGP4 and -5 were found to be inactive.
[c]GME1 also catalyzes the conversion of GDP-α-D-Man to GDP-β-L-Gul (not shown). L-Gul is a 5-epimer of D-Man and 3-epimer of L-Gal.

Figure 3

Summary of the nucleotide sugars that supply pectin biosynthesis and their biosynthetic pathways, showing proven (*bold*) and putative nucleotide-sugar interconversion enzymes and pathways that have been identified in *Arabidopsis*. Nucleotide sugars in red are present in all pectins, those in orange are present in rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II), and those in blue are specific to RG-II and/or other substituted galacturonans. Nucleotide sugars with a gray oval background are also produced via salvage pathways, which involve conversion of free sugars to corresponding nucleotide sugars by the sequential action of sugar-1-P kinases and a pyrophosphorylase. (*a*) Nucleotide-sugar interconversion pathway involving UDP-α-D-Glc. Enzyme abbreviations: UGEs, UDP-Glc 4-epimerases/UDP-Gal 4-epimerases; RHMs, tri-activity enzymes with UDP-Glc 4,6-dehydratase, UDP-4-keto-6-deoxy-D-Glc 3,5-epimerase, and UDP-4-keto-L-Rha 4-keto-reductase activities; UER1, bi-activity enzyme with UDP-4-keto-6-deoxy-D-Glc 3,5-epimerase and UDP-4-keto-L-Rha 4-keto-reductase activities; UGDs, UDP-Glc dehydrogenases; GAEs/UGlcAEs, UDP-GlcA 4-epimerases; UXSs, UDP-GlcA decarboxylases/UDP-Xyl synthases; AXSs, UDP-D-Apif/UDP-D-Xyl synthases; MIOXs, *myo*-inositol oxygenases that catalyze the conversion of *myo*-inositol to GlcA, which is subsequently converted to UDP-α-D-GlcA via the salvage pathway; UXEs, UDP-Xyl 4-epimerases/UDP-Ara 4-epimerases; RGPs, UDP-L-Arap mutases. Alternative names: MUM4, MUCILAGE-MODIFIED4; AUDs, membrane-anchored UDP-D-GlcA decarboxylases; SUDs, soluble UDP-D-GlcA decarboxylases; MUR4, MURUS4. (*b*) Nucleotide-sugar interconversion pathway involving GDP-α-D-Man. Enzyme abbreviations: GMDs, GDP-Man 4,6-dehydratases; GERs, GDP-4-keto-6-deoxy-Man 3,5-epimerases/4-reductases; GME1, GDP-Man 3′,5′-epimerase. Alternative names: MUR1, MURUS1. The dotted red line between GER1 and GMD2/MUR1 indicates protein complex formation between these two enzymes. (*c*) CMP-Kdo synthesis from Kdo by CMP-Kdo synthetase (CKS). (*d*) Nucleotide sugars with no activated forms identified to date. **Supplemental Table 1** provides more detailed information on the nucleotide-sugar biosynthetic enzymes.

GalAT: an enzyme with galacturonosyltransferase activity

HG:GalAT: homogalacturonan:α-1,4-D-galacturonosyltransferase (EC 2.4.1.43)

Nonreducing end(s): end(s) of a polysaccharide with sugar anomeric carbon linked in a glycosidic bond; a linear polysaccharide has one nonreducing end, whereas a branched polysaccharide has multiple nonreducing ends

salvage pathway enzymes are soluble and located in the cytosol, and thus the produced nucleotide sugars must be translocated by nucleotide-sugar transporters into the Golgi lumen to be used by pectic polysaccharide biosynthetic enzymes whose catalytic domains face the Golgi lumen. However, isoforms of UDP-GlcA 4-epimerase (GAE) (53, 104) and UDP-Xyl 4-epimerase (UXE) (18) as well as half the isoforms of UDP-GlcA decarboxylase (UXS) (59, 119, 149) are type II membrane proteins localized to the Golgi apparatus, their products being readily accessible to the wall matrix biosynthetic GTs. Recently, an *Arabidopsis* CMP-Kdo synthetase (CKS) was localized to the mitochondria (78), where Kdo-containing lipid A–like molecules are synthesized (94). It is not known whether the CMP-Kdo produced in the mitochondria is transported to the Golgi apparatus or whether another CMP-Kdo-synthesizing enzyme exists in the Golgi apparatus to accommodate pectin synthesis.

Based on the interconversion pathway, one would expect that defects in the earlier stages of the pathway may limit the availability of multiple nucleotide sugars and thus cause a more global effect on wall polysaccharides. Indeed, this is exemplified by the defective synthesis of UDP-GlcA in the *ugd2 ugd3* double mutant, which results in reductions in multiple wall sugar residues associated with both pectin and xyloglucan (136). In contrast, mutations in the genes that synthesize nucleotide sugars used largely for pectin synthesis have been shown to affect pectin structure and content. For example, the *Arabidopsis mum4* mutants are defective in RHM2/MUM4, which converts UDP-Glc to UDP-L-Rha, and are specifically affected in seed epidermal cells, which produce significantly less seed mucilage with smaller-sized and reduced amounts of RG-I (114, 166, 169). Mutations in *MUR1*, a gene encoding an isoform of GDP-D-Man 4,6-dehydratase that converts GDP-D-Man to GDP-L-Fuc, results in dwarf plants with brittle tissues that contain almost no L-Fuc in RG-II and xyloglucan in aboveground organs and also have reduced RG-II borate-diester cross-linking (111, 120, 137). In *Nicotiana benthamiana*, virus-induced gene silencing of *AXS1*, which encodes UDP-D-Api*f*/UDP-D-Xyl synthase, leads to cell death and thickened walls associated with defects in RG-II side chains (2). Although it is still unclear what role the salvage pathway, compared with the interconversion pathway, has in the synthesis of nucleotide sugars destined for the syntheses of wall polysaccharides and other glycosylated molecules, that the salvage pathway is essential is evidenced by mutants of the promiscuous UDP-sugar pyrophosphorylase. These mutants are male gametophytic lethal owing to the defective pectocellulosic intine layer of pollen grains (148). All these examples demonstrate that nucleotide-sugar biosynthesis is essential for, and may become a limiting factor during, pectin biosynthesis.

KNOWN PECTIN BIOSYNTHETIC TRANSFERASES

The identification and characterization of pectin biosynthetic transferases and the encoding genes are major challenges. Progress has been slow due to, among other things, the difficulties in obtaining pure and active enzyme preparations. As summarized below, numerous enzymatic activities have been demonstrated in crude enzyme preparations. To date, however, enzymatic activity has been biochemically demonstrated for only seven transferase genes, comprising four pectin biosynthetic activities. In addition, several other genes have been assigned putative functions in pectin synthesis (**Table 1**). Most of these proven and putative pectin biosynthetic enzymes have been shown to localize to the Golgi apparatus, and all of them have predicted or proven type II transmembrane topology.

Homogalacturonan Biosynthetic Glycosyltransferases

HG:α-1,4-D-galacturonosyltransferase (HG:GalAT) catalyzes the transfer of D-GalA from UDP-D-GalA onto the nonreducing end of endogenous and exogenous HG acceptors

Table 1 Proven and putative pectin biosynthetic genes in *Arabidopsis*

Gene name	Identifier	CAZy family	Function	Protein complex	Reference(s)
Homogalacturonan (HG)					
GAUT1	At3g61130	GT8	Proven HG:GalAT	GAUT1:GAUT7 HG:GalAT	6, 158
GAUT7	At2g38650	GT8	Proven Golgi membrane anchor of GAUT1	GAUT1:GAUT7 HG:GalAT	6
GAUT8/QUA1	At3g25140	GT8	Putative HG:GalAT		14, 90, 115
QUA2/TSD2	At1g78240		Putative HG:MT		84, 106
QUA3	At4g00740		Putative HG:MT		101
CGR3	At5g65810		Putative HG:MT		60
Rhamnogalacturonan I (RG-I)					
ARAD1	At2g35100	GT47-B	Putative RG-I:α-1,5-AraT	ARAD1:ARAD2	55, 56
ARAD2	At5g44930	GT47-B	Putative RG-I:α-1,5-AraT	ARAD1:ARAD2	56
GALS1	At2g33570	GT92	Proven β-1,4-GalT		96
GALS2	At5g44670	GT92	Putative β-1,4-GalT		96
GALS3	At4g20170	GT92	Putative β-1,4-GalT		96
Rhamnogalacturonan II (RG-II)					
RGXT1	At4g01770	GT77	Proven RG-II:α-1,3-XylT		33, 128
RGXT2	At4g01750	GT77	Proven RG-II:α-1,3-XylT		33, 128
RGXT3	At1g56550	GT77	Proven RG-II:α-1,3-XylT		32
RGXT4/MGP4	At4g01220	GT77	Proven RG-II:α-1,3-XylT		95
Xylogalacturonan (XGA)					
XGD1	At5g33290	GT47-C	Proven XGA:β-1,3-XylT		70

Abbreviations: CAZy, Carbohydrate Active enZYmes database (**http://www.cazy.org**); AraT, arabinosyltransferase; GalAT, α-1,4-galacturonosyltransferase; GalT, galactosyltransferase; MT, methyltransferase; XylT, xylosyltransferase.

(4, 29, 63, 147). Membrane-bound HG:GalAT activity has been identified in multiple species (reviewed in 19), in all of which it strictly requires Mn^{2+} for catalysis. HG:GalAT activity has been localized to the Golgi apparatus, with the catalytic domain facing the Golgi lumen (159). The reaction products generated by tobacco membrane-bound HG:GalAT are ~50% esterified, of which at least 40% are methyl esters (28). Membrane-bound HG:GalATs in microsomes and isolated Golgi bodies can use endogenous acceptors to generate high-molecular-weight (>500 kDa) products (28, 159). In contrast, detergent-solubilized HG:GalATs require an exogenous acceptor for activity, preferably oligosaccharides with a degree of polymerization of 10 or more (4, 29, 63, 113). The elongation of such acceptors by more than 10 GalA residues has been reported (4, 158). Neither detergent-solubilized nor detergent-permeabilized HG:GalATs could initiate de novo polymerization of HG in the absence of an exogenous acceptor substrate or generate high-molecular-weight polymeric products. These results indicate that detergent-solubilized HG:GalATs most likely function in the elongation step of HG biosynthesis. The possibility remains, however, that detergent treatment of HG:GalAT-containing microsomes may negatively impact endogenous HG:GalAT activity and/or biosynthetic complexes and result in loss of subunits, endogenous acceptors, and/or processivity (4, 28, 29, 63, 159).

GAUT: GALACTURONOSYLTRANSFERASE (named after GAUT1, the first identified HG:GalAT)

GATL: GAUT-like

CAZy (Carbohydrate Active enZYmes): a database of carbohydrate active enzymes, including glycosyltransferases and glycosylhydrolases that generate and degrade glycosidic bonds, respectively (**http://www.cazy.org**) (21)

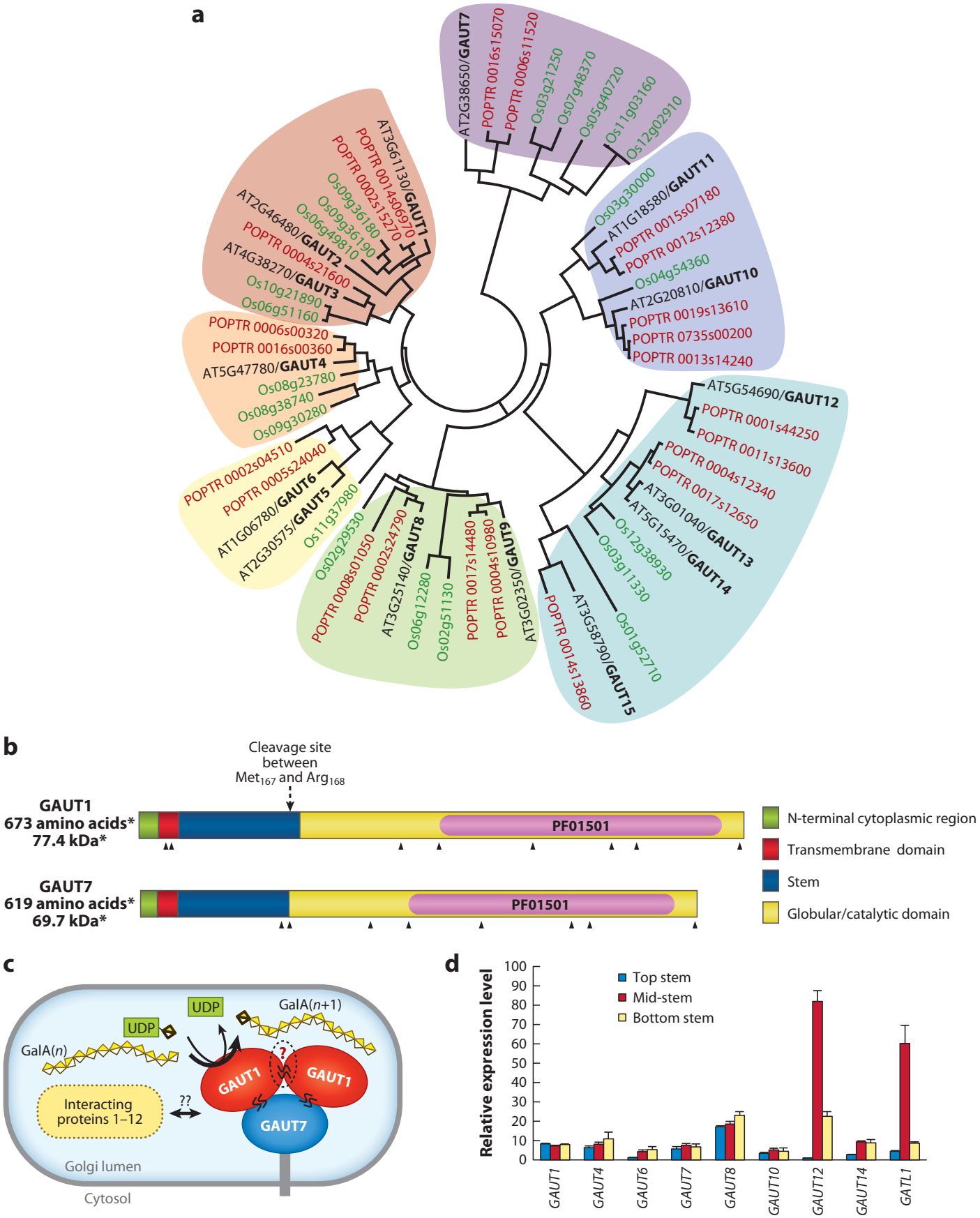
Arabidopsis GALACTURONOSYLTRANSFERASE 1 (GAUT1) was identified via a proteomics analysis of a partially purified, HG:GalAT activity–enriched *Arabidopsis* detergent-solubilized enzyme preparation (158). Transient expression in mammalian HEK293 cells of recombinant GAUT1 (truncated to remove its native transmembrane domain) yielded HG:GalAT activity, and anti-GAUT1 polyclonal antibodies specifically immunoabsorbed detergent-solubilized HG:GalAT activity from *Arabidopsis* suspension-culture cells, providing conclusive biochemical evidence that GAUT1 is an HG:GalAT (158). More recently, it was shown that GAUT1 functions in a protein complex with GAUT7 (see Role of Protein Complexes in Pectin Biosynthesis, below). In silico tertiary structure prediction of *Arabidopsis* GAUT1 through comparison with the solved structure of the GT8 *Neisseria meningitidis* LgtC and rabbit glycogenin (44, 125) indicated regions/motifs that may have roles in substrate binding, catalysis, and protein-protein interaction (175). Proof of these motifs' function will require functional analysis via site-directed mutagenesis.

BLAST analysis of GAUT1 protein sequence against the *Arabidopsis* genome led to the identification of the GAUT (36–68% identity to GAUT1) (**Figure 4*a***) and GAUT-like (GATL) (23–29% identity to GAUT1) families, with 15 and 10 members in *Arabidopsis*, respectively. Together, these GAUTs and GATLs form the GAUT1-related superfamily within the CAZy (Carbohydrate Active enZYmes; **http://www.cazy.org**) GT8 family (21, 158). Other members of the GAUT1-related superfamily have been proposed to also function as GalATs that catalyze the transfer of GalA from UDP-GalA onto HG or other acceptors (158). *GAUT* and *GATL* genes are expressed transcriptionally in all major tissues of *Arabidopsis*, with the exceptions of *GAUT2*, which was suggested to be a nonfunctional truncated homolog of *GAUT1* (20), and *GATL4*, whose expression is restricted to pollen grains and elongating pollen tubes (80).

Sugar composition analysis of *Arabidopsis* transfer DNA (T-DNA) mutant lines revealed eight *gaut* mutants—*gaut6* and *gaut8–14*—having significantly different wall sugar content (in mol%) of GalA, Xyl, Rha, Gal, and/or Ara in comparison with the wild type, with significantly reduced wall GalA content also reported for *gatl3*, *-6*, and *-9*, suggesting that the corresponding genes are involved in wall pectin and/or xylan polysaccharide biosynthesis

Figure 4

GAUT protein family phylogeny, GAUT1:GAUT7 complex, and differential expression of *GAUT* genes in *Arabidopsis* inflorescence stems. (*a*) Phylogenetic tree of the GAUT family members in *Arabidopsis*, rice, and poplar shows division of the GAUT proteins into seven clades. Full-length protein sequences obtained from Phytozome (**http://www.phytozome.org**) were processed essentially as described in Reference 175. Multiple sequence alignment was performed using version 6.925 of the MAFFT program (72), and phylogeny was reconstructed using version 2.1.4 of the FastTree program (132). (*b*) Schematic of *Arabidopsis* GAUT1 and GAUT7 proteins. The N-terminal cytoplasmic domain and the transmembrane domain were predicted using version 2 of the HMMTOP program (164); the stem domain was estimated as approximately 25% of the total protein length. Arrowheads indicate Cys residue positions. PF01501 is the Pfam Glyco_transf_8 domain. Asterisks denote that the protein size was predicted from translated gene sequence; in vivo, GAUT1 cleavage between Met_{167} and Arg_{168} results in a 58.6-kDa protein (6). (*c*) Model of the *Arabidopsis* GAUT1:GAUT7 homogalacturonan:α-1,4-D-galacturonosyltransferase (HG:GalAT) complex that catalyzes the elongation step of HG synthesis. Intermolecular covalent disulfide bonds (SS bonds) exist between GAUT1 and GAUT7, and perhaps also between the two GAUT1 molecules. Bolded residues represent GalA residues before and after transfer from UDP-GalA onto the nonreducing end of the growing HG. (*d*) Transcript expression of selected *GAUT* and *GATL* genes in *Arabidopsis* inflorescence stems, as revealed by real-time polymerase chain reaction analysis. First-strand cDNA was synthesized using 1 μg of total RNA isolated from 1-inch stem segments taken from the top stem (immediately below the inflorescence), mid-stem, and bottom stem (immediately above the soil). Relative gene expression was normalized using *ACTIN2* as the reference gene, and the expression of *GAUT12* in the top stem was set to 1. The value is the mean ± standard deviation of three independent experiments.



GALS: GALACTAN SYNTHASE

ARAD: ARABINAN DEFICIENT

(20, 80). Despite the changes in wall composition, many of the mutants did not show major growth phenotypes compared with the wild type. However, the *gaut8*, *gaut12*, and *gatl1* mutants show severe dwarfed growth and sterility (14, 20, 88, 126, 154). Mutant characterization suggests that *GAUT8/QUASIMODO 1* (*QUA1*) may function in HG biosynthesis, although a marked reduction in xylan synthase activity also suggests a connection to xylan synthesis (see more detail below). Despite efforts, there have been no reports of successful isolation of homozygous mutant lines for *GAUT1* and *GAUT4*, suggesting that these genes encode proteins crucial to plant growth and development whose mutation may be lethal.

Rhamnogalacturonan I Biosynthetic Glycosyltransferases

RG-I biosynthesis requires both GalAT and rhamnosyltransferase (RhaT) activities to generate the backbone as well as multiple galactosyltransferases (GalTs) and arabinosyltransferases (AraTs) to initiate, elongate, and branch the side chains. RG-I backbone synthesis in vitro has not been demonstrated to date, at least in part because of the lack of available donor substrate UDP-Rha. Multiple in vitro studies on the synthesis of galactan side chains in several plant species have demonstrated Golgi-localized β-1,4-GalT activities with different pH optima and acceptor substrate preferences (1, 10, 41–43, 51, 130). The most well-characterized β-1,4-GalT activity specifically elongates the galactan side chains (66, 81, 82, 130). Partially purified detergent-solubilized enzyme from soybean was shown to elongate galactoheptaose acceptors by >25 Gal residues, almost achieving the size of the native soybean β-1,4-galactan (degree of polymerization of 43–47) (81). Very recently, *Arabidopsis* GALACTAN SYNTHASE 1 (GALS1) of GT92 has been demonstrated as a β-1,4-galactan:β-1,4-GalT (96). Loss-of-function mutation and overexpression of the *GALS1* gene result in reduced and increased amounts, respectively, of cell wall galactan, as reflected by sugar residue composition and epitope recognition by an LM5 antibody specific for β-1,4-galactan. Transient expression of *GALS1* in *N. benthamiana* leaves resulted in a recombinant enzyme, which upon purification was able to use UDP-Gal as the donor substrate to elongate β-1,4-galactopentaose, thus biochemically confirming its enzymatic function as a β-1,4-GalT. Mutants of the homologous *GALS2* and *GALS3* also displayed decreased total wall Gal content, implicating these genes as putative β-1,4-GalTs.

Several AraTs involved in the synthesis of arabinan and arabinogalactan side chains have been characterized from mung bean. Although the majority of Ara in RG-I is in the furanose ring form (Ara*f*), mung bean solubilized microsomes were able to transfer a single arabinopyranose residue from the donor substrate UDP-β-L-Ara*p* in β-1,3 and β-1,4 linkages onto the nonreducing ends of α-1,5-arabino- and β-1,4-galacto-oligosaccharide substrates, respectively (64, 67, 110). An α-1,5-arabinofuranosyltransferase activity was also reported that uses UDP-β-L-Ara*f* to elongate α-1,5-arabino-oligosaccharides (83). The recent characterization of *Arabidopsis* T-DNA insertion mutants implicates ARABINAN DEFICIENT 1 (ARAD1) and its homolog ARAD2 in pectin arabinan biosynthesis (55, 56). RG-I isolated from *arad1* mutants, although having a normal number of side chains, was reduced significantly (~70%) in total Ara and 5-linked Ara*f* content compared with the wild type. Immunohistochemical analyses also revealed changes in the pattern of pectin arabinan epitopes recognized in *arad1* and *arad2*. Although very closely related (65% amino acid identity), ARAD1 and ARAD2 are nonredundant because ARAD2 overexpression could not rescue the wall phenotype of *arad1*. Although *arad1*, *arad2*, and the *arad1 arad2* double mutant show no apparent growth phenotypes, the available data support designating these proteins as putative RG-I arabinan:α-1,5-AraTs. Efforts to obtain conclusive biochemical evidence for enzyme function via heterologous expression of these proteins have so far been unsuccessful (56).

Rhamnogalacturonan II Biosynthetic Glycosyltransferases

The complexity of the RG-II structure mandates the involvement of multiple GTs in its biosynthesis, yet none of these have been conclusively identified aside from the RHAMNOGALACTURONAN XYLOSYLTRANSFERASES (RGXTs) that have RG-II:α-1,3-xylosyltransferase activity (see below). Based on defective intercellular adhesion and RG-II-related wall composition phenotypes in the mutant callus line *nolac-H18*, *Nicotiana plumbaginifolia* GUT1 was proposed as an RG-II:β-1,4-glucuronosyltransferase (GlcAT) that incorporates GlcA onto side chain A of RG-II (69). However, this was recently contradicted by studies of the closest *Arabidopsis* homologs of NpGUT1, i.e., IRX10/At1g27440 and IRX10-L/At5g61840. The results strongly suggest roles for these GTs in xylan biosynthesis (17, 174). The enzyme activities of NpGUT1, IRX10, and IRX10-L remain to be demonstrated to resolve this contradiction. A putative *Arabidopsis* Kdo-transferase (KdoT; At5g03770) was also recently identified based on homology to the *Escherichia coli* enzyme involved in lipopolysaccharide biosynthesis; however, the normal RG-II structure of null T-DNA insertion mutants and the mitochondria localization of this putative enzyme make it unlikely to be a putative RG-II:KdoT (152).

Arabidopsis RGXT1–4 have been heterologously expressed in baculovirus-transfected insect cells and *Pichia pastoris* and shown to transfer a Xyl residue from UDP-Xyl onto L-Fuc (32, 33, 95, 128), making a structure unique to side chain A in RG-II (**Figure 2*b***). Specifically, the reaction products of RGXT1–3 were characterized by NMR and enzyme digestion using α- and β-xylosidases and shown to have an α-1,3-linkage (32, 33). RGXT1 and -2 also incorporate [^{14}C]Xyl onto RG-II extracted from *rgxt1* and *-2* mutants but not RG-II from the wild type. RGXT1, -2, and -4 have been localized to the Golgi apparatus (33, 35, 95). *RGXT1* and *-2* are expressed in seedlings and highly expressed in vegetative tissues of mature plants, *RGXT3* is expressed only in leaves and siliques in mature plants, and *RGXT4* appears to be expressed ubiquitously and at a higher level than the other *RGXT*s (32, 33, 35, 95). Mutants of *RGXT1* and *-2* do not have observable growth phenotypes, and no *RGXT3* mutant is available. In contrast, mutation of *RGXT4* severely affects pollen tube and root growth, leading to infertility and seedling lethality (95). Wall analysis of *RGXT4* homozygous mutant seedlings indicates a ~30% reduction in 2-*O*-methyl-D-Xyl of RG-II and a ~23% reduction in RG-II dimerization. The *RGXT4* mutant phenotypes support the function of RGXTs in RG-II synthesis and illustrate the critical wall-strengthening role of RG-II.

RGXT: RHAMNOGALACTURONAN XYLOSYLTRANSFERASE

XGD: XYLOGALACTURONAN DEFICIENT

Xylogalacturonan and Apiogalacturonan Biosynthetic Glycosyltransferases

Mutants of *Arabidopsis* XGA:β-1,3-xylosyltransferase (XylT) XYLOGALACTURONAN DEFICIENT 1 (XGD1) display no growth phenotypes (70). However, analyses of leaf walls indicated defective biosynthesis of low-substituted XGA in the mutants, as shown by a ~25% reduction in total Xyl content, substantial decreases in XGA-characteristic linkages of terminal Xyl and 3,4-GalA in the XGA-enriched fraction, and the absence of XGA-characteristic oligosaccharides in XGA-hydrolase-digested pectin material compared with the wild type. Transient expression of XGD1 in *N. benthamiana* resulted in detergent-treated microsomes capable of transferring [^{14}C]Xyl from UDP-[^{14}C]Xyl onto endogenous oligosaccharides in *N. benthamiana* heat-treated control microsomes and onto exogenous HG oligosaccharides (degree of polymerization of 12–14). The synthesized XGA product was digestible by XGA hydrolase. Although the exact anomeric configuration of the XGD1 reaction product remains to be determined, these data point to XGD1 as the enzyme that catalyzes transfer of Xyl from UDP-Xyl onto GalA residues in the HG backbone to make XGA. Because the *xgd1*

mutant showed no difference from the wild type upon immunolabeling using the LM8 antibody, which is specific for highly substituted XGA, it is likely that other yet-to-be-identified β-1,3-XylTs also exist.

A microsomal membrane preparation from *Lemna minor* contains an AGA apiosyltransferase (ApiT) activity that incorporates [^{14}C]Api*f* from UDP-[^{14}C]Api*f* onto endogenous acceptors, generating a reaction product that is characterized as ammonium oxalate soluble and pectinase degradable and contains apiobiose side chains released by hydrolysis at pH 4 (99, 116). Inclusion of UDP-GalA in the reaction mixture increased the ApiT activity by twofold and also increased the total incorporation of [^{14}C]Api*f* onto endogenous acceptors and the linear range of the reaction, suggesting a cooperation between the syntheses of the HG backbone and the side chain by GalAT and ApiT activities, respectively. Because both the Api*f* residues of the incorporated apiobiose side chains were shown to be radioactive, the ApiT activity may correspond to two separate enzymes catalyzing sequential addition of the Api*f* residues or to a single enzyme capable of consecutively adding two Api*f* residues.

Pectin Methyltransferases

Pectin homogalacturonan:methyltransferase (HG:MT) catalyzes the transfer of a methyl group (CH_3) from the donor substrate *S*-adenosyl-L-methionine (SAM) onto the C-6 carboxyl group of α-1,4-linked GalA residues in HG acceptors. Membrane-associated activities capable of incorporating radiolabeled methyl groups from [^{14}C]SAM onto HG-containing, high-molecular-weight endogenous pectin acceptors have been demonstrated in multiple species (48, 49, 62, 68, 73–75, 146, 167). HG:MT activity is Golgi-localized, with the catalytic domain facing the Golgi lumen (50). SAM is synthesized in the cytosol (168), and its transport into the Golgi lumen and subsequent use in pectin methylation have been demonstrated (62). It is noteworthy that with mung bean and tobacco microsomes, addition of UDP-GalA in the HG:MT reaction increased the incorporation of [^{14}C]methyl groups onto endogenous pectin acceptors (48, 73). However, inclusion of SAM in the HG:GalAT reaction mixture did not stimulate HG synthesis (28, 74), indicating that the polymerization of HG precedes its methylesterification.

Several *Arabidopsis* putative HG:MTs have been described. *Arabidopsis* QUA2 and QUA3 belong to the 29-member QUA2 family characterized by the putative SAM-dependent MT domain PF03141 (106). Allelic mutants of *QUA2* (*qua2* and *tsd2*) display dwarfed growth and cell-cell adhesion defects yet have a normal distribution and degree of pectin methylesterification (84, 106). However, *qua2* has significant reductions in total wall GalA and HG content compared with the wild type.

Detergent-permeabilized microsomes from tobacco BY-2 cells overexpressing QUA3 produce a greater amount of radiolabeled product when incubated with [^{14}C]SAM and HG compared with those of the wild type (101). Although the reaction product was not characterized, the result implicates QUA3 as a putative HG:MT. Downregulation of *QUA3* in *Arabidopsis* suspension-culture cells by RNA interference caused three times higher immunolabeling with the JIM5 antibody (which recognizes low-esterified HG) compared with that of the wild type. These cells had otherwise normal labeling by JIM7 and LM7, antibodies that recognize HG with higher degrees of methylesterification. The increased JIM5 labeling supports a putative HG:MT function for QUA3; however, the unchanged labeling by JIM7 and LM7 is inconsistent with this conclusion. Interestingly, QUA3 has been identified as a putative interacting protein of the GAUT1:GAUT7 HG:GalAT complex (see below), connecting QUA3 to HG synthesis.

The recently reported *Arabidopsis* Cotton Golgi-Related 3 (CGR3) contains SAM-dependent MT domain PF08241 (60), a different MT domain than that in QUA2 and QUA3. Compared with the wild type, mutants of *CGR3* showed changes in immunolabeling of petiole cross sections using HG-specific antibodies,

with *cgr3* knockout lines showing reduced JIM7 epitopes and CGR3–green fluorescent protein (GFP) overexpression lines showing a substantial increase in recognition by JIM7 and LM20. The increased binding by LM20 and JIM7, both antibodies that recognize highly esterified HG, supports CGR3 function as a putative HG:MT. The identity of QUA2, QUA3, and CGR3 as functional HG:MTs awaits biochemical confirmation of the activity of the proteins.

Pectin Acetyltransferases

Membrane-bound homogalacturonan:acetyltransferase (HG:AT) and rhamnogalacturonan I:acetyltransferase (RG-I:AT) activities have been studied in potato suspension-culture cells (121). Microsomal preparations incorporated radioactivity from [^{14}C]acetyl–coenzyme A (CoA) onto endogenous acceptors, at least half of which could be released by endo-polygalacturonase (EPG)/pectin methylesterase (PME) and rhamnogalacturonan acetylesterase treatments. The RG-I:AT activity and the total microsomal AT activity have been partially characterized.

Recently, two *Arabidopsis* putative xyloglucan ATs, AXY4 and AXY4L, were described whose mutations cause reduced acetylation specifically in xyloglucan (45). In contrast, a more general acetylation defect that affects both pectic and nonpectic polysaccharides was reported in the *reduced wall acetylation 2* (*rwa2*) mutant (98). Comparison of these proteins to homologs from bacteria, fungi, and animals indicates a conserved mechanism of polysaccharide *O*-acetylation, suggesting a possible acetyl-donor translocator function for the multi-transmembrane RWA proteins and supporting a putative xyloglucan AT function for the type II AXY4 and AXY4L (46). *AXY4* and *AXY4L* belong to the 46-member *TRICHOME BIREFRINGENCE-LIKE* (*TBL*) gene family (12) proposed to encode wall polysaccharide *O*-ATs. It is thus reasonable to speculate that other *TBL* genes might encode pectin-specific ATs.

ROLE OF PROTEIN COMPLEXES IN PECTIN BIOSYNTHESIS

Detergent solubilization of cell wall biosynthetic transferase activities from membrane preparations often leads to a loss of activity and possible loss of processivity and/or de novo synthetic capability (i.e., synthesis without the need for exogenous acceptors). It has been suggested that these losses may be due to disruption of enzyme complexes (29, 110, 158, 159). The need for one or more protein complex partners may also be a factor in the many unsuccessful attempts to heterologously express enzymatically active plant cell wall transferases. It is therefore perhaps not surprising that the potential role of protein complexes in plant cell wall polysaccharide biosynthesis has become an increasingly common theme. Below, we first summarize what is known about protein complexes in cellulose and hemicellulose syntheses and then describe pectin biosynthetic complexes.

Endo-polygalacturonase (EPG): enzyme that degrades the HG backbone (polygalacturonate) by cleaving within the polymer (as opposed to exo-polygalacturonases, which cleave from the end of the polymer)

Pectin methylesterase (PME): enzyme that removes methyl ester groups from the C-6 position of GalA residues in the HG backbone, thereby exposing the negatively charged carboxyl groups

Cellulose and Hemicellulose Biosynthetic Complexes

The first reported plant cell wall polysaccharide biosynthetic complex was the plasma membrane–located rosette structures now known to participate in cellulose microfibril formation (34). Cellulose synthase (CESA) proteins, thought to be the catalytic subunits of the cellulose synthase complexes, have been shown to associate in the rosettes and to form homo- and/or heterocomplexes in vivo.

Increasing evidence suggests that protein complexes may also function in hemicellulose biosynthesis. A wheat putative glucuronoarabinoxylan synthase complex was reported to contain putative GTs from CAZy families GT43, GT47, and GT75 (181). These GTs coimmunoprecipitate from detergent-solubilized membrane preparations in an apparent complex that exhibits cooperative XylT, AraT, and GlcAT activities. However, a definitive description of the complex is lacking and the catalytic function for each of the GTs has not been assigned. Recently, Chou and coworkers used

bimolecular fluorescence complementation (BiFC) and in vitro pull-down assays of proteins transiently expressed in *Arabidopsis* protoplasts to show homo- and heteroprotein complexes between *Arabidopsis* XXT1, XXT2, XXT5, and CSLC4 involved in xyloglucan biosynthesis (23). Although XXT1 and XXT2 are proven xyloglucan:α-1,6-XylTs, and CSLC4 has been shown to have β-1,4-glucan synthase activity, neither XXT5 catalytic activity nor the activity of the pulled-down complexes has been demonstrated (23, 179). Together, the data suggest a multi-GT enzyme complex that synthesizes the xylosylated glucan backbone.

Pectin Biosynthetic Complexes

The GAUT1:GAUT7 HG:GalAT complex is the first biochemically proven protein complex in the biosynthesis of plant cell wall matrix polysaccharides. GAUT7 is a GAUT family member with 36% amino acid sequence identity and 60% similarity to GAUT1 (158). GAUT7 was coidentified with GAUT1 in the GalAT activity–containing, partially purified *Arabidopsis* solubilized membrane protein preparation used to identify GAUT1. Yet in contrast to GAUT1, GAUT7 did not yield GalAT activity upon transient expression in HEK293 cells. Interaction between GAUT1 and GAUT7 in a protein complex was evidenced by reciprocal coimmunoprecipitations of the two *Arabidopsis* proteins using anti-GAUT1 and anti-GAUT7 antibodies, and by BiFC between GAUT1 and GAUT7 (6). Coimmunoprecipitation of GAUT1 and GAUT7 was eliminated in the presence of both reducing and denaturing agents but persisted in the presence of either agent alone, indicating the involvement of both covalent and noncovalent interactions in holding the complex together. The disulfide-bonded core of the complex was demonstrated by nonreducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and proteomics to be ~185 kDa in size, likely consisting of two GAUT1 subunits and one GAUT7 subunit (**Figure 4*c***) based on normalized mass spectral counts. The GAUT1:GAUT7 complex appears ubiquitous, because promoter:GUS expression analysis showed widespread coexpression of *GAUT1* and *GAUT7* in most tissues and developmental stages of *Arabidopsis* plants, including tissues undergoing both primary and secondary wall formations (see below).

Intriguingly, further characterization of the complex revealed that although both GAUT1 and GAUT7 are predicted to be type II transmembrane proteins, *Arabidopsis* GAUT1 is posttranslationally cleaved, resulting in a mature protein with no transmembrane domain (6) (**Figure 4*b***). It was subsequently demonstrated that GAUT1 devoid of its transmembrane domain requires association with GAUT7 to be retained in the Golgi apparatus, giving GAUT7 a function as a membrane anchor for GAUT1 in the GAUT1:GAUT7 complex (6) (**Figure 4*c***). The concept of requiring complex formation to anchor a pectin biosynthetic protein to the membrane may also apply to the GATL proteins. GATLs are predicted to have only N-terminal signal peptides, thus rendering them soluble, secreted proteins, yet fluorescent protein tagging has shown that some of them localize to the endoplasmic reticulum and/or Golgi apparatus (79, 80, 92).

It will be interesting to determine whether cleavage of GAUT1 and/or complex formation between GAUT1 and GAUT7 influence the catalytic activity of the GAUT1:GAUT7 complex, and whether GAUT7 has an undiscovered catalytic activity. The effects of proteolytic cleavage and complex formation on enzyme activity have previously been reported for plant carbohydrate esterases and for mammalian complexes of closely related GTs in the secretory pathway (3, 77, 107, 173).

Proteomics analysis of the immunoprecipitated GAUT1:GAUT7 HG:GalAT complex also identified 12 proteins as potentially interacting, albeit perhaps transiently, with the GAUT1:GAUT7 complex (6). The interaction of these proteins with the complex will need to be validated by additional methods; however, the identities of these proteins, some of

which are related to wall polysaccharide biosynthesis, provide a new perspective for considering pectin synthesis. For example, QUA3 and one of its homologs are among the identified GAUT1:GAUT7-complex putative interacting proteins, providing another indication of the long-suggested cooperation between HG synthesis and HG modification and making these proteins excellent candidates for further study to verify whether they are functional HG:MTs. KOR1, an endo-β-glucanase thought to be involved in cellulose synthesis (34), was also found to be associated with the GAUT1:GAUT7 complex, which may suggest a more intimate connection between the syntheses of cellulose and pectin than is currently apparent. Two dynamin-related proteins, DRP2A/ADL6 and DRP2B/ADL3, are thought to be involved in cell plate formation and vesicular trafficking (39, 71, 87), and their putative interaction with the GAUT1:GAUT7 complex may provide a clue to begin to unravel the transport and deposition mechanisms for pectic polysaccharides into the wall, a process that appears to involve transport from the Golgi apparatus to the plasma membrane and cell plate via secretory vesicle clusters (163).

Most unexpected are the *Arabidopsis* homologs of ribophorin I and ribophorin II. Both are homologs of subunits of the oligosaccharyltransferase (OT) that catalyzes the transfer of core *N*-glycan oligosaccharides en bloc from lipid precursors onto nascent polypeptides in the protein *N*-glycosylation pathway (76). The identification of OT-complex subunit homologs as potential GAUT1:GAUT7-complex interacting proteins leads us to suggest that pectin synthesis may involve en bloc transfer of pectic oligosaccharide domains, as opposed to sole nonreducing elongation as put forward in the consecutive GT model (**Figure 1**).

The discovery of the GAUT1:GAUT7 HG:GalAT complex was followed more recently by studies on ARAD1 and ARAD2 (56). BiFC and Förster resonance energy transfer (FRET) showed that upon transient expression in *N. benthamiana*, these putative arabinan biosynthetic AraTs form both homo- and heterodimers. When the differentially tagged, coexpressed ARAD1 and ARAD2 were separated on reducing versus nonreducing SDS-PAGE and analyzed by western blotting, the results indicated the occurrence of three kinds of dimers, i.e., ARAD1/ARAD1, ARAD2/ARAD2, and ARAD1/ARAD2, each mediated by disulfide bridges. Whether such dimers are biologically relevant or are artifacts of protein overexpression remains to be determined. However, each type of dimer may serve a somewhat distinct function because, as mentioned earlier, ARAD1 and ARAD2 are not redundant.

PECTIN BIOSYNTHESIS DURING PRIMARY AND SECONDARY WALL FORMATION

Multiple lines of evidence have led to a new view of primary wall architecture and the role of pectin therein. Dick-Pérez and coworkers (25, 26) used multidimensional solid-state NMR to study insoluble and unfractionated primary wall preparations, comparing walls from the wild type with those from the *xxt1 xxt2 xxt5* triple knockout mutant, which contains virtually no xyloglucan. They also analyzed partially depectinated wild-type walls. The data indicate extensive interactions of pectin with both cellulose and hemicellulose but fewer cellulose-hemicellulose interactions than were previously thought to occur. Based on these results, they suggested a single polysaccharide network in which cellulose, hemicellulose, and pectin share the load-bearing function in the primary wall. This is in contrast to the previously held concept of a pectin matrix independent from a cellulose-hemicellulose load-bearing network (5). Pectin load-bearing function in the absence of xyloglucan is also supported by the near-normal growth and development of *XXT* double and triple mutants and by the increased wall-creep response upon treatment of these mutants with pectin-disintegrating agents, e.g., hydrolytic enzymes and a chelating agent (22, 117, 180). Pectin was shown to be the most dynamic polysaccharide, conferring flexibility to

the primary wall (25, 26), and was shown to have roles in the deposition of cellulose microfibrils in conjunction with cortical microtubules (176).

Because pectin makes up a major portion of the primary wall that surrounds expanding cells and is also part of the growing cell plates during cell division, pectin synthesis should be active in meristematic regions and cell cultures. Indeed, the expression of pectin biosynthetic genes such as *GAUT1* and *GAUT7* in such tissues indicates involvement of the encoded proteins in primary wall synthesis (6). Wall deposition of pectin has also been demonstrated during oscillatory growth at the tips of root hairs and pollen tubes, and a role for the degree of HG methylesterification in this process has been suggested (122). The regulation of wall pectin deposition and the regulation of its subsequent demethylesterification by PMEs and PME inhibitors (PMEIs) in the wall were shown to modulate pectin-Ca^{2+} cross-linking (100, 123, 143, 171). These processes appear to control wall stiffness and mediate tip growth in root hairs and pollen tubes as well as primordial development in shoot apical meristems and suggest a close connection between pectin synthesis and modification.

Pectin has also been detected in cells and tissues with secondary walls (150), and there is evidence that it affects secondary wall formation (61, 139, 155, 156). During cotton fiber elongation, a cotton fiber middle lamella forms that consolidates the individual fibers into bundles and thus fosters maximum growth of cotton fibers in the confined space within a boll (156). This adhesive middle lamella, composed mainly of low-esterified HG and partially fucosylated xyloglucan, is eventually degraded to release individual cotton fibers. The unique existence and degradation of the pectin-enriched middle lamella in developing cotton fibers highlights the significance of pectin structures as possible primers or scaffolds for secondary growth. As mentioned above, dynamic postsynthesis modification of pectin structure is catalyzed by PME action. Decreased demethylesterification of pectin through downregulation of *PttPME1* promotes symplastic growth (i.e., diameter expansion) of both vessel and fiber cells and intrusive growth (i.e., length elongation) of fiber cells in poplar plants (155). Conversely, upregulation of *PttPME1* resulted in a reversed phenotype. These results suggest that regulation of pectin demethylesterification affects symplastic and intrusive growth of fiber and vessel cells through control of wall flexibility.

The dynamic modification of pectin has also been visualized in eucalyptus cambium cells in tissues undergoing secondary growth using the antibody JIM5, which recognizes low-methylesterified HG (52). Although abundant JIM5 epitopes were present in the newly formed xylem cells and later remained in the protoxylem region, no JIM5 epitopes were found in the original cambium cells in apical tissues, thereby suggesting active pectin metabolism during xylem and phloem differentiation. Recent analysis of the *Arabidopsis* mutant of *PME35* indicates that a reduction in PME activity along with the accompanying suppression of HG demethylesterification in cortex cell primary walls and in the middle lamella of interfascicular fiber cells leads to a pendant stem phenotype and impaired mechanical strength of the middle and basal stem (61). A direct or indirect involvement of pectin in secondary wall formation and thickening in interfascicular fibers has been proposed.

There is no direct evidence to date that pectin is synthesized as a component of the secondary wall itself. However, the expression of many members of the *GAUT* and *GATL* gene families in tissues undergoing secondary wall thickening has been demonstrated (20, 80, 102), thus supporting a role for multiple GAUTs in pectin biosynthesis in such tissues. For example, *GAUT1* and *GAUT7* expression has been demonstrated via promoter:GUS analysis in vascular tissues as well as in meristematic and reproductive tissues (6), and the expression of both genes remains constant throughout *Arabidopsis* stem development (**Figure 4*d***). In addition, other representatives of each subclade of the GAUT family that we tested showed increased expression in the mid-stems and bottom stems (**Figure 4*d***). Assuming that primary wall synthesis occurs in the elongating young/top

stem, whereas secondary wall synthesis starts in the mid-stem (where elongation ceases) and decreases in the mature/bottom stem, these results suggest roles for many of the GAUTs not only in primary wall synthesis but also during secondary wall formation. However, because stem tissue is composed of multiple cell types, it will be necessary to isolate specific cells such as developing vessels and fiber cells by laser microdissection (109) or to use a xylogenic suspension-culture system (85) to establish direct correlations between the expression of *GAUT* genes and secondary wall thickening.

CONNECTION BETWEEN PECTIN AND XYLAN BIOSYNTHESIS

In dicots, pectin is particularly abundant in primary walls, whereas xylan is a hemicellulose abundant in secondary walls. It is therefore surprising that increasing evidence indicates a connection between pectin and xylan biosynthesis. A priori, it would be expected that most pectin is laid down prior to secondary cell wall xylan deposition. Thus, it is possible that preexisting pectin influences xylan biosynthesis and deposition. It is also possible that regulation of pectin postbiosynthetic modifications such as HG demethylesterification generates pectin structures that could affect xylan deposition during secondary growth.

Mutant studies revealed that pectin defects sometimes lead to a reduction in xylan biosynthesis and a subsequent reduction of xylan in the walls. Based on wall chemical analyses, immunolabeling with JIM5 and JIM7, and cell-cell adhesion phenotype, *qua1* plants (14) and suspension-culture cells (90) have reductions in HG, suggesting that QUA1 has a role in HG biosynthesis. However, studies of *qua1* stem microsomes revealed a reduction in not only HG:GalAT but also xylan synthase activities (115), indicating that the mutation also affects xylan biosynthesis. Similarly, the *atcsld5* mutant has reduced xylan synthase and HG:GalAT activities (11). However, sequence similarity between *ATCSLD5* and *CESA*s and the abundant presence of xylan in *atcsld5* suggest that *ATCSLD5* may be involved in synthesizing noncrystalline cellulose or some other β-1,4-glucan rather than pectin or xylan per se. Hence, the reduction in HG:GalAT and xylan synthase activities in *atcsld5* is likely a secondary effect due to the reduction of a wall polymer that remains to be discovered (11).

Another class of xylan-defective mutants, *irregular xylem 8* (*irx8*)/*gaut12* and *parvus*/*gaolaozhuangren 1* (*glz1*)/*gatl1*, has reduced wall xylan content that causes a collapsed xylem phenotype (15, 16, 124, 126, 127) but normal or even apparently elevated levels of xylan synthase activity in mutant stem microsomes (15, 91, 93). These results indicate that the amount of xylan in muro can be downregulated, owing not to defects in xylan backbone elongation but rather to xylan deposition, possibly due to the lack of an attachment point. Detailed characterization of xylan in both mutants revealed a reduced signal for a xylan reducing-end sequence, 4)-β-D-Xyl*p*-(1, 4)-β-D-Xyl*p*-(1, 3)-α-L-Rha*p*-(1,2)-α-D-Gal*p*A-(1,4)-D-Xyl*p*, which is more abundant in wild-type xylan (124) and has been proposed to act as either a primer or terminator for xylan elongation (177). This xylan reducing-end sequence has a free reducing end when isolated in xylan preparations; however, whether it is free or anchored during biosynthesis is not known (124). Both *IRX8*/*GAUT12* and *PARVUS*/*GLZ1*/*GATL1* encode GTs that may be involved in the synthesis of this glycosyl sequence (15, 124), although no enzymatic activity has been demonstrated. Complications arise as both mutants also have defects in specific pectin fractions. Detailed wall fractionation of *irx8*/*gaut12* stem walls revealed a small loss of GalA in an EPG/PME-released wall fraction, likely an HG fraction, as demonstrated by linkage analyses (126). The *parvus*/*glz1*/*gatl1* mutant has a humidity-dependent dwarf phenotype with indehiscent anthers as well as a reduction in RG-I branching (88). The data do not rule out the possibility that both genes encode GTs that synthesize a specific pectic polymer

or oligomer domain that provides a substructure for xylan deposition. This hypothesis is particularly interesting considering the sequence similarity of both IRX8/GAUT12 and PARVUS/GLZ1/GATL1 to GAUT1, a confirmed pectin biosynthetic HG:GalAT (158).

EVOLUTION OF PECTIN BIOSYNTHESIS

Recent reviews on the evolution of plant cell walls (36, 131, 157) support the postulate that land plants evolved from charophycean green algae belonging to Archaeplastida, a monophyletic eukaryote group thought to have originated from endosymbiosis of a cyanobacterium. This view is supported by the phylogenetic analysis of the GT8 pectin synthesis-related class I proteins, including GAUTs, GATLs, and the lower-plant-specific GAUT and GATL-related (GATR) subfamilies, which placed the green algal (*Ostreococcus lucimarinus*) protein ancestral to the GAUT and GATR clades (175). The study also demonstrated that the *GAUT*, *GATL*, and *GATR* genes were likely acquired from a cyanobacterium (*Synechococcus*) progenitor, had a different evolutionary origin than the other plant GT8 genes, and subdivide into unique protein subclades with predicted functional specialization.

HG has been detected in diverse charophycean green algae, including *Penium margaritaceum* (27), *Netrium digitus* (31), and *Chara corallina* (133), indicating a function in algae prior to the movement of plants onto land. This function appears to involve HG demethylesterification and calcium binding (27), which presumably affects HG-HG interaction and function in the wall. Cell wall structure studies based on biochemical analysis and/or immune reactivity or carbohydrate-binding domain reactivity indicate that HG and RG-I are present in both vascular and avascular plants, including the moss *Physcomitrella patens* (105, 140). RG-II, in contrast, is present in extant vascular plants, including gymnosperms, lycophytes, and pteridophytes, but most likely is not present in mosses such as *Physcomitrella*, suggesting that RG-II evolved as plants adapted to upright growth on land (9).

The later evolution of RG-II compared with HG and RG-I is supported by phylogenic analyses of three classes of proven pectin biosynthetic enzymes (**Table 1**). Analysis of GT77 clade B, which contains RGXT1–4, revealed that the *Physcomitrella* member in this clade is distant from the vascular-plant counterparts and thus is unlikely to function in RG-II synthesis, a conclusion consistent with the absence of the complete RG-II structure in this bryophyte (129). Also, the paucity of *Physcomitrella* representatives in GT47 subgroup C2, which contains XGD1, is consistent with the apparent absence of XGA in this moss (40). GAUT1 orthologs, in contrast, exist in the oldest extant vascular plant, *Selaginella moellendorffii*, and in the avascular *Physcomitrella*, which are a lycophyte (spikemoss) and a bryophyte (moss), respectively (58). These results are in agreement with the presence of pectic HG in both primitive vascular plants and nonvascular plants (57, 86).

Both *Selaginella* and *Physcomitrella* have orthologs in clades with *Arabidopsis* GAUT1–3, GAUT10–11, and GAUT12–14, suggesting possible functions for these proteins in HG and/or RG-I synthesis because both polymers are present in these species (57, 175). Interestingly, *Selaginella* (but not *Physcomitrella*) also has orthologs in clades containing GAUT8–9, suggesting a possible function of GAUT8 and/or GAUT9 during the evolution of primitive vascularization. Furthermore, the acquisition of GAUT4–7 in higher plants suggests that these genes may function in vascularization or other higher-plant-specific wall roles. As mentioned above, GAUT1 has been shown to encode an HG:GalAT, and GAUT7 serves to anchor GAUT1 in the Golgi apparatus. No functional data are available on GAUT2–6, but our preliminary results suggest that at least two of these GAUTs also have HG:GalAT activity (K.H. Caffall, L. Tan, R. Amos & D. Mohnen, unpublished results). We hypothesize that the entire GAUT1–7 subclade is involved in the synthesis of HG domains either enzymatically

or as anchor proteins. We also speculate that the evolution of higher-plant vascularization required acquiring the ability to synthesize specific types of HG domains encoded by the action of specific GAUTs.

TWO CONTRASTING HYPOTHETICAL MODELS FOR PECTIN BIOSYNTHESIS

In the introduction, we presented two hypothetical models for pectin synthesis: the consecutive GT model and the domain synthesis model. Our early pectin synthesis studies were based on a consecutive GT model (**Figure 1*a***) in which an increasingly complex pectic structure is synthesized as it moves through the *cis*-, medial-, and *trans*-Golgi regions by the sequential action of distinct GTs. Indeed, pectin biosynthetic enzymes such as the GAUT1:GAUT7 complex, RGXTs, XGD1, and GALS1, which have in vitro activities that add glycosyl residues consecutively onto the nonreducing ends of pectin acceptors, support this model.

Our more recent results (6, 162) indicate that such a view of pectin synthesis may be inadequate to explain all the pectin biosynthesis and structural data. As described above, HG synthesis—or at least the elongation phase of HG synthesis—occurs via a GAUT1:GAUT7 core complex whose 12 putative interacting proteins include 2 putative MTs, 2 putative subunits of an OT complex, and 2 dynamin-like proteins (6). These GAUT1:GAUT7-complex putative interacting proteins could potentially function, respectively, in the processes of HG modification, en bloc oligosaccharide domain transfer, and transport of pectin to the wall. These data lead us to suggest that pectin synthesis may occur, at least in part, via en bloc transfer of pectin domains onto growing polysaccharides and/or glycoconjugates, as illustrated by the domain synthesis model (**Figure 1*b***). In this model, different pectin domains—e.g., HG and RG-I—would be independently synthesized and elongated by the consecutive action of appropriate GTs (step 1 in **Figure 1*b***). A proposed OT-like activity would transfer the HG domain en bloc onto another HG or RG-I domain to form a growing pectic polysaccharide, or possibly even onto a proteoglycan (step 2). The resulting pectic polymer would subsequently be transported to the wall (step 3) by a secretory vesicle, perhaps in a dynamin-assisted way, and then inserted into the wall. Although some experimental data exist to support or suggest each of these steps, much work is needed to critically test this model.

It is noteworthy that the possible transfer of oligo- and polysaccharide domains during pectin synthesis was postulated in 1967 by Stoddart & Northcote (161). In an elegant series of radioactive pulse-chase experiments using actively growing sycamore cells, these authors showed that "radioactive material passed from the neutral arabinan-galactan fraction to the weakly acidic pectinic acid fraction" (p. 57). Based on the data, they suggested that the pectin fraction received blocks of "arabinan-galactan" during synthesis and that this transfer might "occur before or after deposition of the material in the wall" (p. 58). These findings are consistent with the domain synthesis model, in this case suggesting en bloc transfer of arabinan-galactan domains onto pectin. Whether the consecutive GT model, the domain synthesis model, or both accurately represent the pectin biosynthetic process will require critical analyses of the initiation and elongation stages of pectin synthesis.

SUMMARY POINTS

1. Pectin is a family of structurally complex plant cell wall polysaccharides or glycan domains that contain α-D-GalA linked at the 1 and 4 positions. The pectic polysaccharides include homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), apiogalacturonan (AGA), and xylogalacturonan (XGA).

2. Synthesis of the complex pectin structures requires a large number (≥67) of glycosyltransferases, methyltransferases, and acetyltransferases and is affected by the availability of nucleotide-sugar substrates. Pectin is synthesized in the Golgi apparatus and transported via secretory vesicles to the wall.
3. *Arabidopsis* genes encoding four types of pectin biosynthetic GTs have been functionally identified: *GAUT1* (encoding HG:α-1,4-GalAT), *GALS1* (encoding β-1,4-galactan:β-1,4-GalT), *RGXT1–4* (encoding RG-II:α-1,3-XylT), and *XGD1* (encoding XGA:β-1,3-XylT).
4. The GAUT1:GAUT7 HG:GalAT complex and putative ARAD1/2 arabinan biosynthetic complex(es) serve as precedents for the involvement of protein complexes in pectin synthesis.
5. GAUT1 N-terminal processing, the concomitant loss of its transmembrane domain, and its anchoring in the Golgi apparatus by covalent and noncovalent interactions with GAUT7 provide a mechanism for tethering pectin biosynthetic catalytic subunits in protein complexes.
6. Recent data indicate that pectin plays a more important role in primary walls than previously thought, including conferring load-bearing function and overall wall flexibility; pectin may also influence xylan synthesis and/or secondary wall thickening.
7. Two hypothetical and testable models for pectin synthesis are proposed: the consecutive glycosyltransferase model and the domain synthesis model.

FUTURE ISSUES

1. Development of pectin biosynthetic enzyme assays, including generation of diverse donor and acceptor substrates (especially for RG-I and RG-II), is essential for functional identification of pectin biosynthetic enzymes.
2. Why are there so many members of the GAUT and GATL families? Answering this question will require determining the type of pectic (or other) polysaccharides or glycan domains synthesized by each of the encoded proteins.
3. How are the pectin biosynthetic enzymes organized in the Golgi apparatus, and how widespread are enzyme complexes in pectin biosynthesis?
4. The interactions of the 12 putative interacting proteins with the GAUT1:GAUT7 complex need to be validated and the role of these proteins in pectin synthesis (if any) needs to be demonstrated.
5. A fundamental test of the domain synthesis model requires determining whether a pectin oligosaccharyltransferase activity functions in planta; if so, its structure and functional specificity need to be determined.
6. How is pectin synthesis initiated? Are lipid-linked or other activated-sugar donors involved? Are lipid-linked or protein-linked acceptors involved in the initiation and/or elongation stage?
7. How is pectin biosynthesis regulated?

8. What are the mechanisms of pectin assembly, delivery, and deposition into the wall? Are all pectins synthesized as parts of proteoglycans? When and how are pectin domains incorporated into APAP1?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Drs. Maor Bar-Peled and Alan Darvill for helpful comments during the writing of this review. This work was supported by USDA AFRI 2010-65115-20396, NSF-MCB 0646109, and BioEnergy Science Center grant DE-AC05-00OR22725. The BioEnergy Science Center is a US Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the Department of Energy's Office of Science.

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Volume 64, 2013

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