

Toward a Systems Approach to Understanding Plant Cell Walls

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One of the defining features of plants is a body plan based on the physical properties of cell walls. Structural analyses of the polysaccharide components, combined with high-resolution imaging, have provided the basis for much of the current understanding of cell walls. The application of genetic methods has begun to provide new insights into how walls are made, how they are controlled, and how they function. However, progress in integrating biophysical, developmental, and genetic information into a useful model will require a system-based approach.

Plant cell walls are complex and dynamic structures composed mostly of polysaccharides with high molecular weights (1–4), highly glycosylated proteins, and lignin. As a measure of the complexity, the *Arabidopsis* genome contains more than 730 genes encoding putative glycosyltransferases or glycosyl hydrolases (5) and several hundred additional genes encoding other types of proteins implicated in wall biosynthesis or function. Although their general catalytic activity can often be inferred from sequence, the precise enzymatic function and biological role of most of these proteins are unknown (2). For example, genetic analysis has identified the specific biological role for only two of the more than 170 gene products with similarity to pectin-degrading enzymes (6, 7).

Faced with the prospect of analyzing the function of 1000 or more genes that may contribute to the synthesis and remodeling of cell walls, we explored the idea that a systems approach may provide a useful framework for defining the hierarchy of essential questions. The concept of systems biology has recently emerged as a way of envisioning how multifactorial biological processes operate as a whole (8). The concept is usually applied to understanding networks of genes or gene products but is more broadly applicable. Kitano (8) defines four key elements in a system: the design principles, system structure, the control method, and the system dynamics. Here, we attempted to evaluate the current state of knowledge about the poly-

saccharide components of dicotyledonous plant cell walls in the context of these elements. Not surprisingly, our analysis highlights many major gaps in our knowledge. However, the application of genomics, molecular genetics, and new analytical methods should provide many opportunities to close some gaps in the foreseeable future.

Design Principles

The body plan of a higher plant is essentially like a building made of “osmotic bricks.” Each cell is osmotically pressurized to between 0.1 and 3.0 MPa (1 MPa ~ 145 pounds per square inch). The pressure rigidifies the cells by creating tension in the cell walls. Each cell is glued to adjacent cells by pectic polysaccharides that normally prevent sliding of the cells under large strains. However, cell walls are also capable of controlled modifications that allow cells to expand in a polarized fashion during growth. Because each cell wall is attached to adjoining cell walls, coordinated expansion is necessary. It has been proposed that the role of the brassinosteroid hormones is to coordinate cell expansion (9).

Plant cell division involves the biogenesis and integration of new walls at the plane of division. In this process, two opposing walls form within the mother cell, and then the new walls integrate with the existing wall, and the plasma membrane repositions to form the daughter cells (10, 11). Certain cell types, such as the fiber cells in wood, are subject to mechanical stress and undergo additional cell wall synthesis after the cells have finished dividing and are fully expanded. This “secondary cell wall” is deposited interior to the “primary cell wall.” Thus, the fundamental design principles include strength, expandability, and modularity.

Cell walls also provide a barrier to infection by pathogens. Exogenous application of cell wall fragments to uninfected plants triggers defensive reactions, indicating the existence of glycan-activated signal transduction chains. It has been proposed that some of the structural complexity in plant cell wall composition reflects the presence of latent signal molecules, which trigger defensive responses when they are released during the cell wall degradation that accompanies pathogenesis (12). Several lines of evidence have also implicated cell wall polysaccharide fragments and proteoglycans in developmental processes (13–15). For example, deglycosylation inactivated a proteoglycan named xylogen that mediates intercellular interactions required for xylem differentiation in cultured *Zinnia* cells (14). Thus, the design principles of cell walls cannot be understood solely in the context of mechanical properties.

System Structure

When viewed by electron microscopy (EM) (Fig. 1), cell walls appear to be a network of extended polysaccharides with high molecular weights (16, 17). In higher plants, the visually dominant structural features are cellulose microfibrils with diameters of ~3 nm, which appear to wrap around the cells and are cross-linked by single-chain polysaccharides such as xyloglucons.

Structural analysis of cell wall polysaccharides has resulted in the compilation of “average” structures for the major cell wall polysaccharides (4, 18). These are illustrated in figs. S1 to S6. In brief, the leaf cell walls of a dicot species such as *Arabidopsis* contain three major classes of polysaccharides: cellulose, hemicelluloses, and pectins. Cellulose is present as long unbranched fibrils composed of approximately 30 to 36 hydrogen-bonded chains of β -1,4-glucose. The length of the fibrils is unknown but single glucans containing up to 14,000 glucose units have been observed, corresponding to a fibril length of about 7 μ m. Hemicelluloses are branched polysaccharides containing backbones of neutral sugars that can form hydrogen bonds to the surface of cellulose fibrils. Pectins are defined by the presence of uronic acids as

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major components. The simplest of these is homogalacturonan (HG), an unbranched polymer of (1→4) α -D-galacturonic acid. Rhamnogalacturonan I (RGI) has a backbone composed of alternating (1→2) α -L-rhamnose-(1→4) α -D-galacturonic acid decorated primarily with arabinan and galactan side chains. It has recently been suggested that RGI functions as a scaffold to which other pectins, such as rhamnogalacturonan II (RGII) and HG, are covalently attached as side chains (18).

A representative structure for an *Arabidopsis* leaf primary cell wall that is broadly consistent with more specialized models (18) and with views from EM (16, 17) is presented in Fig. 2. The complexity of the image underscores the challenge associated with understanding the structure, function, and synthesis of plant cell walls. The cellulose microfibrils, which are made at the plasma membrane, are insoluble because the glucan chains aggregate laterally by means of hydrogen bonding and van der Waals forces to produce crystalline structures of parallel chains. The other polymers are secreted as soluble polymers that must unfold and diffuse within the aqueous environment of the wall to their final destination. Because some of the polymers are insoluble when extracted from the wall, we speculate that they may be modified after secretion by the removal of structural components (e.g., branches) that facilitate solubility. Also, it has been proposed that some polymers are assembled into larger (less soluble) polysaccharides following secretion into the wall (19). One of the driving forces for assembly of the overall structure is thought to be the hydrogen bonding of hemicellulose to the surface of cellulose microfibrils (Fig. 2). Somewhat counterintuitively, biophysical studies have indicated that the presence of the hemicellulose cross-links weakens the mechanical strength of cell walls by preventing cellulose aggregation, thereby facilitating cell wall expansion (20).

The factors involved in pectin deposition are unknown. Pectins have been proposed to

be important for control of wall porosity, for adhesion of adjoining cells (21), and in controlling the ionic environment of the cell wall (1). Additionally, analyses of mutations that alter the structure of RGII indicate that borate-diester cross-links between apiose residues in RGII molecules are also important for strengthening of the wall, intercellular adherence, and normal growth in vascular plants (22). Because the borate diester forms spontaneously, it provides a mechanism for forming cross-links after the polymers are assembled in place. Another example of in muro modification is the formation of calcium bridges between the carboxyl groups of HG chains to create interpolymeric adhesion. HG is thought to be made as a fully methyl-esterified polymer in the Golgi (4). Pectin methyl-esterases in the cell wall remove methyl groups, thereby making the carboxyls available to coordinate calcium ions that form interchain salt bridges. The existence of 67 genes for putative pectin methyl-esterases in *Arabidopsis* highlights the importance of this mechanism.

Measurements of the total sugar composition of cell walls from different tissues of *Arabidopsis* revealed that every tissue type has a different polysaccharide composition (23). Immunohistochemical studies with

monoclonal antibodies that recognize polysaccharide epitopes provide examples of spatial and temporal differentiation of wall polysaccharides (24, 25). These and other studies show that the composition of the wall is tightly controlled in different cell types and in relation to growth and development (24, 26). Immunological studies have also shown that the various polymers are not uniformly distributed within the walls. RGII, for example, appears to be enriched near the plasma membrane (27), whereas polysaccharides such as HG are enriched in the middle lamella, where adjoining cell walls abut. The differences between various cell types in cell wall composition and structure could reflect different needs for elasticity, the mobility of various types of molecules in the cell wall, or poise with respect to pathogen signaling.

The observation that each cell type may have a distinct composition makes it problematic to interpret experimental results on the basis of analyses of organs composed of different cell types. The use of isolated *Zinnia* cells, which can be forced to undergo synchronized terminal differentiation to vascular cells in culture, represents a promising system for studying many aspects of cell wall biology (28, 29). Additionally, the large size and layered organization of cambium in

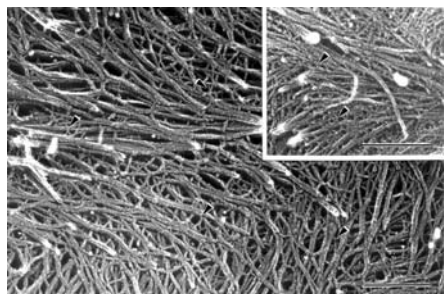


Fig. 1. Electron micrograph of outer cell walls of EDTA-extracted epidermal cells of pea (*Pisum sativum*) plants. Cellulose microfibrils and their cross-links are indicated by arrowheads. The inset shows the walls before extraction. Scale bars, 200 nm. [Image from (16)]

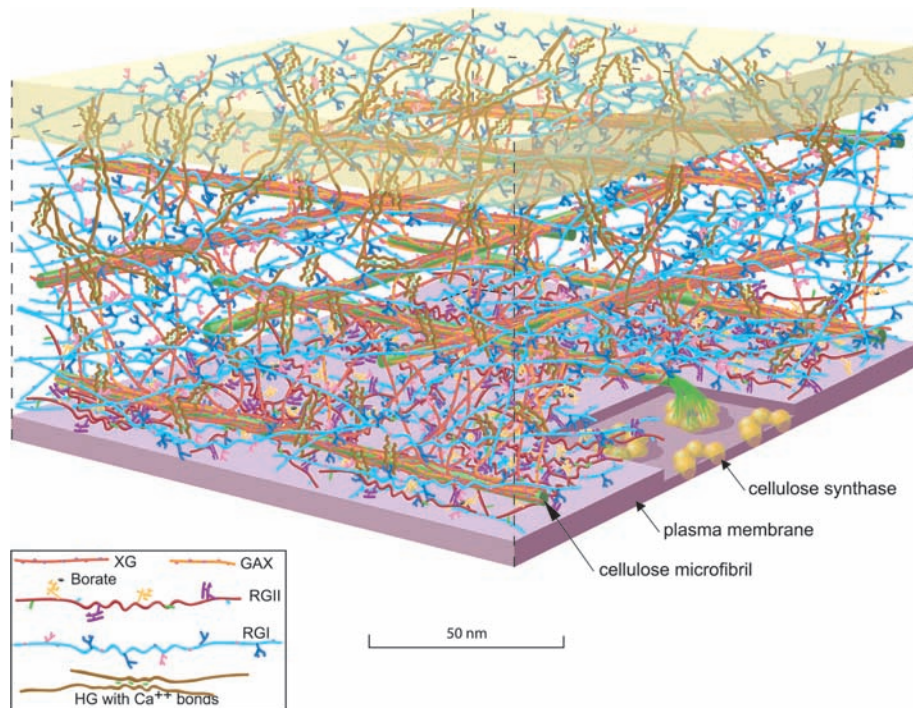


Fig. 2. Scale model of the polysaccharides in an *Arabidopsis* leaf cell. The amount of the various polymers is shown based approximately on their ratio to the amount of cellulose. The amount of cellulose shown was reduced, relative to a living cell (Fig. 1), for clarity. Because of the exaggerated distance between microfibrils, the hemicellulose cross-links [shown in dark orange (xyloglucan, XG) or light orange (glucuronoxylarabinan, GAX)] are abnormally extended. Also, recent solid-state NMR studies have suggested that, in some plants, only about 8% of the surface of the cellulose microfibrils is occluded by XG (89). The figure is an elaboration of a model originally presented by McCann and Roberts (90). The figure was rendered by Abbey Ryan.

poplar trees facilitates sampling of steady-state mRNA levels in specific cell types at various stages of development by cutting thin sections with a cryomicrotome. Analysis of the mRNA on DNA chips and microarrays allowed a system-level analysis of secondary wall formation (30). The recent completion of the poplar genome sequence, and the fact that poplar and *Arabidopsis* have a similar complement of genes, will greatly facilitate the value of this experimental system.

Control, Synthesis, and Assembly

Remarkably little is known about the enzymes that catalyze synthesis of cell wall polysaccharides. Cellulose and callose (β -1,3-glucan) are the only polysaccharides for which proteins involved in the synthesis of the main chains are known. In higher plants, cellulose synthase forms a "rosette" complex in the plasma membrane (31). The complex is one of the largest protein complexes known, with a diameter about equal to that of a ribosome. It is thought that each of the six subunits that comprise a rosette contains five or six CESA proteins, each of which synthesizes one of the β -1,4-glycans that comprise a microfibril in typical higher plants (Fig. 2). In some organisms, such as the red alga *Erythrocladia subintegra*, rectangular complexes of up to 230 nm in length produce ribbons of cellulose rather than fibrils (32).

Arabidopsis has ten cellulose synthase (CESA) genes, three of which are required for primary wall synthesis and at least three of which are required for secondary wall synthesis. It now appears from mutant analysis that the various genes are not functionally redundant; three different CESA proteins must be simultaneously present to produce a functional cellulose synthase (33). It has been hypothesized that this could be due to the geometric constraints associated with assembling 30 to 36 subunits into a planar, membrane-localized complex of approximately 3 million daltons (34).

Genetic screens for mutants of *Arabidopsis* deficient in cellulose have implicated a number of factors other than the CESA proteins. The *KORRIGAN* gene encodes a membrane-localized cellulose (35–37). Bacterial cellulose synthesis also requires a cellulase for in vivo activity but not for in vitro activity, suggesting a role in cellular processes rather than catalysis. As in plants, bacterial cellulose synthase is a membrane complex containing 12 to 25 subunits (38). However, bacteria use cellulose not in their walls but rather to create biofilms and adherence. The *Arabidopsis* *COBRA* gene encodes a glycoposphatidyl inositol (GPI)-anchored protein of unknown function (39). Similarly, the *KOBITO* gene encodes a membrane protein of unknown function (40). The *ectopic deposition of lignin in pith* (*elp*) mu-

tant is defective in a protein with sequence similarity to endochitinases (41). Because higher plants do not synthesize chitin, the ELP protein presumably hydrolyzes another polysaccharide. Mutants deficient in glycosidase I and II, enzymes that catalyze the early steps of N-linked glycan maturation, are severely deficient in cellulose (42, 43). Unfortunately, in the absence of a robust and facile in vitro assay for cellulose synthase activity in *Arabidopsis*, it has not yet been possible to assign specific roles to these gene products.

It is notoriously difficult to convincingly measure cellulose synthase activity in extracts from higher plants. One of the challenges is the presence in plant membrane preparations of a highly active β -1,3-glucan synthase that obscures β -1,4-glucan synthase activity, necessitating detailed structural analysis of the products of assays. However, several groups have observed activity and have made progress toward defining improved assay conditions (44, 45). No exogenous primer was required to initiate synthesis of cellulose in vitro, raising doubts about the proposed involvement of sterol- β -glucoside as a primer (46). However, the discrepancy between in vivo and in vitro requirements for a cellulase in bacterial cellulose synthesis highlights the notion that in vitro conditions may not accurately reflect the in vivo conditions. Similarly, immunohistochemical evidence consistent with the idea that sucrose synthase may channel uridine 5'-diphosphate (UDP)-glucose to cellulose synthase (47) may be challenging to test in vitro.

Several CESA genes appear to be expressed throughout plants (34), even though cellulose synthesis is thought to be largely confined to expanding cells. This raises the possibility that cellulose synthesis is controlled posttranscriptionally. Bacteria, such as *Escherichia coli*, also exhibit constitutive expression of cellulase synthase (38). Enzyme activity is thought to be regulated by small effector molecules [i.e., cyclic diguanosine 5'-monophosphate (GMP)] or through stabilization of the complex by additional proteins (38). Cyclic di-GMP has not been observed in plants, and *Arabidopsis* does not have an obvious homolog of the enzyme that makes cyclic di-GMP.

A second level of control is responsible for the oriented deposition of cellulose fibrils. Cellulose fibrils are generally deposited perpendicular to the axis of elongation restricting lateral swelling and allowing longitudinal expansion. A variety of correlative evidence suggests that the orientation of cellulose deposition is, in some way, regulated by the orientation of microtubules. More than 40 years ago, cells treated with colchicine were observed to display random orientation of cellulose fibrils (48). Consist-

ent with this, the *fragile fiber* mutants encoding a kinesin-like protein (*fra1*) and a katanin-like protein (*fra2*) have been demonstrated to have abnormal orientation of cellulose deposition (49, 50). However, studies of the conditional *mor1* mutant of *Arabidopsis*, which is deficient in microtubule polymerization at the nonpermissive temperature, have shown that ordered cellulose deposition is possible in the absence of assembled cortical microtubules and an existing cellulose template (51, 52). It has also been observed that treatment of protoplasts with the cellulose synthase inhibitor isoxaben prevents characteristic orientation of the microtubules demonstrating cell wall-to-cytoskeleton feedback (53). We believe that these seemingly contradictory lines of evidence may reflect a variable relationship between the cytoskeleton and the cellulose synthase complexes, depending on the stage of cell wall synthesis and expansion. Recent progress in visualizing microtubules in live cells (54), combined with new tools for simultaneously visualizing cellulose synthase, may clarify this relationship.

Most noncellulosic polysaccharides are thought to be synthesized in the Golgi, secreted, and covalently linked in muro into larger polysaccharides (19). The majority of the synthetic enzymes are integral membrane proteins, most of which have been intractable to purification. Genes for pectin synthesis have been particularly challenging to identify. However, mutant screens for variation in cell wall sugar composition (55) or for mutants with phenotypes indicative of defective cell walls (56, 57) have identified candidate genes for several of the enzymes involved. A tobacco mutant, defective in a putative glucuronyltransferase, has altered pectin content and defective intercellular attachment that appears to be due to a defect in RGII synthesis (58). The *quasimodo* mutant of *Arabidopsis* has reduced pectin because of a defect in a family 8 processive glycosyltransferase, which is a candidate for an HG synthase (59). Similar to most genes for enzymes implicated in cell wall synthesis in *Arabidopsis*, *quasimodo* is a member of a large family of related genes. A surprising finding was the discovery that a mutation in one of four isoforms of UDP-D-glucose 4-epimerase, an enzyme that acts in the formation of UDP-D-galactose, affected the synthesis of proteoglycans and polysaccharides but not galactolipids (60). This and several related observations have been interpreted as supporting the concept that substrate channeling may be a broadly important control point in polysaccharide biosynthesis (61).

There have also been important breakthroughs in the identification of enzymes involved in the synthesis of xyloglucan. An α -1,2-fucosyltransferase that adds the termi-

Perspectives and Future Directions

A highly simplified system diagram incorporating the major concepts discussed here is presented in Fig. 3. The cyclical nature of the diagram emphasizes that the expansion of the cell wall and the integration of a new cell plate during cytokinesis are components of the cell cycle. Thus, we infer that many of the genes involved in primary cell wall synthesis and modification will be found to be controlled by factors that control other aspects of the cell cycle. However, cells that are programmed to continue dividing would be expected to have different controls than cells that are terminally differentiated. Each differentiated cell type probably has a different combination of controls to ensure that composition of the wall is compatible with the needs of that cell type. Although not emphasized here, cell walls can be modified in response to environmental stimuli. Thus, the two main inputs are developmental and environmental processes. Indeed, because cell size and cell shape are functions of cell wall expansion, any attempt to understand the mechanics of morphogenesis will ultimately lead to questions about the control of cell wall synthesis and expansion. We speculate that as methods for interrogation of cell wall structure and function improve, large numbers of morphologically abnormal mutants that cannot currently be understood in a developmental context will be found to lie at the interface of morphogenesis, the cell cycle, and cell wall biogenesis.

Viewing cell walls in a developmental context may help explain the large numbers of structurally similar genes for cell wall-related enzymes that are evident in the sequenced plant genomes. It is apparent that for some functions, such as cellulose synthesis, a small number of genes are used in most or all of the roughly 40 cell types in a plant. This is compatible with speculation that cellulose synthesis is not primarily controlled at the transcriptional level. By contrast, the large numbers of structurally related genes in other gene families may suggest that other cell wall-related processes are based on the participation of specialized genes in a tissue or temporal dependent manner. It is also likely that, because polysaccharides are composed of a small number of sugars but a relatively large number of different linkages, the members of large families of structurally similar genes encode enzymes that exhibit linkage- or context-dependent differences in catalysis.

The development of methods for determining where and when each gene is expressed in *Arabidopsis* and other plants (85) is a high priority in moving toward a more refined understanding of how walls are controlled. The power of transcript profiling with DNA chips or arrays to associate genes with processes cannot be fully realized as long as

RNA samples are derived from mixtures of cell types. Hypotheses concerning gene function derived from transcript profiling can be rapidly tested by exploiting the extensive collection of indexed insertion mutations in *Arabidopsis* (86). At present, sequence-indexed insertions are available for approximately 22,600 of the genes in *Arabidopsis* (87).

Although powerful genomic resources are available in *Arabidopsis* (86, 88), they are only a subset of the diverse resources that will be required to permit formulation of a detailed system model of cell walls. The development of tools, such as additional monoclonal antibodies or aptamers, differentiated cell cultures of *Arabidopsis*, diagnostic hydrolytic enzymes for structural analysis, and substrates for enzyme assays, are needed. These tools will facilitate analysis of mutants and will help to elucidate the function of enzymes, individual polysaccharides, and structural motifs that occur in the walls of *Arabidopsis* and other species. New biophysical methods that permit improved imaging and nanoscale interrogation or manipulation of cell walls may also facilitate a deeper understanding of how the components are organized and how that organization results in the observed physical properties. Looking over the horizon, hypothetical methods such as scanning probe nuclear magnetic resonance (NMR) or confocal EM would be very useful for visualizing the fine structure of cell walls.

Finally, the emphasis here on *Arabidopsis* should not obscure the substantial diversity in wall composition between plant species. For instance, in commelinoid monocots, most of the neutral hemicellulose and pectins are replaced by glucuronoarabinoxylan. As experimental methods and resources for studying complex polysaccharides and nanocomposites improve, this diversity will provide a rich source of information about structure-function relationships.

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Figs. S1 to S6

10.1126/science.1102765

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Figure S1. Xyloglucan.

Xyloglucan is composed of subunits of XXXG, XXLG, XXFG, and XLFG in most plant families. Xyloglucans derived from Solanaceous plants and immature podaceous plants contain XXGG subunits and may have small amounts of XGGG and GXGG subunits. Individual xyloglucan molecules range in length from 30 - 400 nm which correspond to roughly 15 - 200 Glc residues. Acetylation?

Vincken JP *et al.* Plant Physiol 114 (1997) 9-13
 Zablackis E *et al.* Plant Physiol. 107 (1995) 1129-1138
 McCann *et al.* J. Microsc 166 (1992) 123-136

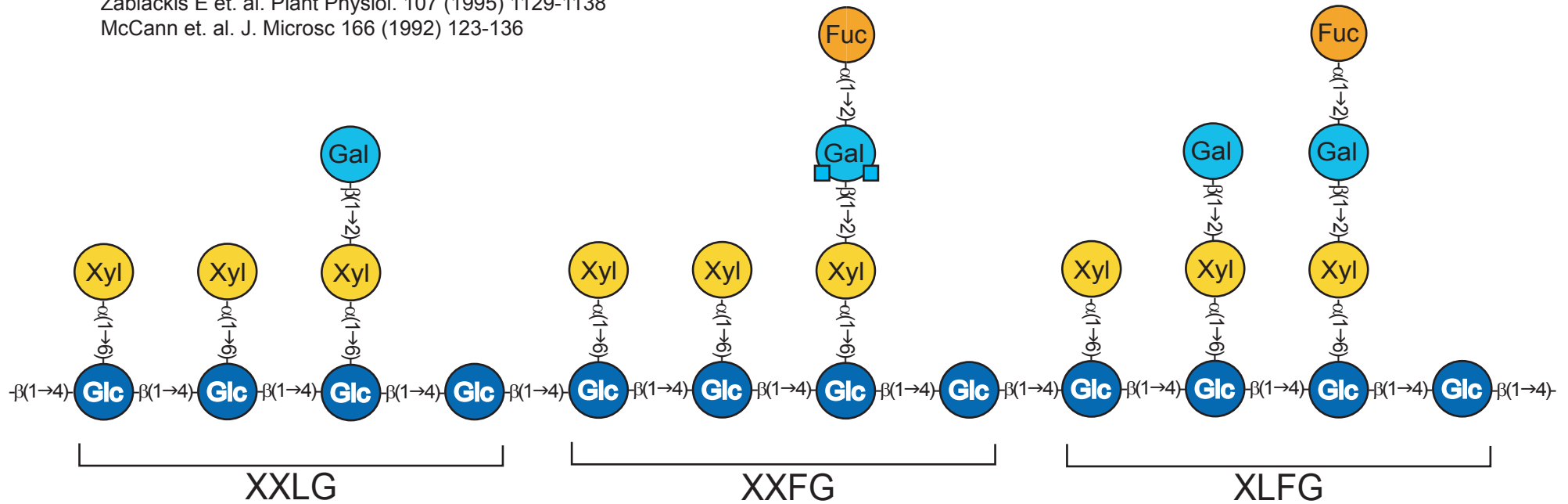


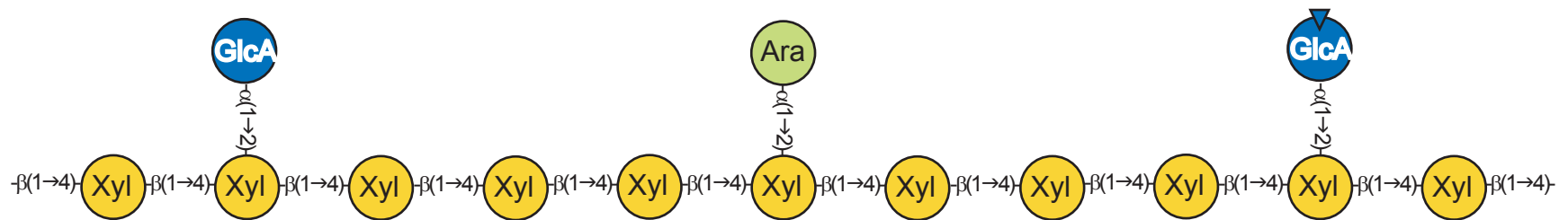
Figure S2. Glucuronoarabinoxylan.

Glucuronoarabinoxylan (GAX) accounts for roughly 5% of the dicot cell wall. Approximately 25% of the Xyl residues are substituted with either Ara, GlcA, 4-O-methyl glcA, or--in some plant families--ferulic acid.

DP?

Zabackis E et. al. Plant Physiol. (1995) 107 1129-1138

Darvill et al. Plant Physiol. (1980) 66 1135-1139



D-Xylopyranose



L-Arabinofuranose



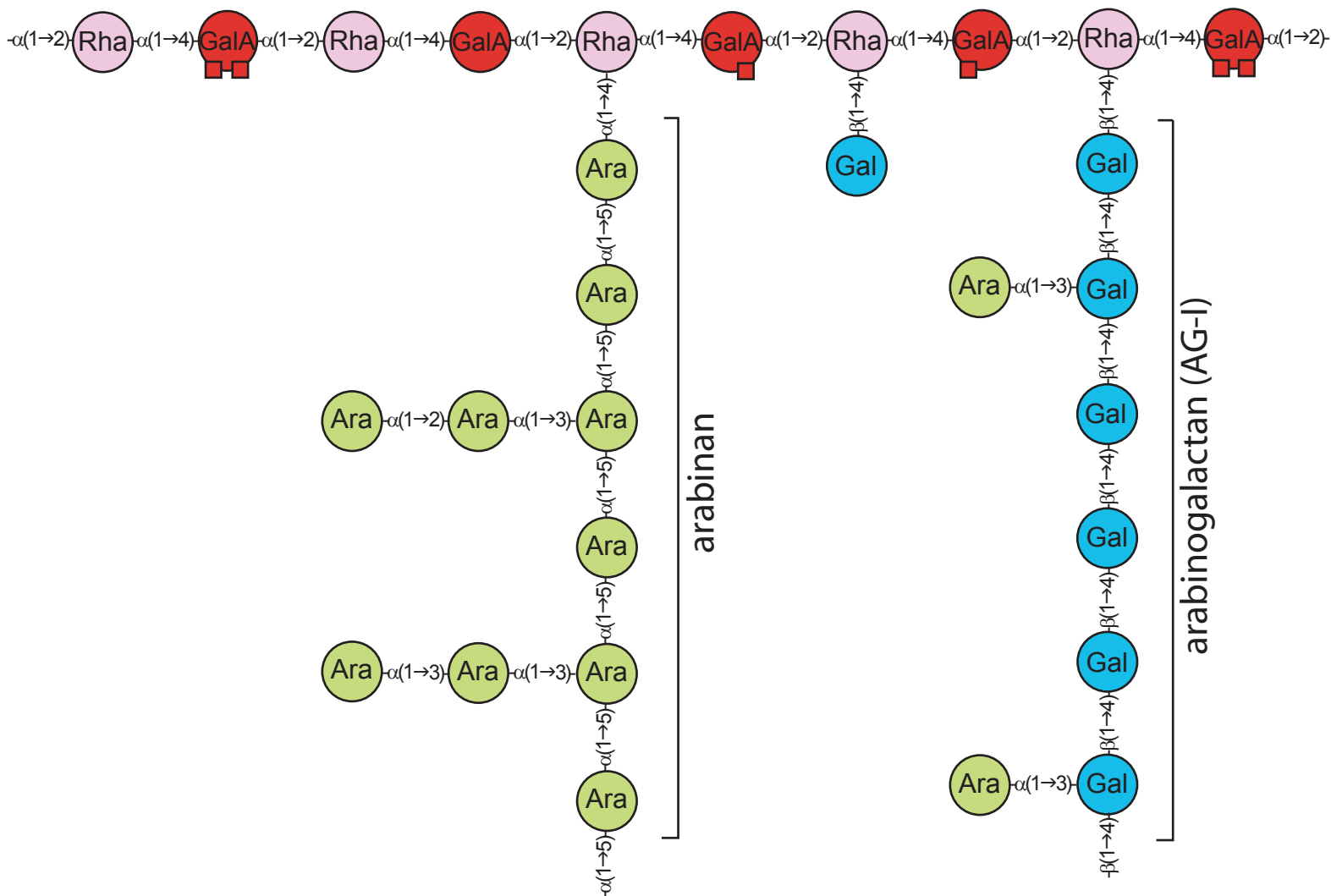
D-Glucopyranuronic acid,
also with 4-O-methyl groups (\blacktriangledown)

Figure S3. Rhamnogalacturonan I (RG-I).

The backbone can be composed of more than 100 $[-2)\text{-}\alpha\text{-L-Rhap-(1}\rightarrow\text{4)-}\alpha\text{-D-GalpA-(1}\rightarrow\text{-}]$ -disaccharide units. Between 20% to 80% of the rhamnosyl residues carry side chains, depending on the source. These side chains can be single unit $[\beta\text{-D-Galp-(1}\rightarrow\text{4)]}$ or arabinan or arabinogalactan I with variable chain length. In smaller amounts, $\alpha\text{-L-Fucp}$, $\beta\text{-D-GlcpA}$, 4-O-Me GlcpA and ferulic and coumaric acid may also be present. It is also suggested that homogalacturonan and xylogalacturonan are attached to RG-I.

Vincken JP *et al.* Plant Physiol 132 (2003) 1781-1789

Ridley BL *et al.* Phytochemistry 57 (2001) 929-967



D-Galactopyranuronic acid
may contain acetyl groups (■)
at O2 and O3



L-Arabinofuranose



L-Rhamnopyranose



D-Galactopyranose

Figure S4. Rhamnogalacturonan II (RG-II).

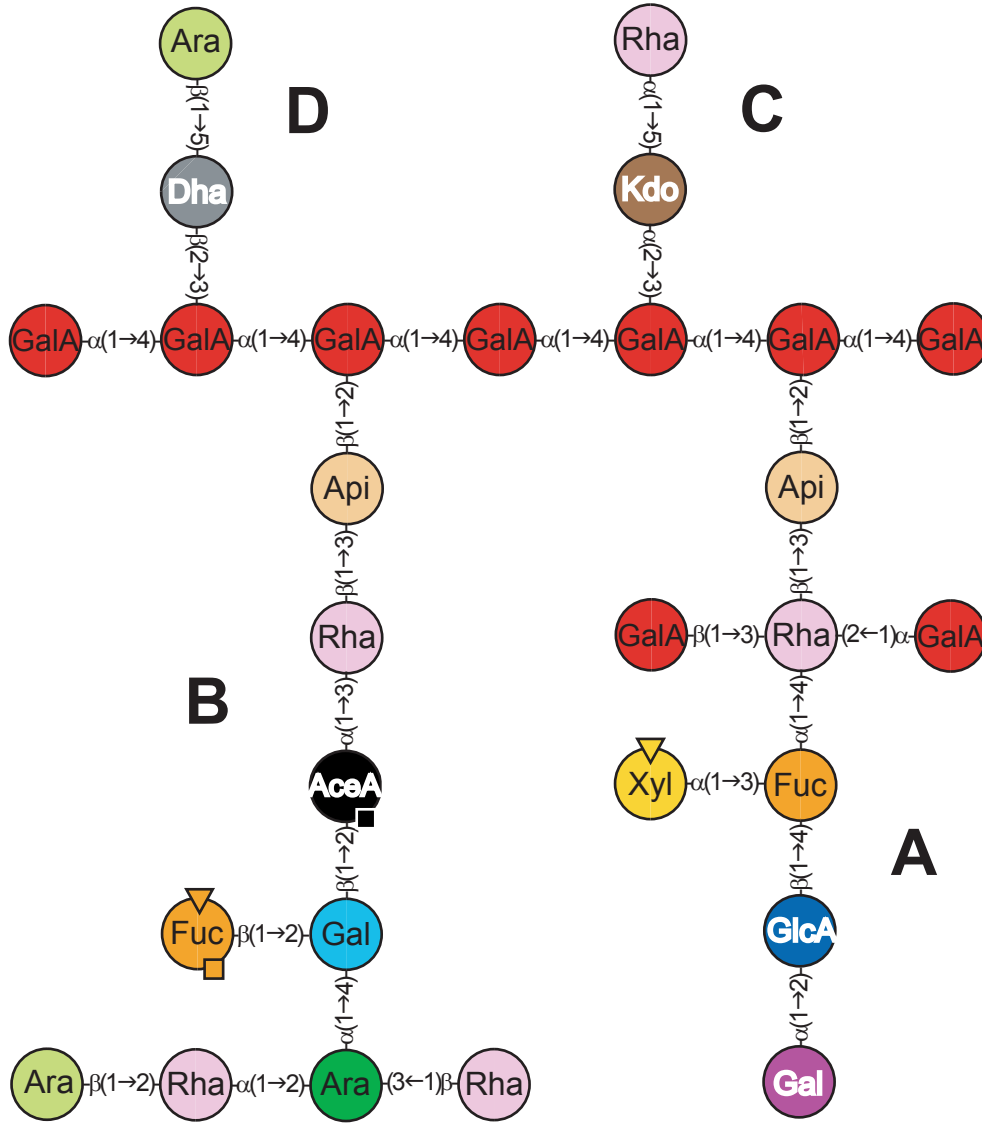
Four side chains (A-D) are linked to a homogalacturonan backbone consisting of at least 7 $[\rightarrow 4)\text{-}\alpha\text{-D-GalpA-(1}\rightarrow]$ residues. The order and the linkages of the glycosyl residues in the side chains as well as the distribution of the side chains have not been unambiguously determined.

O'Neill MA *et al.* Annu Rev Plant Biol 55 (2004) 109-139

Vincken JP *et al.* Plant Physiol 132 (2003) 1781-1789

Ridley BL *et al.* Phytochemistry 57 (2001) 929-967

Du Penhoat CH *et al.* J Biomol NMR 14 (1999) 253-271



GalA	D-Galactopyranuronic acid	Gal	D-Galactopyranose	Fuc	L-Fucopyranose 2-O-methyl (∇) and O-acetyl (\square) groups are indicated	Dha	3-deoxy-D-lyxo-2- heptulopyranosylaric acid
Rha	L-Rhamnopyranose	Gal	L-Galactopyranose	Xyl	2-O-methyl- D-Xylopyranose	Kdo	3-deoxy-D-manno- octulopyranosylonic acid
Api	D-Apiofuranose	Ara	L-Arabinofuranose	AceA	O-acetyl-L-Aceric acid		
GlcA	D-Glucopyranuronic acid	Ara	L-Arabinopyranose				

