Hemicelluloses
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xyloglucan, xylan, mannan, glucomannan, mixed-linkage glucan, plant cell wall

Abstract
Hemicelluloses are polysaccharides in plant cell walls that have β-(1→4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans, and glucomannans, and β-(1→3,1→4)-glucans. These types of hemicelluloses are present in the cell walls of all terrestrial plants, except for β-(1→3,1→4)-glucans, which are restricted to Poales and a few other groups. The detailed structure of the hemicelluloses and their abundance vary widely between different species and cell types. The most important biological role of hemicelluloses is their contribution to strengthening the cell wall by interaction with cellulose and, in some walls, with lignin. These features are discussed in relation to widely accepted models of the primary cell wall.

Hemicelluloses are synthesized by glycosyltransferases located in the Golgi membranes. Many glycosyltransferases needed for biosynthesis of xyloglucans and mannans are known. In contrast, the biosynthesis of xylans and β-(1→3,1→4)-glucans remains very elusive, and recent studies have led to more questions than answers.
INTRODUCTION

A strong wall—composed of polysaccharides, proteins, and in some cells, phenolic compounds—surrounds every cell of terrestrial plants. The type of wall, composed mainly of polysaccharides, probably evolved among the charophyte green algae but is more evidently suited for life on land. The wall provides support and shape for the plant, allowing it to stand upright. Some plants reach heights of more than 100 m, and obviously cell walls in such plants are capable of supporting very large physical forces and are very durable. The plant cell wall also provides a barrier against the environment and potentially pathogenic organisms. However, in spite of the strong, rigid, and seemingly impenetrable properties of cell walls, they are metabolically active, allowing exchange of material and signals between cells, and are capable of expanding. The cells generated in meristems by cell division will typically expand to 100 times their original length, and the primary wall surrounding such cells fulfills the support and barrier functions while expanding with the growing cell. Primary cell walls are the walls that surround growing cells. After cessation of cell expansion, some cells (e.g., fibers and tracheary elements) develop a secondary wall, which gives additional strength. Some cell walls in seeds are highly thickened due to accumulation of polysaccharides that serve as seed storage.

Polysaccharides make up most of the wall, but the wall also contains proteins. Phenolic compounds, notably lignin, constitute up to 30% of some secondary walls. Many kinds of polysaccharides are present in all cell walls, the specific makeup varying in different species and tissues. Classically, cell wall polysaccharides have been grouped into cellulose, hemicelluloses, and pectins. Of these different classes, only cellulose is well defined, consisting entirely of β-(1→4)-linked glucan chains. Pectins are highly heterogeneous polysaccharides, traditionally characterized by being relatively easily extracted with hot acid or chelators and by containing a large amount of galacturonic acid residues. Hemicelluloses traditionally comprise the remaining polysaccharides, which can be extracted with alkaline treatment. These polysaccharides are very different from each other structurally and in physicochemical properties. Definitions based on extractability are not useful. Some pectins can be extracted only with alkaline treatment, and this is particularly true in some species such as lycophytes (50). Similarly, the β-(1→3,1→4)-glucans in grass cell walls and part of the arabinoxylans in cereal endosperm are considered hemicelluloses but are quite readily extracted without alkaline treatment. In this review we have grouped the hemicelluloses into xyloglucan, xylans, mannans and glucomannans, and β-(1→3,1→4)-glucans. Some polysaccharides, such as galactans, arabinans, and arabinogalactans are sometimes included in the hemicellulose group, but since these appear to be part of pectin molecules, at least in the initial synthesis, and do not share the equatorial β-(1→4)-linked...
backbone structure, we think they should not be included in the already heterogeneous group of hemicelluloses. We also consider that callose, which has a backbone entirely composed of β-(1→3) linked glucose residues, should not be considered a hemicellulose.

Hemicelluloses are a heterogeneous group of polysaccharides, and the term was coined at a time when the structures were not well understood and biosynthesis was completely unknown. The term hemicelluloses is therefore archaic and various researchers have suggested that it should not be used. Alternative terms such as cross-linking glycans have been proposed (138, 140), but that has other problems since it is not obvious that cross-linking is a major and common feature of the hemicelluloses. Nevertheless, most workers in the field still use the term hemicelluloses as a convenient denominator for a group of wall polysaccharides that are characterized by being neither cellulose nor pectin and by having β-(1→4)-linked backbones of glucose, mannose, or xylose. These glycans all have the same equatorial configuration at C1 and C4 and hence the backbones have significant structural similarity (Figure 1). We suggest that hemicelluloses should be used to describe only polysaccharides with this configuration.

**STRUCTURE AND DISTRIBUTION**

**Xyloglucan**

Xyloglucan (XyG) has been found in every land plant species that has been analyzed, including mosses, but has not been found in Charophytes (93, 108, 109) (see Figure 2 for an overview of plant phylogeny). XyG is the most abundant hemicellulose in primary walls of spermatophytes except for grasses (Table 1). The basic structure of XyG is shown in Figure 3, but there are many variations to this general pattern. In spite of the variations, XyGs are made of repetitive units, and a special one-letter code is used to denote the different XyG side chains (41). G denotes an unbranched Glc residue, while X denotes α-p-Xyl-(1→6)-Glc. The xylosyl residues can be substituted at O-2 with β-Gal (L side chain) or α-l-Araf (S side chain). A Gal residue substituted at O-2 with α-l-Fuc is designated F. Other less common side chains have other designations.

The branching pattern of XyG is of both functional and taxonomic significance. Less
branched XyGs are less soluble and this may correlate with functional aspects in those families with the lowly substituted XyGs. The most profound difference in XyG structure is charged versus uncharged side chains, with the former found in mosses and liverworts (104) whereas vascular plant XyGs are neutral.

The major divide among vascular plant families is in regard to whether XXGG or XXXG is the predominant, core repeating xylglucan oligosaccharide (142). The less substituted XXGG structure predominates in cell walls of commelinid monocots, which are also distinguished by the low content of XyG in the primary walls, typically 1–5% compared with 20% in dicots (Table 1). These figures refer to expanding cells. For example, elongating light-grown pea internodes contained 19.1% XyG as compared with 8.2% in internodes after cessation of growth (102). Oligosaccharides

Table 1 Occurrence of hemicelluloses in primary and secondary walls of plants

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Amount of polysaccharide in wall (% w/w)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dicot walls</td>
</tr>
<tr>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>Xyloglucan</td>
<td>20–25</td>
</tr>
<tr>
<td>Glucuronoxylan</td>
<td>_</td>
</tr>
<tr>
<td>Glucuronoribosylxylan</td>
<td>5</td>
</tr>
<tr>
<td>(Gluc)mannan</td>
<td>3–5</td>
</tr>
<tr>
<td>Galactoglucamannan</td>
<td>_</td>
</tr>
<tr>
<td>β-(1→3,1→4)-glucan</td>
<td>Absent</td>
</tr>
</tbody>
</table>

aNumbers are typical values; actual values vary between different species and tissue types. Numbers are obtained from several different sources (31, 17, 37, 59, 72, 101, 135, 143, 147, 156; C. Manisseri and H. V. Scheller, unpublished data).

b-, absent or minor; +, present but quantitative data not available.
Figure 3
Schematic illustration of the types of hemicelluloses found in plant cell walls. The letters under the xyloglucan (XyG) molecule illustrate the symbols used for the most common side chains. The structure of the hemicelluloses varies greatly in different plant species and tissue types. “Fer” represents esterification with ferulic acid (3-methoxy-4-hydroxycinnamic acid), which is characteristic of xylans in commelinid monocots.
of the XXGG type are also found in solanaceous XyGs, which are further characterized by lacking fucosyl residues and featuring the arabinose-containing S chain (152). S is also found in XXXG-type units in olive (141). Hoffman et al. (37) have shown that the F and the S side chains coexist in the XyG of Nerium oleander and have compiled an extensive list of XyG-repeating units from a wide range of plant families. Their general conclusion is that while the XyG oligosaccharide module structures correlate with taxonomical groupings, there are several convergent adaptations of XyG structure among Tracheophytes so that the XyG structures do not mirror evolution in any simple way.

The occurrence of the fucose-containing XyG F-chain (see side chain structure in Figure 3) in the grass family is particularly enigmatic. Purified hemicellulose fractions of oat and rice did not contain detectable fucose (65, 73), and such studies led Hayashi (55) to state that grass XyG does not contain fucose. However, fucosylated XyG has been detected in Festuca arundinacea by feeding radioactive fucose to cell suspension cultures (89), and Peña et al. (104) cite unpublished results that low amounts of fucosylated XyG are found by this method in rice as well. These observations can be reconciled if XyG is fucosylated transiently in these grasses during synthesis and most of the fucose is removed during or following deposition in the wall. A transient fucosylation of grass XyG has implications for the assignment of function to grass glycosyltransferases (GTs) of family GT37 (discussed below).

**Xylans**

Xylans are a diverse group of polysaccharides with the common feature of a backbone of β-(1→4)-linked xylose residues (Figure 3). A common modification of xylans is substitution with α-(1→2)-linked glucuronosyl and 4-O-methyl glucuronosyl residues. Xylans dominated with this type of substitution are often known as glucuronoxylans and are the dominating noncellulosic polysaccharide in the secondary walls of dicots. In commelinid monocots (which include grasses and some related species; see Figure 2), xylans are the major noncellulosic polysaccharide in primary walls, constituting about 20% (Table 1) of the wall. These xylans usually contain many arabinose residues attached to the backbone and are known as arabinoxylans and glucuronoxylans (GAXs). The distinction is not clear. Cereal endosperm arabinoxylan has very little glucuronic acid, but heteroxylans in vegetative parts of grasses are often called arabinoxylans, even though they tend to contain more glucuronic acid and 4-O-methyl glucuronosyl residues, making GAX a more appropriate name. Branching patterns in xylan, like those in XyGs, correlate with taxonomy. Arabinofuranose substitutions in grass walls are mostly from O-3 of the backbone xylose residues, and xylose residues doubly substituted with arabinofuranose at both O-2 and O-3 are common in grass endosperm (31). Gymnosperm walls also contain arabinoxylans in relatively high amounts (6). Arabinofuranose substitutions are less frequent in dicot xylans, but they are abundant in sycamore primary walls (27) and in seeds of certain species from diverse taxonomic groups, e.g., flax and psyllium (36, 96). Data by Zablackis et al. (155) suggest that GAX with fewer arabinose substitutions is present in arabinodisp, but unambiguous evidence has not been presented. The arabinofuranose substitutions on dicot xylans are normally at O-2 rather than O-3 as in grasses (27, 155), but doubly substituted xylose residues have been described in flax mucilage (96) and 3-linked arabinofuranose is present in psyllium seeds (27).

Unlike XyGs, xylans do not have a repeated structure, and there are many variations in the structure that are not well known. A common feature of grass xylans is the O-2 linked xylose residues often found as substituents of feruloylarabinofuranosyl side chains (144). Other features have been described, e.g., α-galactose linked to O-2 of glucuronosyl residues in eucalyptus (123) and α-arabinofuranosyl residues linked to O-2 of arabinose residues that are directly bound to the backbone in sorghum (139).
Ferulate esters have been described in gymnosperms, but it is not clear to which polysaccharide they are bound (19). In Amaranthaceae sensu lato, i.e., including Chenopodiaceae, Rhamnogalacturonan-I (RG-I) side chains are the sites of feruloylation, which occurs in both galactans and arabinans (115). The arabinosyl residues are predominantly feruloylated at position 2 (60), but small amounts of feruloylation at position 5 (as in GAX) have been detected (78). Recent studies suggest that feruloylated pectins may be present in many other plant groups but restricted to stomata and therefore perhaps present at a much lower level (64). The feruloyl transferases are unknown both in Chenopodiaceae and in Poaceae, so it is not possible to determine from the structures of the enzymes if they have a common origin. [See also (59) for a comprehensive review of variations in feruloylated polysaccharides.]

Ferulate esters are important because they can be oxidatively cross-linked in a variety of ways. Ferulate dimers are easily detected in grass walls and likely represent intra- and intermolecular linkages in and between GAX molecules. Ferulate can also be cross-linked with lignin (48) and we can therefore assume that GAX and lignin become covalently cross-linked through these linkages. Cross-linking through ferulate esters is widely assumed to render the cell wall recalcitrant to digestion, which would be an obvious benefit as a defense against microorganisms and herbivores. Similarly, the ferulate esters make grass cell walls recalcitrant to enzymatic saccharification prior to fermentation into biofuels (11, 12).

Mannans and Glucomannans

β-(1→4)-linked polysaccharides containing mannose are widely distributed and the main hemicellulose in Charophytes (108, 109). The backbones may consist entirely of mannose, as in mannans and galactomannans, or with mannose and glucose in a nonrepeating pattern as in glucomannans and galactoglucomannans. Mannans and glucomannans are often acetylated.
Mannans have been much studied in their role as seed storage compounds, but they are found in variable amounts in all cell walls. In gymnosperms, galactoglucomannans are major components of the secondary walls (31). Mannans appear to have been very abundant in early land plants and are still abundant in mosses and lycophytes (50, 93). In spermatophytes, mannans and glucomannans are generally much less abundant and it appears that other hemicelluloses have largely replaced them. Nevertheless, mannans play essential roles, as evidenced by the embryo lethal phenotype in an arabidopsis mutant that is lacking the major (gluco)mannan synthase in seeds (47).

β-(1→3,1→4)-glucans

β-(1→4)-linked glucans with interspersed single β-(1→3)-linkages are well known in grasses. These mixed linkage glucans are dominated by cellotriosyl and cellotetrasyl units linked by β-(1→3) linkages, but longer β-(1→4)-linked segments also occur (128). Mixed linkage glucan in primary walls plays a role in cell expansion and the amount is very growth-stage dependent (23, 45, 97).

The β-(1→3,1→4)-glucans have not been found in dicots but are found throughout Poales, including the most basal family, Flagellariaceae (125). They have been suggested to be more abundant in the most derived family, Poaceae (grasses; 125), but the strong growth-stage dependency of β-(1→3,1→4)-glucan content makes such quantitative arguments difficult.

Recent studies have shown that β-(1→3,1→4)-glucans are not restricted to Poales but are present in Equisetum (38, 126), and it has been suggested that they are also present in liverworts (109). Even more recent work has shown the presence of β-(1→3,1→4)-glucans in Charophytes and red algae (Z. Popper and W. Willats, personal communication) (Figure 2). The occurrence of β-(1→3,1→4)-glucans in many primitive taxa could indicate that they represent an ancient trait. However, if β-(1→3,1→4)-glucan in grasses were a conserved ancient trait, then we would have to postulate the independent disappearance in a large number of Spermatophyte taxa that have been investigated. It seems more likely that β-(1→3,1→4)-glucan has evolved independently in grasses. Since at least some of the genes responsible for β-(1→3,1→4)-glucans biosynthesis in grasses are known (see below), it should be readily testable if orthologs are conserved in Equisetum and different algae while missing in dicots.

BIOSYNTHESIS

Xyloglucan

Glycosyltransferases. Many of the biosynthetic enzymes involved in XyG biosynthesis have been identified. The fucosyltransferase was one of the first cell wall biosynthetic enzymes to be identified (106) (see Table 2 for a list of GTs involved in hemicellulose biosynthesis). XyG fucosyltransferase was purified from pea using a traditional biochemical approach, and the arabidopsis ortholog was expressed and assayed in vitro. Subsequently, a fucose-deficient arabidopsis mutant, mur2, was shown to be affected in the same gene (138). Analysis of another fucose-deficient mutant, mur3, from the same mutant collection led to cloning of a gene, which turned out to encode a XyG β-(1→2)-galactosyltransferase (84). The galactosyltransferase has been shown to be highly specific for the third galactose in the repeating XXXG unit of xyloglucan. This is the galactose that is often fucosylated, explaining the fucose-deficient phenotype. The reduction in galactose is harder to detect since galactose is present in many other polymers. The arabidopsis ortholog was expressed and assayed in vitro. Subsequently, a fucose-deficient arabidopsis mutant, mur3, from the same mutant collection led to cloning of a gene, which turned out to encode a XyG β-(1→2)-galactosyltransferase (84). The galactosyltransferase has been shown to be highly specific for the third galactose in the repeating XXXG unit of xyloglucan. This is the galactose that is often fucosylated, explaining the fucose-deficient phenotype. The reduction in galactose is harder to detect since galactose is present in many other polymers. The galactose on the second position in the repeating unit must be incorporated by a different galactosyltransferase. Li et al. (80) have obtained evidence that a GT in subgroup A of the GT47 family is responsible for the activity, but the final evidence has not yet been reported. GT47 subgroup A contains 10 members in arabidopsis and only MUR3 has had the activity confirmed in vitro.
<table>
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<tr>
<th>Activitya</th>
<th>GT name</th>
<th>Identifierb</th>
<th>CAZyc</th>
<th>Reference</th>
<th>Comment</th>
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<td>GT2</td>
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<td>β(1→2)-GalT</td>
<td>MUR3</td>
<td>At2g20370</td>
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<td>ACN67534</td>
<td>GT2</td>
<td>29</td>
<td>From barley, catalytic activity not determined</td>
</tr>
</tbody>
</table>

aKnown and predicted enzymes involved in biosynthesis of the most common structures in hemicelluloses. The less common structures of XyGs and xylans require a large set of additional activities.

Identifiers are given as gene locus IDs for *Arabidopsis thaliana* and as NCBI accession numbers for other species.

cGlycosyltransferase (GT) family in CAZy database.
The xylose residues in XyG are transferred by $\alpha-(1\rightarrow6)$-xylosyltransferases, which are retaining enzymes from family GT34. Two enzymes, XXT1 and XXT2 (originally named XT1 and XT2), have been identified in arabidopsis and shown to be involved in the synthesis of XyG (21, 34). Somewhat surprisingly, a third GT34 enzyme, XXT5 from a separate clade of GT34, has been implicated in the same function, but the activity has not been unambiguously demonstrated (156). The XXT5 clade has two additional, uncharacterized arabidopsis members. In addition, GT34 has two arabidopsis members that are more closely related to galactomannan $\alpha-(1\rightarrow6)$-galactosyltransferase (156).

The backbone of XyG is apparently synthesized by members of cellulose synthase–like proteins belonging to the CSLC family (Figure 5). Arabidopsis has five members of the CSLC subfamily and only CSLC4 has been shown to be involved in XyG biosynthesis (24). It seems likely that the other CSLC members are involved in XyG biosynthesis as well, but it cannot be excluded that some CSLC members have a role in biosynthesis of other polymers.

Hydrolases. The GTs described above are all Golgi-localized enzymes and work together to produce a XyG precursor molecule that is transported to the wall. However, important changes to the XyG molecules take place after the initial synthesis in the Golgi. Thus, it has been shown that specific apoplastic glycosidases are responsible for the trimming of nascent XyG chains and important in determining the heterogeneity of the polymer in the wall (100). The value of such apparently wasteful trimming is unclear, but the higher substitution degree of native hemicelluloses will help to keep them soluble during transport and incorporation into the wall. In general, hydrolases are likely to play an important role in determining hemicellulose structures in the wall, and it may be noted that many hydrolases are coexpressed with polysaccharide biosynthetic enzymes. It is interesting to note that the arabidopsis genome contains about 300 membrane-bound GTs but more than 500 glycoside hydrolases and lyases, many of which are involved in modification of wall polysaccharides. Plant hydrolases have been described in recent reviews (82, 91).

Another important and well-studied modification of XyG is carried out by the XET enzymes. These are enzymes related to hydrolases and carry out a transglycosylase reaction. The role of these enzymes is described below in the section on Biological Function.

Polysaccharide acetylation. XyGs, like xylans, mannans, and pectins, are usually acetylated to various degrees. Acetylation of XyG is on the galactose residues, mostly on O-6 (68). Acetylation of cell wall polysaccharides occurs...
in the Golgi by means of transferases using acetyl-CoA (103). Neither acetyltransferases nor acetyl-CoA transporters required for this process have been identified. A mutant of the fungus Cryptococcus neoformans lacks acetylation of the glucurononanmate coat polysaccharide and the affected gene has been suggested to be an acetyltransferase (62). Arabidopsis has four homologs of the C. neoformans protein, and we have recently found that knockout mutants in one of the corresponding genes, At3g06550, are deficient in wall-bound acetate (Y. Manabe and H. V. Scheller, unpublished data). Presently, it is unclear if the protein is an acetyltransferase, but we think its topological model with multiple membrane-spanning segments suggests a transporter function.

Xylans

The main chain of xylan as shown in Figure 1 may be differently substituted with side chains and equipped with a unique oligosaccharide in its reducing end. Side chain structures vary with taxonomic origin (commelinid monocot versus other flowering plants), and there is some uncertainty as to how conserved the reducing end oligosaccharide is. Biosynthesis is thus dealt with under three subsections: Backbone Synthesis, Reducing End Oligosaccharide, and Side Chains.

Backbone synthesis. Because of the structural similarity of xylan to the β-(1→4)-linked backbones of the other hemicelluloses, it has been widely assumed that the biosynthesis would involve members of the CSL protein families, as has been shown to be the case for the other hemicelluloses. However, investigations of CSL proteins have not provided evidence that any of these proteins are involved in xylan biosynthesis. Instead, characterization of xylan-deficient mutants irx9, irx14, irx10, and irx10-like has indicated that the corresponding GTs belonging to families GT43 and GT47 are responsible for elongation of the xylan backbone. Unlike the CSL proteins with multiple transmembrane segments, the members of GT43 and GT47 are predicted to be typical Type II membrane proteins with a single N-terminal membrane anchor. See Table 2 to associate each GT with the corresponding mutant and CAZy family. Although the GTs affected in these mutants belong to different families, they are all named irx for their irregular xylan phenotype. Xylem cells are under negative pressure, and compromised load-bearing ability is associated with vessel collapse or irregular walls. Secondary walls are rich in xylan in arabidopsis, and mutants in any of these genes are deficient in xylan and xylan synthase activity (2, 9, 10, 75, 77). However, so far no xylan synthase activity has been reported for any of these proteins when heterologously expressed.

Presumably, several researchers have attempted to show xylan synthase activity, but without success. In our own work we have expressed these proteins in E. coli and/or tobacco cells but failed to find xylan synthase activity (A. Suttangkakul, A. Oikawa, H. V. Scheller, unpublished data). Obviously, it is not possible to draw conclusions from negative results, but the non-redundancy of IRX9, IRX14, and IRX10 indicates that they are all required for backbone synthesis, perhaps in a complex containing more than one protein. A homolog of IRX10 named NpGUT1 for glucuronosyltransferase was discovered in a Nicosatiana plum-baginifolia cell adhesion mutant and suggested to be involved in RG-II A-chain biosynthesis (61). IRX10 and IRX10-like appear to be redundant and are unlikely to be orthogonal to NpGUT1. The mutants do not display a cell adhesion phenotype and should rather be named for their pronounced irx phenotype found when both genes are knocked out (9, 149). Selaginella and Physcomitrella have only a single IRX10/IRX10L ortholog (50) confirming that one gene product suffices. For IRX9 the situation is more complex. Arabidopsis contains a homolog of IRX9, At1g27600, the role of which has not been reported. Rice and other higher plants have members of both the IRX9 and the At1g27600 group, while Selaginella and Physcomitrella have orthologs only of At1g27600 (50). The most likely explanation

XET: xylol glucan endotransglucosylases belong to the xylol glucan endotransglucosylase/hydrolase (XTH) group of CAZy (see below) family GH16 that also comprises the xylol glucan endohydrolases (XEH). The XETs cleave xylol glucan (XyG) backbones, retain the energy of the glucosidic linkage, and graft the reducing end of the cleaved molecule onto the nonreducing end of an acceptor XyG. XEH-catalyzed hydrolysis proceeds similarly, with water acting as the acceptor substrate.

CAZy: a comprehensive system and database (www.cazy.org) for classifying carbohydrate active enzymes. Currently, glycosyltransferases and glycoside hydrolases in CAZy are classified into 91 families, 43 classifying CAZy families. These families are classified into 115 different subfamilies, respectively (16).
Reducing end oligosaccharide. As mentioned above, xylans in conifers and several dicots have been shown to have a reducing end with the unique structure β-d-Xylp-(1→4)-β-d-Xylp-(1→3)-α-L-Rhap-(1→2)-α-L-GalpA-(1→4)-d-Xylp (Figure 4) (1, 63, 105). Treatment of xylan with xylanases allows the isolation of the oligosaccharide. A number of xylan-deficient mutants, irx7 (also known as fra8), irx8, and parvus, have been shown to retain in vitro xylan synthase activity while being depleted in the reducing end oligosaccharide (77, 105). In contrast, the reducing end oligosaccharide is present in irx9, irx14, and irx10/irx10L mutants (9, 10, 105, 149). These observations indicate that IRX7, IRX8, IRX9, IRX14, IRX10, and PARVUS are all specifically involved in synthesizing the oligosaccharide.

It is unclear how many GTs are needed to make the oligosaccharide. At the least, there must be a XylT specific for Rha-GalA-Xyl, a RhaT specific for GalA-Xyl, and a GalAT specific for the terminal xylose, but it is possible that additional xylosyltransferases would be required to transfer the reducing end xylose to a nonpolysaccharide primer and to transfer xylose onto the Xyl-Rha-GalA-Xyl acceptor. The Rha-specific XylT and the GalA-specific RhaT are expected to be inverting enzymes, while the Xyl-specific GalAT would be a retaining enzyme. IRX8 (also known as GAUT12) is a homolog of GAUT1, which is a retaining GalA transferase known to synthesize the backbone of homogalacturran (127). Hence, IRX8 is the most obvious candidate for the GalA transferase. PARVUS (also known as GATL1) is also a member of GT8, but belongs to the GATL group rather than the GAUT group (127). Rather than transferring a charged sugar, bacterial homologs of the GATL group are lipopolysaccharide galactosyltransferases. Also, PARVUS has been reported to be located in the ER (77), indicating an earlier biosynthetic step than the subsequent Golgi-localized steps.

Hence, we suggest that PARVUS is an α-xylosyltransferase transferring the reducing end xylose to a primer, which may be a lipid. IRX7 is an inverting enzyme belonging to GT47. It is a close homolog to IRX10, which as mentioned above is implicated as a xylosyltransferase involved in xylan backbone synthesis. Heterologous expression of IRX7 has proved that it can function in vitro as a xylosyltransferase with several different sugars as acceptor (A. Suttangkakul and H. V. Scheller, unpublished data). Hence, IRX7 (and its close homolog F8H; 76) is a candidate for the Rha-specific XylT. At present there is no clear candidate for the rhamnosyltransferase, nor for any additional xylosyltransferases needed in the nonreducing end prior to IRX9, IRX14, and IRX10.

What is the function of the reducing end oligosaccharide? At first sight the most obvious function would be as a primer, since synthesis of cell wall polysaccharides is generally assumed to take place by transfer to the nonreducing end of the growing chain (121). However, York and O’Neill (153) have speculated that xylose may be synthesized in the other direction, i.e., growing in the reducing end and with the oligosaccharide functioning as a terminator sequence. This could explain why irx7 and irx8 mutants have unusually long xylan molecules (105, 153). The fact that xylooligosaccharides that are modified in the reducing end, e.g., by attachment of fluorescent tags, are still excellent acceptors for xylan synthase in vitro suggests that synthesis does in fact proceed in the more conventional way by transfer to the nonreducing end. However, as pointed out by York and O’Neill (153), the modified oligosaccharides could be transferred to the reducing end of a growing chain in the same way that a reducing
end terminator sequence would normally be transferred. The reducing end oligosaccharide has not been reported in grasses, although the genes associated with its biosynthesis seem to be conserved. The absence of the oligosaccharide in grass xylan would also be in agreement with the oligosaccharide being a terminator rather than a primer. A primer would more likely be indispensable. Clearly, much more work is needed before xylan biosynthesis is understood.

Side chains. The most important side chains of xylans should be formed by α-glucuronosyltransferases and α-arabinofuranosyltransferases. Both activities have been detected in vitro (3, 112, 157), but the GTs responsible for the transfer have not been identified. FRA8 was originally proposed to be a xylan glucuronosyltransferase (158), but it is generally agreed that this is not correct and that FRA8 is involved in synthesis of the backbone, Xylan α-glucuronosyltransferase is expected to belong to a retaining GT family, but no obvious candidates have been proposed.

Arabinosyl residues may be linked to position 2 or 3 or to both positions of the xylosyl residues of the backbone. A monosubstituted xylosyl residue is expected to be a very different acceptor compared with an unsubstituted residue, and hence at least three inverting arabinosyltransferase activities are required: an α(1→2) and an α(1→3) arabinosyltransferase and a third for adding the second residue to a monosubstituted xylose (no information is available regarding the order of transfer). Members of GT61 (a family of inverting GTs) are highly expressed in grasses, which also contain many grass-specific GT61 members. The only known activity of GT61 is the β-(1→2)-xylosyltransferase activity involved in synthesis of N-linked glycans in plants (129). Hence, it has been speculated that GT61 members are involved in transferring xylose to feruloyl-arabinofuranosyl side chains of xylan (92). However, the abundant and conserved GT61 members in grasses and the presence of GT61 members in other plants suggest to us that GT61 would more likely include the various arabinofuranosyltransferases needed for xylan synthesis.

The fact that the arabinose residues in xylan are in the arabinofuranose configuration while UDP-arabinopyranose is the nucleotide sugar known to be present in plants has been a conundrum (Figure 6). Intact Golgi vesicles can incorporate arabinofuranose residues into xylan using UDP-arabinopyranose as the substrate (112). Most likely, the mechanism behind this transfer involves formation of UDP-arabinofuranose by UDP-arabinopyranose mutase, as has been shown for arabinan biosynthesis (70, 71). Mutase activity has been found in reversibly glycosylated proteins (RGP) from rice and arabidopsis (71; C. Rautengarten and H. V. Scheller, unpublished data). RGP are abundant proteins, which are known to be located in the Golgi but apparently as extrinsic membrane proteins on the cytoplasmic side of the membranes. The mutase catalyzes a reversible reaction, which favors the pyranose configuration 10:1. Hence, it seems most likely that the mutase would interact with a transporter and/or transferase so that the generated UDP-arabinofuranose can be used in a channeled reaction. The major UDP-xylene-4-epimerase required for generating UDP-arabinopyranose is predicted to be located inside the Golgi (13). Therefore, it is surprising that the mutase is located on the outside of the Golgi, since this would imply that UDP-Arap is moved out of the Golgi, converted to UDP-Araf, and then moved back into...
the Golgi again. On the other hand, isolated wheat Golgi vesicles did not have detectable UDP-xylose-4-epimerase activity (112), so perhaps the topology and localization of the epimerases need to be further investigated. UDP-xylose is known to be synthesized both in the Golgi lumen and in the cytosol (52).

**Hydrolases.** GAX in primary walls has been shown to be synthesized in a more highly arabinosylated form and subsequently trimmed by arabinofuranosidases, resulting in much fewer substituted polymers in mature cells (20, 43, 97). Highly substituted arabinoxylan is more soluble and does not interact with cellulose. Presumably, the soluble form is ideal for initial integration into an expanding wall, while the less substituted polymer functions in the mature wall by interaction between insoluble xylan molecules and cellulose (20).

**Ferulate esters.** As mentioned above, ferulic acid esters are important components of grass arabinoxylans. The feruloyl transferases are not known, but the transfer takes place intracellularly in the Golgi (40, 98). The substrate has not been identified either, but feruloyl-CoA is the most likely substrate, although other evidence has suggested feruloyl-glucoside as a possible substrate (98). In vitro synthesis of feruloylated arabinoxylan has not been convincingly demonstrated, although some activity using feruloyl-CoA as donor and small oligosaccharides as acceptors has been reported (154). Other work using microsomal preparations from wheat or parsley has shown that feruloyl-CoA incubation resulted in feruloylation of a protein rather than a polysaccharide (69, 98). We tend to believe that the feruloylated protein is an intermediate in the feruloylation of arabinoxylan. The ferulic acid in wheat microsomes appeared to be bound to arabinosyl residues on the protein, although the structure was not unambiguously identified (N. Obel and H. V. Scheller, unpublished data), and the protein may be RGP. Most likely, feruloyl-arabinose is transferred as a unit from a precursor, perhaps RGP, onto the acceptor. Mitchell et al. (92) have identified putative acyl-transferases that are candidate feruloyl transferases based on a coexpression study of rice genes. The candidate proteins are predicted to be cytoplasmic, which is consistent with a role in feruloylation of a cytoplasmic intermediate such as RGP but not with feruloylation of arabinoxylan in the Golgi. Very recent data have confirmed that some of the candidate acyl-transferases are involved in xylan feruloylation (107).

**Mannans and Glucomannans**

A mannan synthase involved in making the backbone of galactomannan in guar was shown to be a member of the CSLA family (28). Subsequently, several CSLA members have been shown to have mannan and glucomannan synthase activity, apparently being able to utilize both GDP-mannose and GDP-glucose as substrates (81). Incorporation of glucose and mannose in glucomannan does not follow a strict pattern but is determined primarily by the relative concentration of the substrates. However, this may not necessarily be true in vivo. The functional equivalence of CSLA proteins has also been confirmed in vivo since constructs with 35S promoter used to drive different CSLA homologs could complement the embryo-lethal csla7 mutant in arabidopsis (46).

Preliminary data from our laboratory have indicated that CSLD proteins are also glucomannan synthases (150). These results were obtained with microsomal preparations of arabidopsis CSLD5 expressed in tobacco and should be confirmed with purified protein. The structure and phylogeny of CSLD proteins (Figure 5) (4) have led to suggestions that the proteins would be glucan synthases using UDP-glucose as a substrate, so a GDP-sugar dependent activity is unexpected.

A galactosyltransferase involved in making galactomannan was identified in fenugreek and was the first GT involved in synthesis of plant cell walls for which the activity of the pure enzyme was shown (32). Whereas the fenugreek enzyme, which belongs to GT34, is involved in making seed galactomannan, it appears to
Mixed-Linkage Glucans

The biosynthesis of β-(1→3,1→4)-glucan has recently been shown to involve CSLF and CSLH proteins (Figure 5) (14, 29). The corresponding gene families are absent in arabidopsis and poplar and present in rice and Brachypodium consistent with a grass-specific occurrence. The involvement of CSLF and CSLH was shown by expressing isoforms of the rice proteins in arabidopsis and detecting small amounts of β-(1→3,1→4)-glucan in the transgenic plants. It is surprising that both types of protein can produce β-(1→3,1→4)-glucan alone. Most likely, the heterologously expressed proteins interact with other proteins present in arabidopsis, but obviously the CSLF and CSLH proteins do not need to be present simultaneously for β-(1→3,1→4)-glucan synthase activity to occur. In support of this, CSLF and CSLH genes are not coexpressed. Comparison of Brachypodium, wheat, and barley has recently shown that CSLF6 is the major CSLF gene expressed in wheat and barley seedlings, while other CSLF isoforms and CSLH are expressed at a very low level (23). In contrast, Brachypodium seedlings show a high expression level of CSLH. It will be interesting to investigate whether these species have different β-(1→3,1→4)-glucan fine structures: e.g., perhaps one type of CSL protein is chiefly involved in making the short β-(1→4)-linked segments and the other CSL protein type is chiefly responsible for making the longer segments. The ability of arabidopsis to synthesize β-(1→3,1→4)-glucan when transformed with a single gene related to universally present CSL genes suggests that evolution of β-(1→3,1→4)-synthesis can take place relatively easily and supports the idea that occurrence of β-(1→3,1→4)-glucan in very different taxonomic groups is the result of convergent evolution. Isoforms of CSLH and CSLF have been localized in the Golgi (29, 35), and in vitro activity studies have been consistent with biosynthesis of β-(1→3,1→4)-glucan in the Golgi (44, 56). However, unlike other matrix polysaccharides, β-(1→3,1→4)-glucan has not been detected in the Golgi (148). This would imply either that the β-(1→3,1→4)-glucan epitope is completely masked in Golgi vesicles, perhaps by acetylation, or that some stage of synthesis takes place in the plasma membrane.

The CESA-CSL Superfamily

The cellulose synthase and cellulose synthase-like superfamily of GTs (Figure 5) is involved in synthesis of cellulose (CESA), hemicelluloses XyG (CSLC), glucomannan (CSLA and perhaps CSLD), and β-(1→3,1→4)-glucan (CSLF and CSLH). This raises the question of what the other CSL proteins may do. Most likely, they would all be involved in making hemicelluloses as defined in this review, i.e., β-(1→4)-linked glycans with an equatorial
Expansin: a class of proteins that stimulate creep in cell walls. They are related to hydrolases of CAZy GH45 but have no hydrolytic activity. Their mode of action is thought to be unzipping of hydrogen bonds between wall polysaccharides.

FUNCTION OF HEMICELLULOSES

Biological Function—Hemicelluloses in Cell Wall Models

Primary walls and cell expansion. Cell wall models that are created to illustrate how wall polymers are organized in higher-order structures influence the thinking about biological functions of the hemicelluloses. The first significant cell wall model viewed the wall as a giant molecule, i.e., with covalent linkages between many noncellulosic polysaccharides (66). A notable feature of this model was a glycosidic linkage between XyG and galactan side chains of RG-I. This view contrasted with the sequential extractability of wall polysaccharides that led to the old hemicellulose definition. Talbott and Ray (131) presented a model that emphasized separability features of the wall. According to this model cellulose microfibrils were coated with several sheaths, with the hemicelluloses forming the innermost and most tightly bound sheath; this model is referred to as the multicoat model (25). An attractive feature of the model was that its construction outside the protoplast could be understood in terms of thermodynamically reasonable self-assembly mechanisms of affinities and phase separation (83). At the same time McCann & Roberts (85) presented a cell wall model drawn to scale (Figure 7). This model has become the most influential to date. It is also based on noncovalent interactions but has a particular noncovalent interaction as the central tenet: the importance of tethering glycans that cross-link cellulose microfibrils. It is referred to as the sticky network model (25). Tethering glycans, i.e., XyG in typical primary non-commelinid walls, bind to cellulose microfibrils by two mechanisms: by being trapped in the microfibril during the crystallization just after synthesis, and by multiple hydrogen bonds between XyG and cellulose. The evidence for tethering by XyGs is circumstantial but very strong: The XyG backbone adopts a helical conformation in solution, which together with arrangements of side chains prevents self-association in solution while at the same time favoring adoption of a flat conformation upon interacting with cellulose (79). Endo-glucanases will more easily access substrate in solution than substrate in semicrystalline or adsorbed states and can thus be used for preferential extraction of more solvated XyG domains of the wall. The part of XyG, which is accessible for release from pea cell wall by endoglucanase, differs by the predominance of GXFG (see Figure 3) from the XyG that could only be released by strong alkali (49, 99). The definition and biological significance of these distinct XyG domains are corroborated by the observation that expansins induce creep more effectively in artificial cellulose/XyG composites in which the XyG chains are long enough to tether the microfibrils than in composites made with short XyG fragments (145).

Figure 7
Simplified model of the primary cell wall. Reprinted from McCann and Roberts (85) with permission.
The unequivocal existence of tethering glycans does not mean they are indispensable load-bearing structures of the wall. Celery appears to have dispensed with tethering glycans and features a pure multicoated model wall. With only 2% XyG and 2% xylan, there are not enough hemicellulosic polysaccharides to coat the microfibrils, and no other polysaccharides appear to have taken the place of the hemicellulosic polymers (134). A fully viable mutant devoid of any detectable XyG has been created in arabidopsis (22). Finally, Thompson (136) has calculated that the combined strength of all XyG/cellulose hydrogen bonds in typical dicot walls cannot transmit all the stress that the wall sustains. The solution to the latter problem probably rests in the fact that current models ignore direct cellulose microfibril contacts and entanglement, the structures that sustain by far the major load from turgor and small deformations (146). Another desirable aspect in future models would be evidence for the pectic matrix also tethering microfibrils via RG-I side chains (159, 160). Finally, new models should revisit the issue of covalent linkages between pectin and XyG. The above-mentioned proposal of the original Keegstra et al. (66) model that XyG is synthesized onto an RG-I side chain, e.g., galactan, has more recently received support. The possible occurrence of a base-stable linkage between XyG and RG-I was rediscovered while developing a XyG labeling method (33), and the observations in the following decade pointing to the existence of such a linkage was reviewed by Mort (94). The concurrent insertion of new building blocks should not strengthen the wall so as to impair cell expansion. Whether insertion of new wall polysaccharides even contributes to wall creep is still unresolved (130, 137), but it is widely accepted that the insertion of newly synthesized XyG occurs by transglycosylation catalyzed by XET (26). Attempts to identify transglycosylases that act on other polysaccharides have been met with limited success. A glucomannan transglycosylase has been reported (122), while the search for a homogalacturonan transglycosylase was negative (42). The situation in the grasses is quite enigmatic. β-(1→3,1→4)-glucans are deposited during cell expansion only to be degraded and replaced with GAX as expansion ceases. Measurements of the β-(1→3,1→4)-glucan content in developing wheat, barley, and Brachypodium seedlings during development demonstrate net breakdown as opposed to simple dilution by xylan (23). The hydrolases that are responsible for degradation of β-(1→3,1→4)-glucan belong to CAZy family GH16. XETs, the XyG transglycosylases, are also classified in this family, and it would be conceivable that some of the grass members of GH16 could transglucosylate β-(1→3,1→4)-glucan, but this appears not to be the case (39, 58). Grasses thus appear to rely on a β-(1→3,1→4)-glucan/xylan replacement rather than transglycosylation, and a xylan transglycosylase remains to be identified. In dicots, xylan is mostly deposited in secondary walls, i.e., where expansion has ceased. We speculate that dicots do not have a xylan transglycosylase and hence restrict the use of substantial amounts of xylan to walls that do not expand. Recently, a surprising new class of transglycosylases has been identified. These are the mixed-linkage beta-glucan:XyG endotransglycosylases, or MXEs. These enzymes, which graft β-(1→3,1→4)-glucan onto XyG, have been found in horsetail
and in *Chlorella*, a Charophycean green alga, but not in higher plants (39).

**Secondary walls and xylans.** Cell wall models depicted in textbooks often focus on the primary wall. The reason for this is that primary cell wall models inspire working hypotheses regarding mechanisms required for cell growth. Xylan thus receives less attention though it is a major load-bearing structure in grasses as explained above. The important role of xylans in strengthening secondary walls is very clear from analysis of xylan-deficient mutants. All these contain collapsed xylem vessels and have severely impacted growth and fertility. Since such mutants grow better at high humidity, it seems that the main reason for the poor growth is that water transport is adversely affected by the poorly developed xylem. Xylans are also abundant in fiber cells, which do not have a water-conducting role. Therefore, xylan in this type of cell might be more dispensable, but this has not been tested.

**Mannans.** Apart from the role of galactomannans as seed storage compounds, it is unclear what specific roles the mannans have. Mannans/glucomannans are highly conserved through plant evolution. However, the only known effect of mutating mannan biosynthetic genes is the embryo-lethal phenotype of the *arabidopsis cEL*7 mutant (47). In contrast, deletion of the three glucomannan synthase genes expressed in *arabidopisis* stems resulted in plants lacking detectable glucomannan but without obvious phenotype (46). Mutants in *CSLD* genes show severe effects on tip growth, either in root hair or pollen tubes (5), and mutants in several *CSLD* genes are severely dwarfed (150). However, while preliminary evidence suggests that mannan biosynthesis is affected in the mutants, this still needs additional work.

In conclusion, hemicelluloses play a key role in both primary and secondary walls. XyG holds a special position in the cell wall, as reflected in the repertoire of enzymes available for its manipulation. This applies also to the commelinid monocots, which express a full complement of XETs, though XyG quantitatively is a minor hemicellulose.

**Source of Signal Molecules**

XyG plays an additional role as a source of signal molecules. Breakdown products of XyG, most notably XXFG, were demonstrated to counteract auxin-induced cell expansion (151). This finding was verified and extended in a series of seminal papers by McDougall and Fry (86, 87). It is remarkable that the optimal concentration for the inhibitory activity of XXFG is as low as 1 nM, and the term oligosaccharins was coined for these signal molecules. Other XyG oligomers, XXLG and XLLG in particular, can promote cell expansion, albeit at a 1000-fold higher concentration (88). However, this response is not regarded as signaling but related to the action of XET. Research in XyG oligomers as substrates for XET has received much attention, whereas studies of the oligosaccharin inhibitory activities dwindled at the end of the nineties, and it is thus still not clear how important these responses are in vivo. Mutants in XyG biosynthesis can have very strong phenotypes (132, 133), but as mentioned above, mutants devoid of XyG do not (22). Perhaps the modified XyG in single mutants gives rise to different or increased amounts of signal molecules and hence invokes a stronger response than wild-type plants. On the other hand, the lack of obvious phenotype of the *mur2* mutant, which lacks XXFG (138), and of *xxt1/xxt2* mutants devoid of XyG altogether (22) suggests that if signaling by XyG fragments plays a role in plant growth and development, it is not a very important one. The small amount of XyG present in grass cell walls would seem unlikely to have an important structural role. Perhaps XyG has been maintained in grasses for two reasons that are not mutually exclusive: because the XyG-derived oligosaccharins indeed are indispensable, and because the process of de novo wall formation during cell division generally is more conserved among angiosperm families than is the mature primary wall structure and hence XyG is required for wall assembly during cytokinesis in grasses.
Information on a signaling role for hemicelluloses other than XyG is very limited. In contrast, many studies have shown that fragments of pectin play a role in the signaling of pathogen attack and induce a pathogen response (7, 30).

**Seed Storage Carbohydrates**

Hemicelluloses in the cell wall have the primary role of interacting with other polymers to ensure the proper physical properties of the wall. However, in a large number of cases, hemicelluloses have been recruited to the function of seed storage carbohydrate (90, 116). This has happened independently many times in evolution, and it has been suggested that from a taxonomic viewpoint hemicelluloses are as important as starch is in the role as storage carbohydrate in seeds (90). Much of our knowledge of hemicelluloses comes from the study of seed polysaccharides rather than the polymers in vegetative tissues. XyG is abundant in plants such as nasturtium and tamarind. Galactomannans are known from a large number of economically important plants, e.g., coconut, guar, and locust bean. Galactomannans are especially abundant in the endosperm of legumes but also occur in other seeds. Glucomannan is present in the konjak plant (Amorphophallus konjac); in this case the storage organ is a corm and not the seed. Arabinoxylans are present in seeds of dicots such as flax and psyllium (36, 96) and also in cereal endosperm. Cereal endosperm additionally contains β-(1→3,1→4)-glucans.

**ENGINEERING OF CELL WALLS**

Hemicelluloses have commercial significance. Seed storage hemicelluloses are used directly as products in the food industry, e.g., guar and locust bean gums (galactomannans), konjak gum (glucomannan), and tamarind gum (xyloglucan). In addition, the hemicelluloses give important properties to many food and feed products. In the baking industry, the insoluble arabinoxylans affect baking quality. β-(1→3,1→4)-glucans and arabinoxylans are well-known antinutritional compounds in animal feed, and they can cause filtering and haze problems in the brewery industry due to their viscosity. To alleviate these problems, hemicellulose-degrading enzymes are added to feed and are used in the baking and brewery industries. On the other hand, β-(1→3,1→4)-glucan has a documented cholesterol-lowering effect in hypercholesterolemic humans (67) and daily intake of β-(1→3,1→4)-glucans is recommended by the U.S. Food and Drug Administration. In cellulotic biofuel production, hemicelluloses affect the saccharification of biomass, and the released sugars, largely pentoses, are less desirable for fermentation than hexoses.

It is beyond the scope of this review to discuss all of these industrial applications. However, due to the economic and nutritional importance of hemicelluloses, several researchers have attempted or suggested modification in the hemicellulose composition of plants. Xylan feruloylation has been decreased by expressing ferulic acid esterases in transgenic plants with mixed results (11, 12, 51). To overcome the problem of high-pentose content in biofuel production, it has been suggested that xylan could be replaced with mannan (101). The apparently highly specialized function of xylans in vessels and lignin interactions suggests to us that a complete substitution could be very difficult. Instead, a more specific replacement in fiber cells would seem to have a higher chance of success.

**SUMMARY POINTS**

1. Hemicelluloses are wall polysaccharides, which are characterized by β-(1→4)-linked backbones of sugars in an equatorial configuration. This definition includes xyloglucans, xylans, mannans and glucomannans, and β-(1→3,1→4)-glucans.
2. All the hemicelluloses show differences in structural details between different species and in different cell types within plants.
3. The main role of hemicelluloses is to tether cellulose microfibrils, thereby strengthening the cell wall.
4. Xyloglucans dominate in primary walls of dicots and conifers, whereas (glu-
curonon)arabinoxylans dominate in commelinid monocots.
5. Hemicelluloses are synthesized by glycosyltransferases located in the Golgi membranes. The backbones of xyloglucan, mannan, and β-(1→3.1→4)-glucans are synthesized by members of the cellulose synthase-like gene family, which are multimembrane-spanning proteins.
6. Xylan backbones are apparently synthesized by glycosyltransferases that are Type II membrane proteins with a single membrane-spanning segment, but none of these has been unambiguously identified. Xylans in many, if not all, plants have a unique tetrasac-
charide in the reducing end, which appears to be involved in biosynthesis either as a primer or a termination sequence.
7. Hemicelluloses are important components in food and feed and constitute a major part of lignocellulosic biomass.

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LITERATURE CITED
3. Baydoun EAH, Waldron KW, Brett ET. 1989. The interaction of xylosyltransferase and glucuronyl-
58


35. Fincher GB. 2009. Revolutionary times in our understanding of cell wall biosynthesis and remodeling in the grasses. Plant Physiol. 149:27–37

284 Scheller • Ulvskov


104. Peña MJ, Darvill AG, Eberhard S, York WS, O'Neill MA. 2008. Moss and liverwort xyloglucans contain galacturonic acid and are structurally distinct from the xyloglucans synthesized by hornworts and vascular plants. Glycobiology 18:891–904


111. Popper ZA, Fry SC. 2008. Xyloglucan-pectin linkages are formed intraprotoplasmically, contribute to wall-assembly, and remain stable in the cell wall. Planta 227:781–94


116. Reid JSG. 2005. Tobacco transgenic lines that express fenugreek galactomannan galactosyltransferase constitutively have structurally altered galactomannans in their seed endosperm cell walls. Plant Physiol. 131:1487–95


121. Scheller HV, Doong RL, Ridley RL, Mohnen D. 1999. Pectin biosynthesis: A solubilized $\alpha$1,4-galacturonosyltransferase from tobacco catalyzes the transfer of galacturonic acid from UDP-galacturonic acid onto the nonreducing end of homogalacturonan. Planta 207:512–17


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## Contents

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*Winslow R. Briggs* ................................................................. 1

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*Arendas Möglich, Xiaojing Yang, Rebecca A. Ayers, and Keith Moffat* .................. 21

Auxin Biosynthesis and Its Role in Plant Development  
*Yunde Zhao* ........................................................................... 49

Computational Morphodynamics: A Modeling Framework to Understand Plant Growth  
*Vijay Chickarmane, Adrienne H.K. Roeder, Paul T. Tarr, Alexandre Cunha, Cory Tobin, and Elliot M. Meyerowitz* ...................................................... 65

Female Gametophyte Development in Flowering Plants  
*Wei-Cai Yang, Dong-Qiao Shi, and Yan-Hong Chen* ........................................ 89

Doomed Lovers: Mechanisms of Isolation and Incompatibility in Plants  
*Kirsten Bomblies* ...................................................................... 109

Chloroplast RNA Metabolism  
*David B. Stern, Michel Goldschmidt-Clermont, and Maureen R. Hanson* ........ 125

Protein Transport into Chloroplasts  
*Hsou-miu Li and Chi-Chou Chiu* .................................................. 157

The Regulation of Gene Expression Required for C4 Photosynthesis  
*Julian M. Hibberd and Sarah Covshoff* ............................................. 181

Starch: Its Metabolism, Evolution, and Biotechnological Modification in Plants  
*Samuel C. Zeeman, Jens Kossmann, and Alison M. Smith* ............................ 209

Improving Photosynthetic Efficiency for Greater Yield  
*Xin-Guang Zhu, Stephen P. Long, and Donald R. Ort* .................................. 235

Hemicelluloses  
*Henrik Vibe Scheller and Peter Ulvskov* .................................................. 263

Diversification of P450 Genes During Land Plant Evolution  
*Masaharu Mizutani and Daisaku Obta* .................................................. 291
Evolution in Action: Plants Resistant to Herbicides
Stephen B. Poyles and Qin Yu ................................................................. 317

Insights from the Comparison of Plant Genome Sequences
Andrew H. Paterson, Michael Freeling, Haibao Tang, and Xiyin Wang ................. 349

High-Throughput Characterization of Plant Gene Functions by Using
Gain-of-Function Technology
Youichi Kondou, Mieko Higuchi, and Minami Matsui .................................. 373

Histone Methylation in Higher Plants
Chunyan Liu, Falong Lu, Xia Cui, and Xiaofeng Cao .................................. 395

Genetic and Molecular Basis of Rice Yield
Yongzhong Xing and Qifa Zhang .................................................................. 421

Genetic Engineering for Modern Agriculture: Challenges and
Perspectives
Ron Mittler and Eduardo Blumwald ......................................................... 443

Metabolomics for Functional Genomics, Systems Biology, and
Biotechnology
Kazuki Saito and Fumio Matsuda ............................................................... 463

Quantitation in Mass-Spectrometry-Based Proteomics
Waltraud X. Schulze and Björn Usadel ........................................................ 491

Metal Hyperaccumulation in Plants
Ute Krämer .................................................................................................. 517

Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake
and Metabolism and Mitigation Strategies
Fang-Jie Zhao, Steve P. McGrath, and Andrew A. Meharg ................................ 535

Guard Cell Signal Transduction Network: Advances in Understanding
Abscisic Acid, CO₂, and Ca²⁺ Signaling
Tae-Houn Kim, Maik Böhmer, Hongbong Hu, Noriyuki Nishimura,
and Julian I. Schroeder ............................................................................. 561

The Language of Calcium Signaling
Antony N. Dodd, Jörg Kadla, and Dale Sanders ........................................... 593

Mitogen-Activated Protein Kinase Signaling in Plants
Maria Cristina Suarez Rodriguez, Morten Petersen, and John Mundy ................. 621

Abscisic Acid: Emergence of a Core Signaling Network
Sean R. Cutler, Pedro L. Rodriguez, Ruth R. Finkelstein, and Suzanne R. Abrams ... 651

Brassinosteroid Signal Transduction from Receptor Kinases to
Transcription Factors
Tae-Wuk Kim and Zhi-Yong Wang ................................................................. 681
Directional Gravity Sensing in Gravitropism

Miyo Terao Morita ................................................................. 705

Indexes

Cumulative Index of Contributing Authors, Volumes 51–61 ......................... 721
Cumulative Index of Chapter Titles, Volumes 51–61 .................................. 726

Errata

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