Structure and Function of the Polysaccharides in Plant Cell Walls

Plant Cells Are Surrounded by a Cell Wall
The Division and Growth of Plant Cells Are Controlled by the Cell Wall

Growing Plant Cells Are Surrounded by a Primary Cell Wall

Nature Reviews | Molecular Cell Biology
Plant Cells that Provide Mechanical Support or Form Vascular Tissue Are Surrounded by a Secondary Cell Wall

After deposition of the secondary cell wall, the cell usually dies and loses its contents (e.g., to form a vascular “tube”).

The secondary cell wall is made in successive layers. Each layer contains strong cellulose microfibrils in parallel arrays. The direction of cellulose microfibrils in each layer is varied, as shown in the right.

The secondary cell wall has three layers (S1, S2, and S3) in which the cellulose microfibril orientation differs.
Cellulose Microfibrils Are Crystalline Arrays of Glucan Chains Surrounded by Matrix Polysaccharides

The Monosaccharides That Comprise the Plant Cell Wall
Pectins Have α-1,4-linked GalA in Their Backbones

Homogalacturonan (HG) has a backbone consisting entirely of α-1,4-linked GalA residues.

Rhamnogalacturonan I (RG-I) is actually a family of related polysaccharides with a backbone consisting of alternating Rha and GalA residues. The Rha residues bear sidechains consisting of primarily of Gal and Ara residues. GlcA, 4-O-methyl-GlcA and Fuc residues are also found in the sidechains.
Homogalacturonans Can Also Have Sidechains

Substituted Homogalacturonans

**A**

Substituted Homogalacturonans

**B**

Apiogalacturonan

Rhamnogalacturonan II (RG-II) is a “Substituted Homogalacturonan”

**Side chain A**

\[
\alpha-L-Rhap(1\rightarrow2)\alpha-L-Arap(1\rightarrow4)\beta-D-GalpA(1\rightarrow2)\alpha-L-Araf(1\rightarrow3)\beta-D-Arap(1\rightarrow)
\]

**Side chain B**

\[
\beta-L-Arap(1\rightarrow5)\beta-D-Kdo(1\rightarrow)
\]

**Side chain C**

\[
\beta-L-Arap(1\rightarrow5)\beta-D-Dha(1\rightarrow)
\]

**Side chain D**

Sidechains A and B are initiated with apiosyl residues

Sidechains C and D are initiated with acidic residues structurally related to sialic acid

RG-II is the most complex polysaccharide known. Its structure is highly conserved among all vascular plants. Mutations that lead to loss of RG-II are lethal.
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**Rhamnogalacturonan II (RG-II)** is a “Substituted Homogalacturonan”

RG-II in the Cell Wall is Found Predominantly in a Dimeric Form Where an Apiosyl Residue in One Molecule is Linked to an Apiosyl Residue in the Other Molecule Via a Borate Di-Ester

Most of the borate (an element essential for plant growth) in the plant is found in RG-II crosslinks. Boron deficiency produces symptoms very similar to the phenotype of mutations that cause subtle changes in RG-II structure.
**Requirement of Borate Cross-Linking of Cell Wall Rhamnogalacturonan II for Arabidopsis Growth**

O’Neill et al., 2001 *Science* 294: 846-849

The mur1 mutation causes the L-fucose in RG-II to be replaced with L-galactose, resulting in severe growth defects. Such mutations lead to inefficient RG-II crosslinking. Supplying the mutant plant with additional borate or with an exogenous source of fucose restores RG-II crosslinking and rescues the growth phenotype.
Vascular Plants Have Highly Specialized, Xylan-Rich, Lignified Secondary Cell Walls

Localization of Xylan in Monocot (Switchgrass) and Woody Dicot (Poplar) Cell Walls

CCRC-M138, a xylan directed antibody, shows that xylan is a component of almost all cell walls in grasses, but is restricted to cells with secondary cell walls in dicots.

Images by Utlu Avci, Hahn Lab at CCRC, uavci@ccrc.uga.edu
3/19/15

**Xylan Is a Major Component of Secondary Cell Walls**

Structure of glucuronoxylan typically found in the secondary cell walls of most plants.

- O-Acetates (not shown) are the most abundant decoration of the backbone.
- The chain length of glucuronoxylan is tightly controlled (about 100 residues).
- The acidic structure at the reducing end appears to be involved in controlling chain length during biosynthesis.
- The glycosyl transferases involved in xylan biosynthesis were characterized only recently*. These include:
  - XYS1 (Xylan Synthase 1), a family 6T47 glycosyltransferase that catalyzes elongation of the xylan backbone
  - XOAT1 (Xylan O-Acetyl Transferase 1), which catalyzes transfer of O-acetyl groups from acetyl-CoA to O-2 of the backbone xylose residues.
- These two enzymes are sufficient to catalyze the generation of O-acetylated xylan in vitro.


**XYS1 Catalyzes Elongation of the Xylan Backbone**

**XOAT1 Catalyzes Acetylation of the Xylan Backbone**

(a) Catalytic reaction scheme:

\[ R_1 = \text{xylene or xylitol-2AB} \]
\[ R_2 = \text{xylose} \]

(b) MS spectra showing relative intensity for Xyl-2AB and acetyl-CoA.

(c) Graph showing the increase in acetylated xylans over time.

*Urbanowicz et al. (2014) Plant J. 80: 197-206

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**XYS1 and XOAT1 Are Sufficient to Catalyze the Generation of O-Acetylated Xylan In Vitro**

(a) IR spectra showing Xyl-2AB and IRX10-L.

(b) MS spectra showing various Xylan derivatives.

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**Immunolabeling with Monoclonal Antibodies Reveals Xylan Epitopes in the Axillary Hair Cells of the Moss *P. patens***

LM11 (Panel b) and CCRC-M137 (Panels c and f) bind branched xylans. LM10 (Panel d) preferentially binds "unsubstituted or low-substituted" xylans. CCRC-M88 (Panel e) binds xyloglucan.

**Axillary Hairs Constitute a Very Small Proportion of the *Physcomitrella* Leafy Gametophore**

GUS-promoter fusion analysis of an axillary hair-specific gene

Monoclonal Antibodies that Bind Xyloglucan Strongly Label Most *P. patens* Gametophore Cells, But Weakly Label Axillary Hair Cells

Axillary hair cell walls have chemical similarity to secondary cell walls in vascular plants.

Ameya Kulkarni, et al., *Glycobiology* in Review

Vascular Plants Have Highly Specialized, Xylan-Rich Secondary Cell Walls

Flowering Plants
Seed Plants
Coniferids (500) - Pinus
Gnetidra (120) - Ephedra
Ginkgoes (1) - Ginkgo
Cycads (145) - Zamiaceae

Gymnosperms (12,400)
Polypodiales (12,000) - Ceratopteris
Equisetopsida (15) - Equisetum
Marattiales (190) - Marattia
Ophioglossidae (110) - Botrychium
Pteridaceae (10) - Pteris

Lycopoda (1240)
Isoetales (140) - Isoetes
Selaginellales (700) - Selaginella
Lycopodiales (500) - Lycopodium

Bryomorpha (16000) - Physcomitrella
Anthoceromorpha (100) - Anthoceros
Marchantiomorpha (800) - Marchantia
Charales (250) - Chama
Coleochaetales (15) - Coleochaete
Zygmenates (800) - Spirogyra
Other green algae (6000) - Volvox
The Secondary Cell Walls of Vascular Plants contain Xylan with Methyl-Glucuronic Acid Sidechains

**Physcomitrella**

**Selaginella**

**Equisetum**

**Arabidopsis**

Both Me-GlcA (Residue I) and GlcA (Residue G)

__GXMT1 Catalyzes the Transfer of Methyl Groups to O-4 of the GlcA Residues of Glucuronoxylan__

A) MeGlcA (%)

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B) CBM5b1-2

C) CBM55

GXMT1 Catalyzes the Transfer of Methyl Groups to O-4 of the GlcA Residues of Glucuronoxylan

Xyloglucans Are Highly Branched Hemicelluloses with a Cellulosic Backbone
Xyloglucans Bind to the Surface of Cellulose by Adopting a Complementary Conformation

Twisted conformation – in solution

Flat conformation – bound to cellulose surface

The Genes Encoding the Glycosyltransferases Responsible for Xyloglucan Biosynthesis

The enzymes that extend the sidechains (adding Gal or Fuc) are REGIOSPECIFIC

Plants lacking XXT1 and XXT2 do not produce xyloglucan
Xyloglucan has a Key Role in Root Hair Growth

The mur-3-3 mutant xyloglucan lacks fucose-galactose sidechains. xxt1 xxt2 mutant lacks xyloglucan.

Cavalier, Plant Cell 2008

Root Hair Xyloglucan Has a Distinct Structure

CCRC-M1 (recognizes fucosylated xyloglucan)

Cross-sections of A. thaliana roots
Arabidopsis Root Hairs Contain an Acidic Xyloglucan

Structure of the Root Hair Xyloglucan

GalA Containing Sidechains

Not present in *mur3-3* plants
**At1g63450 Is a Root-Hair Specific Homolog of MUR3 that Encodes a Xyloglucan-Specific GalA Transferase**

**Root Hair Elongation is Reduced In the xut1 Mutant**

The root hair phenotypes of Ler-0, xut1, and complemented xut1 seedlings grown on nutrient agar.

1H NMR spectroscopy shows that xut1 root xyloglucan lacks β-GalpA residues.

Peña, et al., *Plant Cell* 2012
Role of the Acidic Xyloglucan in Root Hair Tip Growth

Heterologously Expressed XUT1 and FUT1 Generate the Y and Z Sidechains in Vitro

MALDI-TOF spectra of oligosaccharide fragments of the products formed using UDP-GalA and GDP-Fuc as donor substrates and polymeric xyloglucan (from the mur3-3 xlt2 double mutant) as the acceptor.
Xyloglucans Containing Fucosylated XXXG Subunits Are Cell-Specific Structures in Maize Root Hairs But Not in Cortex Cells

Calcofluor Cellulose  CCRC-M88 Any Xyloglucan  CCRC-M1 Fucosylated Xyloglucan

MUR3 Encodes a Regiospecific Galactosyltransferase
Dysfunctional Xyloglucan Structure Leads to Dwarfism in the Arabidopsis mur3-3 Mutant

The Xyloglucan of mur3 Loss-of-Function Mutant Lacks the F Side Chain

The oligosaccharides generated by xyloglucan-specific endoglucanase fragmentation of the 4M KOH-soluble xyloglucan were analyzed by three techniques.
Several MUR3 Alleles Have Been Identified But Only Loss-of-Function Lines Have a Cabbage-Like Phenotype

The mur3-3 xxt1 xxt2 Triple Mutant Does Not Produce Xyloglucan and Does Not Have a Cabbage-Like Phenotype

Glycome profiling confirms that mur3-3 xxt1 xxt2 walls lack xyloglucan