

Plant Polysaccharides

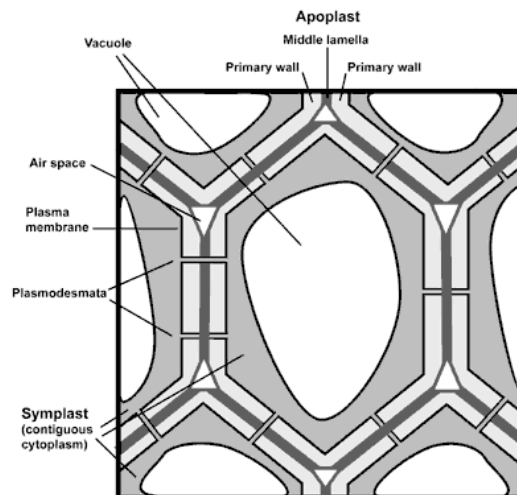
BCMB 8020

- overview of plant glycobiology
- plant cell walls
- types of plant cell wall polysaccharides: structure and biosynthesis
- RG-II: a curious case in plant glycobiology
- special functions of plant polysaccharides
- biofuel

- green plants constitute about half of the living matter on earth
- plants synthesize many of the same types of oligosaccharides that are found in animals but also produce a wide variety of unique sugar chains
- many of the earliest studies on carbohydrates were done in plants



General Organization and Features of a Plant Cell



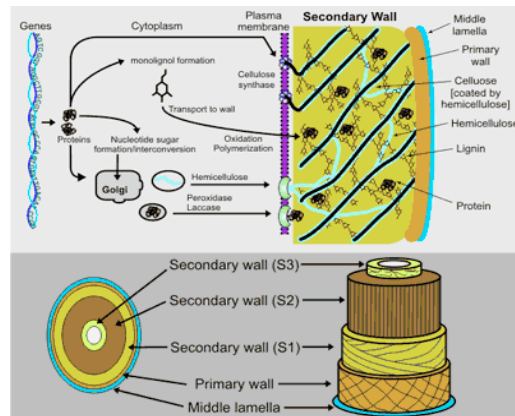
vacuole = lysosome/storage granule
 symplast = shared cytoplasm

plant cells were first described by Robert Hooke who noted that slices of cork looked like “cells” in a monastery under his early microscope

Plants form two types of cell walls: a primary cell walls that differ in composition and function:

Primary cell wall: highly flexible, found in growing plant cells, polysaccharide-rich (80-90% polysaccharide; 10-20% protein)

Secondary cell wall: thicker and stronger, contains majority of plant biomass, lignin, a macromolecule of cross-linked phenolic structures, is a major component of the secondary wall, polysaccharides are also present but in relatively lower proportion and altered content

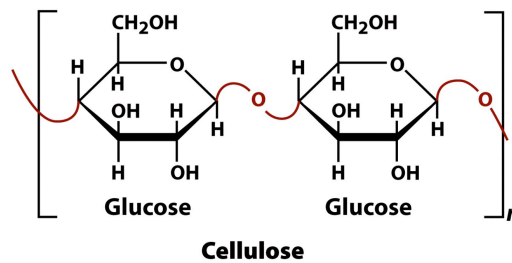


Primary cell wall is the extracellular matrix of plants

- contains many types of polysaccharides; main polysaccharides are:
 - cellulose
 - hemicellulose
 - pectins
- plant cells also contain several structural glycoproteins that are rich in hydroxyprolines and heavily modified by serine and threonine-linked oligosaccharides (*O-linked*)
 - extensins
 - proline/hydroxyproline-rich glycoproteins
 - arabinogalactans

Cellulose: structure and biosynthesis

- cellulose is a long, linear polysaccharide consisting of β 1,4 linked glucose residues; the most abundant biopolymer in nature
- forms a crystalline microfibril via extensive hydrogen bonding between individual cellulose polysaccharides
- biosynthesis of cellulose occurs at the plasma membrane using cytoplasmic UDP-glucose generated from sucrose
- cellulose provides tensile strength to plant cell matrix (similar to role of collagen fibers in animal cells)



Cellulose biosynthesis involves nearly 40 different CESA proteins at the plasma membrane and is functionally tied to microtubules

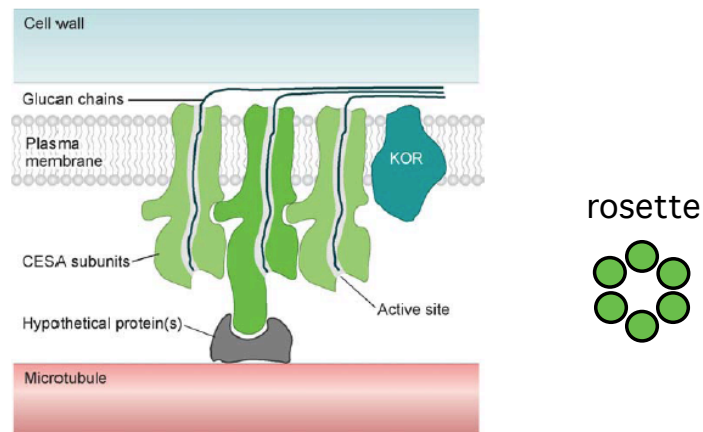


Figure 1

Schematic model of cellulose synthesis. Cellulose synthesis takes place in the plasma membrane. The plasma membrane is tightly appressed to the cell wall so that most of the cellulose synthase is in or below the plane of the membrane, which minimizes friction as the enzyme moves through the plasma membrane in response to elongation of the growing glucan chains by addition of glucan moieties from cytoplasmic UDP-glucose. The cellulose synthase complex is thought to contain as many as 36 CESA proteins, only a subset of which are illustrated. That three types of CESA proteins are required to form a functional complex suggested that different types of CESA proteins perform specific functions, such as interacting with the cortical microtubules.

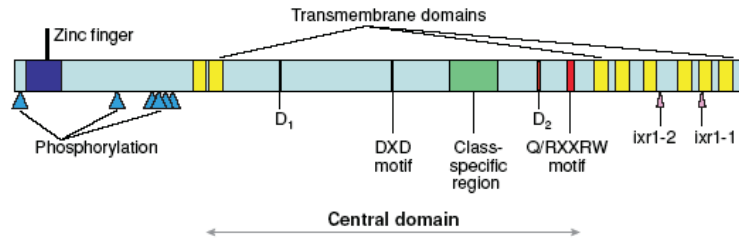


Figure 2

Illustration of the structure of CESA3, a typical CESA protein. Two motifs that have been implicated in activity of related glycosyltransferases are shown as well as the locations of several aspartate residues (D₁ and D₂) that are thought to participate in the enzyme's catalytic activity. The locations of two point mutations that confer resistance to the herbicide isoxaben are shown, as are the sites where the protein has been found to be phosphorylated.

Annu. Rev. Cell Dev. Biol. 2006. 22:53-78

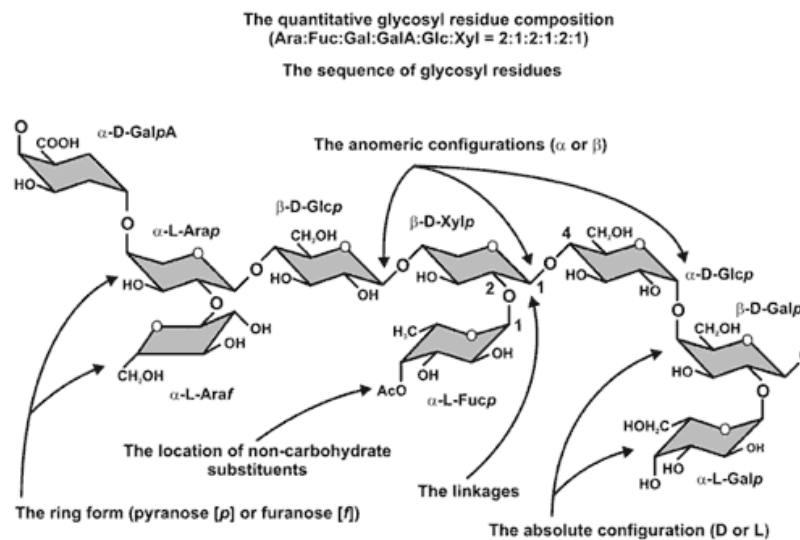
Hemicellulose: structure and biosynthesis

- hemicellulose consists of β 1,4 linked glucose residues (like cellulose) that are substituted with other sugars; xyloglucan is the predominant hemicellulose - others include glucuronoxylan, arabinoxylan, glucomannan, and galactomannan
- coat and cross-link cellulose fibers in the primary cell wall
- as cells increase in volume, H-bonds that link cellulose and hemicellulose loosen, allowing the internal osmotic pressure of the cell to push apart the cellulose microfibrils; this process is critical for plant cell growth and is helped by enzymatic cleavage of xyloglucan chains

Pectins: structure and biosynthesis

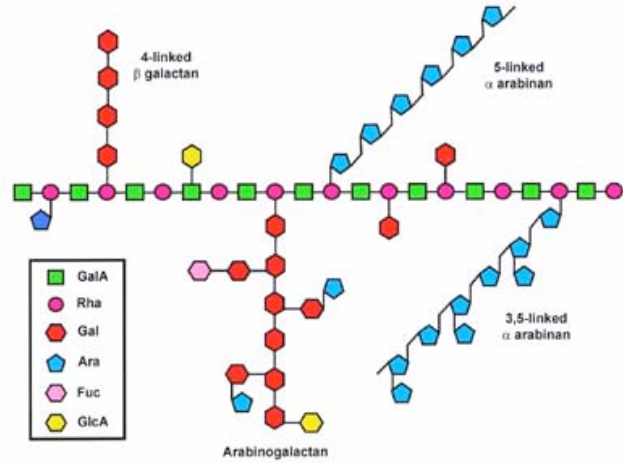
- pectins are complex acidic polysaccharides that contain 1,4-linked α -D-galactosyluronic acid residues; they resemble glycosaminoglycan (GAG) chains
- pectins that have been characterized include homogalacturonan (65% of plant pectins), substituted galacturonans like apiogalacturonan and xylogalacturonan and rhamnogalacturonan I and II
- embedded within the cellulose/hemicellulose network, pectins provide hydration and additional strength to the primary wall
- pectins can be modified by methyl esterases; extent of methylation can determine porosity and stiffness of cell wall

Determination of pectic polysaccharide structure requires the full range of available analytical techniques



Rhamnogalacturonan I (RG-I): structure and biosynthesis

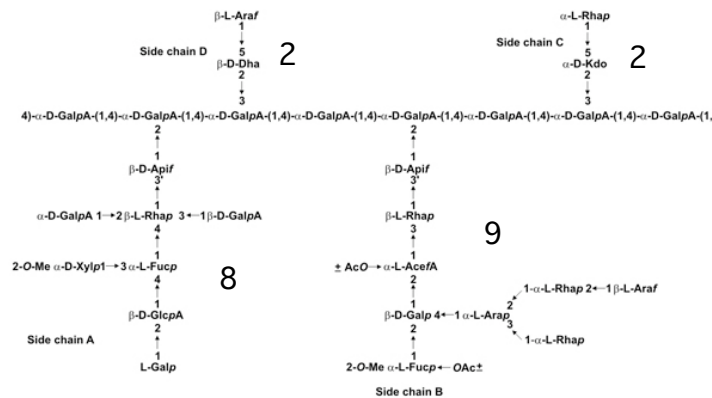
RG-I consists of a 4- α -D-GalpA-(1,2)- α -L-Rhap backbone



GalA residues are not usually substituted in RG-I

Rhamnogalacturonan II (RG-II): structure and biosynthesis

RG-II consists of a 1,4- α -D-GalpA backbone



- RG-II is substituted with four highly complex and conserved side chains that contain 12 different sugars in more than 20 different linkages

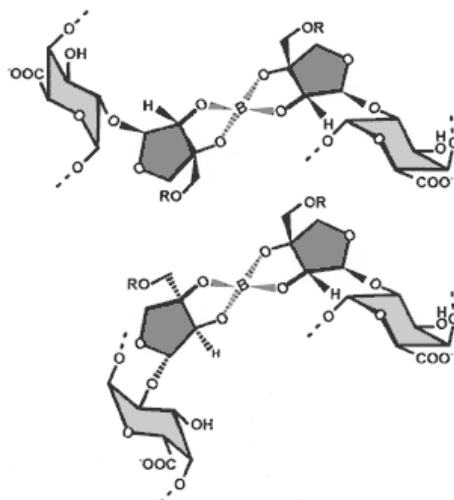
RG-II is a major polysaccharide component of red wine

- 1 liter of red wine may contain between 100 and 150mg of RG-II (white wine typically contains 20 - 30 mg of RG-II per liter)
- differences result from the different processing used to make these wines
- white wines are made by fermenting grape juice which contain little of the cell wall but red wines are made by fermenting grape berry pulp
- the cell wall accounts for a large portion of the pulp and the RG-II is solubilized during the fermentation process
- RG-II is extremely resistant to known microbial polysaccharide degrading enzymes and thus is not utilized as a carbon source during fermentation



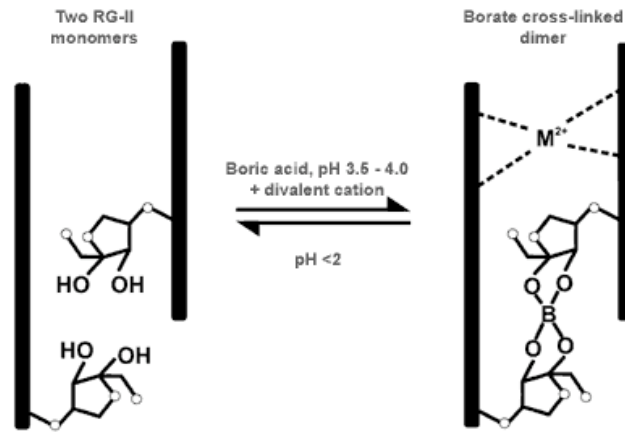
Why do higher plants invest so much energy and effort in producing such a complicated structure in their wall?

- first clue came from observations that RG-II exists as boron-cross-linked dimer (between two apiose residues on the side chain A of an RG-II monomer)



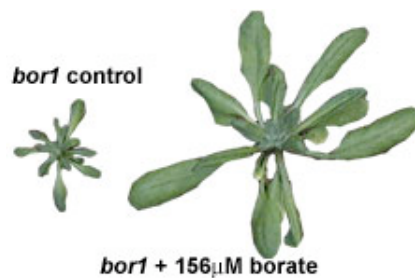
two stereoisomers of borate-linked RG-IIs

Formation of the borate ester cross link



Changes in boron content affect plant growth

Bor1 mutant: mutation in a boron transporter



- WT and bor1 RG-II have similar glycosyl residue compositions but bor1 has much lower levels of cross-linked monomers
- boron (and other metals) are important components of plant cell walls and play a role in cross-linking pectin
- does this prove that RG-II is also important for cell growth?

Does the absence of RG-II dimers cause growth defects?

- to address this question, different mutants in Arabidopsis were studied including the pleiotropic mutant, mur1
- mur1 mutant identified by screening hydrolysates of chemically modified plants; characterized by fragile cell walls and dwarfed size
- found to be deficient in GDP-mannose-4,6-dehydrase (enzyme responsible for GDP-fucose synthesis)

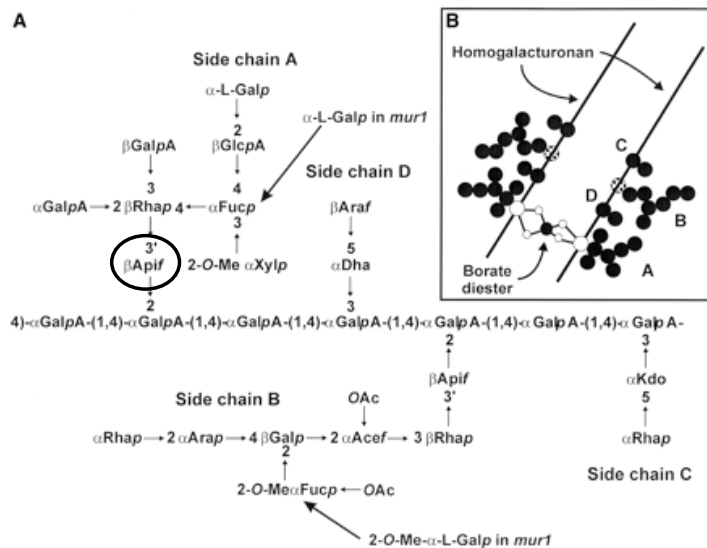
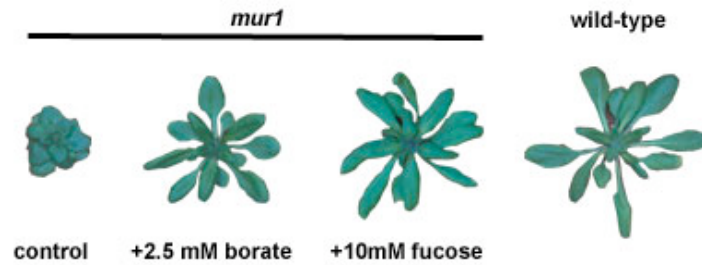
Problem: fucose is not only found in RG-II (xyloglucans and glycan side chains of many proteins contain fucose)

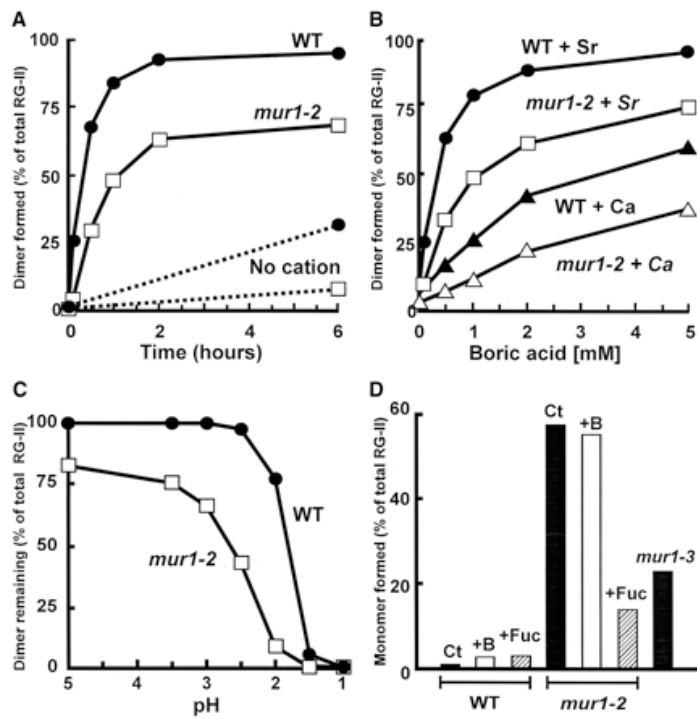
Which altered polysaccharides are responsible for the growth defect in the mur1 mutant?

- another mutant cgl1 entirely lacks fucose in its glycoproteins but surprisingly does not show any phenotypes
- mur2 mutants synthesize xyloglucans with only 2% of normal fucose But also have no growth defect
- effects on RG-II in mur1?

Arabidopsis mutants with altered RG-II structure (cont.)

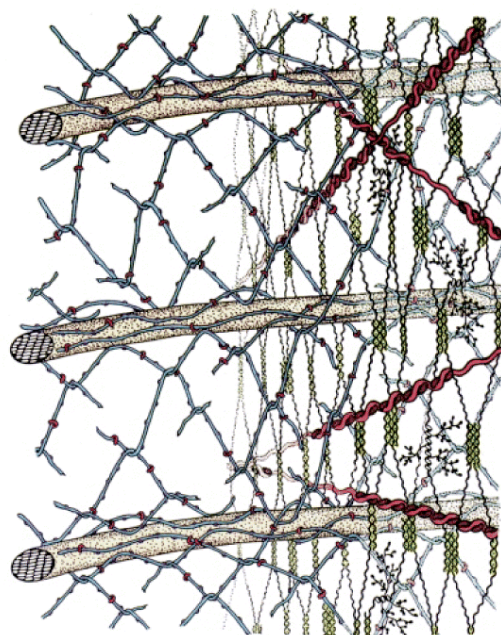
- L-galactose is substituted in place of fucose; this leads to reduced amounts of RG-II dimer, less stable dimers and a slower rate of dimer formation
- mutant can be partially rescued by watering plants in fucose or borate
- provide evidence for crucial role of RG-II in plant growth and the necessity of boron (and other metals) in plant biology





Science 26 October 2001:
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Model of Primary Cell Wall



cellulose microfiber



Xyloglucan



PGA junction one

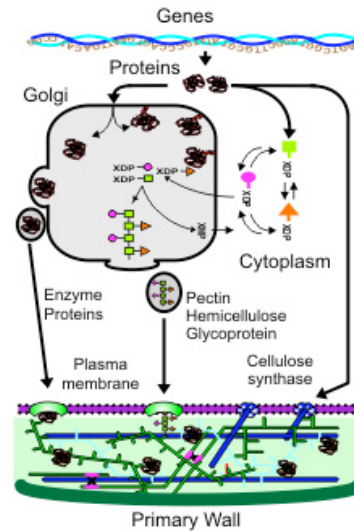
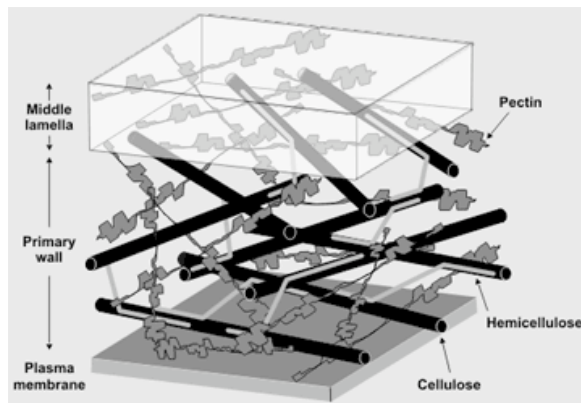


RG I with arabinogalactan side-chains



Extensin

Many aspects of plant cell wall biosynthesis and organization are not understood



Some of the functions of the primary wall:

- * structural and mechanical support
- * maintain and determine cell shape
- * resist internal turgor pressure of cell
- * control rate and direction of growth
- * ultimately responsible for plant architecture and form
- * regulate diffusion of material through the apoplast
- * carbohydrate storage - walls of seeds may be metabolized
- * protect against pathogens, dehydration, and environmental factors
- * source of biologically active signaling molecules
- * cell-cell interactions

Oligosaccharides serve as signals to regulate plant development, plant defense and its interactions with microorganisms

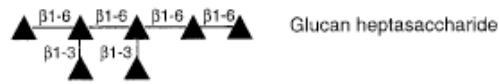
roles in development

- xyloglucan fragments from plant cell walls act as signals to trigger the expansion of cell walls
- expansion of cell walls must occur in a highly regulated fashion due to the high turgor pressure of the cell
- arabinogalactans are found abundantly in conditioned plant cell culture media and have recently been shown to have an important role in cell fate determination and somatic embryogenesis

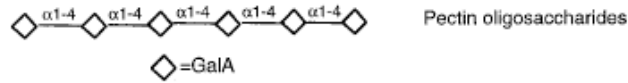
Numerous defense mechanisms have evolved in plants to prevent infection by pathogenic fungi

- fungi facilitate their colonization of plant tissue by producing glycanases (e.g. galacturonases, xylanases and glucanases) that fragment plant cell wall polysaccharides
- oligosaccharides generated by these glycanases provide the fungus with a carbon source but also elicit defense responses
- plants produce proteins that inhibit fungal glycanases and thereby increase the life time of the biologically active oligosaccharides
- fragmentation of the fungal cell wall by plant-derived endo 1,3 β -glucanases also generates oligosaccharides that induce plant defense responses
- the fungus itself may produce glucanase inhibitor proteins to prevent the degradation of its own cell wall and limit its perception by the plant

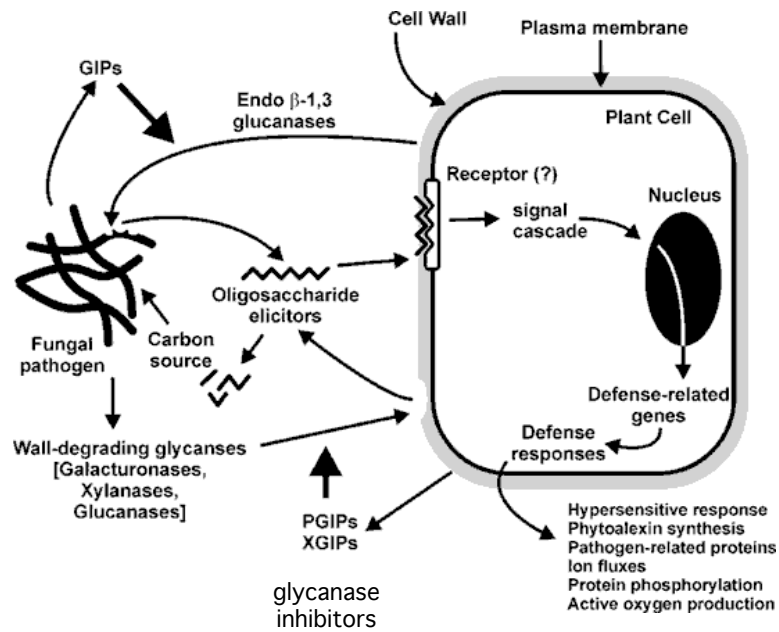
Elicitor oligosaccharides from the fungal cell wall



Elicitor oligosaccharides from the plant cell wall

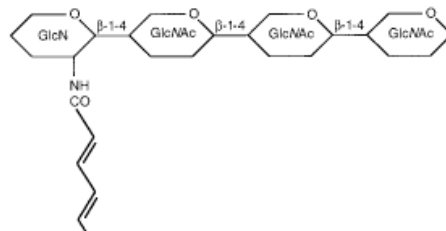


Plant Cell Wall and Pathogens: a delicate balance



Glycan signals are important for plant-bacteria symbiosis

- nitrogen-fixing *Rhizobium* bacteria and legumes form a mutual symbiosis
- to establish a symbiotic relationship with legumes, soil-dwelling rhizobia must invade the roots of the plants and induce the formation of nodules
- nodules are specialized organs in which the bacteria multiply and fix atmospheric nitrogen while deriving energy and carbon skeletons from the photosynthetic products supplied by the plant
- flavonoids released by plant roots induce bacterial nod genes that encode enzymes involved in the synthesis of Nod factors, lipochitooligosaccharides (LCOs)
- Nod factors stimulate deformation of root hairs and the formation of nodules



Nod factor

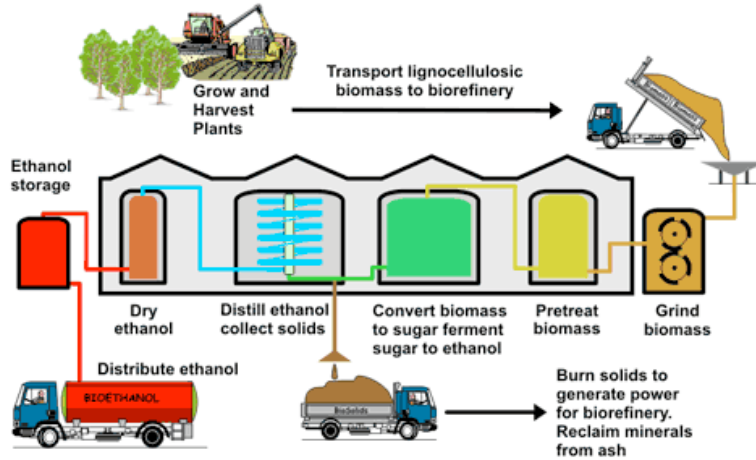
Biofuels

- lignocellulosic biomass (secondary wall) is derived from trees and grasses and from agricultural residues and is one of the most abundant organic materials in the world
- value of this biomass as a energy source was first exploited over 700,000 years ago when early humans mastered the use of fire
- humans have used biomass as building materials, as animal food, for clothing and for making paper
- biomass is renewable (unlike fossil fuels)

GOAL: to generate liquid fuels (i.e. ethanol) from biomass in a way that is efficient and cost-competitive with gasoline

Converting biomass to ethanol will involve many steps

Figure 2 The conversion of biomass to fuel involves numerous technologies



- most biofuel production is being done with corn and sugar cane but these are not ideal sources

corn is a high-maintenance crop that requires water, fertilizer, insecticide and it is FOOD!
sugar cane needs a tropical environment

Switchgrass and hardwoods like Poplar would be ideal sources of biomass

Advantages:

- renewable and low maintenance
- high-yield
- no impact on food crop production

Disadvantages:

- costly processing
- recalcitrance of biomass (difficulty of enzymatically releasing sugars from biomass)

Why is plant biomass so recalcitrant?

cell walls have evolved to be resistant to the action of hydrolytic enzymes to enable plants to survive in an environment where they are under constant threat from micro-organisms and animals

structural features of lignocellulosic biomass that make it difficult to enzymically convert the polysaccharides to sugars are:

- crystalline nature of cellulose
- presence of hemicelluloses that coat cellulose fibers
- presence of lignin
- presence of waxes and phenolic compounds
- multi-layered architecture of cell walls

How is UGA involved in the biofuel initiative?

- understand composition and structure of the cell wall
- modify the wall composition to overcome recalcitrance
- develop technologies to make production of biofuels cost-effective

