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Research Interests

Modification of Plant Cell Walls to Improve Biomass for Biofuel Production

Pectin Biosynthesis

Anti-Cancer Effects of Pectin

Function of Pectin in Plants

Plant Cell Walls as Sources of Biofuel and Bioproducts

The cell wall of plants is a polysaccharide and protein rich macromolecular structure that is essential for plant form and function and is the first entity encountered by plant symbionts and pathogens. The first wall laid down by plant cells, the primary wall, consists of three types of polysaccharides: cellulose, hemicelluloses and pectins³⁰. As some cells differentiate they form secondary cell walls which generally have reduced levels of pectin, increased amounts of cellulose and hemicellulose, and may or may not, be lignified. Plant cell walls have multiple functions in the plant including providing cell structure, participating in cell-cell adhesion, and involvement in plant growth, development and disease responses. Plant cell walls also serve numerous functions for humankind ranging from providing so-called lignocellulosic biomass used for biofuel production, to bioproducts such as paper and pulp products, timber, fine chemicals, adhesives, polymers for implantation devices, drug delivery, nutraceuticals, and pharmaceuticals.

A significant part of the research in the Mohnen lab is devoted to identifying and characterizing plant cell wall biosynthetic enzymes that impact the recalcitrance of biomass for biofuel production. Mohnen is Plant Cell Wall Biosynthesis Lead in the DOE-funded BioEnergy Science Center (BESC, <http://www.bioenergycenter.org/>), and thus coordinates those research efforts as well as those in her lab dedicated to reducing the recalcitrance of biomass (i.e. modifying plant walls to make them easier to deconstruct), and thereby increasing the effective use of plant biomass for biofuel production. Her research in this area is centered on increasing our basic understanding of the genes and proteins that carryout and regulate cell wall synthesis so as to enable the systems biology level understanding of wall production and modification required to modify plant walls to provide better biomass for biofuel production and bioproducts with enhanced functionality. A considerable past and present effort is dedicated to understanding how pectin, the plant's (and likely the world's) most complicated family of polysaccharides is synthesized. Since recent results suggest that pectins are intimately associated with other wall polymers, including hemicelluloses, this research effort spans all the plant wall polymers.

What is Pectin?

Pectin is a major polysaccharide component of all plant primary walls. Anabolic and catabolic changes in pectin metabolism are associated with fruit ripening, organ abscission, plant defense responses, growth, and development. Oligosaccharides released from pectin induce plant defense responses and regulate plant development^{1,9}. Pectin is also a food fiber in fruits and vegetables and an economically important nutritional and gelling agent in foods. Pectin has beneficial effects on human health² including the lowering of blood cholesterol and serum glucose levels³, and the potential inhibition of cancer growth and metastasis^{4-8,29}. The goals of research in the Mohnen laboratory are to understand the biosynthesis³¹ and the biological functions of pectin. The main strategy is to study pectin biosynthetic enzymes and their genes to elucidate how pectin is synthesized. The long term goal is to use that knowledge, and transgenic plants that produce modified pectin, to determine the function of pectin *in planta*, to study how pectin is synthesized, and to alter pectin structure so as to produce pectins with novel health and nutritional properties and produce plants with improved agricultural value.

Biosynthesis of Pectin.

Galacturonosyltransferases

The main research project in the Mohnen laboratory is based on the premise that the most direct way to elucidate the biological functions of pectin is to understand how pectin is biosynthesized^{9,10-11}. Current efforts center on how the pectic polysaccharide homogalacturonan (HG) is synthesized. HG is a linear polymer of α -1,4-linked galactosyluronic acid that makes up ~60% of the pectic polysaccharides (Fig. 1).

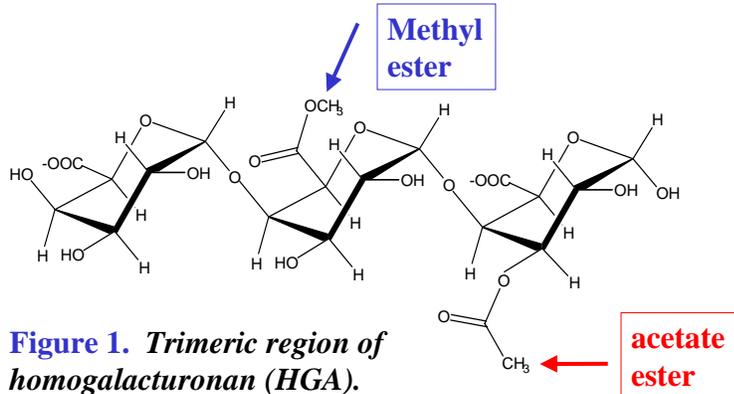
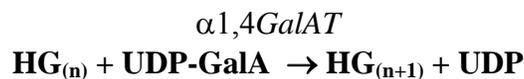


Figure 1. Trimeric region of homogalacturonan (HGA).

We previously identified a 4- α -galacturonosyltransferase (GalAT) that transfers UDP-GalA (and UDP-[¹⁴C]GalA)¹² onto HG or HG oligosaccharides (oligogalacturonides, OGAs) using membrane preparations from tobacco¹³, radish, pea¹⁴ and *Arabidopsis thaliana*²⁸. The product synthesized onto endogenous acceptors by membrane bound tobacco GalAT is ~105 kDa and contains up to 89% HG,

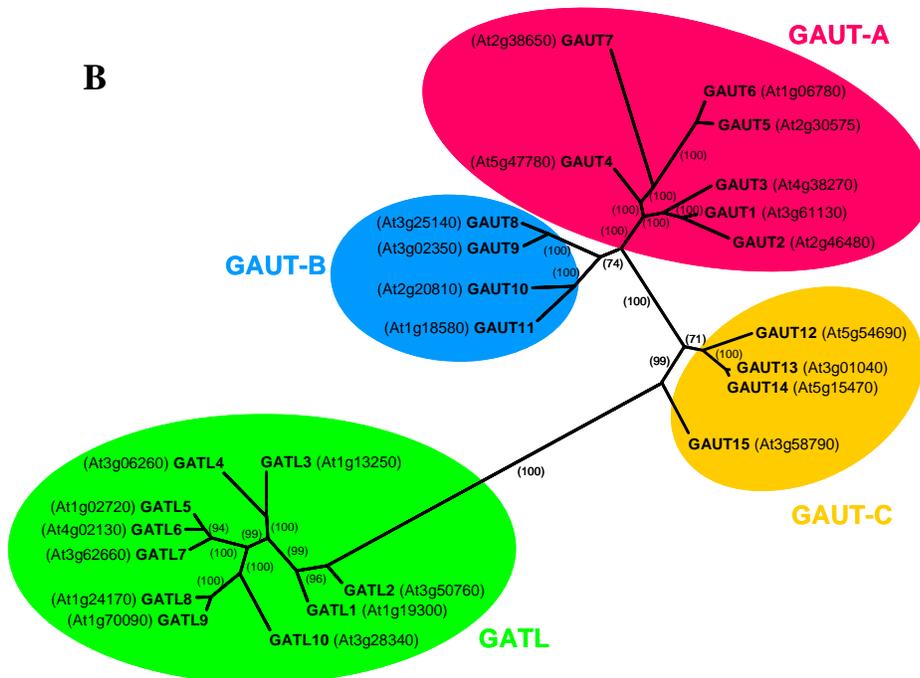
of which at least 50% is esterified¹³. Detergent-solubilized tobacco GalAT¹⁵ transfers GalA from UDP-GalA onto the non-reducing end¹⁶ of exogenous OGAs with degrees of polymerization (DP) of >9¹⁵.



We showed that in pea GalAT is localized to the Golgi with its catalytic site in the Golgi lumen¹⁴. These results are consistent with a type II membrane protein topology¹⁷ for GalAT. Under low UDP-GalA concentrations, solubilized GalAT adds predominantly one galacturonic acid onto the non-reducing end of exogenous OGA acceptors (e.g OGA of DP 15 to DP 16), while at

higher concentrations of UDP-GalA (~mM), OGAs can be extended by more than one GalA residues, suggesting that at least *in vitro* the enzyme acts non-processively²⁸.

We partially purified GalAT from *Arabidopsis*, trypsinized the partially purified protein, and determined the amino acid sequence of candidate GalATs by tandem mass spectrometry sequencing²⁸. Expression of one of the candidate GalAT genes (JS36) in human embryonic kidney cells gave low levels of GalAT activity in the recombinant cells²⁸, leading us to name this gene *GAUT1* (galacturonosyltransferase1). Blast analysis of *GAUT1* with the *Arabidopsis* genome identified 14 genes with $\geq 36\%$ sequence identity and ≥ 56 sequence similarity to *GAUT1*. We propose that these 15 genes comprise a GAUT gene family²⁸. An additional 10 *Arabidopsis* genes with slightly lower sequence similarity, but similar conserved domains, were also identified and named the GAUT-Like or GATL-like family²⁸. Phylogenetic analysis of the *Arabidopsis* GAUT1-related gene family revealed four clades (see below). Our current efforts center on proving the function of the GAUT genes and, with our collaborator Michael Hahn, the GATL genes, by studying gene mutants, and by identifying the specific enzyme activity, substrate specificity, and *in vivo* function of these 25 proposed pectin and wall biosynthetic genes. We propose that these genes encode multiple GalATs involved in the synthesis of the pectins HG, rhamnogalacturonan I (RG-I) and rhamnogalacturonan-II (RG-II) and other wall polymers³².



***Arabidopsis* Galacturonosyltransferase1-related (GAUT1-related) gene family consisting of 15 GAUT genes and 10 GAUT-Like (GATL) genes (Sterling et al., 2006).**

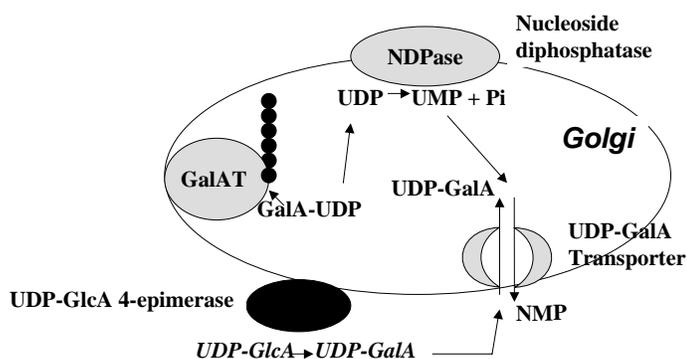
Pectin methyltransferases

The enzyme that methylates HG at the C6 carboxyl, HG-methyltransferase (HG-MT), contributes to HG function by modifying the charge on HG and thus, the ionic and structural properties of pectin, including its gelling properties. We previously localized tobacco HG-MT activity^{18,19} to the Golgi and showed that its catalytic site faces the Golgi lumen²⁰, suggesting that HG-MT and HG-GalAT are both localized in the same subcellular compartment.

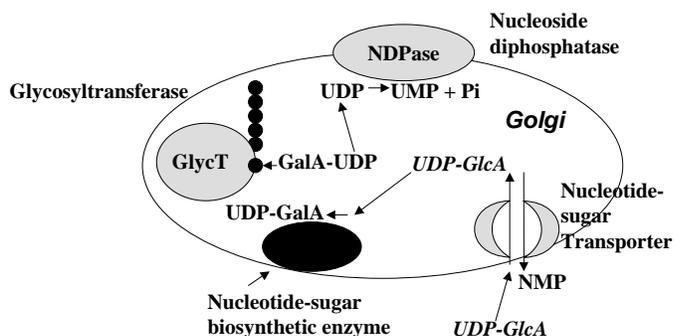
Synthesis of UDP-GalA

The location of pectin synthesis in the Golgi leads to the question of where the nucleotide-sugar substrates are synthesized and of how the substrates gain access to the enzyme. We have proposed that the UDP-GalA synthesizing enzyme, UDP-GlcA 4-epimerase, is located on the cytosolic side of the Golgi and that the UDP-GalA is transported into the Golgi lumen by a UDP-GalA:UMP antiporter¹⁴ (model A below). While this model is consistent with the topology of some nucleotide-sugar biosynthetic enzymes in animals and plants, there are also indications that some nucleotide biosynthesis enzymes, such as UDP-glucuronic acid decarboxylase, may actually reside in the Golgi (model B below)¹⁰. Thus, until the definitive subcellular location of UDP-GlcA 4-epimerase is confirmed experimentally, two models for the location of UDP-GlcA 4-epimerase, must be considered. Our preliminary data confirm that UDP-GlcA 4-epimerase co-fractionates with Golgi membranes (Adams and Mohnen), suggesting that the epimerase is membrane bound and not free in the cytosol. If UDP-GalA is synthesized on the cytosolic side of the Golgi, it is likely transported into the Golgi via a UDP-GalA:UMP antiporter in the Golgi membrane. Regardless of whether the UDP-GalA is synthesized on the cytosolic or luminal side of the Golgi, the UDP released upon transfer of the GalA from UDP-GalA onto HG would be hydrolyzed by a Golgi-localized nucleotide-5'-diphosphatase (NDPase) into UMP and inorganic phosphate. The nucleoside monophosphate would then presumably be transported out of the Golgi by the nucleotide-sugar:nucleoside monophosphate antiporter.

(A) Model showing UDP-GlcA 4-epimerase located on the cytosolic face of the Golgi



(B) Model showing UDP-GlcA 4-epimerase in the Golgi lumen



Biological activity of Pectin in Humans and Animals

A developing research area in the Mohnen lab is the investigation of the beneficial effects of pectin on human health. Pectin has multiple beneficial effects on human health² including the lowering of blood cholesterol and serum glucose levels³, and the potential inhibition of cancer growth and metastasis^{4,29}, and the inhibition of fibroblast growth factor-receptor interactions²¹. Some of these effects appear to occur via the induction of apoptosis and/or the interfering with ligand:receptor interactions. However, neither the specific pectin structure with these activities nor the precise molecular mechanisms of pectin's activities are known. In a collaborative project with Vijay Kumar²² (VA Medical Center, Augusta, Georgia), we are studying the effects of different pectins on cell apoptosis and on cancer metastasis²⁹. Prostate cancer is the most common malignancy and the second leading cause of death in American men. Specifically we are studying the effects of different pectins on apoptosis in several different human prostate cancer cell lines and identifying the specific structure(s) in pectin that induce apoptosis (programmed cell death) in human prostate cancer cells. The goal of these studies is to determine the molecular mechanism(s) by which pectin induces apoptosis in prostate cancer cells. A longer-term goal is to develop recommended diet changes and/or pectin-based nutraceutical or pharmaceutical strategies to combat the incidence and lethality of prostate cancer, and other types of cancer, and to promote human health.

Biological Activity of Pectic Oligosaccharides in Plants.

A long term research interest of the Mohnen lab is how the biologically active oligosaccharide fragments released from pectin - oligogalacturonides (OGAs) - regulate plant development^{23,24}. OGAs with a degree of polymerization (DP) >9 regulate *in vitro* morphogenesis and *de novo* meristem formation in tobacco thin cell-layer explants (TCLs)^{25,26,27}. OGAs inhibit root formation and/or induce *de novo* flower shoot formation in TCLs, with a half-maximum morphogenesis response at ~400 nM. TCLs cultured in the presence of OGAs also show enhanced polarity of tissue enlargement and organ formation at the basal end of the explant and correspondingly less at the apical end. Thus, OGAs regulate both the polarity of TCL morphogenesis and the type of meristems formed on the tissue explants.

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Acknowledgements

Dr. Mohnen gratefully acknowledges support for her research from the following funding agencies: the U.S. Department of Agriculture, the National Science Foundation, the Georgia Cancer Coalition, and the U.S. Department of Energy.