

## Mechanisms of Plant Cell Wall Polysaccharide Biosynthesis: A Two-Phase Model for the Non-Processive Biosynthesis of Homogalacturonan Polysaccharides by the GAUT1:GAUT7 Complex

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**Project Goals: The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.**

Hydrolysis and fermentation of plant cell wall polysaccharides produces fuel products from biomass feedstocks. Cell walls in mature plant tissues contain a heterogeneous mixture of cellulose, hemicellulose, and pectin as well as arabinogalactan protein-linked polysaccharides. Each of these cell wall components contributes to a highly-crosslinked matrix of glycan polymers that resists enzymatic and chemical depolymerization.

Pectic polysaccharides are deposited early during cellular development and are enriched in the adhesive middle lamella between adjacent cells and in the primary wall. Pectic crosslinking interactions control cell wall stiffness, porosity, cellular adhesion, and cell expansion. Recent efforts within CBI and the BioEnergy Science Center have shown that transgenic modification of the expression of pectin biosynthetic genes in woody and grass feedstocks (poplar and switchgrass) affect phenotypes related to growth and recalcitrance.

The pectic glycan, homogalacturonan (HG), is synthesized by the galacturonosyltransferase (GAUT) gene family. Two members of this family, GAUT1 and GAUT7, form a heteromeric enzyme complex in Arabidopsis. Here, we established a heterologous GAUT expression system in HEK293 cells and show that co-expression of recombinant GAUT1 with GAUT7 results in the production of a soluble GAUT1:GAUT7 complex that catalyzes elongation of HG products *in vitro*.

Study of GAUT1:GAUT7 activity *in vitro* revealed that the complex synthesizes high-molecular-weight polymeric HG (> 100 kDa). Unlike cellulose synthase, GAUT1:GAUT7 is a non-

processive enzyme, and tight control of the size of the HG products can be achieved by varying substrate concentrations during *in vitro* reactions. Unexpectedly, small changes to the degree of polymerization (DP) of acceptor oligosaccharides resulted in major differences in reaction rates and in the apparent mechanism of synthesis. GAUT1:GAUT7 displayed > 45-fold increased catalytic efficiency with DP11 acceptors relative to DP7 acceptors, and reactions primed with short-chain acceptors resulted in a bimodal product distribution of glycan products that has previously been reported as evidence for a processive model of GT elongation.

As an alternative to the processive glycosyltransfer model, we propose a two-phase, non-processive elongation model. A slow phase, which includes the *de novo* initiation of HG and elongation of short-chain acceptors, is distinguished from a phase of rapid elongation of intermediate and long-chain acceptors. Upon reaching a critical chain length of DP11, GAUT1:GAUT7 elongates HG to high molecular weight products.

Non-processive GTs also synthesize several other classes of extended plant cell wall polysaccharide chains, particularly xylans, arabinogalactans, and the RG-I backbone. Key enzymes in these biosynthetic pathways have been identified, but the mechanisms of high MW polysaccharide elongation remain to be studied. The preference for longer acceptor substrates has been observed in other GT families, but the mechanistic significance of these findings has not been previously appreciated. We propose that the two-phase, non-processive mechanism observed during the study of GAUT1:GAUT7 activity may apply to the biosynthesis of other polysaccharides. Establishment of HEK293 cells as a high-yield heterologous expression system for GTs should enable the study of enzymatic mechanisms of plant cell wall synthesis, functional redundancy among homologous enzymes, polysaccharide elongation length control, and the role of GT complexes in the synthesis of high MW polymers. Mechanistic understanding of the synthesis of HG, with its critical roles in plant growth, biomass yield, and cell wall architecture will support the development of high-yielding, robust bioenergy feedstocks.

## References

1. Amos, R.A., Pattathil, S., Yang, J.-Y., Atmodjo, M.A., Urbanowicz, B.R., Moremen, K.W., Mohnen, D., A two-phase model for the non-processive biosynthesis of homogalacturonan polysaccharides by the GAUT1:GAUT7 complex. (2018) *J. Biol. Chem.* 293:19047-19063.

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