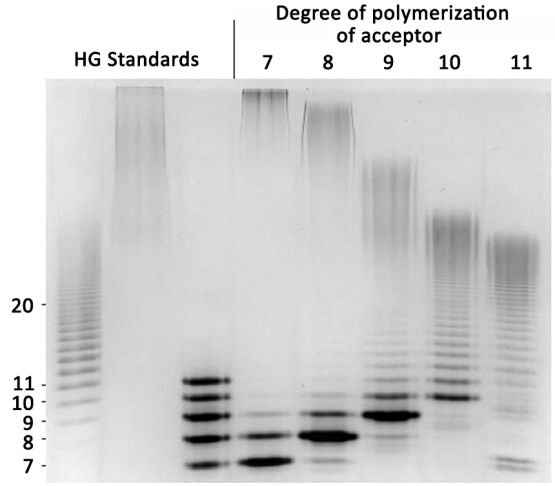
A two-phase model for the non-processive biosynthesis of pectic homogalacturonan polysaccharides by the GAUT1:GAUT7 complex

Background

- Understanding the mechanism of pectin biosynthesis is necessary to explain why reductions in specific pectin biosynthetic genes lead to increased plant growth and reduced recalcitrance to deconstruction.
- The most abundant pectic glycan, homogalacturonan (HG), is synthesized by members of the *Galacturonosyltransferase* (*GAUT*) gene family. GAUT1 and GAUT7 function as a disulfide-linked protein complex that catalyzes the synthesis of HG oligosaccharides.

Approach

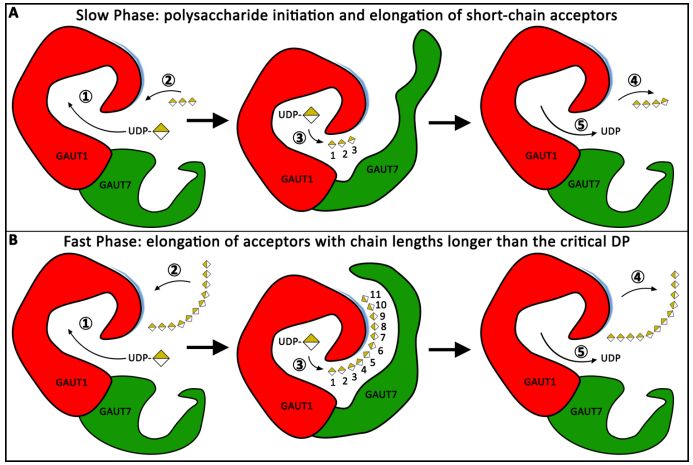
- The GAUT1:GAUT7 complex was purified following heterologous co-expression of GAUT1 and GAUT7 in eukaryotic HEK293 cells.
- The mechanism of HG synthesis was determined using multiple enzyme assays and product analysis techniques to measure the rates of HG elongation and the product distributions catalyzed by GAUT1:GAUT7 in the presence of HG acceptors of varying chain lengths, respectively.



Elongation of short-chain (DP7) HG acceptors by GAUT1:GAUT7 results in an unexpected product distribution including long-chain polysaccharides.

Outcomes

- GAUT1:GAUT7 elongates HG acceptors of all sizes and can initiate HG synthesis *de novo*. HG oligosaccharide acceptors with a degree of polymerization (DP) of DP11 are elongated with 45-fold greater efficiency than shorter-chain acceptors (DP7). The low catalytic efficiency of short-chain acceptors results in an unexpected bimodal product distribution, in which high molecular weight polysaccharide products are observed.
- Elongation of HG chains by GAUT1:GAUT7 is non-processive. A two-phase distributive elongation model was proposed in which the rapid elongation of HG acceptors to high molecular weight products occurs only after the product reaches a critical chain length of DP11.



A two-phase non-processive HG biosynthesis model is proposed in which short-chain acceptors bind inefficiently and are elongated slowly. Rapid elongation to long-chain polymers occurs after the HG acceptor reaches DP11.

Significance

- This study is the first *in vitro* demonstration of the non-processive mechanism by which GAUT1:GAUT7 synthesizes long HG polysaccharides. Other cell wall matrix polysaccharides, including xylan, mannans, and xyloglucan, are also long polymers and may be synthesized through similar mechanisms.

Amos et al., "A two-phase model for the non-processive biosynthesis of homogalacturonan polysaccharides by the GAUT1:GAUT7 complex", *J. Biol. Chem.* 2018 in press. doi:10.1074/jbc.RA118.004463

THE CENTER FOR BIOENERGY INNOVATION