135. GONST1 and 2 are GIPC Specific GDP-Mannose Transporters

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Project Goals:
We want to understand how glycosylation is controlled in plants, and to use this knowledge to engineer plants with improved glycan profiles for bioenergy, industry and human health. Our focus is the mannan biosynthetic pathway. Mannan is a C6 polysaccharide, the most abundant polysaccharide after cellulose in softwoods such as pine. However, in most crop plants, as well as the model plant Arabidopsis, it is a relatively minor polymer. The glycosyltransferases responsible for its synthesis have been identified, but attempts to engineer increased amounts have had limited success.

We are identifying and characterizing other components of the mannan synthetic pathway. This allows us to use JBEI-developed plant synthetic biology tools to re-engineer the cell wall. Our initial goal is to develop plants which have an increased accessible hexose content in biomass. This will reduce downstream processing costs for bioenergy, due to microbial preferences for hexoses over pentoses during fermentation.

In this project, the aim was to identify Golgi-localized nucleotide sugar transporters which provide substrates for mannan biosynthesis. Characterization of these transporters will allow us to boost substrate availability for the glucomannan synthases.

Abstract:
Nucleotide sugar transporters (NSTs) translocate the substrates for cell wall biosynthesis and other glycosylation processes into the Golgi from the cytosol. NSTs responsible for the cell wall precursors GDP-Fuc, GDP-Glc, GDP-Man and GDP-L-Gal have yet to be identified. The Arabidopsis thaliana protein GOLGI-LOCALIZED NUCLEOTIDE SUGAR TRANSPORTER 1 (GONST1) and its close homolog GONST2 have been both previously identified as GDP-Man transporters. In vitro, GONST1 and GONST2 can both rescue a yeast GDP-Man transporter mutant1. In a liposome assay, GONST1 can transport all four plant Golgi GDP sugars (GDP-Man, GDP-Glc, GDP-Fuc, and GDP-L-Gal)2. GONST1 and 2 were therefore predicted to supply substrates for glucomannan biosynthesis in vivo. However, our analysis showed that the plants were unaffected in glucomannan biosynthesis, or any other cell wall polymer.

Sugars also decorate glycosphingolipids, and this process is also believed to occur in the Golgi. Glycosylinositolphosphoceramides (GIPCs) are the most abundant sphingolipid in the plant plasma membrane. We demonstrated that Arabidopsis GIPCs contain mannose sugar decorations that are dramatically decreased in gonst1. gonst2 shows a small reduction in GIPC mannosylation and gonst1 gonst2 plants completely lack mannosylated GIPCs, which indicated GONST1 and GONST2 have redundant function in GIPC mannosylation. We conclude that GONST1 and GONST2 specifically transport GDP-Man as substrates for GIPC biosynthesis. gonst1 displays a constitutive hypersensitive response. gonst2 has normal growth but gonst1 gonst2 is severely stunted with leaf lesions and an early
senescence phenotype. The characterization of these mutants demonstrates that the loss of mannose from GIPCs can have a severe effect on plant development and immunity.

The link between GIPC glycosylation and immunity was previously unrecognized. The identification of new members of the biosynthetic pathway will contribute to the understanding of GIPC function.

References
2. Mortimer et al. 2013, Plant Cell

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