The glycosylation of plant sphingolipids affects cellulose crystallization, plant defense signaling and nitrogen fixation ability

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Project goal: The aim of this project was to characterize plant Golgi glycosyltransferases (GTs), as the majority of these proteins are involved in cell wall biosynthesis. The understanding of the plant cell wall is critical to understanding feedstock recalcitrance and enabling the predictive engineering of biomass. However, the GT we characterized turned out to have a role in sphingolipid glycosylation, and in turn to affect cellulose crystallinity. This project has enabled us to discover an unexpected role for membrane lipids in cellulose biosynthesis.

The plasma membrane acts as an interface between the plant cell wall and the inside of the cell. It is the site of cellulose biosynthesis and a range of signal transduction complexes. Glycosylinositol phosphorylceramides (GIPCs) are a class of glycosylated sphingolipids found only in plants, fungi and protozoa. They are extremely abundant in the plant plasma membrane, estimated to form ~25% of the total lipids. Little is known about the glycosylated headgroup, but two recent papers have indicated a key role in plant signaling and defense, and shown that it is synthesized in the Golgi. The Golgi apparatus is also the site of cell wall polysaccharide biosynthesis, with the exception of cellulose and callose, but it is not clear how pools of substrates are directed to different glycans. Here, we identify a Golgi-localized *Arabidopsis thaliana* glycosyltransferase, GIPC MANNOSYL TRANSFERASE1 (AtGMT1), and demonstrate that it is a GIPC-specific mannosyl-transferase.

Previously, we identified a GIPC-specific GDP-mannose transporter mutant *gonst1*, which had dwarfed stature and a constitutive defense response[1]. We obtained three alleles of *gmt1*, which displayed a very similar phenotype to *gonst1*. Sphingolipid analysis revealed that *gmt1* almost completely lacked hexosylated GIPCs. *gmt1* has elevated production of salicylic acid and H_2O_2, suggesting that GIPC sugar decoration plays a role in plant defense signaling. Unexpectedly, we also found a reduction in crystalline cellulose content in the *gmt1* plants, suggesting significant misglycosylation can impact cellulose crystallinity. We are now investigating the role of these proteins in other species, and have found that a homolog of AtGMT1 in *Medicago truncatula*

may have a role in nodulation and nitrogen fixation. Our future work will focus on understanding how GIPC glycosylation is able to control these varied phenotypes.

References

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