225. GUX and IPUT Members of Arabidopsis Glycosyltransferase Family 8 are Glucuronosyltransferases Involved in Cell Wall and Glycosphingolipid Synthesis

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Project Goals: The Cell Wall Biosynthesis group at the Joint BioEnergy Institute is focused on developing a better understanding of plant cell wall biosynthesis and the role of cell wall polymers in plant growth and development. One of our specific aims is to identify and study the structures and functions of glycosyltransferases and other enzymes involved in cell wall synthesis. Our eventual goal is to use this knowledge to modify the composition of plant cell walls for better plant growth and biofuel yields.

Plant cell walls are a major sink for fixed carbon and a potentially important source of feedstocks for renewable energy. Cellulose microfibrils, the main load-bearing component of cell walls, are cross-linked by highly branched polysaccharides called hemicelluloses. In biofuel crops such as switchgrass, Miscanthus, and poplar, the major hemicellulosic polymer is xylan (Figure 1).

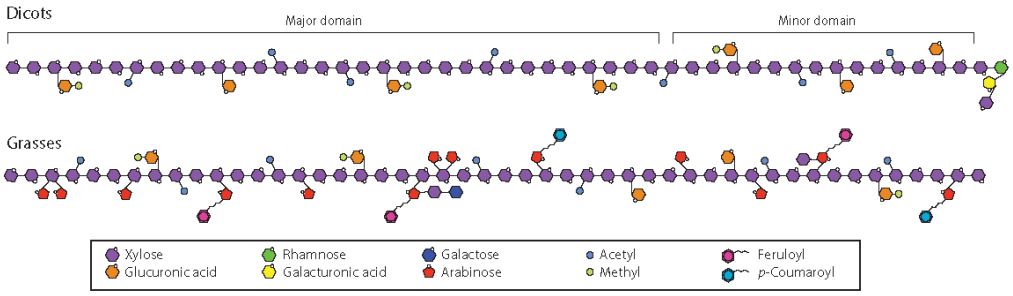

Figure 1. Structures of xylan in dicots and grasses. From (1)

We have previously shown that three members of Arabidopsis Glycosyltransferase Family 8, named Glucuronic Acid Substitution of Xylan (GUX)1, GUX2, and GUX4, are glucuronosyltransferases responsible for synthesizing glucuronic acid side chains on xylan.[2] Here, we characterized the substitution patterns with which GUX proteins transfer glucuronic acid to xylan. This analysis showed that *in vitro*, GUX1 transfers glucuronic acid to alternating xylose residues while GUX2 and GUX4 do not distinguish between odd or evenly spaced residues. These preferences closely match proposed roles for GUX1 and GUX2 *in planta*, where they are thought to synthesize distinct glucuronoxylan domains with different substitution patterns.[3] These results indicate that the GUX proteins are capable of recognizing subtle differences in xylan backbone structure, leading to related but separate enzymatic functions. Further

characterization of these proteins will help us understand how GUX proteins alter specific domains of the xylan backbone and how these alterations affect xylan functions in plants. In addition, we characterized a closely related protein that we have named Inositol Phosphorylceramide Glucuronosyltransferase 1 (IPUT1). Surprisingly, we found that this protein does not synthesize cell wall polymers but instead glycosylates an abundant class of sphingolipids called glycosyl inositol phosphorylceramides (GIPCs). We used a synthetic biology approach to reconstruct the plant GIPC synthesis pathway in yeast, allowing us to demonstrate the function of IPUT1. The identification of this new enzymatic activity sheds light on the synthesis of an important group of plant lipids. In addition, it will help us understand how the GUX/IPUT clade of proteins is able to recognize and differentiate between specific acceptor substrates such as glycolipids and xylan polymers.

References

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This work was supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Lab and the U.S. Department of Energy.