Hemicellulose Biosynthesis is Becoming Crystal Clear

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are testing large numbers of natural variants and generating specific and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways. BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

The synthesis and assembly of polysaccharides to form the plant cell wall is a complex process. Glycosyltransferases (GT) are the ubiquitous enzymes responsible for creating the diverse and complex array of oligosaccharides and glycopolymers found in nature. Although several of the key glycosyltransferases involved in plant polysaccharide synthesis have been identified, none have been crystallized to date. The structure and mechanism of cellulose synthase (CesA), perhaps the most widely studied glycosyltransferase involved in cell wall biogenesis, is becoming clearer as a result of crystal diffraction analysis of bacterial orthologs, but the plant CesA and other glycosyltransferases involved in the biosynthesis of plant cell walls have resisted crystallographic analysis.

Recently, we have developed methods to express highly active forms of several glycosyltransferases involved in the biosynthesis of the hemicelluloses xylan and xyloglucan, which are abundant components of plant biomass. We are currently focusing on understanding the mechanisms by which these enzymes catalyze the highly substrate-specific and regio-specific transfer of sugar residues to the growing polysaccharides. The first enzyme we have studied in depth is *At*FUT1, a glycosyltransferase in the GT37 family that catalyzes the regio-specific transfer of fucosyl residues to the sidechains of xyloglucan. We have crystallized this enzyme alone, as a complex with its donor substrate (GDP-fucose), and with a xyloglucan octasaccharide acceptor substrate (XXLG). This resulted in the first crystal structures for any plant glycosyltransferase involved in cell wall polysaccharide biosynthesis and the first crystal structure of a GT37 enzyme. We have also used site-directed mutagenesis to modify specific amino acids in the *At*FUT1 sequence that are critical for its catalytic activity, biochemically characterized these mutants, and interpreted the results in the context of the crystal structure. We are currently using the combined structural and biochemical information, along with molecular dynamics simulations, to gain insight into the

key mechanisms by which *At*FUT1 and other members of the GT37 enzyme family fulfill their biochemical functions. By providing the archetypal crystal structure for GT37 enzymes, these studies also facilitate the structural modeling of other members of this family.

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