

Structural Comparison of Plant Glycosyltransferases

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Project Goals:

The project aims to develop a fundamental understanding of the function of glycosyltransferases involved in cell wall biosynthesis. A fundamental understanding of the structure-function relationship of this class of enzymes will enable the development of tools for engineering of plants with modified cell walls and improved properties for processing to biofuels.

Plant cell walls are composed primarily of structural polysaccharides including cellulose, hemicelluloses and pectins. These complex polysaccharides are synthesized by glycosyltransferases (GTs) – a family of enzymes that transfer a sugar residue from an activated donor substrate, usually a nucleotide sugar, to an acceptor such as a growing oligosaccharide. GTs generally have narrow substrate specificity, and are highly stereo- and region-specific. The GTs involved in hemicellulose and pectin biosynthesis are membrane proteins located in the Golgi apparatus. Plants have a large number of such proteins, e.g. more than 300 in Arabidopsis, most of which have an unknown function.

Predicting the function of a putative GT based on sequence similarities is problematic and many closely related sequences have different catalytic activities. GTs appear to share a limited number of protein fold types and only two structural folds, GT-A and GT-B, have been identified to date. However, for many GT families – particularly those specific to plants – no structure has been solved, so it is not clear if other fold types exist.

Crystallization and structural comparison of the catalytic domains could help to find conserved motifs involved in substrate recognition of the many GTs in plants. We have selected a diverse group of rice and Arabidopsis GTs potentially involved in cell wall biosynthesis. Using bioinformatics and modeling, secondary structures were predicted for optimal construction of truncation variants suitable for crystallization. The protein variants were expressed in *E. coli* with fusion protein tags for improvement of solubility and expression and for purification. More than 70 proteins were expressed at high levels as soluble proteins and some were selected for initial crystallization efforts. Crystals have been obtained and results of the analysis will be reported.

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