THE ENZYMATIC ORIGIN OF PENTAFURANOSES IN BACTERIAL POLYSACCHARIDES

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The remarkable diversity of carbohydrate backbone structures in bacterial polysaccharides results from the activities of glycosyltransferase enzymes with precise donor and linkage specificities. The most prevalent glycosyltransferases are classical Leloir enzymes that use sugar nucleotide donors to assemble lipid-linked oligo- and polysaccharides. These enzymes can often be identified (and activities sometimes assigned) by sequence and predicted protein structure data. However, examination of genetic loci encoding enzymes required for the biosynthesis of ribofuranose (Rib*f*)-containing glycans identified no obvious candidate glycosyltransferase for incorporating this sugar, suggesting it may require an enzyme with different type of donor or mechanism. Using two lipopolysaccharide (LPS) O antigen polysaccharides from *Klebsiella pneumoniae* as prototypes, we discovered cytoplasmic dual-catalytic domain enzymes that incorporate β-linked Rib*f* into glycan backbones [1]. These enzymes use phosphoribosyl-5′-phospho-D ribosyl-α-1-phosphate (PRPP) as the donor substrate in a 2-step Rib*f*-transferase reaction. In the first step, a *glycosyl*-phosphoribosyltransferase (*g*PRT) module transfers Rib*f*-5-phosphate to a specific acceptor. The reaction sequence is completed by removal of the 5′-phosphate by a promiscuous phosphoribosylphosphate (PRP) module. We solved the crystal structure of a homolog from a thermophilic bacterium and found the *g*PRT domain represents a new glycosyltransferase fold that diverges substantially from previously known PRTs, while the PRP module is structurally related to phosphatases belonging the haloacid dehalogenase family. This characterization allowed assignment of *g*PRT-PRP pairs involved in biosynthesis of diverse β-Rib*f*-containing polysaccharides, from a wide range of bacteria with different lifestyles. However, this mechanism did not explain the presence of α-linked Rib*f* in some glycans. Investigation of prototypes from *Citrobacter youngae* revealed a more complex extracytoplasmic mechanism for incorporating α-Rib*f* or α-Xyl*f* [2]. In these systems, PRPP is used by a polyprenylphosphoribose synthase enzyme to generate polyprenol phosphate-linked Rib*f*, which can be further epimerized to produce the Xyl*f* derivative at the cytosol-membrane interface. These lipid-linked sugars are then flipped across the cytoplasmic membrane by a dedicated exporter, where they provide the direct glycosylation donor substrate for an integral membrane GT-C-fold glycosyltransferase. The overall process resembles glycosylation pathways that incorporate arabinofuranose and hexose residues into glycostructures from mycobacteria and some Gram-negative bacteria, respectively. In summary, investigation of Rib*f* incorporation has provided fundamental new insight into the biochemical foundation of microbial glycobiology and antigenic diversification, and provides a structural and bioinformatic framework to discover new enzyme representatives from diverse bacterial species.

References:

1. S.D. Kelly, D.M. Williams, J.T Nothof, T. Kim, T.L. Lowary, M.S. Kimber and C. Whitfield. *Nat. Chem. Biol.* **2022**, *18*, 530-537.
2. S.D. Kelly, N.H. Dong, J.T Nothof, T.L. Lowary and C. Whitfield. *Proc. Nat. Acad. Sci. USA.* **2024**, *121*, e2402554121.