Department of Molecular and Cellular Biology

Graduate Seminar MCB*6500

Friday, March 1, 2024 @ 12:00 p.m.

presented by:

Manitabhai Govind

(Advisor: Dr. Matthew Kimber)

" A structural and functional characterization of a new family of retaining β-Kdo transferases from the capsular polysaccharide of Escherichia coli K13"

Kdo (3-deoxy-D-manno-oct-2-ulosonic acid) is an unusual sugar found predominantly in bacteria. Kdo is an essential constituent of the lipopolysaccharide core but also occurs in capsular polysaccharides (CPS) which are major virulence factors for pathogenic bacteria. Previous research from the Kimber lab illustrated that glycosyltransferases which transfer β-Kdo (WbbBGT99) operate through a rare double displacement mechanism involving a covalent enzyme-substrate intermediate. β-Kdo occurs as a ribose-β-Kdo repeat in *Escherichia coli* K13 CPS; however, the enzyme responsible for its incorporation is unknown. Inspection of the kps gene locus revealed a large uncharacterised open reading frame with a domain which I termed as KdoX¬¬, sharing distant structural similarity with WbbBGT99. Furthermore, KdoX conserves key catalytic residues with WbbBGT99 including the covalent adduct forming nucleophile Asp193. However, low global sequence identity of KdoX with proven β-Kdo transferases suggests that KdoX represents a new family of glycosyltransferases. I hypothesize that KdoX is a β-Kdo transferase which adds β-Kdo to ribose through a double displacement mechanism. Activity assays using its donor, CMP-β-Kdo and a synthetic acceptor will be monitored by thin layer chromatography to confirm the proposed activity. Mass spectrometry will be used to confirm the formation of the Asp193-Kdo adduct. I also plan to structurally characterize KdoX using X-ray crystallography to obtain structural snap-shots of the catalytic cycle to support the double displacement mechanism. Overall, this research will deepen our understanding of diversity in β-Kdo transferases, while also providing a foundation for the development of vaccines and therapeutics targeting *E. coli* K13 CPS.

.