Department of Molecular and Cellular Biology

Graduate Seminar MCB*6500

Friday, April 12, 2024 @ 12:45 p.m.

presented by:

Mallory Wright

(Advisor: Dr. Georgina Cox)

"Dissecting the connection between the efflux machinery, lipopolysaccharide transport and cell envelope integrity in Escherichia coli to uncover novel targets and chemical leads for antimicrobial development"

The highly impermeable outer membrane (OM) and multidrug efflux pumps are innate traits of Gram-negative bacteria that challenge the identification of new antimicrobials. Developing a more comprehensive understanding of these traits will be conducive to overcoming intrinsic antimicrobial resistance in Gram-negative pathogens. To facilitate the study of *Escherichia coli* efflux pumps, the Cox Lab generated efflux-deficient mutants – Efflux KnockOut-35 (EKO-35) and the Tripartite Efflux (TE) mutant. The antimicrobial susceptibility of these mutants was expected to be comparable to the widely utilized efflux-deficient *ΔtolC* strain, but instead *ΔtolC* was found to be hypersusceptible to several synthetic compounds with unknown targets. Investigations into the causal mechanism of these susceptibility differences revealed that the *ΔtolC* OM is compromised. Subsequent work identified that one synthetic compound inhibits an essential transporter involved with lipopolysaccharide biosynthesis, revealing that *ΔtolC* is sensitive to perturbations in cell envelope biogenesis. Taken together, this implicates a role for TolC in maintaining OM integrity. The other synthetic compounds active against *ΔtolC* are predicted to target other components of cell envelope biogenesis. By generating spontaneous resistance mutants to identify the targets of the synthetic compounds, I intend to further demonstrate TolC's role in this biological process. Additionally, I intend to identify other essential genes that *ΔtolC* becomes sensitized to upon CRISPRi-mediated genetic repression to show that TolC is associated with biological processes expanding beyond cell envelope biogenesis. Overall, this work aims to enhance our understanding of TolC and substantiate it as an integral component of many biological processes in *E. coli*.