Robert McKee

“The c-di-GMP regulatory network in Clostridium difficile and its role in modulating surface adherence and persistence in the mammalian gut.”

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1131 Bioinformatics

Dissertation Advisor: Dr. Rita Tamayo

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ABSTRACT
Robert McKee: “The c-di-GMP regulatory network in Clostridium difficile and its role in modulating surface adherence and persistence in the mammalian gut (Under the direction of Dr. Rita Tamayo)

Clostridioides difficile (Clostridium difficile) is a spore-forming bacterial pathogen responsible for hundreds of thousands of infections each year in the United States. C. difficile outbreaks are common in hospitals because C. difficile spores can persist for months on surfaces and are resistant to many disinfectants. Despite the significant disease burden that C. difficile represents, we know surprisingly little about the factors necessary for C. difficile to colonize and persist in the mammalian intestine. Previous work demonstrated that the signaling molecule cyclic diguanylate (c-di-GMP) regulates a variety of processes in C. difficile including production of the toxins that are required for disease symptoms. Using monolayers of human intestinal epithelial cells, we demonstrate that c-di-GMP promotes attachment of C. difficile to intestinal epithelial cells. We also demonstrate that regulation of type IV pili (TFP) by c-di-GMP promotes prolonged adherence of C. difficile to epithelial cells in vitro. C. difficile mutants lacking TFP were cleared more quickly than the parental strain during single strain mouse infections and were outcompeted by the parental strain during in vivo competition experiments in mice. Thus, our data provides evidence that TFP promote persistence of C. difficile in the intestine. To determine what other genes c-di-GMP regulates in C. difficile, we performed RNA-sequencing comparing the transcriptome of C. difficile with elevated c-di-GMP to that of C. difficile with basal levels of c-di-GMP. We demonstrate that c-di-GMP regulates the expression of 166 genes greatly expanding the known members of the c-di-GMP regulon. We demonstrate that c-di-GMP regulation of several transcripts in C. difficile is dependent on c-di-GMP sensing riboswitches present in the 5’ untranslated regions of these transcripts. Our results also show that c-di-GMP regulates a number of cell envelope proteins in addition to TFP and flagella. These data suggest a broader role for c-di-GMP in remodeling the C. difficile cell surface.