(1) Submission ID#1539063

CHARACTERIZATION OF THE NEISSERIA GONORRHOEAE TDFJ PROMOTER, WHICH IS DUALLY REGULATED BY ZINC AND IRON

Author(s)

Sandhya Padmanabhan, MS

Ph.D Candidate

Georgia State University

Julie Stoudenmire, PhD

Assistant Professor of Research

Georgia State University

Alexis H. Branch, n/a

PhD Candidate

Georgia State University

Nicholas Noinaj, PhD

Associate Professor

Purdue University

Cynthia Cornelissen, PhD

Professor

Georgia State University

Background

Neisseria gonorrhoeae (Ngo) is a human-specific pathogen that causes the STI, gonorrhea. The WHO estimates that 106 million new cases of gonorrhea occur every year. Both men and women can be infected; however, women suffer from increased morbidity due to the asymptomatic nature of the infections. In women, these infections can lead to pelvic inflammatory disease and infertility. Infection with Ngo does not result in protection and no effective vaccine has been developed, leaving antibiotics as the only way to mitigate the disease. Emergence of strains with high levels of antibiotic resistance has resulted in an urgent need for development of novel therapeutics. During colonization, the host combats infection by the process of nutritional immunity. However, Ngo uses nutrient acquisition systems such as TonB-dependent transporters (TDTs), for metal piracy. These TDTs are well conserved and important for Ngo's survival in the host making them promising vaccine candidates. TDTs TdfH and TdfJ have been characterized as zinc importers and are regulated at the molecular level by the zinc-sensing regulator, Zur. TdfJ is further induced by iron, indicating dual regulation by Zur and Fur.

Aim/Methods

We characterized the Zur regulon of Ngo with respect to zinc and interrogated the mechanism of zinc and iron

regulation of the tdfJ promoter. We used RT-qPCR and RNA Seq to study gene expression under excess and low zinc in wild-type and zur mutant strains. We further characterized the promoter elements upstream of tdfJ by 5'RACE. We utilized a DNA-Protein Interaction ELISA (DPI-ELISA) to characterize the specific binding of two regulators, Zur and Fur, to the tdfJ promoter.

Results

We found that tdfJ was significantly differentially regulated under low vs. high zinc conditions using RNA Seq and confirmed via RT-qPCR. tdfJ promoter analysis identified the transcriptional start site and putative upstream Zur and Fur-binding sites. Purified Ngo Fur and Zur bound specific sequences within the tdfJ promoter in the DPI-ELISA. Further, consensus Fur and Zur binding site sequences were computed from RNA Seq analysis of Ngo.

Conclusions

The mechanism of dual regulation of the tdfJ promoter involves both an iron-dependent Fur induction and a zinc-dependent Zur repression.