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The gonococcal znuCBA locus is transcribed from multiple zinc- and Zur-regulated promoters

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Background

Neisseria gonorrhoeae (Ngo) and its human host both require transition metals for biological functions including cell signaling and metabolism, gene regulation, enzymatic processing, and oxidative stress resistance. During infection, the host employs nutritional immunity, in which metal sequestration proteins including Calprotectin and S100A7 are deployed to starve the pathogen of metals thus inhibiting bacterial growth. In response to zinc and manganese limitation, Ngo expresses the high-affinity zinc and manganese uptake system, ZnuABC, which is encoded by the znuCBA operon and is predicted to be zinc-repressed by the zinc uptake regulator, Zur.

Aim/Methods

Our aim was to characterize the znuCBA operon under metal-deplete conditions, which resemble the metal limitation experienced by Ngo during infection. To map the operon, the wild type and Zur mutant strains were grown in Chelex-treated chemically defined media (CDM): metal-deplete CDM alone, CDM plus zinc, or CDM plus zinc chelator. RNA was isolated and purified for RNA-seq analysis of znuCBA expression and for promoter prediction, and the results were confirmed by RT-PCR.

Results

RNA-seq analysis revealed putative promoter elements upstream of znuC and znuA. Additionally, the znuA transcript exhibited a higher fold difference in expression relative to the znuC and znuB transcripts when Ngo was grown in zinc-replete verses zinc-deplete conditions. The RT-PCR data showed that the znuCBA locus is transcribed from multiple promoters in metal-deplete conditions: a single message through the entire operon at low levels and a znuA alone transcript at higher levels.

Conclusions

znuA is expressed from two transcripts, as a part of the znuCBA polycistronic operon and as a single znuA only transcript. The redundancy in znuA transcription suggests a critical role for ZnuA, during metal-depleted

growth conditions. Previous work has demonstrated that ZnuA is required to utilize zinc from the nutritional immunity protein, S100A7, which is enriched at the mucosal epithelium. Transcriptional control is likely conferred by promoter elements identified upstream of znuA in addition to one upstream of znuC. Future directions include characterizing the Zur binding sites upstream of and within the znuCBA locus using DNA-Protein ELISA (DPI-ELISA).