PUBLISHABLE SUMMARY: INVESTIGATING sRNAs AS THE MASTER ON/OFF SWITCH OF *VIBRIO CHOLERAE* VIRULENCE

INTRODUCTION:

In *Vibrio cholerae*, the bacterium that causes cholera, the infectious cycle is controlled by four small regulatory RNAs (sRNAs) in a cell density-dependent manner. At low cell density, at the onset of infection, sRNAs Qrrs1-4 are synthesized and, with the aid of the RNA-binding protein Hfq, base-pair with specific mRNAs to turn on virulence genes. At high cell density, at the end of infection, the Qrrs are no longer synthesized, virulence genes are turned off and the bacterium is released from the host. This critical sRNA-mRNA interaction functions as the virulence on/off switch in *V. cholerae* and its components are attractive antimicrobial targets.

PROJECT OBJECTIVES:

The goal of the project was to elucidate the molecular details of the interactions between the Qrr sRNAs, their mRNA targets and Hfq in order to further understand the mechanism of *V. cholerae* virulence and provide a foundation for designing new strategies for the treatment and prevention of cholera.

WORK PERFORMED AND RESULTS:

Characterization of Hfq: *V. cholerae* Hfq was cloned, expressed and purified and its biochemical and biophysical properties were compared to those of Hfq from the model organism *Escherichia coli*. Both Hfq proteins are hexameric in solution and low resolution structural data revealed that they adopt a similar star-shaped conformation with disordered C-terminal regions (CTRs) extending outwards from a folded core. The *V. cholerae* hexamer is less resistant to denaturants than that of *E. coli* and truncated proteins and domain-swap constructs were prepared to determine the origin of the stability difference. Sequence analysis indicated that the protein core is highly conserved between *V. cholerae* and *E. coli* while the CTRs differ in both length and composition. However, both the core and CTR account for the difference in hexamer stability suggesting a different inter-protomer interface in the two proteins.

Characterization of Hfq-Qrr sRNA Interactions: *V. cholerae* Hfq was found to bind to each Qrr sRNA with 1:1 stoichiometry and low nanomolar affinity. Binding experiments were also performed with the truncated and domain-swap Hfq proteins and suggested that the CTR is important for maintaining the stoichiometry of binding. Low resolution structural studies showed that the Qrrs bind predominantly to one face of Hfq. RNA-binding mutants were prepared based on homology to *E. coli* and revealed that the Qrrs bind primarily to the proximal face. The Qrrs also undergo a conformational change upon complex formation with Hfq while the protein structure remains unchanged.

Characterization of sRNA-mRNA Interactions: A novel method for the surface immobilization of RNA was developed in order to quantify RNA-RNA interactions by surface plasmon resonance. This sensitive assay revealed that there are preferences with regard to which Qrr binds to which mRNA target.

CONCLUSIONS AND IMPACT:

The work completed to-date has provided valuable insights into the mechanism of *V. cholerae* virulence at the molecular level, particularly with regard to the Hfq-sRNA interactions. The goal is to publish the findings in international journals during the next year. The researcher has been retained by the host institution to continue working on the project in collaboration with the scientist in charge and will expand the studies to characterization of the Hfq-sRNA-mRNA ternary interactions. It is anticipated that both the current and future results will be beneficial for designing antibacterial strategies to combat cholera.