

KATHRYN M. RAMSEY, PH.D

Assistant Professor

DEPARTMENT OF CELL AND MOLECULAR BIOLOGY

DEPARTMENT OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

Center for Biotechnology and Life Sciences, 120 Flagg Road, Kingston, RI 02881 USA p: 401-874-2932

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Dear Editor,

Enclosed is a manuscript entitled “Ribosome heterogeneity results in leader sequence-mediated regulation of protein synthesis in *Francisella tularensis*” that we are submitting for publication in the Journal of Bacteriology as a Research Article.

It is becoming increasingly appreciated that changes in ribosome composition can result in changes to gene expression, yet it is not well understood how these impacts are mediated. We previously demonstrated that changes in ribosome composition in the human pathogen *Francisella tularensis* impact gene expression and virulence (Trautmann and Ramsey, JBact, 2022). In this manuscript, we investigate the mechanism by which this may occur.

Our work reveals that the ribosomal protein bS21-2 influences translation of specific mRNAs in a leader sequence-dependent manner. Using translational reporters, we investigated the sequence requirements for these effects, finding that bS21-2 only positively influences translation in the absence of strong Shine Dalgarno-anti-Shine Dalgarno interactions. We also identified a specific 6-nucleotide sequence in a particular mRNA leader necessary for bS21-2-mediated translation. This manuscript provides evidence that bS21-2 exerts its effects by influencing translation initiation and we speculate that this may occur via specific interactions between bS21-2 and leader sequences. Our results are consistent with a model in which bS21 homologs, in *F. tularensis* and other bacteria, interact directly with particular mRNA leader sequences to modulate initiation of protein synthesis and govern gene expression. These findings are novel yet also consistent with other work demonstrating that bS21 is involved in translation initiation.

In the course of these studies, we also investigated the potential for effects of bS21-2 to be mediated indirectly by the RNA binding protein Hfq. While we determined that bS21-2 and Hfq both influence production of a key virulence factor, the type VI secretion system (T6SS) in *F. tularensis*, they do so via distinct pathways. These results are important because they offer additional evidence regarding the mechanism by which Hfq influences T6SS in *F. tularensis*. Prior published studies reported conflicting models and our work is consistent with, and offers additional evidence for, a model in which Hfq negatively controls the abundance of a subset of transcripts corresponding to T6SS proteins.

Our findings that bS21-2 in *F. tularensis* function controls translation initiation in a leader sequence-dependent manner has significant implications for how bS21 may influence protein synthesis in other bacteria and in phage, which can encode bS21 homologs. Accordingly, we believe our study will be of interest not only to those studying *F. tularensis* or bS21, but also those studying gene regulation, ribosome biology, phage biology, ribosome heterogeneity, and ribosome-mediated antibiotic resistance.

Thank you very much for your consideration of this manuscript.

Yours sincerely,



Kathryn Ramsey