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Dear Editor,

Enclosed is a manuscript entitled "A ribosomal protein homolog regulates gene expression and virulence in a bacterial pathogen" that we are submitting for publication in the Journal of Bacteriology. In the manuscript we show that a specific homolog of a ribosomal protein acts as a post-transcriptional regulator of gene expression and is important for intramacrophage growth of *Francisella tularensis*. Our findings suggest that incorporation of specific ribosomal protein homologs into *F. tularensis* ribosomes, i.e., ribosome heterogeneity, may lead to modification of gene expression. This would represent an important, yet previously unappreciated role for ribosome heterogeneity in gene regulation.

The ribosomal protein we have investigated, bS21, plays a poorly-defined role in translation initiation and yet is implicated in control of many cellular processes in diverse bacteria. In addition, bS21 has been found in thousands of bacteriophage genomes and is proposed to aid in viral replication by hijacking the ribosome for viral protein production. To the best of our knowledge, our data represent the first study of how bS21 impacts bacterial gene expression on a genome-wide scale and is the first to investigate how multiple homologs of bS21 encoded by the same organism impacts translation.

Our findings indicate that a specific bS21 homolog, bS21-2, influences the abundance of approximately 160 proteins in a manner that cannot be explained by changes in transcript abundance. Proteins that are positively regulated by bS21-2 include the type six secretion system (T6SS) components, which are essential for intramacrophage survival. Consistent with reductions in T6SS proteins, cells without bS21-2 have an intramacrophage growth defect. While expression of another homolog of bS21, bS21-3, can restore T6SS protein abundance, only bS21-2 can complement the intramacrophage growth defect. This suggests that bS21-2 regulates not only the T6SS proteins, but other proteins(s) which contribute to survival in host cells. We propose that bS21 homologs in *F. tularensis* are exerting their effects by influencing translation initiation, via specific interactions with the 5' untranslated regions of a specific set of mRNAs.

Our findings that bS21 homologs in *F. tularensis* function, either directly or indirectly, as post-transcriptional regulators, have significant implications for how bS21 functions in other bacteria. Additionally, 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, ribosome heterogeneity, and ribosome-mediated antibiotic resistance.

Thank you very much for your consideration of this manuscript.

Yours sincerely,

Kathryn Ramsey