
JB00140-23 Decision Letter

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To: kramsey@uri.edu

Sat, May 20, 2023 at 7:00 PM

Dr. Kathryn M Ramsey
University of Rhode Island
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Re: JB00140-23 (Ribosome heterogeneity results in leader sequence-mediated regulation of protein synthesis in *Francisella tularensis*)

Dear Dr. Ramsey:

Thank you for submitting your manuscript to Journal of Bacteriology. Two reviewers have critiqued your study, and both recommend only minor alterations that will not require any additional experimentation. Both reviewers have several suggestions that will help with the flow of the manuscript and make it easier to read, and some improvements to the figures. These alterations should not take long. I look forward to receiving your revised manuscript.

Below you will find the comments of the reviewers.

Preparing Revision Guidelines

To submit your modified manuscript, log onto the eJP submission site at <https://jb.msubmit.net/cgi-bin/main.plex>. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

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Thank you for submitting your paper to JB.

Sincerely,
Laurie Comstock
Editor, Journal of Bacteriology

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Reviewer comments:

Reviewer #1 (Comments for the Author):

This paper investigates the action of the *Francisella tularensis* ribosome protein bS21-2, which is one homolog of bS21 and whose deletion results in a changed proteome. The authors show that 1) being responsive to bS21-2 correlates to sequences within the 5'UTR and with a weaker Shine-Dalgarno sequence, 2) for one of the affected proteins, mraY, the deletion of a hexamer sequence 58 nt upstream of the AUG start, eliminates the bS21-2 effect, and 3) for certain bS21-2-responsive T6SS genes, the RNA chaperone Hfq acts to decrease transcript abundance, while bS21-2 works to increase translation. The work has been performed well. These are interesting findings that would be interesting to those in the translation field and to those investigating regulation of bacterial virulence.

My main concern with the paper is its overall organization and presentation of the data, because as written, I believe that it is quite difficult for the reader to follow. Consequently, these findings are not as impactful as they should be.

I suggest the following:

1. Start with a discussion of the previously found bS21-2 proteome and the analyses in Fig. 2 and 3D, showing that the 5' UTR and, in general, a weaker SD site correlates with bS21-2 responsiveness.
2. Show the full analysis of *mraY* combining the info in Fig. 3C, 4, 5B, and 6 to show what is not and what is needed for bS21-2 responsiveness with this gene. This would then combine the complete mutational analysis of *mraY* in one section. Please highlight the GACUCU sequence throughout these figures, both in the sequence and in the secondary structures. Please add an analysis of whether this site is present in the other bS21-2 responsive genes (apparently it is not) and show the sequences of the other genes in a supplemental table.
3. Show the analysis of the T6SS genes, and the conclusion that Hfq and bS21-2 work in different ways.

I believe that this organization would make the paper much easier to read and would greatly enhance its impact.

Other comments:

Line 138 should read 'on both operons'

Line 161 - Increase looks more like 10-fold and 23 -fold? Please explain.

Fig. 1. Please make the bar colors consistent throughout the panels (same color for same gene) In Fig. 1E, please show from 0 to 10 as most of the graph and then have a double line to indicate the higher 25 value.

Please move Fig. S3 to the main text as this is important.

Line 213 should read Fig. 2B?

Line 217 says C1529 while Fig. 3A says C1539.

Fig. 3B What sequence in *pdpA* do you consider to be the SD? Is it the AAGUAG that is too close to the AUG or the AUUAAG that is in the correct position? Please mark in 3B and in 3C. Also please indicate the absolute b-gal units in the legend. This is important since the 'BadSD' probably gives very low units.

Line 275 I did not see a Fig. S5.

Discussion. Much of the Discussion is a recapitulation of the results. I would shorten this and then discuss what is known about bS21-2 in other systems and how and if your findings relate to this.

Reviewer #2 (Comments for the Author):

Review of Trautmann et al 2023

Here Trautmann and colleagues have investigated the mechanisms by which the ribosomal protein bS21-2 affects expression of T6SS genes in *Francisella tularensis*. They found that 5' UTRs from some of the affected genes were sufficient to make reporter genes affected by bS21-2. The 5' UTR features that produce this effect appear to be complex. The effect was abrogated by optimization of the SD sequence, suggesting that bS21-2 promotes translation of genes with weak SD sequences. Overall, the study is well done and well presented and will be a useful contribution to the field.

My comments are all minor, as follows:

It would be helpful to include a schematic of the two T6SS operons in the first figure to make it easy to see which of the genes tested in various places in the paper belong to which operon.

Fig 1: Please put the panels in order A-E to improve readability.

Fig 3 and others that show beta-gal activity or fluorescence: Please show the data as arbitrary units,

not normalized to the WT version of each gene. The expression levels of the constructs may vary, and sometimes this is important, as is shown in one of the supplementary figures with respect to the different impact of bS21-2 for constructs with strong SD sequences. Rather than put this in the supplement, it would be cleaner and more informative to omit the normalization from all the figures and thus retain data that may be interesting and important.

Fig 5: Please make the secondary structure figures much larger so the numbers and bases can be read.

Fig 6: Please add shading or dashed lines connecting the allele names to the sequence to make it easier for the eye to follow.

Fig S1: Change the word "opening" in the legend to "open."

Fig S3: The figure legends state that fluorescence readings were normalized to a non-fluorescent strain. However, the methods section states that readings from non-fluorescent strains were subtracted (which to this reviewer makes more sense). Please correct the language as needed.

Line 12, it is unclear what is meant by the term "in contrast." In contrast to what? I suggest revising the sentence.

Line 29, replace "is" with "are."

Line 55, the word "in" is missing.

Line 60, "mRNA" should be singular.

Line 68, I suggest putting the reference at the end of this sentence rather than at the end of the paragraph.

Line 101, replace the word "genes" with the word "transcripts," assuming that is what is meant.

Line 103, it is confusing whether the effect being referred to is a transcript abundance effect, a protein abundance effect, or both.

Lines 114-115, was the tag integrated in the chromosome at the native locus? Specify this (or what was done if something different) to help the reader understand that the native regulatory sequences were controlling the tagged copy of the gene.