Examining the Mechanism of the Self-Regulation of *rpsU2* in *Francisella tularensis*

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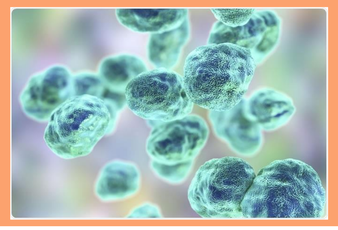
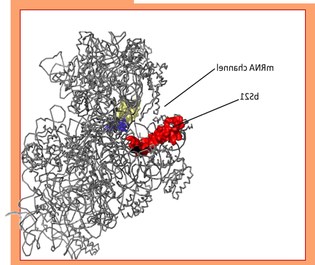
Introduction

Francisella tularensis

- Gram negative
- Intracellular pathogen
- Causative agent of tularemia
- Potential bioweapon



bS21: a small subunit ribosomal protein involved in translation initiaion



bS21 depicted in the small subunit of the *E. coli* ribosome

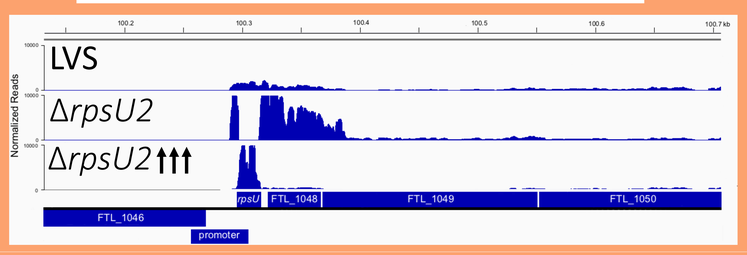
F. tularensis:
Three homologs
- Potential lead to ribosome heterogeneity

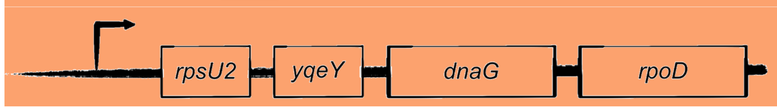
Loss of bS21-2
- Influences virulence factors

Study Goals

1. Understand the regulation of bS21 homologs
2. How does bS21-2 affect itself? How do the homologs bS21-1 and bS21-3 affect bS21-2?

bS21-2 Regulates its Own Expression



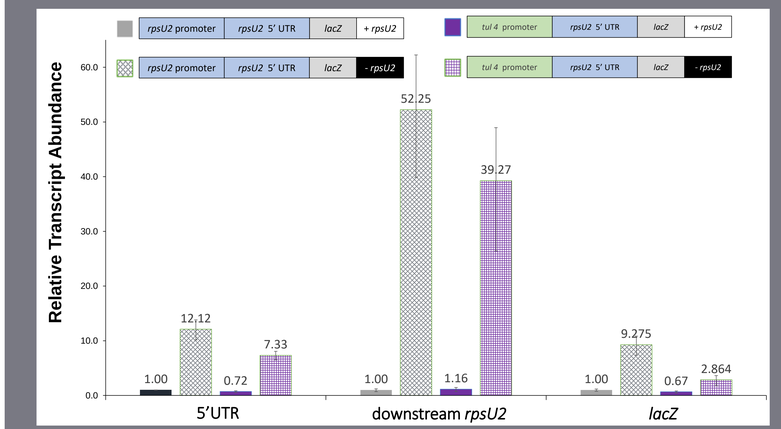


Results

Constructs and Predictions

			If in cells **with** bS21-2 (WT)	If in cells **without** bS21-2 (Δ*rpsU2*)
rpsU2 promoter	*rpsU2* 5′ UTR	*lacZ*	⊕	⊕⊕⊕⊕⊕
tul 4 promoter	*tul 4* 5′ UTR	*lacZ*	⊕⊕⊕	⊕⊕⊕
rpsU2 promoter	*tul 4* 5′ UTR	*lacZ*	?	?
tul 4 promoter	*rpsU2* 5′ UTR	*lacZ*	?	?

rpsU2 5′UTR and P*tul4* Permits Regulation by bS21

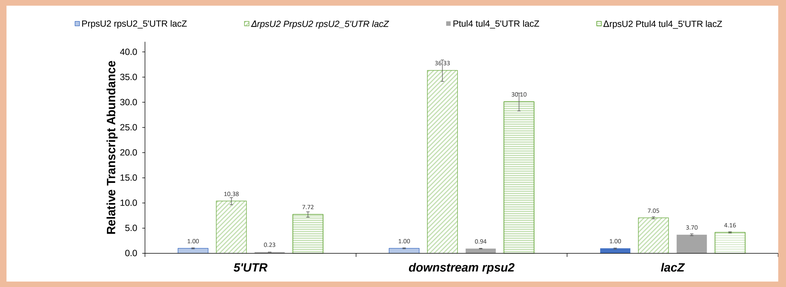


Native Context: P_{rpsU2} 5′UTR_{rpsU2} *rpsU2* *yqeY*

Tn7 Site: P_{tul4} 5′UTR_{rpsU2} *lacZ*

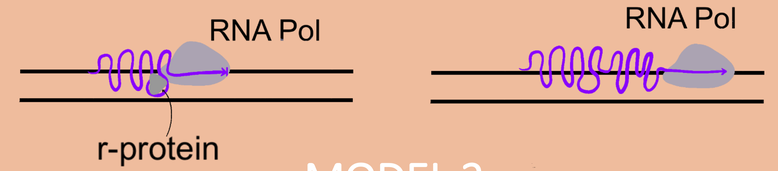
Results Cont.

A Title Eventually

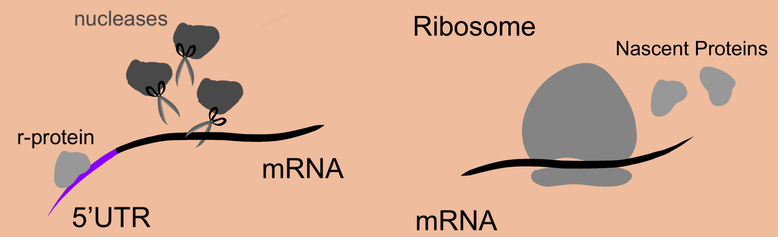


Models for r Protein Regulation

MODEL 1: Attenuation



MODEL 2: Post-Transcriptional Control



Conclusions

- The 5′UTR of the *rpsU2* gene is a candidate as a bS21 target for transcriptional regulation
- The *rpsU2* promoter is not necessary for the regulation of the operon

Future Directions

- Test the *rpsU2* promoter's contribution to the regulation of *bS21-2*
- Assess the relationship between transcript abundance and protein activity with *lacZ*
- Examine post-transciprtional model via degradation assays

References

Burgos, H. L., O'Connor, K., Sanchez-Vazquez, P., & Gourse, R. L. (2017). Roles of Transcriptional and Translational Control Mechanisms in Regulation of Ribosomal Protein Synthesis in Escherichia coli. *Journal of bacteriology*, *199*(21), e00407-17. https://doi.org/10.1128/JB.00407-17

Lindahl, L., Archer, R., & Zengel, J. M. (1983). Transcription of the S10 ribosomal protein operon is regulated by an attenuator in the leader. *Cell*, *33*(1), 241–248. https://doi.org/10.1016/0092-8674(83)90353-7

Nomura, M., Gourse, R., & Baughman, G. (1984). Regulation of the synthesis of ribosomes and ribosomal components. *Annual review of biochemistry*, *53*, 75–117. https://doi.org/10.1146/annurev.bi.53.070184.000451

Zengel, J. M., & Lindahl, L. (1994). Diverse mechanisms for regulating ribosomal protein synthesis in Escherichia coli. *Progress in nucleic acid research and molecular biology*, *47*, 331–370. https://doi.org/10.1016/s0079-6603(08)60256-1