Investigating the regulation of a protein required for virulence in a bacterial pathogen

Christina Surace

Project Start Date: September 3, 2024

Project End Date: December 20, 2024

Amount of Funding Requested: $1,000

Introduction

Francisella tularensis is a highly infectious intracellular pathogen which causes the disease tularemia, also known as rabbit fever. As an intracellular pathogen, growth and replication inside a cell is essential for *F. tularensis* to cause disease. *F. tularensis* is considered as a potential bioweapon because of key features such as: it can be easily aerosolized, very few cells are required for infection, and it has the potential to cause lethal disease. In Dr. Kathryn Ramsey's laboratory, we work with a specific strain, *F. tularensis* subspecies *holarctica* LVS (live vaccine strain). LVS is an attenuated strain that does not cause disease in humans but maintains its ability to cause disease in animal models. The Ramsey lab is interested in finding out how genes important for virulence are regulated.

Previous findings of the Ramsey lab have established that the ribosomal protein, bS21-2 is important for virulence of *F. tularensis* (Trautmann and Ramsey 2022). The protein bS21-2 is encoded by the gene called *rpsU2* in the LVS strain. The gene *rpsU2* is located in an operon with multiple other genes (Figure 1). An operon is a cluster of genes that are transcribed at the same time into a single mRNA and are controlled by the same promoter.

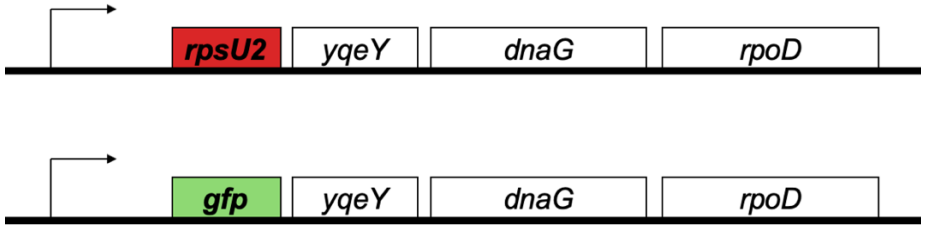

Figure 1. Illustration of the *rpsU2* operon. **Top:** the *rpsU2* gene (represented by the red box labeled "*rpsU2*") is encoded in an operon with multiple other genes (*yqeY*, *dnaG*, *rpoD*) which are all controlled by the same promoter (arrow). **Bottom:** Representation of the reporter strain I will generate by replacing the *rpsU2* gene with the gene for green fluorescent protein (*gfp*).

Notably, when cells lack the bS21-2 protein, there is an unusual change in the amount of mRNA transcript and protein corresponding to the *rpsU2* operon. Specifically, compared to cells with bS21-2, there is a large increase in *rpsU2* operon transcript amount but no apparent change in protein abundance (Figure 2). According to the Central Dogma of Molecular Biology, DNA is transcribed into RNA, which is then translated into proteins. Due to this theory, any changes in transcript amount should be proportional to changes in protein amount in a cell. We hypothesize that the lack of translation of the additional *rpsU2* operon transcript into more protein is due to an unknown regulatory factor limiting translation.

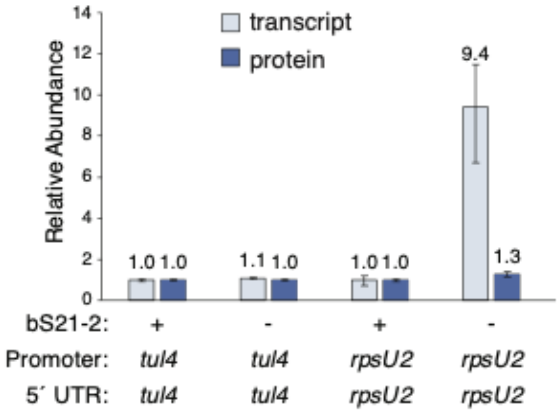

Figure 2. The above figure indicates the relationship between the presence of bS21-2 in the cell and its effect on the relative abundance of *rpsU2* operon transcript and protein in the cell. Cells lacking bS21-2 have more transcript, yet no change in relative protein amount.

Project Objectives

The goal of this research project is to further investigate the regulation of a protein important for pathogenesis in *F. tularensis*, bS21-2 (encoded in the *rpsU2* operon). Specifically, I aim to identify what gene(s) influence translation of the *rpsU2* operon in cells lacking bS21-2. To do this, I will first create a *F. tularensis* LVS reporter strain to easily assess *rpsU2* operon protein abundance, then mutagenize this strain to identify what mutations may lead to increased *rpsU2* operon protein abundance. I expect to find that the reporter strain will be similar to other cells lacking bS21-2, which have more *rpsU2* operon transcript but no corresponding increase in relative protein amount. In the mutants of interest, I will be looking for cells that defy this regulation of translation and in which the increased mRNA amount leads to higher reporter protein abundance. This increase in protein abundance could indicate that inhibitory factors of translation were removed. Through my research, my goal is to identify these factors limiting *rpsU2* operon protein translation in cells lacking bS21-2.

Project Plan

I will modify the *F. tularensis* LVS genome by replacing the gene encoding bS21-2 (*rpsU2*) with the reporter gene encoding green fluorescent protein (*gfp*). In these cells, I expect to find that there will be increased transcription of the *rpsU2* operon, which will contain the *gfp* transcript, but no relative increase in GFP protein abundance. The use of GFP as a reporter will allow us to easily and directly measure the abundance of *rpsU2* operon protein abundance in the cell by measuring the amount of fluorescence. In order to modify the genome and create this reporter strain, I will first clone a plasmid that contains the *gfp* gene in place of *rpsU2*, as well as a selection marker for kanamycin resistance and the *sacB* gene which is a sucrose sensitivity gene. After creating the plasmid, I will use it in a well-established protocol called allelic exchange to create a strain of *F. tularensis* that lacks the *rpsU2* gene but contains the *gfp* gene in its place.

Once I have successfully replaced *rpsU2* with *gfp*, I will create mutants of this strain using a well-established system of transposon mutagenesis. When the mutants are created, I will pick

which ones to investigate by visually looking for more GFP. Colonies that appear greener in color will indicate a higher amount of green fluorescent protein in the cell. I will study these colonies and identify which genes have been mutated. I expect these genes might encode factors such as translation inhibitors, whose loss would lead to this increase in protein abundance in the cell. Upon completion of this project, I hope to identify factors that control the production of the virulence factor bS21-2 in *F. tularensis*.

Timeline

As of Spring 2024, I have begun creating the plasmid, and plan to have it completed by the end of the semester. For Fall 2024, I will create the *F. tularensis* LVS strain lacking the *rpsU2* gene, instead having the *gfp* in its place. This should be completed within the first month of the Fall 2024 semester. The remainder of the semester will be dedicated to the transposon mutagenesis and investigation of mutants with high GFP production.

Contribution

As a microbiology enthusiast with a particular interest in pathogenic bacteria, I came across Dr. Kathryn Ramsey's lab website while searching for research opportunities on campus. I reviewed Dr. Ramsey's research descriptions and sent her an email which expressed my interest in the lab's research on pathogenic gene expression in *F. tularensis*. Soon after, I met with her, and she offered me an opportunity to work on my own project in her lab regarding the regulation of the virulence gene *rpsU2.* I was intrigued and excited by the opportunity to uncover some of the mystery behind the pathogenic mechanisms of this potentially lethal pathogen. I decided that I wanted to further understand this mechanism in attempts to prevent human infection. In my career as a research scientist, I hope to work on projects that advance scientific knowledge in a way that can also improve human health.

Expected Outcomes

The knowledge acquired from the completion of this project will impact the general understanding of gene regulation for pathogenic mechanisms in *F. tularensis.* This will benefit the scientific community, especially those studying similar pathogens, as we will be able to provide insight into factors important for virulence which may be present in other species.

Not only will this project advance scientific knowledge and human health, but it is also of immeasurable value to me. I am very passionate about the biomedical sciences and hope to dedicate my career to helping people by performing research in these disciplines. This project aligns with my moral values as well as my career goals. To continue working on biomedical research at a high level, I plan on pursuing a PhD in the biological and biomedical sciences. To be accepted to graduate school in this field, having research experience as an undergraduate is a necessity. Being able to work on my own project provides me with invaluable experience where I am able to gain technical skills as well as skills in critical thinking and problem solving, scientific communication, and data analysis.

References

Trautmann, H. S., & Ramsey, K. M. (2022). A Ribosomal Protein Homolog Governs Gene Expression and Virulence in a Bacterial Pathogen. *Journal of Bacteriology*, *204*(10), e0026822. https://doi.org/10.1128/jb.00268-22

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March 29, 2024

Undergraduate Research Initiative Committee
University of Rhode Island
Kingston, RI 02881

Dear Committee Members,

I am extremely pleased to offer my support to Christina Surace for her undergraduate research project, "Investigating the regulation of a protein required for virulence in a bacterial pathogen." My laboratory studies the molecular mechanisms that lead to pathogenicity of the human intracellular pathogen *Francisella tularensis*. While this organism is highly pathogenic to humans, my laboratory takes advantage of the model organism *F. tularensis* subsp. *holarctica* LVS (Live Vaccine Strain), which does not infect or cause disease in humans and we do not work with any of the highly pathogenic strains.

Christina is an academically excellent upper-level student with a double major in Biotechnology and Cell and Molecular Biology (on the microbiology track) and is also a MARC U*STAR trainee. The goals of the MARC U*STAR program include training students to become critical thinkers, capable researchers, and future scientists, as well as develop a culture of excellence and community in undergraduate research at URI. Acceptance into the MARC U*STAR program is competitive and trainees are well-prepared to begin research. During the fall semester, Christina participated in "rotations," or trials periods, in multiple research laboratories to identify a scientific group to join for her undergraduate research. Christina ultimately chose to join the Ramsey laboratory; we are extremely pleased to welcome her to our group and we are committed to helping her achieve her research and training goals.

When discussing ongoing research in the Ramsey laboratory, Christina expressed interested in a project involving regulation of a gene important for virulence. Previous undergraduates and a graduate student had all made the same unusual observation about expression of this virulence protein, bS21-2. Specifically, in mutant cells that lack bS21-2, there is an increase in the mRNA coding for the bS21-2 protein. This is not surprising, as there are well-established mechanisms for proteins to control their own expression and, when protein levels are low, to increase protein production. What was unexpected is that this increase in mRNA abundance does not lead to corresponding increases in protein abundance. This suggests that other regulatory factors are controlling translation of this mRNA into protein. These are the first studies to examine what controls production of this specific virulence protein in *F. tularensis* and, since Christina is interested in regulation of virulence, she became interested in this unusual mechanism of regulation. Given her interest in understanding why more of this specific mRNA doesn't necessarily lead to more protein, her project goal will be to use a straightforward genetic screen to identify factors limiting translation of this mRNA. It is exciting to me that she is interested in this topic because her work will provide insight into regulation of translation in the human pathogen *F. tularensis*, an understudied topic, and particularly into regulators of translation that could significantly impact virulence. I enthusiastically support the continued development and undertaking of her independent research project.

Together with my graduate students, I will supervise all the proposed work in this project to ensure safety, rigor, and reproducibility. The success of this project will be based on Christina's

ability to (i) learn and implement research protocols, (ii) critically analyze the resulting data, (iii) make connections between the resulting data and the current scientific literature, and (iv) effectively communicate research findings, both within and outside our research group.

Christina is an outstanding student with great enthusiasm for laboratory research. Her undergraduate research experience in my laboratory, including the exciting work proposed here, will prepare her to achieve her future goal of entering graduate school in biomedical research. She is extremely well-prepared to carry out the proposed experiments and I support her application for an undergraduate grant for original student research without reservation and with enthusiasm.

Sincerely,

Kathryn M. Ramsey, PhD
University of Rhode Island
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