Studying the Function of Heterogeneous Ribosomes Using Antibiotics

Oli Horyn[1], Hannah Trautmann[2], Kathryn M. Ramsey[1,2]

[1]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881

[2]Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02881

Antibiotic resistance is an ever-growing global threat to our ability to treat bacterial infection. A potential bioweapon, the Gram negative bacteria *Francisella tularensis* is highly infectious and has the potential to cause lethal disease. The ribosomal protein bS21 is important for translation initiation and the *F. tularensis* genome encodes three homologs: *rpsU1*, *rpsU2*, and *rpsU3*. We are interested in bS21 as we suspect that differential use of these homologs may impact translation initiation by altering ribosome structure. These three bS21 homologs were investigated individually using isogenic strains in which all three native *rpsU* genes were deleted but contain a single homolog at a neutral location. These strains, named Tn7::*rpsU1*-V, Tn7::*rpsU2*-V, Tn7::*rpsU3*-V, respectively, were used to determine if ribosomes incorporating different bS21 proteins have different susceptibilities to ribosome-targeting antibiotics. The only antibiotic found with altered efficacy among these strains is kasugamycin, a drug that inhibits translation initiation. We were able to confirm differences in kasugamycin resistance among the strains with different bS21 content and also discovered resistant mutants during the process. We determined that all the kasugamycin-resistant mutants have predicted inactivating mutations in the *ksgA* gene. In this project, we validated the differences in susceptibility to kasugamycin among *F. tularensis* strains with altered bS21 content and identified mutants with increased resistance in a known kasugamycin resistance-determining gene. The differences we identified in kasugamycin sensitivity suggest structural differences among ribosomes with altered bS21 content, suggesting that we may be able to exploit heterogeneity in ribosomes to develop antibiotics that target different ribosome populations.