Testing the function of newly identified small proteins in the mycobacterial ribosome

Problem / Question

Current models of translation initiation in bacteria require a 5' UTR and Shine Dalgarno sequence for ribosome assembly and start codon selection. However one quarter of transcripts in mycobacteria are Leaderless and lack both a 5' UTR and Shine Dalgarno. The mechanism of how this happens is poorly understood.

Hypothesis

Recently discovered ribosomal proteins, encoded in Mycobacterium smegmatis by Msmeg0945 and Msmeg1916, facilitate Leaderless initiation in mycobacteria.

Project Overview

We created knockouts of Msmeg0945 and Msmeg1916. The viability of knockout strains indicated that the small ribosomal proteins they encode were not essential for survival under standard laboratory conditions. To determine whether they contribute to Leaderless translation efficiency, we generated matched luciferase reporter plasmids. Leadered and Leaderless reporter plasmid pairs were created for each of three independent promoters. These were then individually electroporated into each knockout strain as well as a wild type strain. Luciferase production was measured in a luminometer to determine the Leaderless initiation efficiency relative to Leadered control for each promoter type.

Msmeg0945 and Msmeg1916

50S 30S
Msmeg0945
MGSVIKKRKRMSKKKHKLRRTRVQRRKLGHK
Msmeg1916
MAKRGRKKDRHKSNAKNGKRPNA

Both proteins are small, basic, and very well conserved

(155)

Promoters used:
• Rv1133c
• Synthetic
• Msmeg0945

After electroporating reporter plasmids into knockout and wild type cells, luciferase assays were used to measure the relative amounts of luciferase production. The amount of luciferase produced in each cell is correlated with how frequently the Leadered or Leaderless transcript from that plasmid is being successfully translated by the cell.

Conclusion

• The results of this initial experiment were inconclusive, and must be repeated to either support or refute the hypothesis
• These strains will also be tested under conditions that stress the ribosome, with antibiotics and in minimal media
• Other ribosomal functions for these proteins are being tested

Works Cited


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