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

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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.

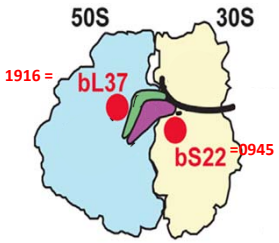
Hypothesis

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



(Hentschel et al., 2017)

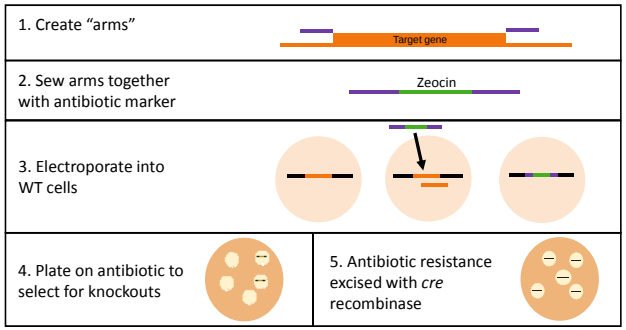
Msmeg0945
MGSVIKKRRKRMSKKKHR
KLLRRTRVQRRKLGK

Msmeg1916
MAKRGRKKRDRKHSKANH
GKRPNA

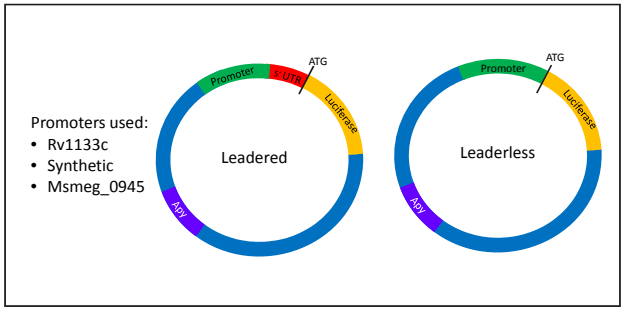
Both proteins are small, basic, and very well conserved

Procedure

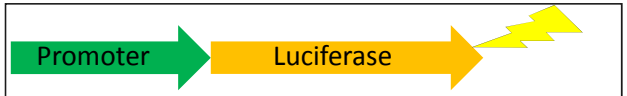
Step 1- Create knockouts



Step 2- Create reporter plasmids



Step 3- Luciferase assay



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.

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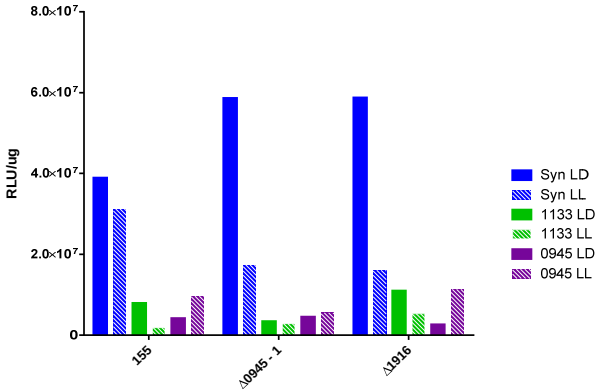
Results



- **Synthetic promoter:** This is the strongest promoter. Cells lacking either Msmeg0945 or Msmeg1916 have reduced Leaderless translation initiation efficiency relative to Leadered initiation compared with wild type *M. smegmatis*.
- **Rv1133c promoter:** Cells lacking Msmeg0945 had slightly improved Leaderless initiation efficiency relative to Leadered when compared to wild type ratios. Msmeg1916 ratios were similar to wild type.
- **Msmeg0945 promoter:** Cells lacking Msmeg0945 had slightly decreased Leaderless initiation efficiency relative to Leadered when compared to wild type ratios. Msmeg1916 ratios were similar to wild type.

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

Jendrik Hentschel, Chloe Burnside, Ingrid Mignot, Marc Leibundgut, Daniel Boehringer, & Nenad Ban. (2017). The complete structure of the mycobacterium smegmatis 70S ribosome. *Cell Reports, 20*(1), 149-160. doi:10.1016/j.celrep.2017.06.029

Shell, S. S., Wang, J., Lapierre, P., Mir, M., Chase, M. R., Pyle, M. M., . . . Gray, T. A. (2015). Leaderless transcripts and small proteins are common features of the mycobacterial translational landscape. *PLoS Genetics, 11*(11), e1005641. doi:10.1371/journal.pgen.1005641