MOI Project 7

Studies on the redundant role of highly redundant small non-coding RNAs in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis, the causative agent of tuberculosis in humans, is the most important bacterial pathogen on a global perspective, causing around 1.5 million deaths each year. Many of its virulence traits that allow replication during infection in face of a strong immune response remain poorly understood. Non-coding RNAs (ncRNAs) have attracted increasing attention in recent years with regard to the factors that may be involved in the regulation of gene expression underlying adaptation and virulence processes in bacterial pathogens. The general mechanism of ncRNA-based regulation is based on base pairing with target mRNAs or association with proteins, resulting in activation or interruption of gene expression. Cis-acting ncRNAs regulate mRNAs derived from the opposite DNA strand and therefore have fully complementary properties with the specific target structure. Intergenic, trans-acting ncRNAs have a broader spectrum of target structures and exert their effect despite imperfect base pairing with the target RNAs. Several pathogens like *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been described to control different regulatory networks employing ncRNAs. In contrast, very little is known about the function of ncRNAs in *M. tuberculosis*.

We have previously started to characterize the role of two highly-abundant ncRNAs in *M. tuberculosis* that accumulate during stationary phase. Phenotypic characterizations of individual site-specific gene deletions mutants revealed no discernable effects. However, genome-wide transcriptomic and proteomic profiling of the mutants surprisingly showed strong overlaps in the differentially expressed transcripts and proteins, with particular strong enrichment of ribosomal proteins in the stationary growth phase. These findings indicate that the two investigated ncRNAs have a partially redundant function despite different base sequences.

In this project, we aim to continue our studies on highly-abundant ncRNAs by generating a double gene deletion mutant to characterize the effects of simultaneous absence of both partially redundant ncRNA species. Phenotypic characterization will be done to reveal the intrinsic role of the studied ncRNAs for growth and viability of the bacterial cells but also addressing potential extrinsic functions to manipulate infected host cells. The elucidation of mechanisms will be approached by combining various complementary methods. These include, among others methods, the generation of site-directed *M. tuberculosis* gene deletion mutants employing phage transduction, transcriptomic and proteomic profiling, and genetic interaction mapping employing Tn-seq.