

## ABSTRACT

Bacterial transcription is a fundamental process within the cell, essential for converting the genetic code into functional products. This process is regulated by many transcription factors and small RNAs that are necessary for adapting to environmental changes, stress response and cellular survival. Transcription process is mediated by the key enzyme RNA polymerase (RNAP). The primary goal of this Thesis was to identify and characterize new transcription factors and small RNAs that interact with RNAP. The main focus was on mycobacterial transcription regulators, including i) regulatory RNAs such as Ms1 RNA and its homologs (**Publication I and II**); ii) transcription factors such as CarD, RbpA and CrsL (**Manuscript II**); MoaB2 (**Manuscript I**); and HelD (**Manuscript III**). My research interests were also focused on other bacterial species in which we performed RIP-seq, such as *Bacillus subtilis*, *Corynebacterium glutamicum* and *Streptomyces coelicolor*.

Using computational searches, Ms1 RNA homologs were predicted in more than 800 actinobacterial species. We experimentally confirmed Ms1 RNA presence in *Streptomyces coelicolor* (scr3559 sRNA) (**Publication I**). With RIP-seq, we identified several species-specific RNAs interacting with the bacterial transcription machinery, including MTS2823, *sigA*, *sigB*, *recO*, *rny*, scr0792 and CoRP RNA (**Publication II**). We also showed that MoaB2 binds to  $\sigma^A$  in *M. smegmatis* (**Manuscript I**). Additionally, we showed that mycobacterial HelD associates with highly expressed genes and has a global effect on gene expression (**Manuscript III**).

Lastly but more importantly, we have identified a novel transcription factor in *M. smegmatis*; CrsL, and show that it binds to CarD and RNAP. We performed ChIP-seq to identify CrsL-associated genes and combined these data with RNA-seq from *crsL* gene knockdown. We revealed that CrsL regulates the expression of many genes and possibly acts as a transcription enhancer (**Manuscript II**). We showed that Ms1 expression is associated not only with RNAP level, but also with CarD and RbpA levels (**Manuscript II**).

Collectively, this Thesis provides novel insights into the bacterial transcriptional machinery, thereby enhancing our knowledge of its regulatory networks.

**Keywords:** bacterial transcription, small RNAs, mycobacteria, RNA polymerase, CarD