Synopsis

Adaptation to a rapidly fluctuating environment is the key to the survival of an organism. Bacteria sense and respond to stress by an overall reprogramming of the cellular processes to shut down the energy-consuming processes and switch to pathways that ensure the survival under stress. One of the strategies utilized by bacteria is to mount 'stringent response' which is mediated by the second messengers (p)ppGpp. (p)ppGpp governs a multitude of phenotypes in Mycobacteria and for a long time the bifunctional Rel was believed to be its only (p)ppGpp synthetase. A serendipitous detection of (p)ppGpp in a *Mycobacterium smegmatis* strain devoid of Rel led to the discovery of a second (p)ppGpp synthetase thereby broadening the horizon of stringent response in Mycobacteria (Murdeshwar and Chatterji, 2012). This unique protein contained a RNaseH domain along with the (p)ppGpp synthesis domain suggesting a role distinct from that of Rel. Subsequent characterisation of the protein revealed that neither domain is active in isolation raising a question about the link between these activities. Due to the crucial role played by (p)ppGpp, it becomes essential to analyse the (p)ppGpp null phenotype. Several bacterial species like *Bacillus subtilis* have short alarmone synthetases in addition to Rel and they have been proposed to be activated under particular stress conditions underlining the need to delineate the role of these (p)ppGpp synthetases (Nanamiya et al., 2008). Our study proposes a role for MS_RHII-RSD *in vivo* and deals with the phenotypic characterisation of the Δ*rel* Δ*ms_rhII-rsd* strain.

Chapter 1 reviews the available literature in the field of stringent response and provides the rationale behind this study. The discovery of (p)ppGpp and the plethora of functions regulated by it is explained along with a description of the key players in the (p)ppGpp metabolism. The chapter stresses upon the need to investigate the significance of a second (p)ppGpp synthetase in Mycobacteria and the scope of the current study.

Chapter 2 deals with the elucidation of the *in vivo* significance of MS_RHII-RSD in *M. smegmatis* and proposes a role for the protein in R-loop removal during stress which requires both RNaseH activity and (p)ppGpp synthesis. The *in vitro* R-loop hydrolysis assays along with evidence for R-loop removal in *M. smegmatis* have been discussed along with the strategy used for the generation of the Δ*ms_rhII-rsd* strain.

Chapter 3 explores the interdependence between the RNaseH and (p)ppGpp domains in MS_RHII-RSD in an attempt to unravel the necessity of the RNase H activity in a (p)ppGpp synthetase. The generation of active-site mutants of RNaseH and RSD along with their functional and biophysical characterisation has been described in detail. Oligomerisation studies with MS_RHII-RSD revealed the importance of a hexameric form for the protein.

Chapter 4 further elaborates upon the link between the RNaseH activity and the (p)ppGpp synthesis activity and reveals a possible regulation of (p)ppGpp synthesis activity by RNA. Furthermore, the differing substrate specificities between Rel and MS_RHII-RSD are discussed. A possibility of the presence of pGpp due to MS_RHII-RSD in Mycobacteria has been outlined.

Chapter 5 describes the attempts at generating a (p)ppGpp-deficient strain of *M. smegmatis* and reveals the surprising presence of yet another (p)ppGpp synthetase. The generation and characterisation of the *Δrel Δms_rhII-rsd* strain was performed and the physiological role of MS_RHII-RSD in biofilm formation and antibiotic tolerance has been highlighted.

Chapter 6 summarizes the results of the study and points out the future directions for the work.

Appendix 1 gives a comprehensive list of strains and plasmids used in this study.

Appendix 2 provides a list of growth differences in antibiotics between the wild type and knockout strains of *M. smegmatis* obtained by Phenotype microarray.
Appendix 3 is a commentary on the Pup-proteasome regulation in Mycobacteria.