

Synopsis

Nucleotide based second messengers are known to regulate wide variety of processes in all domains of life. Two such bacterial second messengers are (p)ppGpp (guanosine tetra- or pentaphosphate) and c-di-GMP (cyclic dimeric guanosine monophosphate). The alarmone (p)ppGpp is synthesized by bacteria to face any kind of stress; while the signalling nucleotide c-di-GMP is synthesized principally to switch from motile (planktonic) to sessile (biofilm) life style. Apart from mediating the said functions, these nucleotides also regulate transcription, translation, replication, virulence and pathogenicity of the several bacterial species. In this work, we have tried to uncover novel functions or phenotypes that are governed by the second messengers (p)ppGpp and c-di-GMP in *Mycobacterium smegmatis*. In *M. smegmatis*, (p)ppGpp and c-di-GMP are synthesized and degraded by the bifunctional proteins Rel_{Msm} and DcpA, respectively. The architecture of both the proteins is similar; the synthesis and hydrolysis domains for the second messengers occur in tandem. The knockout mutants of *rel_{Msm}* and *dcpA* genes, Δrel_{Msm} and $\Delta dcpA$, have been used in this study to uncover the novel functions of these second messengers in mycobacterial physiology.

Chapter 1 provides is an overview of the current literature pertaining to (p)ppGpp and c-di-GMP. An historical perspective with regard to the discovery of the (p)ppGpp and c-di-GMP is given. The metabolism of these second messengers has been discussed. This has been followed by the description of various functions governed by the second messengers. Finally, the scope of the current work has been outlined.

Chapter 2 investigates the effect of disrupting (p)ppGpp and c-di-GMP signalling on the antibiotic sensitivity in *M. smegmatis*. Using Phenotype Microarray (PM) technology, the

growth of Δrel_{Msm} and $\Delta dcpA$ knock out strains was compared to those of the wild-type and respective complemented strains in 240 different antimicrobials. It was found that the knockout mutants displayed enhanced survival in the presence of multiple antibiotics. The PM data was corroborated by the independent determination of minimum inhibitory concentrations of seven different antibiotics. Finally, the plausible reasons for the multidrug resistance of Δrel_{Msm} and $\Delta dcpA$ strains have been discussed.

Chapter 3 explores how the impairment of (p)ppGpp and c-di-GMP alters the cell wall of *M. smegmatis*. Thin layer chromatography analysis of cell wall fractions such as glycopeptidolipids (GPLs), mycolic acids, polar and apolar lipids was carried out. It was found that the amount of GPLs and polar lipids were reduced in the Δrel_{Msm} and $\Delta dcpA$ knockout strains.

Chapter 4 explores the effect of (p)ppGpp and c-di-GMP on the growth, cell morphology and cell division in *M. smegmatis*. It was found that the Δrel_{Msm} and $\Delta dcpA$ knockout strains have slow growth compared to those of the wild type and respective complemented strain. The overproduction of (p)ppGpp and c-di-GMP, achieved through overexpression of Rel and DcpA proteins, encased the overexpression strains *relOE* and *dcpAOE* in a biofilm like matrix. The higher levels of (p)ppGpp and c-di-GMP caused *M. smegmatis* assume coccoid morphology. Microscopy analyses revealed that the Δrel_{Msm} and $\Delta dcpA$ strains are elongated, multinucleate and multiseptate.

Chapter 5 explores effects of (p)ppGpp and c-di-GMP on the global gene expression profile in *M. smegmatis*. Many genes were shown to be differentially expressed in the Δrel_{Msm} and $\Delta dcpA$ knockout strains. Genes regulating cell division, cell wall biosynthesis, superoxide metabolism or reactive oxygen species metabolism and genes encoding transporters were differentially expressed in the Δrel_{Msm} and $\Delta dcpA$ knockout mutants. The microarray data

were corroborated by quantitative real-time PCR. Gene expression data explained the multidrug resistance, the reduction in the level of GPLs and polar lipids, slow growth, changes in cell morphology and defective cell division exhibited by the Δrel_{Msm} and $\Delta dcpA$ knockout mutants.

Chapter 6 summarizes the entire work embodied in the thesis.

Appendix 1 lists the 240 antimicrobials compounds and their mode of action for which antibiotic sensitivity of the Δrel_{Msm} and $\Delta dcpA$ knockout mutants was tested.

Appendix 2 lists the growth differences among the knockout, wild type and complemented strains in the form of area under curve values.

Appendix 3 lists the genes that were differentially expressed in the Δrel_{Msm} and $\Delta dcpA$ knockout strains.

Appendix 4 is a comprehensive review on the kinetic and thermodynamic parameters governing the sigma factor competition in *Escherichia coli* and how (p)ppGpp and anti-sigma factors regulate this competition among sigma factors for the limited pool of core RNA polymerase in *E. coli*.