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SUMMARY OF THE THESIS

Pathogenic bacteria are able to adapt rapidly in the host by up-regulating expression of gene products necessary for survival in the specific environmental niche defined by the site of infection. In the human host, environments encountered by bacteria can vary widely from the external skin to mucosal surfaces of the airway or the intestine, or deeper tissues and the bloodstream. Therefore, adaptation and survival of the bacteria hinge on their ability to probe the environment and respond appropriately. *Mycobacterium tuberculosis* (Mtb) is a successful intracellular pathogen that can adapt to changing environmental conditions encountered within the host. Although, very little is known about the global regulatory mechanisms that coordinates environmental changes and intracellular gene expression in Mtb, it seems likely that signal transduction and transcriptional regulatory mechanisms contribute to the ability of the organism to be so well adapted to various changes in the environmental conditions.

As phosphate is often a limiting nutrient in various environmental niches, its import in bacteria is accomplished through several parallel transport systems. Two component systems (TCSs) PhoB-PhoR and PhoP-PhoR, in *Escherichia coli* and *Bacillus subtilis* respectively, regulate phosphate transport and govern adaptation to phosphate-limiting conditions. Also, phosphate transport apparatus was found to be critical for Mtb growth in macrophages and important for establishing persistent infection in animal models (Rengarajan *et al.*, 2005). Interestingly Mtb harbors a locus which appears to encode a TCS with significant similarity to *B. subtilis* PhoP-PhoR system. Although, these pair of genes in Mtb were annotated as *phoP/phoR* based on the sequence similarity, genes regulated by PhoP and the signal sensed by PhoR remains unclear in Mtb.

A growing body of evidence in recent years has emphasized role(s) of *phoP-phoR* TCS in Mtb growth and survival in animal and cellular models. Mtb *phoP* gene, which codes for a putative transcription regulator of the *phoP-phoR* TCS, has been shown to regulate intracellular growth of the bacteria in mouse macrophages and is likely to play a key role in the bacterial physiology and pathogenesis. A *phoP* knockout mutant of Mtb strain shows severe growth attenuation in human bone marrow-derived macrophages (Perez *et al.*, 2001, Walters *et al.*, 2006 and Asensio *et al.*, 2006). Additionally, the mutant Mtb strain lacks sulfatides, diacyltrehaloses and

polyacyltrehaloses in the cell envelope, suggesting involvement of PhoP in the regulation of virulence and complex lipid biosynthesis. Although, activation of *phoP-phoR* could not be detected under variety of stress conditions examined, global gene expression profiling indicates that more than 100 hundred genes are either up-regulated or down-regulated by PhoP in Mtb. Given the relationship that exists between the virulence-associated PhoP and Mtb growth in macrophages and animal models (Perez *et al.*, 2001, Walters *et al.*, 2006 and Asensio *et al.*, 2006) on one hand and between genes encoding phosphate transport apparatus and Mtb growth (Rengarajan *et al.*, 2005) on the other hand, we sought to investigate role of PhoP on the control of phosphate-regulated genes in the tubercle bacilli.

In order to define the functional role and regulatory capabilities of PhoP in Mtb, we asked the following questions. Whether Mtb PhoP and PhoR proteins constitute a functional signal transduction circuit? In other words, do they form a communication module based on cognate phosphorylation? Is PhoP a transcriptional regulator? Which gene(s) are regulated by virulence-associated response regulator PhoP in Mtb? Is it by direct binding to a specific sequence of single or multiple promoters? With the broad objectives to begin characterizing the contribution of the PhoP-PhoR system to Mtb physiology, a functional characterization of the *phoP* and *phoR* gene products was initiated in this study.

The thesis is divided into four chapters. Chapter 1 consists of general introduction and literature review describing Mtb as a pathogen, synergy of tuberculosis with HIV and the emergence of multidrug-resistant (MDR) and extreme-drug-resistant (XDR) strains of Mtb, in relation to its regulatory capabilities including TCSs that contribute to the survival and adaptation of Mtb as a successful pathogen. This chapter also describes phosphotransfer chemistry employed by the TCSs, their genomic distribution and their role(s) in different organisms. At the end of the chapter scope and objectives of the present study has been outlined.

Chapter 2 describes purification and molecular characterization of PhoP-PhoR TCS from Mtb H37Ra. Since, there was no information available on the molecular and biochemical characterization of *phoP-phoR*, we sought to investigate if Mtb *phoP* and *phoR* gene products encode for a functional TCS. This chapter describes over-

expression and purification of both the RR (PhoP) and HK (PhoR) proteins, their phosphorylation conditions along with identification of primary phosphorylation sites in both the proteins.

In Chapter 3 we focused our attention characterizing RR PhoP, its DNA binding properties and the effect of phosphorylation on DNA binding. Signal transduction systems function as intracellular information-processing pathways that link external stimuli to specific adaptive responses. Despite great diversity in stimuli and responses, a relatively small number of molecular strategies are used for signaling. Protein phosphorylation is one such fundamental strategy and response regulators such as PhoP, are typically found at the ends of such phosphotransfer pathways. There they function as phosphorylation-activated switches that regulate output responses. These proteins usually have a two- (or more) domain structure with a conserved N-terminal regulatory domain and a variable C-terminal effector domain(s) with a winged-helix-turn-helix DNA binding motif involved in DNA binding and transactivation via RNA polymerase. In this chapter, we show that *phoP* is transcribed via autoregulation and the basis of autoregulation i.e. the binding of PhoP to the *phoP* promoter region, was examined in great details. Further investigation of PhoP-DNA interaction by footprinting assay revealed three 9-bp direct repeat units with the consensus binding site $^{1}AC^{T}/_{G}{}^{T}/_{G}{}^{T}/_{G}P_yAP_uC^{9}$, where P_y is a pyrimidine and P_u is a purine within the PhoP protected DNaseI footprint of the *phoP* promoter region. Thus, the body of work described in this chapter clearly shows that Mtb *phoP* and *phoR* genes encode for proteins which constitutes a functional signal transduction circuit. Strikingly, PhoP has been shown as the only RR in Mtb which auto-represses its own activity. In sharp contrast all other auto-regulatory features involving RRs of Mtb have demonstrated auto-activation of its own promoter(s).

The objective of Chapter 4 was to identify sequence determinants recognized by PhoP. Furthermore, DNA binding by Mtb PhoP was dependent on orientation of the repeat subunits and the intervening spacer length separating the repeat subunits (DR1 and DR2). We also established that repression of *phoP* involves recruitment of two PhoP protomers. To complement the finding that repression of *phoP* involves

recruitment of two PhoP protomers on adjacent repeat subunits, we determined Lys141-Arg247 as the functional C-terminal domain (PhoPc) that binds to the target DNA. Evidence is presented that the N-terminal domain is not merely a regulator of phosphorylation-dependent protein-protein interactions of PhoP protomers, but is also a modulator of DNA binding functions. Towards the end we present a model that accounts for the DNA binding and protein-protein cross-linking data. Our model suggests that PhoP organizes two protomers in head-to-head orientation on adjacent repeat subunits. Together, characterization of the sequence determinants recognized by PhoP and functionality of the protein provides important insights into the mechanism of transcription regulation by the key regulator.