Summary:

The present work emphasizes on the complex regulation of virulence mechanism (s) by *Mtb* PhoP. While we introduce the subject in chapter 1, in the 2nd chapter, we focussed on understanding the molecular mechanisms by which PhoP controls ESAT-6 secretion. We observed that a) lower expression of *espACD* operon (important for ESAT-6 secretion) in Δ*phoP* and b) constitutive expression of *espACD* operon in Δ*phoP* resumed ESAT-6 secretion. Further, chromatin-immunoprecipitation and EMSA experiments identified the PhoP binding region within *espACD* promoter, in close proximity to that of EspR. Interestingly, we observed the protein-protein interactions between PhoP and EspR; we subsequently show that EspR is unable to bind to *espACD* promoter in Δ*phoP*. These results unravel the mechanism of regulation of ESX-I-dependent ESAT-6 secretion. Chapter 3 focuses on the role of PhoP in acid response. We observed Δ*phoP* was growth defective compared to wild type *Mtb* during *in vitro* acidic condition. Further we observed no induction expression of acid inducible genes including *whiB3* in Δ*phoP*. Subsequent experiments identified the direct binding of PhoP to *whiB3* promoter in a phosphorylation dependent manner. In addition, core PhoP binding sites within *whiB3* promoter was identified by DNaseI foot printing assay. Further experiments need to be performed to understand the relationship, if any, between PhoP and WhiB3 during acidic stress and how they contribute to *Mtb* survival under this condition. In Chapter 4, we extended our work to identify the sensory signal that activates the *phoPR* TCS. Previously, low [H^+] and and increased [Cl^-] were suggested as the ligands for the PhoPR TCS (Abramovitch et al., 2011; Tan et al., 2013). We constructed Δ*phoR Mtb* to identify the activating signal and understanding the role of *phoR* in *Mtb* pathogenesis. Importantly, we observed comparable cell shape, morphology and cording features of Δ*phoR* to that of Δ*phoP*. Also, we noted reproducibly lowered expression of acid inducible genes in Δ*phoR* compared to WT. However, the fact that the activation of acid inducible genes was not completely abrogated in Δ *phoR* suggests that PhoP might be phosphorylated by other regulators. Also PhoR has been shown to interact with other regulator Rv0260c. Together, we undertook an effort to investigate phosphorelay and protein-protein interactions using both PhoP and PhoR with the objective of identifying the signal activates PhoP and understand functional importance of interaction with other response regulators.