ABSTRACT

Bacteria face the challenging task of compacting their chromosomes to accommodate them in a small cytoplasmic volume and at the same time maintaining the nucleoids in a highly organized and dynamic state for transcription, DNA replication, and chromosome partitioning to take place with accuracy and speed. DNA also has to be protected from damage to preserve the genetic information. The structure and organization of the bacterial chromatin is shaped by compacting forces, such as DNA supercoiling, macromolecular crowding, and by nucleoid-associated proteins (NAPs). The NAPs are often referred to as histone-like proteins due to their functional similarity with eukaryotic histones. In *Escherichia coli,* a dozen proteins have been identified as NAPs. The well characterized members of this family are HU, H-NS, IHF, Lrp, and Fis. NAPs carry out wrapping, bridging, and bending of the bacterial DNA, resulting in its compaction and topological rearrangements. Most NAPs display broad specificity toward DNA and can thus have a global effect on gene expression. NAPs are known to influence the virulence and pathogenesis of different pathogenic bacteria. In spite of their critical role in bacterial physiology and virulence, very limited information is available on NAPs of *Mycobacterium tuberculosis* (*Mtb*). The work presented in this thesis describes the functional characterization of two important NAPs of *Mtb* namely, Rv3852 and Rv2986c (HU) and understanding their role in genome organization and topology modulation.

Chapter 1 of the thesis provides introduction on bacterial nucleoid and its architectural organization. The importance of nucleoid-associated proteins in maintenance of genome architecture and supercoiling have been discussed. Further, the functions of some of the well characterized NAPs have been described with specific examples. Finally, a brief overview of *Mtb* genome, disease epidemiology, and pathogenesis is presented along with the description of the initial studies on mycobacterial NAPs.

In **Chapter 2** studies have been directed to functionally characterize Rv3852, a NAP of *Mtb,* conserved among the pathogenic and slow growing species of mycobacteria. Data presented in this part show that the NAP binds DNA in a sequence independent manner and ectopic expression of the protein in *Mycobacterium smegmatis* cells causes spreading of the nucleoid. The protein has both DNA binding and membrane anchoring properties and is predominantly localizes in the cell membrane. The carboxyl terminal region of the protein has the propensity to form transmembrane helix which is shown to be necessary for its membrane localization. The protein is involved in genome organization and its ectopic expression in *M. smegmatis* results in defects in biofilm formation, sliding motility and change in a polar lipid profile. The study demonstrates the crucial role of Rv3852 in regulating the expression of KasA, KasB and GroEL1 proteins which are in turn involved in controlling the surface phenotypes in mycobacteria.

Chapter 3 describes the studies on an essential NAP, Rv2986c, the homologue of histone-like protein HU in *Mtb* (MtHU). HU plays an important role in maintenance of chromosomal architecture and in global regulation of DNA transactions in bacteria. The work described in this chapter reports the functional characterization of HU from *Mtb*. Although HU is essential for growth of *Mtb*, there have been no reported attempts to perturb MtHU function with small molecules. Based on the crystal structure, a core region within the MtHU-DNA interface has been identified that can be targeted using stilbene derivatives. These small molecules specifically inhibit MtHU-DNA binding, disrupt nucleoid architecture and reduce *Mtb* growth. The stilbene inhibitors induce gene expression changes in *Mtb* that resemble those induced by HU deficiency. The results indicate that HU is a potential target for development of therapeutics against tuberculosis.

The work presented in **Chapter 4** focuses on understanding the role of MtHU in maintenance of DNA topology. The topological homeostasis of bacterial chromosomes is achieved by the balance between compaction and topological organization of genomes. Two classes of proteins play major roles in chromosome organization: The NAPs and topoisomerases. The NAPs bind DNA to compact the chromosome, whereas topoisomerases catalytically remove or introduce supercoils into the genome. The data presented here demonstrates that MtHU specifically stimulates the DNA relaxation ability of mycobacterial topoisomerase I (TopoI) at lower concentrations but interferes at higher concentrations. A direct physical interaction between MtHU and TopoI is necessary for enhancing the enzyme activity both *in vitro* and *in vivo*. The interaction is between amino terminal domain of MtHU and carboxyl terminal domain of TopoI. Binding of MtHU does not affect the two catalytic trans-esterification steps but enhances the DNA strand passage, requisite for the completion of DNA relaxation, a new mechanism of regulation of topoisomerase activity. An interaction deficient mutant of MtHU is compromised in enhancing the strand passage activity. The species specific physical and functional cooperation between MtHU and TopoI may be the key to achieve DNA relaxation levels needed to maintain optimal superhelical density of mycobacterial genomes.