Synopsis

The eubacterial genome is maintained in a negatively supercoiled state which facilitates its compaction and storage in a small cellular space. Genome supercoiling can potentially influence various DNA transaction processes such as DNA replication, transcription, recombination, chromosome segregation and gene expression. Alterations in the genome supercoiling have global impact on the gene expression and cell growth. Inside the cell, the genome supercoiling is maintained judiciously by DNA topoisomerases to optimize DNA transaction processes. These enzymes solve the problems associated with the DNA topology by cutting and rejoining the DNA. Due to their essential cellular functions and global regulatory roles, DNA topoisomerases are fascinating candidates for the study of the effect of topology perturbation on a global scale. Genus Mycobacterium includes a large number of species including the well-studied *Mycobacterium smegmatis* (*Msm*) as well as various pathogens–*Mycobacterium leprae*, *Mycobacterium abscessus* and *Mycobacterium tuberculosis* (*Mtb*), the last one being the causative agent of the deadly disease Tuberculosis (TB), which claims millions of lives worldwide annually. The organism combats various stresses and alterations in its environment during the pathogenesis and virulence. During such adaptation, various metabolic pathways and transcriptional networks are reconfigured. Considering their global regulatory role, DNA topoisomerases and genome supercoiling may have an influence on the mycobacterial survival and adaptation. Biochemical studies from our laboratory have revealed several distinctive characteristics of mycobacterial DNA gyrase and topoisomerase I. DNA gyrase has been shown to be a strong decatenase apart from its characteristic supercoiling activity. Similarly, the mycobacterial topoisomerase I exhibits several distinct features such as the ability to bind both single- as well as double-stranded DNA, site specific DNA binding and absence of Zn^{2+} fingers required for DNA relaxation activity in other Type I enzymes. Although, efforts have been made to understand the biochemistry and mechanism of mycobacterial topoisomerases, in *vivo* significance and regulatory roles remain to be explored. The present study is aimed at understanding the mechanism, *in vivo* functions, regulation and genome wide distribution of mycobacterial topoisomerases.

Chapter 1 of the thesis provides introduction on DNA topology, genome supercoiling and DNA topoisomerases. The importance of genome supercoiling and its regulatory roles has been discussed. Further, the regulation of topoisomerase activity and the role in the virulence gene regulation is described. Finally, a brief overview of *Mtb* genome, disease epidemiology, and pathogenesis is presented along with the description of the work on mycobacterial topoisomerases.

In **Chapter 2,** the studies are directed to understand the DNA relaxation mechanism of mycobacterial Type IA topoisomerase which lack Zn^{2+} fingers. The N-terminal domain (NTD) of the Type IA topoisomerases harbor DNA cleavage and religation activities, but the carboxyl terminal domain (CTD) is highly diverse. Most of these enzymes contain a varied number of Zn^{2+} finger motifs in the CTD. The Zn^{2+} finger motifs were found to be essential in *Escherichia coli* TopoI but dispensable in the *Thermotoga maritima* enzyme. Although, the CTD of mycobacterial TopoI lacks Zn^{2+} fingers, it is indispensable for the DNA relaxation activity of the enzyme. The divergent CTD harbors three stretches of basic amino acids needed for the strand passage step of the reaction as demonstrated by a new assay. It is elucidated that the basic amino acids constitute an independent DNA-binding site apart from the NTD and assist the simultaneous binding of two molecules of DNA to the enzyme, as required during the strand passage step of the catalysis. It is hypothesized that the loss of Zn^{2+} fingers from the mycobacterial TopoI could be associated with Zn^{2+} export and homeostasis.

In **Chapter 3,** the studies have been carried out to understand the regulation of mycobacterial TopoI. Identification of Transcription Start Site (TSS) suggested the presence of multiple promoters which were found to be sensitive to genome supercoiling. The promoter activity was found to be specific to mycobacteria as the promoter(s) did not show activity in *E. coli.* Analysis of the putative promoter elements suggested the non-optimal spacing of the putative -35 and -10 promoter elements indicating the involvement of supercoiling for the optimal alignment during the transcription. Moreover, upon genome relaxation, the occupancy of RNA polymerase was decreased on the promoter region of *topoI* gene implicating the role of DNA topology in the Supercoiling Sensitive

Transcription (SST) of TopoI gene from mycobacteria. The involvement of intrinsic promoter elements in such regulation has been proposed.

In **Chapter 4**, the importance of TopoI for the *Mtb* growth and survival has been validated. *Mtb* contains only one Type IA topoisomerase (Rv3646c), a sole DNA relaxase in the cell, and hence a candidate drug target. To validate the essentiality of *Mtb* topoisomerase I for bacterial growth and survival, conditionally regulated strain of *topoI* in *Mtb* was generated. The conditional knockdown mutant exhibited delayed growth on agar plate and in liquid culture the growth was drastically impaired when TopoI expression was suppressed. Additionally, novobiocin and isoniazid showed enhanced inhibitory potential against the conditional mutant. Analysis of the nucleoid revealed its altered architecture upon TopoI depletion. These studies establish the essentiality of TopoI for the *Mtb* growth and open up new avenues for targeting the enzyme.

In **Chapter 5,** the influence of perturbation of TopoI activity on the *Msm* growth and physiology has been studied. Notably, *Msm* contains an additional DNA relaxation enzyme– an atypical Type II topoisomerase TopoNM. The TopoI depleted strain exhibited slow growth and drastic change in phenotypic characters. Moreover, the genome architecture was disturbed upon depletion of TopoI. Further, the proteomic and transcript analysis indicated the altered expression of the genes involved in central metabolic pathways and core DNA transaction processes in the mutant. The study suggests the importance of TopoI in the maintenance of cellular phenotype and growth characteristics of fast growing mycobacteria having additional topoisomerases.

In **Chapter 6,** the ChIP-Seq method is used to decipher the genome wide distribution of the DNA gyrase, topoisomerase I (TopoI) and RNA polymerase (RNAP). Analysis of the ChIP-Seq data revealed the genome wide distribution of topoisomerases along with RNAP. Importantly, the signals of topoisomerases and RNAP was found to be co-localized on the genome suggesting their functional association in the twin supercoiled domain model, originally proposed by J. C. Wang. Closer inspection of the occupancy profile of topoisomerases and RNAP on transcription units (TUs) revealed their co-existence

validating the topoisomerases occupancy within the twin supercoiled domains. On the genomic scale, the distribution of topoisomerases was found to be more at the *ori* domains compared to the *ter* domain which appeared to be an attribute of higher torsional stress at *ori*. The reappearance of gyrase binding at the *ter* domain (and the lack of it in the *ter* domain of *E. coli*) suggests a role for *Mtb* gyrase in the decatenation of the daughter chromosomes at the end of replication.