Synopsis

Adaptation to external environmental conditions is essential for the survival of a bacterial cell. Bacteria have thus evolved multiple mechanisms to sense environmental stimuli and to couple this information into an appropriate cellular response. The cellular response, in its simplest sense involves a change in the cellular content which is achieved by regulating gene expression. Gene expression in bacteria is primarily regulated at the first step, also referred to as transcription initiation. Extensive studies on this mechanism over the past two decades provide a molecular picture of this process. The main enzyme in this process is the DNA dependent RNA polymerase (RNAP). The RNAP enzyme however lacks a specificity factor that dictates which genes are to be selectively expressed. Selectivity is enforced by a dissociable subunit, the sigma (σ) factor. σ factors have specificity determinants that allow recognition of promoter sequences in the DNA. The sequence features on DNA include the –10 elements (Pribnow box), –35 elements, the extended -10 region, the spacing between –10 and –35 elements and the upstream sequence to –35 promoter element. σ factors recognize a few or several of these promoter sequence features thereby providing an efficient mechanism for RNAP recruitment at a specific promoter. σ factors substantially differ in sequence and structural elements. The principal σ factor of the σ70 family has several domains. This includes specific domains that interact with –10 and –35 promoter elements and the sequence between these two promoter elements. The N-terminal domain of σ factors of the σ70 family also encodes regions enabling auto-regulation of this initiation factor. A defining feature that distinguishes σ70 members from other σ factors (σ38 and σ54) is that σ70 does not require ATP for its activity. The number of σ factors in a bacterial cell varies across species. For example Streptococcus pneumonia has one σ factor, Lactococcus lactis has two, Haemophilus influenza has four while Mycobacterium tuberculosis has thirteen σ factors. The exceptions are Streptomyces coelicolor with 65and Sorangium cellulosum with 109 σ factors. The number of σ factors in a bacterial cell is suggested to be correlated with the diverse environmental conditions encountered by the bacterium and the genomic size. The focus of work reported in this thesis is on M. tuberculosis σ factors.

There are large variations in the number of σ factors in different mycobacterial species. While M. leprae has two, M. tuberculosis has thirteen σ factors. This variation in the number of σ factors has widely been believed to aid rapid signal transduction of environmental stimuli into changes in gene expression. While other transcription factors enable the recruitment of RNAP to specific promoter sequences, repressors that abrogate transcription and effectors that modulate transcription also dictate transcription levels. The intra cellular levels of a σ factor is often the primary determinant of the expression profile. A specific group of σ factors referred to as Extra Cytoplasmic Function (ECF) σ factors, govern the cellular response to specific environmental stimuli. The ECF family of σ factors has been shown to be regulated by diverse mechanisms. These include transcriptional, translational and post translational control by protein – protein interactions. The focus of this thesis was to understand the regulatory mechanism that involves σ factor interacting proteins. ECF σ factors that are governed by protein – protein interactions are often co-expressed with a regulator protein, the anti-σ factor. In several cases, they are a part of the same operon. This ensures similarity in the expression levels of σ factors and their cognate anti-σ factors. The selective dissociation of an inactive σ factor from a σ/anti-σ complex is also effected by diverse mechanisms. These include structural changes in response to environmental stimuli, conformational changes brought about by

the binding of metabolites or by targeted proteolysis of anti-σ factors. The cellular concentration of an activated σ factor is thus often subject to the rate at which it is released from an inactive complex. The notion of specific σ/anti σ factor pairs has recently been challenged with a suggestion that a σ factor could also make non-specific interactions with other anti-σ factors. This finding has implications for the widely accepted model for bacterial gene expression that relies on a cellular estimate of free active σ factors. In this model, referred to as the partitioning of σ factor space model of bacterial transcription, σ factors compete for a limited pool of apo-RNAP and recruit the enzyme to target promoter elements. Two aspects of the mechanism that governs σ factor activity were explored in the course of the studies reported in this thesis. The first is recognition of a particular stress by structurally similar anti-σ factors. This is described in the second chapter of this thesis.

The relative sensitivity of redox sensors plays an important role in providing a calibrated response to environmental stimuli and cellular homeostasis. This cellular machinery plays a crucial role in the human pathogen M. tuberculosis as it encounters diverse microenvironments in the host. The redox sensory mechanism in M. tuberculosis is governed by two component and one component systems, alongside ECF σ factors. ECF σ factors that govern the cellular response to redox stimuli are negatively regulated by forming a complex with proteins called zinc associated anti – σ factors (ZAS). ZAS proteins release their cognate σ factor in response to oxidative stress. The relative sensitivity of the ZAS sensors to redox processes dictates the concentration of free ECF σ factors in the cell. However, factors governing the redox threshold of these sensors remain unclear. The molecular characterization of three σ factor/ZAS pairs - σL/RslA, σE/RseA and σH/RshA using a combination of biochemical, biophysical and electrochemical techniques revealed the differences in redox sensitivity in these proteins despite apparent structural similarity. This finding can potentially rationalize the hierarchy in the activation of the cognate ECF σ factors under oxidative stress. Put together, the study described in chapter 2 provides a basis for examining sequence and conformational features that modulate redox sensitivity within the confinement of a conserved structural scaffold. The other aspect that was examined in the course of this study, described in chapter 3 of this thesis, was on crosstalk between σ/anti σ factors. In this study, we evaluated the affinity and specificity for σ/anti-σ factor interaction. We then analyzed the conformational determinants that enforce fidelity in σ/anti-σ interactions. The experimental data that we obtained on interactions between cognate and non-cognate σ/anti-σ pairs provide a template to evaluate tolerance between specific and non-specific interactions. The results from this analysis suggest non-cognate interactions are feasible. These interactions are likely to govern the extent to which different σ factors are activated in response to a particular environmental stimulus. It appears likely that this mechanism could provide a route to bacterial survival, perhaps allowing phenotypic diversity that help circumvent an environmental insult.

Another aspect we addressed in the course of this work was the evaluation of specific targeting of a prokaryotic transcription initiation factor. Peptide ligands of a M. tuberculosis σ factor, targeting the RNAP-σ interface were evaluated biochemically and biophysically. A sixteen residue long helical peptide from the core RNAP that could interact with σ4 region and suitably designed control peptides are validated in vitro. Consistent findings from in silico and in vitro observations validated the design strategy that can also be extended across σ factors. This study suggests that designed peptide binders can be explored as chemical tools for studying the regulation of transcription. The fifth chapter of this thesis provides a summary of the findings from the three research themes

described in this thesis. This thesis has three appendices. The first two report projects that were discontinued as they were not feasible from the perspective of structural characterization. While Appendix I reports preliminary studies on hemagglutinin-antibody complex, Appendix II describes structural studies on Escherichia coli toxin–anti toxin pairs to evaluate a conformational rationale for protein–protein interactions. Appendix III is a compilation of sequence and structural data that was used for the bioinformatics analysis presented in chapter 3. Put together the studies described in this thesis reveal exquisite adaptation strategies employed by M. tuberculosis to ensure survival under diverse micro-environments in the host.