Synopsis

RNA polymerase (RNAP) is the key enzyme in transcription and it is a multi-subunit enzyme made up of alpha, beta, beta prime and omega subunit in the stoichiometry of $\alpha_2\beta\beta\omega$. Except for the smallest subunit ω (*rpoZ*), all the subunits are essential for the cell survival. No clear phenotype was observed for the Δ*rpoZ* strain of *E. coli* for many years. Recently we isolated several dominant negtaive mutants of ω. These ω mutants were found to be structured as compared to the native ω which is unstructured. Mutant RNAP with the structured ω was found to be defective at the initiation step of transcription. This study showed the structural importance of ω subunit. Also, ω is linked to stringent response and its role is associated with key players of the stringent response i.e. ppGpp and protein DksA. ppGpp and DksA have been extensively studied with respect to the role played by them in cell survival under the stress. DksA and ppGpp show a more pronounced effect *in vivo* as compared to that of *in vitro*. ω has been found to be involved in binding of sigma factors and ppGpp to RNAP and its role has been evaluated in the present study in a more detailed manner. Our studies revealed both the structural and functional role of ω. The functional role of ω in stress response and its role in the distribution of RNAP across the *E. coli* genome has been studied. The importance of the unstructured ω in maintaining the catalytic activity of RNAP has been analysed. Also, the importance of flexible ω in ppGpp and σ factors binding to RNAP has been deciphered.

Chapter 1 gives a brief introduction about the functional modulation of RNA polymerase. Transcription modulators which interact with RNA polymerase to orchestrate the transcription of genes are discussed.

Chapter 2 presents our findings on the functional and structural role of ω subunit in the interaction of ppGpp to RNAP and its physiological importance in *E. coli*.

Chapter 3 documents the assembly of the wild type ω and its dominant negative variant, ω_6 with reconstituted RNAP (core1: $\alpha_2\beta\beta'$). Subsequently, the interaction of σ-factors with reconstituted RNAP (core2: $\alpha_2\beta\beta'\omega$; mutated core2: $\alpha_2\beta\beta'\omega_6$) has been described.

Chapter 4 provides a broader perspective of the role played by ω in transcriptional machinery by looking into the gene selection pattern of ω-less RNA polymerase. Growth phenotype of ω deleted strain with various carbon substrates and its tolerance to different environmental stress like osmotic stress, pH and antibiotic, using phenotype microarray has been examined.

Chapter 5 summarises the work that has been documented in this thesis.

Appendix-

Chapter 6 describes the co-immunoprecipitation studies which were done to analyse the binding profile of RNA polymerase in Δ*rpoZ* and Δ*dksA* strains. Phenotypic microarray and promoter activity assay were done to analyse the correlation of these factors *in vivo.*

Appendix-

Chapter 7 describes the differential role played by ω subunit in Gram-positive and Gram-negative bacteria.