SYNOPSIS

The bacterial transcription machinery requires certain specific proteins, called the sigma factors, to reversibly associate with the core RNA polymerase. Though sigma factors are clearly responsible for promoter recognition by RNA polymerase, multiple switches seem to ensure that the free sigma factors do not bind to the promoter DNA, the exact mechanism of which remains unclear. In our study we have chosen the *M. tuberculosis* alternative stress response sigma factor SigF for structural studies.

The treatment of proteins with different denaturing agents, usually alter the secondary structure of proteins revealing information about their folding/unfolding kinetics and the occurrence and stability of folding intermediates. Two well known denaturants guanidinium hydrochloride and urea show stabilizing effects on SigF secondary structure, at concentrations up to 2 M, and denaturing effects at higher concentrations. The polar kosmotrope ammonium sulfate which reversibly precipitates proteins without altering their structure, surprisingly, was found to destabilize the secondary structure of SigF. The osmolyte betaine was found to have no effect on the protein secondary structure. Another well known stabilizer, the amino acid arginine, was also found to have no effect on our protein. It is thus evident that SigF behaves unusually towards classical protein secondary structure stabilizers and destabilizers, reflecting that the protein exists in complex conformational states.

In $\sigma^F$ of *M. tuberculosis*, emission spectra and fluorescence resonance energy transfer between sole tryptophan at position 112 and probes placed in different regions suggest a compact conformation for a major part of the N-terminal half. Fluorescence anisotropy measurements suggest significant flexibility in the C-terminal half and the extreme N-
terminal region. The compactness of the N-terminal region was further supported by acrylamide quenching data of the single tryptophan. It was concluded that the free sigma factors may be in equilibrium between two conformations; one pre-dominantly compact one, in which the promoter interacting motifs are trapped in wrong conformation and another minor one with a more open conformation in which the C-terminal half is away from the N-terminal domain(s) and is more solvent exposed and perhaps partially disordered. Implications of these conformations for function of sigma factors have been discussed.

The distribution of various transcription factors over the entire bacterial genome when they are not at their target sites, or whether their location on the genome plays a role in finding their target site is not clearly understood. The Gal repressor-operator system of *E. coli* was chosen for studying binding interactions of each of the possible single base operator mutants with the repressor protein *in vitro* using fluorescence anisotropy measurements. Single base pair operator mutations only at a few positions seem to keep the binding energy largely unchanged from the wild type operator while most positions of the operator are important for the interaction. From the energy values thus obtained, the total binding energy difference between any arbitrary sequence and the specific operator sequence could be estimated provided there is additivity. Using these binding energy results a program was used to find the propensity of this repressor for occupancy of all the 16-basepair sites on the genome. We conclude that very tight non-specific interaction of the protein with the bacterial genome is not favored energetically and there are no clusters of preferred energy sites in the genome where the protein can be found to be concentrated.