Title of Thesis: “Structural and functional studies on the transcriptional activator FlrC, implicated in flagellar synthesis and colonization of Vibrio cholerae

Abstract:

Transcriptional regulation of the flagellar system of Vibrio cholerae acts as an important regulating factor that assists in colonization. The flagellar synthesis in V.cholerae is regulated by σ^{54}-dependent transcriptional activator FlrC. It comprises of an N-terminal regulatory (R) domain, central AAA^+/σ^{54} interaction domain and C-terminal DNA binding domain and binds the enhancer elements located downstream of the transcription start sites of the promoters.

To understand the molecular basis of the oligomeric states and the functional regulations, we determined the crystal structures of the central AAA^+ domain (FlrC^C) in nucleotide (Nt) free and AMP.PNP bound states. Our results provide the first structural evidence for an AAA^+ ATPase implicated in flagellar synthesis that forms heptamer both in Nt-free and bound states without any Nt dependent subunit remodeling. A novel cis-mediated ‘all or none’ ATP binding occurs in the heptameric FlrC^C. A unique ‘closed to open’ movement of Walker A motif, assisted by trans-acting ‘Glu-switch’ E286, facilitates ATP binding and hydrolysis. Fluorescence quenching and ATPase assays on FlrC^C and mutants revealed that while R349 of sensor II, positioned by trans-acting E286 and Y290, acts as a key residue to bind and hydrolyse ATP, R319 of a7 anchors ribose and controls the rate of ATP hydrolysis by retarding the expulsion of ADP. Heptameric state of FlrC is restored in solution. Structural results and pulldown assays indicated that σ^{54}-FlrC interaction independent of Nt binding. Fluorescence quenching studies have established high binding affinity (K_d = 0.21 pmole) between FlrC and the downstream enhancer element of flaA promoter with 1:1 stoichiometric ratio. We have also investigated the influence of the promoter DNA on the interactions of FlrC with σ^{54} and the results showed that although FlrC has capability to recognize σ^{54} even in the absence of ATP, the interactions between these two becomes strong and sustainable only in the presence of specific downstream promoter DNA fragment.

Collectively, our results underscore a novel mechanism of ATP binding and σ^{54} interaction that strives to understand the transcriptional mechanism of the bEBPs, which probably interact directly with the RNA polymerase-σ^{54} complex without DNA looping.