SUMMARY

In this study, CysB from *Salmonella typhimurium* has been purified to homogeneity and is characterized using spectroscopic, structural and biochemical approaches. The protocol for full length CysB purification was standardized to isolate tetrameric protein. The purified CysB tetramer was confirmed to be stable and properly folded in solution. While attempting to crystallize full length CysB, we crystallized the CysB-LBD which exists as a dimer in solution. In order to understand the mechanism of ligand binding, we resolved crystal structures of CysB-LBD in apo as well as in complex with three different ligands i.e. sulphate, OAS, and NAS. The most important findings obtained from crystal structures are that CysB has two distinct ligand binding sites and these are allosterically coupled. First, the canonical, inter-domain ligand binding site is shared by sulphate and NAS. Second, the novel secondary binding site, identified in this study from first crystal structures of CysB with ligands, is shared by NAS and OAS. Interestingly, specific binding of OAS in the ligand induced secondary binding site remodels the primary active site to accommodate NAS and active release of sulphate easily. This induced-fit mechanism of NAS facilitates CysB to overcome the rate limiting step of primary active site dynamics and allows us to propose a mechanism of orthogonal DNA binding activity for CysB.

In pursuit of our interest, we characterized CysB-DNA interactions with its own promoter DNA and the results suggest that one tetramer of CysB binds to one ds DNA in 4:1 molar ratio of CysB: ds DNA. The equilibrium dissociation constant for complex formation is estimated to be ~ 1 μM. Systematic standardization was done to obtain CysB-DNA complex crystals. Results of agarose gel electrophoresis and partial refinement of X-ray diffraction data confirmed the presence of DNA in the CysB-DNA complex crystals. Although increased cell volume and extra electron densities clearly indicate the presence of DNA in the unit cell and shifting of symmetry

related dimers suggests DNA binding induced conformational changes. However, further phasing experiments are required for unambiguous tracing of N-terminal DNA binding domain and the complete DNA molecule to make correct model. While investigating the effect of ligand binding on CysB-DNA interaction, we show that CysB-DNA complex dissociates in the presence of both NAS and OAS, however NAS mediated dissociation follows simple, non-cooperative type, as expected for 1:1 binding and dissociation, and the effective dissociative constant of NAS, $K_{I, NAS} \sim 7$ mM. On the contrary, OAS mediated dissociation of CysB-DNA complex is cooperative and the effective dissociative constant of OAS, $K_{I, OAS} \sim 16$ mM. nterestingly, the CysB-DNA complex dissociates more efficiently in the presence of both NAS and OAS. In the presence of fixed amount of OAS, the effective dissociative constant of NAS lecreases to ~ 1-3 mM, demonstrating functional role for OAS. These results are consistent with our structural observations which reveal that binding of OAS to a helper, secondary site which open up the primary site to facilitate NAS binding. Thus the two ligands, OAS and NAS, act synergistically to enhance the activity of CysB. Additionally mutagenesis studies of primary binding site mutants, T100, T102 and E150 reveal altered DNA binding patterns suggesting lifferential modes of recognition. However interestingly, E150 mutant behaves as ligand non-esponsive mutant to NAS but not OAS. This corroborates well with the structural analysis of CysB-LBD-apo and ligand bound forms which suggest that E150 may act as a gate to regulate igand exchange through primary binding site.

Functional studies on *cysB* suggest its role in growth and survival of *Salmonella typhimurium*. Single gene knockout of *cysB* in *Salmonella typhimurium* LT2 result in delayed growth rate of the bacterium and presence of longer Δ*cysB Salmonella* cells suggest incomplete

segregation.
invasion but
in number of
type. Irregul
properties of
processes in S

In sun
state, structur
coupling mec
infection of n
mechanisms o

gregation. *Ex vivo* infection studies in murine macrophages (RAW264.7) show efficient ·asion but attenuation in intracellular replication. This is primarily due to significant increase number of macrophages engaged in internalizing the Δ*cysB Salmonella* as compared to wild e. Irregular colony morphology and altered lipid profiles suggest changes in membrane perties of Δ*cysB Salmonella*. Thus we show here that *cysB* controls the fundamental cesses in *Salmonella* which are responsible for it survival and pathogenicity.

In summary, this is the first study that presents a detailed characterization of assembly e, structure and mechanism of ligand induced conformational changes, a novel of orthogonal pling mechanisms in transcription, and physiological importance of *cysB* gene in growth and ction of macrophages. In addition, the study opens a new avenue to investigate the novel hanisms of gene regulation using *in vivo* and *in vitro* approaches.

HNOLOGY
ok beyond
ges of the
ring off or
-wise da-
nce which
at the time
on, if any
ld strictly
and shall