PREFACE

Mycobacterium tuberculosis is a highly successful pathogen primarily because of its ability to persist in the human host for many years in a latent/persistence state avoiding the host immune system. Our lab has targeted many proteins that are up-regulated in *in vitro* models designed to mimic tuberculosis persistence/latency. A combination of biochemistry, molecular biology, X-ray crystallography and *in silico* tools are used to understand the structure and function of the proteins. The present study mainly involves the structural and functional analysis of MtbLrp (Rv3291c), a transcription regulator that belongs to Lrp/AsnC family. Additionally, the preliminary characterization of a 1-4, α-glucan branching enzyme, (Rv1326c/GlgB), is also reported in a separate chapter.

When the work was initiated, *Rv3291c* had been tentatively annotated in the databases as a member of the Lrp/AsnC family of transcriptional regulators. No crystal structures of the protein from a bacterial source were available although a couple of structures of its archaeal homologs were there. The work was initiated with a sequence analysis followed by cloning, expression, purification and crystallization of the gene product. Subsequently a mutational analysis of the protein was also carried out.

GlgB is an enzyme that catalyzes the third step of the biosynthesis of glycogen and catalyzes the cleavage of an α-(1,4)-glucosidic linkage. It subsequently transfers the cleaved oligosaccharide to form a new α-(1,6) linkage. We started our work with cloning and purification of the enzyme. Experiments were conducted to probe the mechanistic aspects and the present studies have formed the basis for other ongoing studies in the lab.

The thesis is divided into six chapters whose contents are briefly described below:-

Chapter 1 introduces the reader to the Lrp/AsnC family of transcriptional regulators that are also known as Feast/famine regulatory proteins

Chapter 2 covers the various techniques and experimental approaches used to clone, purify and characterize the proteins/peptides in the present work. It also deals with the methods used to solve the structure of the MtbLrp, its complexes and mutational analysis.

Chapter 3 The results obtained while cloning, purifying and characterization of the protein are detailed here. The preparation of the binding site mutants is also reported here.

Chapter 4 deals with the crystallization of wild type MtbLrp. The structure solution and analysis of the protein structure is also reported here.

Chapter 5 deals with the identification of the ligand/effector binding sites as also the crystallization and structure solution of the MtbLrp-complexes. The functional implications of the effector binding to the two binding sites of the protein are explained against the backdrop of the novel results involving the protein-amino acid complexes. The results of the mutational analysis are also detailed and rationalised.

Chapter 6 deals with the cloning, purification and homology modelling of glycogen branching enzyme from *M. tuberculosis*. Effect of chemical and thermal denaturation along with conservation of catalytic residues is reported here.

A part of the results of this thesis have already been reported in the following publications:

- Cloning, expression, purification and crystallization of a transcriptional regulatory protein (Rv3291c) from *Mycobacterium tuberculosis H37Rv*.
  Tripti Shrivastava, Sandeep Kumar Srivastava, and Ravishankar Ramachandran
  Acta Cryst. Sect D Biological crystallography D60, 1874 - 1762004.

- Mechanistic insights from the crystal structures of a feast/famine regulatory protein from *Mycobacterium tuberculosis H37Rv*.
  Tripti Shrivastava and Ravishankar Ramachandran
  Nucleic Acids Research 35, 7324-35, 2007.

Co-ordinates submitted to the Protein Data Bank (http://www.rcsb.org)

2IVM	MtbLrp
2VBW	MtbLrp complex with Phenylalanine
2VBX	MtbLrp complex with Histidine
2VBY	MtbLrp complex with Tyrosine
2VBZ	MtbLrp complex with Tryptophan
2VC0	MtbLrp complex with Leucine
2VC1	MtbLrp complex with Metheonine