# CHAPTER 41

# MICROBIOLOGY

## Doctoral Theses

01. DASHORA (Vishal)
**Towards identifying cis-acting and trans-acting elements regulating Leishmania donovani transcription.**
Supervisor: Prof. Swati Saha
Th 28728

*Abstract*

Transcription in Leishmania is atypical, where functionally unrelated genes are arranged into unidirectional polycistronic transcription units that are coordinately transcribed from transcription start regions (TSRs). Due to the lack of recognizable orthologs of cis- and trans-acting factors, this polycistronic mode of transcription is believed to be constitutive. Challenging this belief, a second tier of transcriptional activation in L. donovani was identified by our laboratory, where a small subset of genes was getting activated in a cell cycle-dependent manner by regions lying immediately upstream of the gene. Building on this knowledge, the first part of the study focused on investigating probable TSRs within the polycistronic gene clusters of L. donovani Chromosome 2. Nuclear run-on assays using synchronized cells revealed that five genes were getting hyperactivated at specific cell cycle stages. The upstream regions of three genes were able to drive eGFP reporter gene expression. Deletion mutant analyses localized the probable TSRs within the ~225bp region immediately upstream of two candidate genes. The second section of the study focused on identifying proteins involved in modulating L. donovani transcription. By carrying out immunoprecipitations with LdHAT1 protein as bait, coupled with mass-spectrometry, Ld320060 was selected to analyze its role in transcription. Ld320060 was annotated as 'LdEpl1' on the basis of its structural similarity with yeast Epl1 protein and essentiality of epl1 gene was confirmed by using homologous recombination. Depletion of one epl1 allele led to modest growth defects which were partially restored by LdEpl1 episomal expression. epl1 mutants were found to be more vulnerable to genotoxic stress agents when compared to wildtype. LdEpl1 depletion did not impact parasite's ability to infect host macrophages but significantly compromised its ability to survive within the macrophages, suggesting that LdEpl1 confers a protective effect on the parasite's response to oxidative stress.

*Contents*

1. Introduction and Review of Literature 2. Identification of probable transcription start regions lying internally within polycistronic gene clusters across L. donovani chromosome 3. To identify proteins involved in modulating L. donovani transcription 4. Summary. Bibliography and Appendices.

02. GAUR (Sharad Kumar)
**Structural and Functional Analysis of Peste Des Petits Ruminants Virus (PPRV) Coded Transmembrane Proteins Using Biophysical and In-Silico Approaches.**
Supervisor: Prof. Rajeev Kaul
Th 28398

*Abstract*

Livestocks contribute immensely to the economy of a country and there are innumberable viruses that are known to cause serious illness in these animals. One such disease is goat plague also known as Peste des petits ruminants disease, caused by PPR virus. According to Baltimore classification of viruses, PPR virus belongs to group V owing to its peculiarity of having a negative sense ssRNA genome. Virion particle is enveloped and the genome codes for six structural proteins, out of which two are transmembrane proteins namely Hemagglutinin (H) and Fusion (F) proteins. Being an important structural protein, host generates an effective humoral immune response against these surface glycoproteins. The structural characterization of such immunodominant protiens would aid in the development of effective DIVA vaccines that would be crucial for effective eradication of this disease. Moreover, structural analysis of these surface glycoproteins can also be used to understand the pathogenesis of this virus. The current study focuses on biophysical and functional characterization of PPRV Hemagglutinin and Fusion protein using various in-silico and biophysical approaches. Computational tools like PsiPred and SOPMA were used to estimate secondary structures of the proteins and were confirmed using Circular dichroism. Both the viral proteins were modelled using Swiss Modeller and their structural conservation was studied. The epitopes against the PPRV-F protein were identified using the immune epitope database. To fish out the cellular interacting partners, a pulldown assay was also performed disclosing that PPRV-F ectodomain interacts with various host proteins which might modulate host immune responses.

*Contents*

1. Introduction 2. Review of Literature 3. Biophysical characterization of PPRV coded hemagglutinin using in-silico approaches 4. To deduce the structural and functional properties of PPRV coded fusion protein using various approaches 5. Discussion. 6. Conclusions and future prospects 7. Bibliography.

03. SINGH (Ashish)
**Identification of Cell Cycle Stage-Specific Transcriptional Start Regions (TSRs) in Leishmania Donovani.**
Supervisor: Prof. Swati Saha
Th 28399

*Abstract*

Leishmania spp belonging to the family Trypanosomatidae, are responsible for causing a spectrum of diseases called leishmaniases, manifested in three major forms – cutaneous, mucocutaneous and visceral leishmaniasis (VL). In Indian subcontinent, VL or "kala-azar" is the most prevalent form of the disease caused by L. donovani. These parasite does not comply with the typical paradigm of eukaryotic gene transcription. Functionally unrelated genes are organized in large unidirectional clusters which are co-ordinately transcribed as long polycistronic units, typically initiating from divergent strand switch regions (dSSRs), constitutively. Recently, a second tier of transcriptional activation regulated in a cell cycle stage-specific manner was unearthed by our laboratory. Based on this finding, we tried to examine this cell cycle stage-specific transcriptional activation of genes at a chromosome-wide level across chromosome 14. Nuclear run-on assays with cells synchronised at early S, mid S, G2/M and G1 phases, identified eleven genes that were getting transcriptionally activated at specific cell cycle stages. Reporter analyses with ~1.5kb upstream regions (URs) of these activated genes exhibited promoter

activity. Detailed analyses of two candidate URs identified the probable transcriptional start regions (TSRs) within the ~500 bp stretches of URs, with critical elements lying between 250 and 500 bp upstream of the gene start codons. In the second part of the study, we analysed the role of an identified trans-acting protein - LdEaf6 in regulating transcription in L. donovani. Genomic replacement of two of the three eaf6 alleles by homologous recombination revealed the gene to be essential for survival with its depletion resulting in slower parasite growth, longer generation time, protracted progression across G1 phase and poor survival within the host macrophages. RNA Seq analyses suggested that depletion of eaf6 does not have a direct impact on global gene expression, with only a small subset of genes getting differentially regulated.

*Contents*

1. Introduction and Review of Literature 2. Investigating cell cycle stage-specific transcriptional activation of genes across chromosome 14 in leishmania Donovani 3. Investigating the possible involvement of an Identified transacting factor in transcription regulation in Leishmania Donovani 4. Summary. Bibliography and Appendices.