REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR-IF : **Dr. Radheshyam Maurya,** Assistant Professor

2. Address:

   Department of Animal Biology, School of Life Sciences, **University of Hyderabad**, Hyderabad-500046, TS.

3. Frontline area of research in which training/research was carried out:

   Epigenetic regulation in *Leishmania donovani*; A comparative studies of histone protein modification in different stages of promastigotes parasite of *Leishmanis donovani*.

4. Name and address of Professor and host Institute:

   Dr. David Sacks, Intracellular Parasite Biology Section, LPD, NIAID, NIH, Bethesda, Maryland 20892.

5. Duration of Fellowship with exact date : 6 Months (28 Jan 2020 to 17 July 2020)

6. **Highlights of work conducted:**

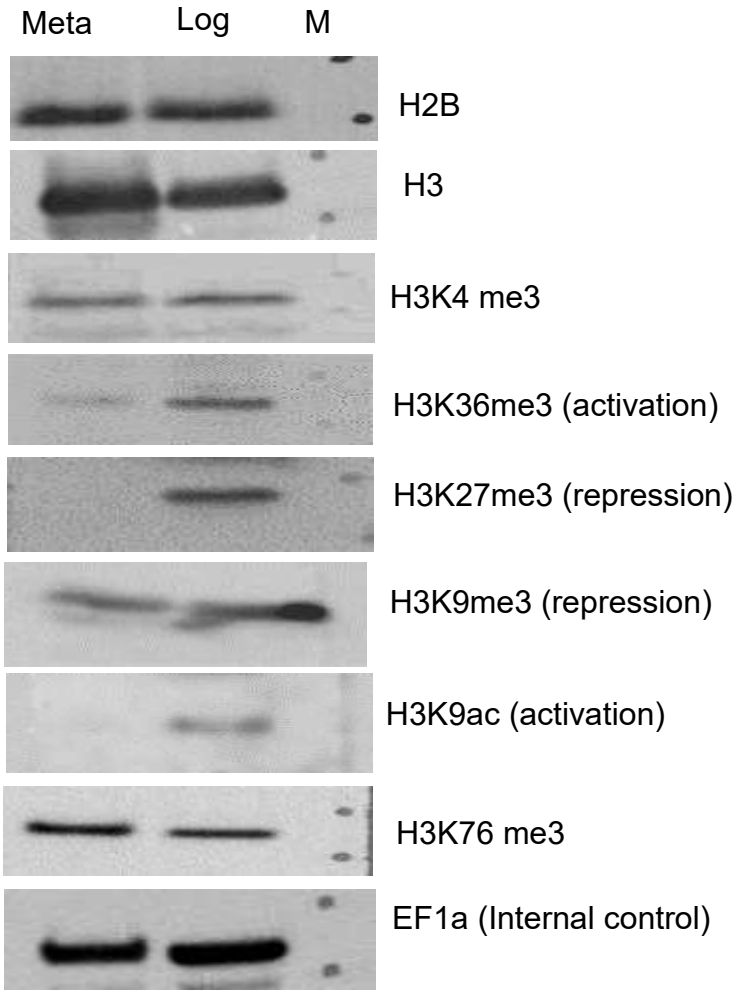
   i) Technique/expertise acquired:

The mechanism of cell programming is often involved epigenetic changes by chromatin remodeling, histone modifications, DNA methylation, etc. leading to cellular gene expression for normal development and cellular differentiation. Epigenetic modification is crucial for cell commitment and fate. A precise expression of lineage-specific genes is essential for the proliferation and differentiation of normal cell development. The aberrant transcriptional regulation can result in malignant transformation and eternal progression. Transcription factors are tightly coordinated with DNA and histone proteins to form chromatin structure. Epigenetics concerns are not mutations in DNA but rather alter the chromatin structure by making genes more or less accessible while also recruiting proteins that alter the chromatin structure and promote transcription. Does parasite adopt epigenetic strategies to manage their survival in two different hosts as a digenetic life cycle?

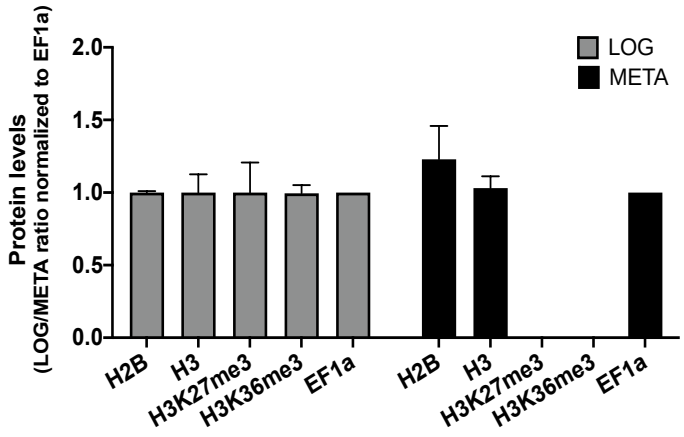
Therefore, in these studies we have analysed the several histone modifications (H2B, H3, H3K4me3, H3K9me3, H3K9ac, H3K36me3, H3K27me3, H3K76me3 and eF1a internal control) in two different stage of promastigotes parasites of *Leishmania donovani* by western

blot. The preliminary results of western blot **(Figure 1A and B)** showed a significant change in histone modification in the following histone proteins:

1A) Western blot analysis of various histone modification*:* Meta: Infective Metacyclic promastigote and Log: Procyclic promastigote of Leishmania donovani



1B) Histone Protein ratio after normalization with EF1a internal control



Trimethylization of H3K36 histone protein

The trimethylation of lysine 36 on histone 3 (H3K36me3) is an abundant and highly conserved chromatin modification that is enriched at gene bodies of transcriptionally active genes and also at centromeric regions as well as associated with open chromatin. The presence and distribution of H3K36me3 domains at actively transcribed genes are conserved from human to yeast, suggesting that this mark is extremely important for proper cellular function. Important regulatory activities linked to the H3K36me3 mark include transcription elongation, prevention of cryptic start sites, as well as pre-mRNA splicing and processing. In addition to transcription, H3K36me3 also plays a vital role in recruiting DNA repair machinery to mismatch regions. Actively transcribed genes pre-marked with H3K36me3 are specially protected from DNA damage.

Trimethylization of H3K27 histone protein

Trimethylation at lysine-27 of histone H3 (H3K27) is mediated by Polycomb repressive complex 2 (PRC2) and associated with transcriptional repression. The distribution of H3K27me3 throughout the genome has been analysed by deep chromatin immunoprecipitation sequencing (ChIP-seq) revealing an inverse relation between H3K27me3 level and transcriptional activity for various subsets of genes, such as encoding Hox proteins, cell-cycle regulators, and transcription factors. It is suggested that H3K27me3 plays a crucial role in transcriptional repression, though it remains unclear how genomic regions covered with H3K27me3 are specified and regulated.

Trimethylization of H3K9 histone protein

The trimethylation of (H3K9) is an epigenetic modification in the DNA packaging protein of Histone H3 protein. H3K9me3 indicates that tri-methylation at the 9th lysine residue of the histone H3 protein and is often associated with heterochromatin. Heterochromatin is the condensed, transcriptionally inactive state of chromatin. It can be facultative or constitutive. Heterochromatin marked with H3K9me3 has a pivotal role in embryonic stem cell development during lineage commitment and also a role in normal cellular growth. H3K9me3 is also acting as a repressor of inappropriate lineage genes expression and maintaining early cell integrity and genomic stability.

Acetylation of H3K9 histone protein

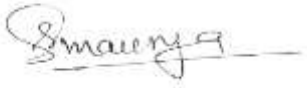
The acetylation of histone complexes (H3K9) is another epigenetic modification in the DNA packaging protein Histone H3. H3K9ac is a mark indicating the acetylation at the 9th lysine residue of the histone H3 protein. The H3K9 histone has two jobs. Genes get turned on if this mark is acetylated and are silenced them if this mark is methylated. H3K9ac is an important acetylation connected with active promoters of particular genes. H3K9ac also has a high co-occurrence with H3K14ac and H3K4me3 which together are the hallmarks of active gene promoters.

ii) Research results, including any papers prepared/submitted for publication:

Overall, we observed among 8 histone proteins 4 were transcriptionally active in the log phase compared to the infectious metacyclic phase (late stage of the parasite), suggesting their role is silent in the metacyclic stage parasites. Further studies are required to understand the functions of these modification in the parasite stage differentiation.

iii) Proposed utilization of the experience in India :

Although the training was halted due to COVId-19 Pandemic, we have defined the proof of concept by analysing the histone protein modification in two different stages of promastigotes parasites. We analysed several histone proteins by western blot and found that many histones are silent or acetylated in one or other stages of parasite development. Moreover, the emergence of drug-resistant Leishmania parasites has been one of the major failures in treatment, prevention and eradication of leishmaniasis in endemic areas. Therefore, the utilization of such training will help to design a new research proposal to understand epigenetic regulation in drug resistant leishmania parasites, which is an important challenge to control leishmaniasis.



Signature of ICMR-IF