Synopsis

The epigenotype of a genetic locus is mediated by modifications including DNA methylation and histone modifications. In mammals, DNA methylation takes place at cytosines predominantly in the CpG (Bird, 2002) and to some extent at the CpH dinucleotides (Lister et al., 2009). As epigenetic modifications are known to modulate the expression profile of a cell in accordance with the environmental cues (Jaenisch and Bird, 2003), it is possible that pathogenic organisms might target this host machinery for a successful infection. A few studies have emerged in the recent past that support this contention. For example, maternal oral infection caused by C. rectus or P. gingivalis has been found to lead to hypermethylation in the promoter region of the imprinted Igf2 gene (Bobetsis et al., 2007). DNA methylation changes have been observed in response to the protozoan Leishmania donovani infection in human macrophages (Marr et al., 2014). The mechanisms highlighted here mostly involve indirect interaction of pathogen’s factors with the host epigenome. Bacterial proteins like RomA of Legionella pneumophila (Rolando et al., 2013) and NuE of Chlamydia trichomatis (Pennini et al., 2010) have been shown to directly modify the histone H3 at lysine14 while proteins like LLO of Listeria monocytogenes (Hamon et al., 2007) are known to induce a signaling cascade that eventually alters the chromatin. The AnkA protein of Anaplasma phagocytophilum was found to repress transcription of CYBB gene in myeloid cells by binding to the AT-rich promoter and recruiting HDAC1 (Garcia-Garcia et al., 2009). Existence of histone mimics (influenza viral protein NS1) has also been reported (Marazzi et al., 2012).
A direct approach to target the host methylome by a pathogen’s protein would mean that the protein should have the potential to be secreted by the pathogen, the ability to localize to the host nucleus and interact with the chromatin remodelling machinery. Further, it may possess either a DNA/RNA methylation activity or histone modification activity.

In an effort to decipher the epigenetic dynamics of a *Mycobacterium tuberculosis* infected macrophage methylome, this thesis reports the identification of a novel cytosine DNA methyltransferase from *mycobacterium* that plays an important role in modulating the host epigenetic response.

Chapter I, ‘Environment and Epigenetics’, introduces the concept of the epigenetic interface in the interaction of a host cell with the environment and elaborates on the literature that have examined this interface during host-pathogen interaction. Recent evidences of epigenomic dynamics in response to environmental cues have been mentioned with a special emphasis on studies pertaining to epigenetic changes in response to a pathogenic insult. DNA methylation being the crux of the present study, the chapter starts by examining the functional implications and effector proteins of DNA methylation have been discussed.

Chapter II, ‘Cloning and screening for mycobacterium methyltransferases’, describes the initial efforts in identification of a mycobacterial protein as a candidate effector of changes in host methylome changes has been discussed. The screening began with a list of 30 putative methyltransferases from mycobacterium and concluded with the identification of three putative
DNA cytosine methyltransferases. One of the proteins, Rv2966c, was taken up for characterization.

This study for the first time reports a cytosine methyltransferase from *Mycobacterium tuberculosis*. Chapter III, ‘Characterization of mycobacterial protein, Rv2966c, as a candidate methyltransferase’, describes the efforts towards characterization of this novel protein and established Rv2966c to be a secretory protein that can methylate cytosine in a non-CpG dinucleotide context. Interestingly, the results also show that the enzymatic activity of Rv2966c but not its secretion, was influenced by post-translational modifications.

In chapter IV, ‘Interaction of Rv2966c with the host epigenome’, the functional consequences of the interaction of Rv2966c with the host epigenome have been examined. The nuclear localization potential of Rv2966c was found to be dependent on its C-terminal residues. Not only did we find that Rv2966c binds to specific regions of the THP1 genome *in vitro* and *in vivo* but also showed interaction with histone H3 and H4. The interaction of Rv2966c with the host epigenome was found to be dictated by the presence of specific histone marks. Analysis also showed DNA hypermethylation to be in the non-CpG context that lead to repression of the hypermethylated gene upon infection with *M. tuberculosis*.

Having established that Rv2966c can modulate the host methylome, it was imperative to examine if the host macrophages indeed undergo DNA
methylation changes upon infection with *Mycobacterium tuberculosis*. Chapter V, ‘Modification of host macrophage methylome upon infection with *M. tuberculosis*’, describes these DNA methylation changes in the infected THP1 macrophages. Majority of the regions showing methylation changes were hypermethylated and less than 17% were hypomethylated. Hypermethylated regions were concentrated in specific hotspots that predominantly localized on chromosome 1 and chromosome 6. A novel motif enriched within these DMRs was also identified. Several genetic loci affected by these methylation changes contained genes involved in cell signaling, immune response and phagosome maturation, metabolism, miRNA processing, membrane trafficking and transport, cell cycle and cell death. Several ncRNA genes (miRNA, lncRNA, piRNA, snoRNA) among others were also targets of differential DNA methylation. Importantly, methylation changes analysed by bisulfite sequencing were found to be in the non-CpG dinucleotide context. Based on our finding that the *M. tuberculosis* cytosine methyltransferase Rv2966c methylates the host DNA in infected THP1 macrophages, preliminary results show that these DMRs could be a cumulative effect of host and pathogen protein activities.

The last chapter, ‘Discussion: The road ahead’, discusses the relevance of the host DNA methylation by Rv2966c with respect to manipulation of the host cellular machinery. Also discussed are the implications of observed global reprogramming of host methylome in response to *Mycobacterium tuberculosis* infection.
References


