Chapter 3

PREMISE: AIMS & OBJECTIVES
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Experimental Approach of the present Study:

Infectious disease is the result of an intimate relationship between the pathogen and host, which involves complex cross-talk. Understanding this complex cross-talk between host and pathogen is essential to improve our understanding of infectious disease. Genome sequencing projects and the advent of global approaches such as proteomics and transcriptional profiling have opened up exciting new possibilities and led to the creation of a new field of biology - functional genomics and proteomics. *Mycobacterium tuberculosis* can infect a variety of cell types and establishes a persistent infection. Comprehensive profiling of the transcriptional and translational status of host and pathogen genes can be exploited in several ways. DNA microarray technology has made it possible to analyze the *M. tuberculosis* global transcriptional response to different stimuli. Experiments have been carried out in broth culture, using conditions that may mimic the macrophage environment (i.e., low pH, cell wall stress, starvation, hypoxia, heat shock, etc.) in resting or activated mouse macrophages, and in vivo, using the mouse lung model of infection. The complete gene expression profile of *M. tuberculosis* growing in mouse macrophages was defined by Schnappinger *et al.*, (2003) and by Rachman *et al.*, (2006). More recently Patricia Fontan *et al.*, (2008), found that 585 genes expressed differentially by intracellular *M. tuberculosis*. Recently, Ludovic and colleagues (2008) have shown the extraordinary plasticity of host cell and pathogen responses to infection, and provide a solid framework to further understand the complex mechanisms involved in immunity to *M. tuberculosis* and in mycobacterial adaptation to different intracellular environments (Tailleux *et al.*, 2008). Most recently two studies by Kumar *et al.*, 2010, and Tobin *et al.*, 2010, where they used complementary genetic approaches to uncover critical host factors that control the response of macrophages when they came into contact with mycobacteria. Specifically, these studies identified a collection of macrophage genes that alter the capacity of mycobacteria to replicate and survive inside the host during the initial stages of infection.

However, very few of the proteome studies have analyzed protein modifications induced by Mycobacterial pathogens in the host cell. This might be due to the high complexity of host cells and the relatively low sensitivity and resolution of common
proteome techniques. Quantitative proteomics, employing stable isotope labeling and high resolution mass spectrometry has gained success in deciphering diverse biological processes due to its high level of coverage proteome, accurate quantification and high through put platforms (Ong and Mann, 2005). New methods have been developed to widen its application to animal organs or microorganisms (Nirmalan et al., 2004; Ishihama et al., 2005), yet they are not as readily used as chemical labeling approaches. Recently a study of Quantitative proteomic profiling of Murine Macrophage responses to Mycobacterium tuberculosis lipids has been done by Wening and colleagues (Shui et al., 2009). This was the first initiative till date to study the host pathogen interaction by using state-of-the-art quantitative approaches- chemical isobaric tagging iTRAQ.

In the context of the above discussion on background literatures, we studied the global modulation of host cell responses following Mycobacterium tuberculosis infection through transcriptomic and proteomic approaches.

**Main objectives of this study are:**

1) In order to broaden the range of proteins under investigation, we have employed “Quantitative proteomics” strategy-“isobaric tagging for relative and absolute quantification” (iTRAQ)- that relies on the labeling of primary amino groups in proteolytically digested proteins over different time points of infection, i.e. 0 hrs, 12 hrs, 24 hrs, 48 hrs and 96 hrs and followed by MS/MS analysis to investigate the global proteome modulation of infected cells. We have hypothesized that by following the pattern of gene expression at different time points, it is possible to elucidate as to which host genes are up- or downregulated over the course of infection.

2) Transcriptional profiling using microarrays provide a unique opportunity to decipher host pathogen cross-talk on the global level. So, to analyze the transcriptional status we have performed microarray experiments at different time points i.e. 0 hrs, 8 hrs, 16 hrs, 48 hrs and 90 hrs.

3) Finally, we have carried out intensive analysis of the host cell expression profiles in terms of its proteome as well as transcriptional status, in order to correlate the mRNA and protein expression profiles with respect to the significant molecules as identified by the patterns of gene expression at different time points in both experimental approaches.