Synopsis
Leishmaniasis comprises a group of diseases, caused by protozoan parasites of the genus *Leishmania*. At least 20 different species of *Leishmania* are responsible for various forms of disease in human and other mammals in the tropics and subtropics that vary in symptoms and severity. Clinical manifestations of the disease range from self-curing cutaneous infection (caused by *L. major*, *L. mexicana*, *L. tropica* & *L. aethiopica*) to mucocutaneous (caused by *L. braziliensis*) to a chronic and often fatal form, visceral leishmaniasis (caused by *L. donovani*, *L. chagasi* & *L. infantum*). Treatments that are currently available for the disease are less effective, toxic when used for a prolonged period of time or poor efficacy. This is partly explained by the complexity of the transmission cycle and lack of our knowledge about *Leishmania* biology, pathogenesis and its virulence mechanisms. Thus, it is the need of the hour to investigate and find out more efficient drug target for treatment of a disease that affects & claims lives of millions of people annually all over the world.

*Leishmania* exist as extracellular, flagellated, less infective procyclic promastigotes within the mid gut of the sandfly. These procyclic promastigotes undergo developmental changes and become highly virulent or metacyclic, and thereafter, migrate to the mouthparts of the sandfly. This process of differentiation is called as metacyclogenesis. When the sandfly takes the blood meal, metacyclic promastigotes get transferred to the bloodstream of mammalian host where they are phagocytosed by macrophages. Promastigotes differentiate into non-motile amastigotes within the phagolysosomes of the macrophage. Amastigotes multiply in the macrophage, rupture it and infect the surrounding macrophages. The life cycle of the parasite completes when released amastigotes are taken up by sandfly vector in a subsequent blood meal and differentiate into flagellated promastigotes in the sandfly mid gut. During the digenetic life cycle of the parasite, it has to survive and multiply successfully in biologically two disparate environments. This is accomplished by profound biochemical and developmental changes during the parasite's life cycle. The parasite adopts various mechanisms by which it is able to survive in such hostile environments. For successful survival and evasion of host immune response, the parasite expresses several 'virulence factors'. Promastigote culture can be grown *in-vitro* therefore there is an easy access to promastigotes
specific material and like many pathogen; *Leishmania* has a tendency to lose virulence during *in-vitro* culture. In our lab clonally identical line of virulent and attenuated line of *L. donovani* has been generated.

The aim of this work to identify the genes that are differentially expressed between the virulent and *in-vitro* generated attenuated lines of *L. donovani* which may be responsible. In our lab, Differential Display-PCR (DD-PCR) carried out between amastigote and promastigote forms of the parasite have yielded several differentially expressed fragments. The strategy I have used is to screen these fragments between virulent and attenuated line of the parasites by reverse northern to check their roles in virulence of *Leishmania*.

This thesis describes the characterization of a novel pair of paralogs in *L. donovani*, their expression analysis and differential regulation at different stages of the parasite, their cellular localization and effects of over expression in *Leishmania*. At the last I have tried to explain how this pair of genes might play a role in regulating the virulence of the parasite.

In chapter 1, I give a short description about *Leishmania* pathogen and leishmaniasis followed by an overview of genome organization, transcription and its regulation. At the end I also discuss about the different concept of virulence and what is known about the virulence mechanisms and factors of *Leishmania*.

In chapter 2, I describe the details of specialized methods that have been used during the study. Routine methods are listed or referenced.

In chapter 3, the first chapter of results section, I present the data for identification of several attenuation specific cDNA fragments from the reverse northern screen. Then after I present the data for characterization of a differentially regulated gene (DRG) whose expression in *Leishmania* cells are associated with the loss of virulence. Further analysis of their expression pattern shows that they are differentially regulated in virulent and attenuated line of parasite. Also genome organization of DRGs in *L. donovani* confers by southern blot and sequencing of *LdDRG* locus. Bioinformatic analysis of DRG protein shows the transmembrane domains at N-terminal of protein is highly conserved either in *Leishmania* genus or *Trypanosoma* genus. Whereas, the second predicted transmembrane domain at C-
terminal shows high degree of conservation in family Trypanosomatidae. Further expression of recombinant DRG in bacterial system and generation of polyclonal antiserum is also discussed in this chapter.

In chapter 4, I analyze the cellular localization and expression pattern of DRG proteins by using chimeras of DRG1 with GFP and DRG2 with mRFP. Further analysis shows that DRG1 is labile protein and migrates through distinct cellular locations including a perinuclear site and an endosomal organelle, termed Multivesicular Tubule (MVT), in a time dependent manner. In contrast, DRG2 protein is stably expressed but in a MVT that is distinct from DRG1. These data first time show the evidence for the presence of multiple MVTs in *Leishmania*.

In chapter 5, I examine the difference between virulent and attenuated promastigotes on several criteria. The data shows that attenuated live have bitter survival at different stress condition. Moreover over expression of DRG1 cause increased survival whereas over expression of DRG2 causes early death in leishmania. Many properties of *Leishmania* cells over expressing DRG1 are similar to attenuated promastigote; indicate its association with the attenuation. Early death by over expression of DRG2 may also lead to low virulence condition.

In summary, two new findings regarding *Leishmania* biology and its virulence emerge from this work. Firstly, unique endosomal organelles can coexist and depending on their molecular constituents, participate in distinctive cellular processes, including survival. Also a novel pair of paralogs, *Differentially Regulated Genes, DRG1* and 2 is identified that contribute differentially to *Leishmania* survival. The DRG proteins differ at just 3 of the last 6 amino acids; yet differ in stability, localization and function. DRG1 is labile protein whereas DRG2 protein is stably expressed. These two proteins localize in similar looking but distinct Multivesicular Tubule (MVT). Secondly, attenuation plays an important role in regulating virulence of the *Leishmania* by altering its survival. Also virulent and attenuated promastigote differ in the survival at different stress conditions. These differences in survival can push the cells to compromise on their virulence. Also over expression of individual DRGs has a contrary effect on *Leishmania*: DRG1 enhances survival while DRG2
causes death. It shows that DRG1 and DRG2 both may play an important role in attenuation hence regulating the virulence of the parasites.