Chapter 6

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The study of host-parasite interaction has attracted considerable attention in recent years because of the realization that much needs to be identified at the level of host-parasite contact to provide a proper understanding of successful pathogenesis. Since the initial interaction between host and the pathogen is dependent on cell surface associated molecules in both organisms, characterization of surface linked proteins and glycoproteins have been of significant interest. *Leishmania* spp., the parasites that cause leishmaniasis have a digenic life cycle where the disease is transmitted to the vertebrate host by infective metacyclic promastigotes residing in the pharynx of the invertebrate vector. Molecules selectively upregulated in metacyclic promastigotes could be associated with changes prior to or during host-parasite interaction because mammalian infections are primarily caused by parasites in this stage of the life cycle. Prohibitin is one such molecule that is upregulated during the metacyclic stage. This thesis presents a comprehensive account of identification and characterization of prohibitin in *Leishmania donovani* and proposes some functional role of the molecule in *Leishmania* life cycle.

Data presented in this thesis show that prohibitin is localized on parasite surface at the aflagellar pole as well as the flagellar pocket, aflagellar pole being the region through which the parasite binds to the host. Prohibitin was also localized to distinct vesicular structures intracellularly which were not lysosomes or autophagic vacuoles. Cloning, sequencing and subsequent bioinformatic analysis of the *Leishmania* prohibitin gene showed that all the species formed closely knit clusters with the trypanosomatids having the closest homology of approximately 75% with *Leishmania donovani* prohibitin which is in tune with the taxonomic closeness between the two species. Sequence similarity with mammals was around 40% that provides an opportunity to identify areas of sequence unique to the parasite for development of possible diagnostic methods. A signal sequence was present at the N-terminus with a probable cleavage site between positions 23-24 with a GPI anchor site at position 252 and the prohibitin domain extended between amino acids 23-214. PIPLC treatment could remove surface prohibitin confirming the presence of a GPI-link.

Having established the localization and biochemical characteristics of *Leishmania* prohibitin, possible functional role of the surface protein was probed for its role in host parasite interactions. Anti-prohibitin antibody present in the media during murine macrophage and parasite interactions reduced binding of the parasite to the host cell as compared to the group exposed to
unrelated antibodies. To further confirm the role of prohibitin in host-parasite interactions, prohibitin was overexpressed in promastigotes in two forms, one was the overexpression of the wild type protein and another was the overexpression of a protein with a mutation at the GPI anchor site, so that expression of surface prohibitin is compromised. When the transfected cells described above were used for infecting macrophages, wild-type prohibitin transfected cells showed higher ability to establish infection as compared to cells expressing mutant prohibitin. Essentially, the above data demonstrates that prohibitin overexpressing promastigotes were able to infect macrophages more efficiently than only vector transfected or N252D mutant transfected cells.

Since it was evident that the parasites used prohibitin to bind to macrophages, possible binding partners for prohibitin in macrophages were investigated. For this, the protein was synthesized in vitro using *Leishmania* prohibitin DNA in a transcription-translation coupled rabbit reticulocyte system where macrophage membrane preparation was added post prohibitin synthesis to the total mixture and subjected to immunoprecipitation with anti-prohibitin antibody. Macrophage membrane HSP-70 co-immunoprecipitated with *Leishmania* prohibitin suggesting that both proteins interacted. Surface staining of macrophages confirmed presence of cell surface HSP-70. This data therefore identifies a possible binding partner for prohibitin on macrophage cell surface and opens up areas for investigation on signaling pathways involved in post prohibitin binding.

Conversion of promastigotes to amastigotes is the most important event in *Leishmania* life cycle because amastigotes are the disease causing forms. However, we observed a significant expression of prohibitin during conversion of promastigotes to amastigotes with the host cells. During this period, changes in shape and a fourfold decrease in size occurs. Evidently, a considerable sculpting of the cell structure occurs in both the stages and therefore, processes relevant to changes in cellular architecture are important. It has been suggested that autophagic activities have a role in architectural changes during development and differentiation. Our observations that autophagosome formation increases during conversion to stationary phase which is coincident with an increase in prohibitin levels suggest that prohibitin could be linked to autophagy. Interestingly, HSP-70 also increased during the same period suggesting some interaction of HSP-70 in the process of autophagy. Our studies using another model where paclitaxel was used for tubulin polymerization, showed an increase in autophagy with concomitant increase in HSP-70 and prohibitin. Since in this model, inhibition of both HSP-70 and prohibitin in isolation using a specific inhibitor and antisense oligonucleotide respectively resulted in reduction in autophagy, it implied that both molecules were possibly involved in regulation of the autophagic
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process. Immunoprecipitation studies show that both HSP-70 and prohibitin interact with each other, during autophagic events suggesting a close co-operation between the two molecules.

Since prohibitin was present on parasite surface and therefore more accessible to the immune system, it was possible that leishmaniasis patients could have circulating antibodies against the protein if it was immunogenic, therefore, patient sera were tested for the presence of anti-prohibitin antibodies. Sera from VL patients were checked for the presence of anti-prohibitin antibodies using a peptide designed from a region of the Leishmania prohibitin that was not present in the mammalian host or the most frequent pathogen invading the human, the Plasmodium bergei. Reactivity of the peptide antigen to the sera was significant in all 40 samples of sera from leishmaniasis patients tested showing that the protein was immunogenic.

Interpreting the results presented in the thesis, we propose that leishmanial prohibitin is an important entity involved in host-parasite interactions and can be viewed as a target for drugs or used as a diagnostic marker.