Chapter 2

Review of Literature
2. Review of Literature
Part 1: Leishmaniasis – The Disease
2.1.1. Leishmania species and Leishmaniasis

Major William Boog Leishman, a Scottish pathologist identified Leishmania parasite from post mortem of an English soldier posted in Bengal who seems to have died of a mysterious ‘fever’ in 1901. He described them as “oval bodies” 2μm in diameter present in the splenic smears. The findings were further substantiated by a similar observation made by Captain James Donovan at Madras in the splenic smears of an Indian patient. But it was Ronald Ross who named the protozoan parasite as the Leishman donovan body, which later became Leishmania donovani, the causative agent of visceral leishmaniasis (VL). The parasite is primarily zoonotic in nature; infecting a large number of vertebrates throughout the world. The parasite belongs to the order Kinetoplastida and family Trypanosomatidae (WHO).

Leishmaniasis is endemic to 88 countries in the world putting 350 million people at risk and it affects 2 million people annually with about 12 million people affected worldwide. The major regions for susceptibility are the tropical and subtropical regions of the world (Fig. 2.1). In the eastern hemisphere, five species are prominent cause of infection namely; Leishmania donovani, L. aethiopica, L. infantum, L. major and L. tropica. The situation being more complex on the western side where 14 species play a role, each species name reflecting occasionally its geographical distribution; L. amazonensis, L. aristedesi, L. braziliensis, L. chagasi, L. colombiensis, L. garnhami, L. guyanensis, L. lainsoni, L. mexicana, L. naiffi, L. panamensis, L. peruviana, L. pifanoi and L. venezuelensis (Sacks and Noben-Trauth, 2002; Rajalingam and Rudel, 2005).

Figure 2.1: Geographical distribution of Visceral Leishmaniasis throughout the world. The regions marked in red represent areas endemic for leishmaniasis.

2.1.2. Clinical manifestations

The clinical symptoms of leishmaniasis are skin sores which erupt within weeks to months after the individual is bitten by sand flies. Other consequences, which can manifest anywhere from a few months to years after infection, include fever, damage to the spleen and liver, and anaemia. In the medical field, leishmaniasis is one of the famous causes of a markedly enlarged spleen, which may become larger even than the liver. There are four main forms of leishmaniasis, namely:

1) **Visceral leishmaniasis** – This is the most serious form and potentially fatal if untreated.
2) **Cutaneous leishmaniasis** - The most common form which causes a sore at the bite site, which heal in a few months to a year, leaving an unpleasant looking scar. This form can progress to any of the other three forms.
3) **Mucocutaneous leishmaniasis** - Commences with skin ulcers which spread causing tissue damage to nasal and mucosal lining.
4) **Post Kala Azar Dermal leishmaniasis** - This is the relapse of visceral leishmaniasis after treatment, resurfacing as cutaneous leishmaniasis.

2.1.3. Leishmaniasis and HIV co-infection

Leishmaniasis as a growing opportunistic infection in patients of Acquired Immunodeficiency Syndrome (AIDS) is the new emerging threat, with about 33.2 million people being affected by the AIDS epidemic (UNAIDS, 2007). In countries like India, Brazil and Eastern Africa, the overlap of endemic regions of visceral leishmaniasis and regions of HIV infection poses a very serious threat to public healthcare (Alvar et al., 2008). Where leishmaniasis occurs in urban areas, conditions often favour explosive epidemics thereby transforming leishmaniasis from a sporadic to an epidemic threat. In persons infected with HIV, leishmaniasis accelerates the onset of AIDS by cumulative immunosuppression and by stimulating replication of the virus. The epidemiological significance of asymptomatic carriers of the parasite has also been amplified by the advent of HIV, as co-infection rapidly activates disease in parasite carriers. Sharing of needles by intravenous drug users contributes to the spread of leishmaniasis as well as HIV (UNAIDS, 2007). Till date, 34 countries worldwide have reported cases of leishmaniasis and HIV co-infection (Fig. 2.2), approximately 70% of cases of leishmaniasis in Europe have co-infection of HIV, the sharing of drug needles being the major cause.
2.1.4. Treatment of Leishmaniasis

The treatment for leishmaniasis is primarily chemotherapeutic with a lot of drugs becoming ineffective due to development of resistance or severe adverse effects. The present drugs for treatment of leishmaniasis are listed below with their most common drawbacks:

1) **Pentavalent Antimony compounds** - The Pentavalent antimony compounds e.g. sodium stibogluconate (Pentostam) and Meglumine antimoniate (Glucantine) still form the first line of treatment for leishmaniasis. The mechanism of action is still not well understood but is believed to be via inhibition of metabolic pathways leading to cellular apoptosis (Sudhandiran and Shaha, 2003; Mehta and Shaha, 2004; Mehta and Shaha, 2006). The drug in clinical use is becoming ineffective due to increased resistance developed by the parasite believed to be due to at least two ABC transporters (Coelho et al., 2003; Haimeur et al., 2000).

2) **Pentamidine** – It is essentially used in cases of leishmaniasis refractory to Pentavalent Antimony compounds; however its use is rapidly declining due to increased resistance to it (Sundar et al., 2000c). Pentamidine is shown to accumulate in the mitochondria (Basselin et al., 2002) and mitochondrial respiratory chain complex II inhibitors can greatly enhance its potency (Mehta and Shaha, 2004). The use of pentamidine is restricted as it is known to cause severe side effects like hypotension, hypoglycemia, diabetes and nephrotoxicity.

3) **Amphotericin B** – Amphotericin B was developed primarily as an antifungal drug known to disrupt fungal membrane due to its close similarity to ergostane based sterols; this explains its efficacy as leishmanial membranes are rich in ergostane based sterols (Goad et al.,
Amphotericin B is primarily used in areas of high antimony resistance but is known to be highly toxic though the lipid formulations are less toxic but expensive limiting general public use (Sundar et al., 1999a; Sundar et al., 2000a).

4) Miltefosine – Miltefosine (hexadecylphosphocholine) was developed as an anti-cancer drug but showed good potential as anti leishmanial drug in vitro (Croft et al., 1987) and in Phase III clinical trials against L. donovani (Sundar et al., 1999b; Sundar et al., 2000b; Sundar et al., 2002). It is believed to change alkyl lipid metabolism and phospholipid biosynthesis (Lux et al., 2000), leading to apoptosis (Paris et al., 2004). No clinical resistance is seen but in vitro resistance is developed due to increased expression of P-glycoprotein pumps (Perez-Victoria et al., 2001b; Perez-Victoria et al., 2001a). Though miltefosine is considered a major breakthrough in anti-leishmanial chemotherapy, the ease of generating resistance in vitro is worrying, hence it is presently only used in combination with other drugs to suppress development of resistant strains.

From the above discussion it is apparent that the chemotherapeutic approach to leishmaniasis is limited due to toxicity of drugs and emergence of resistance. Safe and effective prophylactic treatment is difficult due to adverse effects of the above drugs for people living in endemic regions. These above treatment strategies are further complicated by emergence of Leishmania-HIV co-infection which limits usage of many drugs, thereby complicating the situation further.

2.1.5. Vaccine candidates

No vaccines for leishmaniasis are in use presently, but the genome sequencing efforts have given rise to 14 novel candidates for DNA vaccines which are under study. Another six clones were identified namely, glutamine synthetase, a transitional endoplasmic reticulum ATPase, elongation factor 1gamma, kinesin K-39, repetitive protein A2, and a hypothetical conserved protein as antigens for T cell are presently being developed (Myler and Fasel, 2008).
Part 2: Leishmaniasis – The Biology

2.2.1. *Leishmania* species Life Cycle

*Leishmania* parasites are transmitted by the bites of infected female *Phlebotomus* sandflies, which inject a small number of infectious-stage, highly motile flagellated metacyclic promastigotes into the skin. These forms are opsonized efficiently by serum components and taken up by the macrophages, where they reside in phagolysosomes and transform into replicating non-motile amastigotes (Fig. 2.3).

![Figure 2.3: The Digenic life cycle of the parasite showing the various Sandfly and Mammalian host stages (Sacks and Noben-Trauth, 2002).](image-url)

Infected macrophages are taken up by sandflies during blood feeding; they are lysed in the fly midgut, releasing parasites that transform into rapidly dividing, non-infectious-stage promastigotes. These forms undergo a process of attachment to the midgut wall, release and anterior migration that is accompanied by their differentiation to non-dividing, metacyclic promastigotes (Fig. 2.3), that can be transmitted when the sandfly takes another blood meal (Sacks and Noben-Trauth, 2002)

2.2.2. Parasite – Sandfly Vector Interactions

The above given life cycle is an oversimplification of the complexities involved in the development and survival strategies used by the parasite as neither the digestive enzyme filled gut of sandfly nor the acidic protease filled phagolysosomal compartment of macrophages is congenial environment for growth and proliferation. The parasite shuttles between poikilotherm invertebrate insect vectors from where it is transferred to the homeotherm vertebrate mammalian host during a blood meal, such a life cycle in two entirely different species calls for effective
invasion and survival strategies to be developed by the parasites, especially the surface molecular changes which are the interface of communication to the host cells (Santos et al., 2006). Development of successful strategies by parasites to increase infectivity is dependent on the generation of molecular complexes and interactions, which can assist binding or subvert host responses to assist successful invasion. Some of such interactions are detailed below but many are yet to be explored.

2.2.2.1 Life cycle in Sandfly Vector

Sandflies are poor feeders; they insert their saw like mouthparts into the mammalian host and agitate them to produce a small wound containing blood from superficial blood vessels (Dillon and Lane, 1993). The movement of mouthparts leads to the release of skin macrophages and also freed amastigotes followed by their subsequent uptake into the sandfly gut. The change in conditions of moving from mammalian host to insect vector (decrease in temperature, increase in pH) triggers development of parasite in these vectors (Bates and Rogers, 2004; Kamhawi, 2006). During this phase, the blood meal is surrounded by peritrophic matrix that protects the parasites from the digestive enzymes of the gut. The peritrophic matrix is secreted by the insect vector and is a sac of proteins and glycoproteins held together by chitinous microfibrils, forming a barrier that protects sandfly midgut from abrasive particles and microbes slowing down the diffusion of digestive proteases into the endoperitrophic space (Bates, 2007; Rogers and Bates, 2007). The onslaught of proteases peak at 18-48 hrs leading to killing of 50% of the parasite (Sacks and Kamhawi, 2001; Volf et al., 2001; Pimenta et al., 1997).

The first stage of development is the replicative procyclic promastigotes. After a few divisions the parasites begin to slow their replication and differentiate into elongated, highly motile nectomonad promastigotes. These nectomonad promastigotes then break open the peritrophic matrix and move to the anterior midgut (Fig. 2.4) (Kamhawi, 2006). Their migration to the anterior midgut is followed by their attachment to the microvilli of midgut epithelium until they reach the stomodeal valve guarding the junction between the foregut and midgut. These parasites now mark the successful infection (persistence beyond blood meal and avoidance of expulsion during defecation) and the sandfly as the true disease transmitting vector. The attachment of the parasite is crucial at this step and is aided by lipophosphoglycan (LPG) binding to galectin on surface of the sandfly gut (Sacks and Kamhawi, 2001), though non LPG mediated binding has also been shown recently (Rogers et al., 2004; Svobodova et al., 2006; Volf and Myskova, 2007; Myskova et al., 2007).
On reaching the stomodeal valve the nectomonads transform to shorter leptomonads that resume cell division (Fig. 2.4), these form also secrete promastigote secretory gel (PSG). Some of the nectomonads/leptomonads also adhere to the cuticle lining of the valve and differentiate into haptomonad promastigotes. The form of attachment being entirely different from the midgut epithelium and is mediated by expansion of the flagellar tip into hemi-desmosome-like structure (Wakid and Bates, 2004). Some of the leptomonads transform into mammalian infective metacyclic promastigotes leading to transmission of disease during the next blood meal (Rogers et al., 2002).

The injection of metacyclic promastigotes into the mammalian host is via both inoculation as well as regurgitation. The regurgitation process is assisted by the formation of PSG plug (leading to blockage of sandfly gut followed by regurgitation) with the haptomonads facilitating the regurgitation process (Rogers et al., 2002). The plug essentially is a cell pellet embedded with promastigotes which become actively motile when PSG dissolves. The metacyclic promastigotes are at the poles of the PSG plug ideally positioned for transmission. During egestion three components play together for a successful infection; namely, the metacyclic promastigotes, the sandfly saliva and the PSG plug. Both the sandfly saliva and the PSG plug components have been known to be important for the disease exacerbation (Rohousova and Volf, 2006; Kamhawi et al., 2000), with the PSG plug having a larger impact (Rogers et al., 2004). Recent report of prevention
of *Leishmania* infection in hamsters after immunization with sandfly salivary protein underlines the importance of such factors (Gomes *et al.*, 2008).

### 2.2.2.2 Metacyclogenesis

Metacyclic promastigotes are highly adapted for successful invasion and survival in the mammalian host. In the sandfly, metacyclogenesis refers to a masking of sugar residues involved for binding in the midgut and elongation of LPG molecules (Sacks and Kamhawi, 2001; Dobson *et al.*, 2003a; Dobson *et al.*, 2003b). The mechanism triggering metacyclogenesis are not well understood *in vivo*; *in vitro* it can be induced by low pH (Zakai *et al.*, 1998), anaerobic conditions (Mendez *et al.*, 1999) and a decline in levels of tetrahydrobiopterin (Cunningham *et al.*, 2001). Many genes upregulated during metacyclogenesis are identified but remain poorly understood, the META gene cluster being known to increase virulence in the mammalian host (Ramos *et al.*, 2004). The others like small hydrophilic endoplasmic reticulum-associated proteins (SHERP) are thought to function in the vector only as they are expressed in metacyclics only and null mutants of hydrophilic, acylated surface protein (HASP)/SHERP proteins are as virulent as wild type parasite in macrophage invasion and cell survival (Knuepfer *et al.*, 2001; McKean *et al.*, 2001).

### 2.2.3. Parasite – Mammalian Host Interactions

#### 2.2.3.1 The Invasion

The rule of maxim does not apply to *leishmania* infections as the phlebotomine sandflies regurgitates only 1-1000 metacyclic parasites (Warburg and Schlein, 1986) which results in successful infection. This is due to the fact that the metacyclic promastigotes are more resistant to complement mediated lysis as compared to the procyclic promastigotes (Franke *et al.*, 1985; Sacks and Perkins, 1985; Sacks, 1989; Dominguez *et al.*, 2002) and react differently to the complement machinery (Puentes *et al.*, 1988). The C3 complement molecule binds in its active form to the metacyclic promastigote lipophosphoglycans but is unable to mediate lysis due to altered structure of the lipophosphoglycans (altered sugars and more subunit) resulting in thicker coats impenetrable to C5-9 complex lytic pore formation (Puentes *et al.*, 1990; Pimenta *et al.*, 1991; McConville *et al.*, 1992). In the absence of lysis, these complement coated parasites get easily phagocytosed, the resistance to complement not being absolute but time dependent; hence the kinetics of IgM mediated uptake of the parasite is much faster than the complement mediated lysis (Dominguez *et al.*, 2002). The initial parasite density as a result is as low as 0.03-0.1 parasite per phagocyte, this low density assist the escape of parasite from host counter measures due to ignorance (de Almeida, 2002). During initial phases of infection; low antigen concentration,
inappropriate antigen presentation by non professional antigen presenting cells and low number of reactive cells all seem to favor Leishmania infection (Chang et al., 2003).

2.2.3.2 The Interaction

As described above the first contacts of the parasite with the host cells are extremely crucial for survival and proliferation of the parasite. The surface of parasite shows immense changes depending on the developmental stage, for e.g. the glycocalyx (glycoproteins and related products) of procyclic promastigote is only 5nm thick increasing to 17nm thickness in metacyclics and becoming absent in amastigotes (Pimenta et al., 1991) suggesting the differential requirement of cell surface chemistry at different stages of differentiation of the parasite. LPG and GP63 are few of the well understood components of the leishmanial surface during invasion. Both of them seem to complement each other. GP63 is a 63 kDa glycoprotein, a zinc dependent metalloprotease known to play a role in amastigote survival and host cell modulation (Matlashewski, 2001).

The LPG on the other hand is known to inhibit fusion of phagosome to lysosome providing the parasite sufficient time for adaptation (Dermine et al., 2000; Desjardins and Descoteaux, 1997). LPG treatment of bone marrow derived macrophages also prevent apoptosis in these macrophages (Moore and Matlashewski, 1994). The LPG – C3b, C3bi complement mediated uptake of the parasite allows them to reach the monocyte/macrophage sanctuary using CR1 and CR3 doors (Rosenthal et al., 1996). The uptake process via C3 complement suppresses the important TH1 cytokine IL-12 aiding the parasite survival (Reiner et al., 1994; Marth and Kelsall, 1997; Sutterwala et al., 1997). The LPG interaction is also known to alter the activities of protein kinases (Prive and Descoteaux, 2000), inhibition of protein kinase C activity (McNeely et al., 1989) and disruption of phagolysosomal maturation (Lodge and Descoteaux, 2005).

2.2.4. Role of Autophagy and Apoptosis in Leishmania Virulence

Autophagy and Apoptosis are the two biological processes of programmed cell death that have been shown recently to have great importance for the shaping of Leishmania inoculum for increased virulence. The differentiation of procyclic to metacyclic in the sandfly vector and then to amastigote requires an extensive modification of the intracellular structures of the parasite (Fig. 2.5) (Besteiro et al., 2007).
Autophagy is defined as the process of recycling of cellular components by the proteases of the cell and is important for survival under starvation and modification of intracellular structures (Reggiori and Klionsky, 2005). It mainly consists of three types; chaperone mediated autophagy, microautophagy and macroautophagy. Chaperone mediated autophagy is a response to starvation stress and results in degradation of cytosolic proteins in the lysosomes of mammalian cells; microautophagy is marked by the invagination of lysosomal membranes to sequester cytoplasm and nuclear components, both of these form of autophagy are not well understood.

Macroautophagy is very well studied and involves the formation of double membrane compartments called autophagosomes containing cytoplasm and organelles to be degraded on fusion with lysosomes resulting in the formation of autophagolysosome (Reggiori and Klionsky, 2005). The formation of autophagosome is believed to originate from endoplasmic reticulum (Mijaljica et al., 2006) and in Leishmania has been shown to contain cytosolic proteins (Williams et al., 2006), multi-vesicular bodies and acidocalcisomes (Vannier-Santos and Lins, 2001). There exists a possibility of glycosomes being degraded in autophagosome (Herman et al., 2006). The fusion of autophagosome to the lysosomes leads to the degradation of the components by various peptidases including cysteine peptidase A and B (Williams et al., 2006). The role of autophagy becomes important during metacyclogenesis with the increase in number of autophagosomes (Besteiro et al., 2006; Williams et al., 2006) and increased protein degradation.

Figure 2.5: Schematic representation of changes in intracellular organelle in L. donovani on conversion from promastigote (left) to amastigote (right) form.
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(Alves et al., 2005) preceding the transformation of procycles into metacycles. The simultaneous upregulation of both the processes leads to parasite remodeling during differentiation. The cysteine peptidase mutants show a normal proliferation but impaired metacyclogenesis and transformation into amastigotes suggesting that the parasite can survive accumulation of non recycled components (Williams et al., 2006) but protease activity is important for remodeling of cellular structure. Whereas the expression of dominant negative ATPase VPS4 essential for endosomal sorting, in leishmania cells prevention of endosomal sorting, viability and differentiation of parasite into metacycles by blocking autophagy (Besteiro et al., 2006).

Recently apoptosis of leishmania has come into prominence, though the apoptotic machinery of leishmanial cells is not as well defined as that of its mammalian counter part. But certain hallmarks of apoptosis like DNA laddering, mitochondrial destabilization and phosphatidyl serine exposure are observed (Mukherjee et al., 2002). The exposure of phosphatidyl serine on the cell surface and its subsequent interactions with phagocytes leads to production of transforming growth factor beta (TGF-β1) leading to inhibition of inflammatory responses both in autocrine and paracrine manner (McDonald et al., 1999). In the amastigote stage of the parasite, the exposure of phosphatidyl serine is known to enhance TGF-β1 secretion and interleukin mRNA production, neutralization of these exposed phosphatidyl serine using annexin V has been shown to reduce infectivity by 50%. Recently it has been demonstrated that a part of Leishmania inoculum has apoptotic cells and these cells are essential for disease development as depleting these cells from virulent innoculum leads to loss of disease inducing ability, the authors suggest the role of TGF- β1 secretion as a plausible explanation (van Zandbergen et al., 2006).

The emerging trypanosomatid genome database identifies various proteins related to cell-cycle regulation and death and also differentially upregulated proteins during the various developmental stages. One such protein upregulated during Leishmania metacyclogenesis is prohibitin, known to be a cell cycle regulator in mammalian cells and at the same time plays an important role in mitochondrial function. The functional role of this protein in Kinetoplastid parasite is virtually unknown although identified as a protein increased during infective stage.

Part 3: Prohibitin: The Protein

Prohibitin protein is conserved across a wide cross section of organisms from plants to higher eukaryotes. A variety of functions have been attributed to it, however, the relationship between prohibitin and various kinds of cell death remains to be explored. Prohibitin in
the mammalian system is found to be localized in various subcellular compartments; the most common being mitochondrial but is also nuclear in prostate cancer cell lines, cell surface in epithelial cells and extracellular (secretory) in adipose cells (Gamble et al., 2004; Sharma and Qadri, 2004; Wang et al., 2004). A summary of literature below details the knowledge available on the functional role of prohibitin in lower eukaryotes and mammals.

2.3.1 Mitochondrial Prohibitin

In yeast and higher eukaryotes, prohibitin is known to be localized in the inner mitochondrial membrane and processes newly synthesized mitochondrial proteins (Rajalingam and Rudel, 2005; Nijtmans et al., 2002). Two forms of prohibitin namely Phb1p and Phb2p in yeast; and as prohibitin and Bap 37 in mammalian cells, are present as large complexes in the inner mitochondrial membrane (Tatsuta et al., 2005). Loss of any one of them leads to a decrease in life span due to the decline of mitochondrial function and subsequent oxidative damages that seems to get exacerbated in absence of prohibitins (Piper et al., 2002a). The loss of yeast prohibitin is reported to shorten their replicative (budding) life span but not their G0 (stationary phase) survival but these cells have inefficient mitochondrial electron transport. In the mammalian system these prohibitin complexes are known to interact with stomatin family proteins like SLP-2 which prevents proteolysis of prohibitins and subunits of respiratory complex I and IV (Da Cruz et al., 2008), prohibitin is also implicated indirectly for its stabilizing role to F0/F1 complex (Osman et al., 2007) and for controlling cellular senescence and apoptosis by regulating mitochondrial cristae morphogenesis via dynamin like GTPase OPA1 (Merkwirth et al., 2008). Another report suggests the role of prohibitin in the organization and stabilization of mitochondrial nucleoids (Kasashima et al., 2008). Prohibitin is also known to protect both yeast and mammalian cells from apoptosis in conditions of metabolic stress (Piper et al., 2002a; Piper and Bringloe, 2002). In mammalian system, many diseases have been implicated to the imbalance of prohibitin levels; IL -6 is known to transcriptionally regulate prohibitin expression in intestinal epithelial cells (Theiss et al., 2007b), the increased expression of prohibitin is known to decrease accumulation of reactive oxygen metabolites (Theiss et al., 2007a). The activation induced upregulation and phosphorylation of the prohibitin in the mitochondria of T cells is reported to play a role in T cell survival (Ross et al., 2008).

2.3.2 Nuclear Prohibitin

Prohibitin in higher eukaryotes shows multiple functions; the most well studied is the role as a mitochondrial membrane chaperone with its homolog BAP37 (Nijtmans et al.,
On the other hand nuclear localized prohibitin seems to be colocalized with E2F and p53 (Fusaro et al., 2003). Prohibitin mRNA microinjection in Hela cells is known to block cell cycle progression which is reverted by its antisense, but the observation seems to occur because of the 3' untranslated region of prohibitin mRNA and not because of the protein (Manjeshwar et al., 2003). Alkylation of prohibitin is reported to cause G1 cell cycle arrest (Bouchon et al., 2007). Prohibitin is known to be upregulated in non replicative cells; this upregulation seems to block the cells in G1-S phase of growth by repressing E2F mediated transcription (McClung et al., 1995). It has been shown that the coiled coil domain of prohibitin can physically interact with E2F leading to its repression and is also able to recruit histone deacetylase I (Wang et al., 1999; Wang et al., 2004). In cancerous cells prohibitin is found to be colocalized with E2F and p53 and this association stimulates apoptosis (Fusaro et al., 2003). In this context, prohibitin has been shown to directly interact with p53 leading to enhancement of p53 mediated transcription and the p53 and E2F1 interaction is known to modulate caspase 7 levels (Joshi et al., 2007). Recently, a putative nuclear export signal has been located in the C - terminus of prohibitin which leads to translocation of both p53 and prohibitin from nucleus to mitochondria on treatment of transformed cells with camptothecin (Rastogi et al., 2005). Prohibitin has also been shown to interact with Rb and modulate Rb-E2F mediated transcription by repression of E2F mediated transcription (Wang et al., 1999), likely by recruiting co-repressors (Rastogi et al., 2006). The binding site of prohibitin to E2F is different from that of Rb and in Rb–E2F mediated repression, both Rb and prohibitin respond to different stimuli. While c-Raf and Raf1 can successfully revert prohibitin mediated E2F repression, signaling molecules like E1A, p38 kinase, cyclinD and E are required to revert the Rb mediated pathway but have no effects on the prohibitin pathway (Wang et al., 1999). Brg1-Brm complexes are also believed to play a role in the repression of E2F mediated transcription (Wang et al., 2002), the SWI/SNF ATPase subunit being important for this repression (Zhang et al., 2007). Androgens are known to repress prohibitin upregulation at the level of transcription and promote cell cycle progression. It is now believed that anti estrogenic behavior of estrogen antagonists is mediated via JNK pathway to the recruitment of Brg1-Brm to Prohibitin-E2F promoter complexes hence leading to blockage in cell cycle progression (Wang et al., 2004).

2.3.3 Soluble, Cell surface and Synaptic Prohibitin

Prohibitin has been shown to be present on the human synapses and was found to be overexpressed in chronic Schizophrenic patients (Smalla et al., 2008). In sperm mitochondria, the existence of ubiquitinated forms of prohibitin is believed to be a molecular tag for paternal
mitochondrial degradation after fertilization allowing maternal mitochondrial inheritance (Thompson et al., 2003). Prohibitin has been also demonstrated to be present on the cell membrane of intestinal epithelial cells and binds to Vi antigen of S. typhi leading to a MAP kinase pathway associated decrease in IL-8 (Sharma and Qadri, 2004). Prohibitin is present on the cell surface of adipose tissue and acts as a repressor of pyruvate carboxylase and attenuates insulin mediated glucose and fatty acid oxidation (Vessal et al., 2006), targeting of proapoptotic peptide to prohibitin on adipocyte membranes lead to ablation of white fat but not of other tissues (Kolonin et al., 2004). Prohibitin secreted by adipocytes in lipid droplets is known to interact to complement C3 in the blood (Mishra et al., 2007).

### 2.3.4 Trypanosomatid Prohibitin

The emerging Trypanosomatidae database reveals the presence of prohibitin in several members of the family. The recently completed Leishmania major database reports prohibitin nucleotide sequence as an 807 bp gene with a predicted amino acid sequence that code for a 268 amino acid long protein. Although the gene has been annotated in several of the completed Trypanosomatid genome sequences, very little is known about its functional role in the life cycle of these parasites. For example, it is reported to be upregulated in conditions of ConA induced stress in *T. brucei* but the study failed to provide any insights into the biomolecular reasons for this upregulation (Welburn and Murphy, 1998) and flagellar proteome analysis of *T. brucei* identify prohibitin as a surface associated flagellar protein (Broadhead et al., 2006). Also, prohibitin transcripts are shown to be higher during metacyclogenesis (Almeida et al., 2004). No other report is available on the role of this protein in the survival and functioning of parasites of this group. The ability of prohibitin to regulate cell division and mitochondrial function in several mammalian model systems, makes it an attractive candidate protein for analysis of its role in parasite life-cycle with a view to identify a novel drug target or a pathway suitable for interference with parasite survival.

The above literature presents a comprehensive summary of prohibitin in different organisms. It is evident from the above survey that knowledge of kinetoplastid prohibitin is extremely limited even though it is modulated during crucial life-cycle stages.

There are several reasons why investigating the role of prohibitin in *Leishmania* parasite biology is important;

a) A cDNA array based study suggests that prohibitin is upregulated during metacyclogenesis, thereby suggesting a role of prohibitin in infective activities.
b) Prohibitin upregulation during apoptosis suggests a possible function of the protein in cell death and earlier studies from the laboratory the existence of apoptosis in *Leishmania* using an oxidative stress model provides necessary background to investigate the role of the protein in *Leishmania* apoptosis.

c) Association of prohibitin with mitochondrial function in higher eukaryotes coupled with observations from this laboratory that *Leishmania* mitochondria is vital for apoptotic death in *Leishmania*, merits an investigation into the possible role of prohibitin in *Leishmania* mitochondria.

Bibliography


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