# Ubiquitin-Proteasome System- a target to control pathogenic protozoa

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Ubiquitin-proteasome system (UPS) is an ATP-dependent proteolytic machinery of eukaryotes. Analogous systems have been characterized in eubacteria and archaea as well. Although the structure and functions of this machinery is well-documented in yeast and vertebrates, knowledge of this machinery is rudimentary for unicellular parasitic protozoa. This chapter summarizes the current knowledge regarding the UPS of *Plasmodium*, *Entamoeba*, *Trypansoma*, *Leishmaina* and *Giardia*, including the effect of proteasomal inhibition on these parasites. The unique variations of the UPS of each of these parasites have been highlighted as they may serve as potential therapeutic targets.

**Keywords** Ubiquitin, proteasome, parasite, lactacystin, inhibitor

## 1. Introduction

The proteasome is a multisubunit complex specialized in performing protein degradation functions and thus maintains cellular protein homeostasis. Given the vital nature of the function performed by this complex, its presence has been detected not only in eukaryotes, but also in archaea and actinobacteria [1, 2]. In eukaryotes approximately 70-90% of misfolded proteins, as well as proteins whose functions are no longer needed are degraded by this machinery [3, 4]. In eukaryotes, this complex is located both in the nucleus as well as the cytosol [5]. The complex is also associated with the cytosolic face of the endoplasmic reticulum (ER), where it functions in the degradation of misfolded proteins of the ER via the ERAD (ER associated degradation) pathway [6, 7]. Recent findings also suggest the presence of a similar MAD (mitochondria associated degradation) pathway for the quality control of mitochondrial proteins [8].

The proteolysis function of the proteasome is not random; rather proteins destined for degradation are marked by the attachment of ubiquitin moieties. Ubiquitin is a small protein, generally 76 amino acids long, which is largely conserved across all eukaryotes [9]. The canonical signal for proteasome-mediated degradation is the addition of a poly-ubiquitin chain in which at least four ubiquitins are joined to an internal lysine residue of the substrate that is to be degraded. However, non-canonical ubiquitin assemblies, which also serve as signals for proteasomal degradation, have been documented. The cellular machinery catalyzing the addition of ubiquitin to substrate proteins, followed by the targeting to and degradation by the proteasome is termed the ubiquitin proteasome system (UPS). This chapter will provide a brief overview of the biochemical and structural aspects of the UPS and will summarize the present day knowledge about the involvement of UPS in infectious protozoan parasitic diseases. We will also review literature regarding inhibition of proteasome in controlling such infectious agents and discuss the prospects of using proteasome inhibitors for the treatment of these diseases.

## 2. Biochemistry of ubiquitination

Ubiquitin is covalently attached to a substrate by the concerted action of three different enzymes. The first enzyme of this cascade is ubiquitin activating enzyme (E1). E1 catalyzes the formation an adenylate [10] and then transfer of ubiquitin to a sulfydryl group in the enzyme. E1 then transfer the activated ubiquitin moiety to ubiquitin conjugating enzyme or E2 [11]. The transfer of the activated ubiquitin moiety to the substrate is catalyzed by the next enzyme in this cascade, which is termed the ubiquitin ligase or E3 [12]. E3s can be classified in two major categories. The first group, whose members are termed the HECT E3 ligases, has a signature cysteine residue towards its C-terminus that participates in the transfer of activated ubiquitin to the substrate [13]; the N-terminal part of the protein is responsible for substrate recognition. The second category of E3 ligases is termed the RING family and these ligases bridge the E2 and the substrate without the E3 itself forming any covalent intermediates with ubiquitin [14]. In humans, RING finger ligases outnumber HECT E3 ligases.

As mentioned earlier, ubiquitin is covalently attached to an internal lysine residue of a substrate by an isopeptide bond. This bond is formed between the ϵ-amino group of the internal lysine and the carboxyl group of the C-terminal glycine (G76) of ubiquitin [15]. The next ubiquitin is attached via another isopeptide linkage that involves an internal lysine residue (preferably K48) of the ubiquitin that is already conjugated and C-terminal glycine of the incoming ubiquitin [16, 17]. This linkage is repeated to generate the ubiquitin chain. As previously mentioned, the canonical poly-ubiquitin chain, which targets substrates for proteasomal degradation, consists of a minimum of four ubiquitins linked through K48 residues of ubiquitin. However non-canonical poly-ubiquitin chains that perform the same function have also been documented [18]. These non-canonical chains include chains where ubiquitin residues are joined linearly end to end (C-terminal glycine of the incoming ubiquitin attaching to the N-terminal methionine of the existing

 

ubiquitin) [19, 20, 21], heterologous chains consisting of other ubiquitin-like modifiers (eg. SUMO) and ubiquitin [22], multiply branched chains where more than one ubiquitin attaches to a previous ubiquitin [23, 24] and oligoubiquitin chains that contain only two ubiquitin residues [25].

Attachment of ubiquitin to substrate proteins is not an irreversible process as there are deubiquitinating enzymes (DUBs) that remove the ubiquitin tag from the substrate [26]. The DUBs are classified into two major classes; the type1 DUBs cleave ubiquitin of small substrate whereas type2 DUBs specifically cleave polyubiquitin chains functioning as degradation signals [27]. Eukaryotic DUBs may either be papain-like thiol proteases or metalloproteases. Thiol protease DUBs may contain any of the following domains: UCH (ubiquitin C-terminal hydrolase), USH (ubiquitin specific protease), OTU (ovarian tumor domain) and MJD (Josephin domain). Examples of zinc-dependent metalloprotease DUBs include those with JAMM (JAB1/MPN/Mov34 metalloenzyme) domain [28]. Apart from deubiquitinating substrates prior to proteasomal degradation, DUBs are also involved in other cellular processes such as endocytosis, cell cycle regulation, DNA repair, histone modification etc. [28].

## 3. Structure of proteasome

Eukaryotic 26S proteasome is a multisubunit, multicatalytic protease that is composed of mainly two different parts- a barrel-shaped 20S core particle that is associated with degradation function, and the 19S regulatory particle (RP) that caps the 20S core at one or both ends [5]. The terms '26S', '20S' and '19S' denote the sedimentation coefficient of these particles [5]. The 20S core is made up of stacked homologous rings of non-identical α and β subunits. The α (α1→7) and β (β1→7) subunits form heptameric rings [29, 30]. The 20S barrel consists of two heptameric β rings sandwiched between two heptameric α rings, giving rise to an α-β-β-α assembly [5, 30]. While the access of the partially unfolded protein into the catalytic core is directed by the α subunits (α2, α3 and α4) [31], the proteolytic center stays near the inner chamber formed by stacked β subunits with proteolytic functions performed by β1, β2 and β5 subunits [5]. β1 has caspase-like activity i.e. cleaving after acidic amino acids; β2 has trypsin-like activity i.e. cleaving after basic residues; the β5 has chymotrypsin-like activity thus cleaving after neutral amino acids [32, 33]. Although the proteasome can cleave the peptide bonds at will, it has been documented that the oligopeptides formed due to proteasomal degradation are of length between 2-35 residues, with an average of 8-12 residues [5, 34, 35].

The 19S RP is composed of two main parts: a hexameric ring, that is proximal to the α subunit and a distal lid that recognizes and binds polyubiquitinated substrate. The hexameric ring at the base is composed of six non-identical regulatory particle ATPase subunits (Rpt1→6) [5]. The lid contains nine subunits of regulatory particle non-ATPase (Rpn), which are Rpn3, Rpn5-9, Rpn11, Rpn12 and Rpn15 [5]. The other Rpn subunits, *viz.* Rpn1, Rpn2, Rpn10 and Rpn13, are located at the junction of the base and lid where they are involved in crucial protein-protein interactions necessary for stabilizing the proteasome structure [36]. In 19S RP complex, Rpn10 and Rpn13 are the two principal subunits that recognize polyubiquitinated substrates [37]; Rpn11 is a DUB that deubiquitinates the substrate before it is directed to the interior of the proteasome for degradation [38]. The Rpt subunits (Rpt2, Rpt3 and Rpt5) play pivotal roles in gate opening of attached α subunits of core complex [39].

Although the 26S proteasome is the most abundant form that is present across all eukaryotes, other forms also exists across genera. Apart from the more abundant 19S RP, an 11S regulator or PA28 is found to be associated with the mammalian 20S core. The 11S regulator is a heterohexameric or heteroheptameric complex made up of two different subunits, α and β, which are homologous to nuclear Ki antigen of unknown function [40]. These α and β subunits can have α3β3 or α4β3 or α3β4 arrangements where alternate α and β subunits form ring-like arrangements [27]. Later on another subunit (γ) was also detected in this complex [41]. Unlike the 26S proteasome, the 11S-20S assembly degrades substrates in ATP-independent manner leading to the possibility that only the unfolded proteins are substrates of this complex. A hybrid proteasome was reported by Tanahashi et al., which contains both 19S and 11S assemblies, one at each end of the 20S cylinder [42]. Another type of cap subunit, Blm10 or PA200, is also attached with 20S core particles of yeast and mammals. Similar to the 11S assembly, this Blm10-20S proteasome also degrades substrates without consuming ATP [5].

## 4. Conservation of UPS across life forms

As mentioned before, besides eukaryotes, proteasomes have also been detected in archaea and actinobacteria. However, there appears to be a progression in the complexity of this proteolytic machinery during the course of evolution. Archaea also have a barrel-like proteasome that is remarkably similar in structure to its eukaryotic counterpart wherein a core particle, harboring proteolytic activity, is present in between assemblies of proteins with ATPase activity [43]. The core particle is formed by identical α and β subunits that form heptameric rings and these rings stack together. Actinobacteria proteasomes too have a similar architecture [44]. These prokaryotic proteasome homologues are termed HslV and are composed of a homododecamer with a size of 240 kDa [45, 46, 47]. Although the assembly is different but the protein fold of HslV is very similar to the archaeal and eukaryotic counterparts [27]. Thus, compared to the archaeal and eukaryotic proteasomes, HslV appears to be the simplest form of proteasome extant today [45]. Although

the core particle is conserved across all life forms, there is lack of knowledge regarding 19S or 11S cap in eubacteria or in archaea.

Though ubiquitin is one of the most conserved eukaryotic proteins, it is absent in prokaryotes indicating that the proteasome is more ancient than ubiquitin [27]. However, small proteins that can be attached to other proteins post-translationally are present in both archaeal and eubacterial genomes; while archaea encode Samps (small archaeal modifiers proteins [48], actinobacteria encode Pup (prokaryotic ubiquitin-like protein) [49].

## 5. Disease states associated with UPS

UPS has been associated with quality control as well as maintaining life span of proteins and hence play crucial role in regulation of gene expression, cell cycle progression, immune responses, oxidative stress responses [50]. Due to the involvement of the system in vast array of cellular processes, it is evident that malfunctioning of UPS components is sure to provoke pathological conditions. This is observed in neuropathological conditions, which arise due to accumulation of protein aggregates, termed Lewy bodies inside neurons [50, 51]. Such conditions are associated with cognitive decline and movement disorders. Lewy bodies are found in neurons of the substantia nigra region in patients suffering from Parkinsons disease [52]. Large amounts of ubiquitin-conjugated proteins have been found in Lewy bodies thus indicating a possibility of malfunctiong of UPS [50, 52, 53]. The underlying mechanism for this condition was discovered with the identification of mutations that mapped to genes encoding key components of the UPS, a ubiquitin ligase that was subsequently termed Parkin and a DUB, UCHL-1[53, 54]. Proteasomal malfunctioning is not only documented in case of neurodegenerative diseases, its functional insufficiency is also linked to the development of a range of heart diseases. Cardiacproteinopathy has been associated with accumulation of misfolded proteins in cardiomyocytes, which resulted from an inhibition of proteasomal degradation [55]. Like inhibition of proteasomal activity, atypical activation of proteasomal function also leads to different pathological symptoms. In muscle atrophy caused by uremia, the rate of protein degradation increases in muscles leading to muscle wasting. Muscle atrophy is a common phenomenon that is the underlying reason for the loss of weight in diseases like diabetes, chronic kidney disease etc. [50].

## 6. Proteasome in protozoan parasites

Proteasomes also play a central role in parasites, many of which cause diseases in humans. While several human parasites are medically important, the scope of this discussion will be limited to only the proteasomes of protozoan endoparasites. Besides the involvement of the proteasome in various cellular process discussed in the previous section, the proteasome has an additional important role in the pathogenicity of these parasites. Most of the parasites discussed here undergo morphological state changes that are crucial for the infectious cycle. Thus, the proteasome is likely to be play a vital role as switching from one morphological state to another involves changes in the proteome. This proteomic change not only involves the synthesis of new proteins, but also the turnover of proteins that are no longer needed by the cell.

Diseases caused by such parasites place a heavy toll on society. Malaria alone accounts for close to 225 million infections a year, with 781,000 deaths reported in 2009 [56]. Other fatal diseases include leishmaniasis, African trypanosomiasis, Chagas disease and amoebiasis. While other diseases caused by protozoan endoparasites, such as giardiasis and trichomoniasis, may not be fatal, they do contribute indirectly to mortality. For example, the high parasitic burden in the gut of children suffering from giardiasis has been shown to result in a failure to thrive because of impaired intake of nutrients and trichomoniasis has been documented to cause an increased risk of HIV transmission [57, 58].

The incidence of these parasitic diseases is especially high in developing nations, particularly amongst the socio-economically deprived populations. In the absence of any successful vaccine against any parasitic infections, chemotherapy remains the mainstay for combating these diseases. However, most of the drugs for treatment of parasitic diseases that are currently available in the market suffer from several drawbacks, including high toxicity, rapid induction of drug resistance, non-specificity and high cost of treatment. These diseases have long been neglected in pharmaceutical research. This is partly because given that the target population is economically-challenged, the prospect of returns from the sale of such drugs is not attractive.

### 6.1. Plasmodium

*Plasmodium* parasites, the causative agents of malaria, are the focus of intense research because of the high mortality rate of this disease. Five species of these parasites are known to cause the disease in humans, *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*. While the prevalence of infection by these species may vary according to geographical locations, they have very similar life cycle stages. The parasite has a two-host life cycle, one in the mosquito (definitive host) and the other in vertebrates (secondary host). The sporozoites enter the vertebrate host as they are present in the saliva of the biting mosquito. These sporozoites migrate to the liver and enter hepatocytes. The

 

liver phase of this parasite's lifecycle includes first asexual reproduction through exoerythocytic schizogony and finally merozoite transformation. Merozoites are released into the bloodstream, where they invade erythrocytes and are transformed into ring form. Later the ring form gives rise to a schizont, which is capable of producing large number merozoites. The merozoites are released by rupturing the erythrocytes. Upon release, they infect and rupture more erythrocytes [59]. This rupture of erythrocytes is the hallmark of malaria and lack of timely therapeutic intervention may lead to the death of the infected host. Treatment for this disease includes administration of drugs such as quinolone, pyrimethamine, and artesunate groups [60]. These drugs have been in use for decades and there are several reports of drug resistance [61].

Transcriptome analysis had shown that during transformation from late trophozoite to schizont stage, genes related to ubiquitin-proteasomal degradation are upregulated thereby indicating the importance of this pathway in the parasite [62, 63]. In support of this hypothesis, several reports indicate that the proteasome of *Plasmodium* is likely to serve as an excellent drug target as the parasite is susceptible to chemicals that inhibit proteasome function. Various proteasome inhibitors have been tried in *Plasmodium* for the last two decades with reasonable success. Gantt et al. were the first to report that treatment with lactacystin, a well-known proteasome inhibitor, led to the inhibition of the development from exoerythrocytic to erythrocytic phases of malaria parasite [64]. Subsequent report also documents sensitivity towards another proteasome inhibitor, MLN-273, as it could block *Plasmodium's* erythrocytic development at an early ring stage as well as exoerythrocytes progression to schizonts [65]. A recent study has shown that *Plasmodium* is sensitive towards various proteasome inhibitors like epoxomicin, MG132, MG115, Z-L3-VS, Ada-Ahx3-L3-VS, lactacystin, bortezomib (Velcade®), gliotoxin etc., with the greatest sensitivity being exhibited towards epoximicin. Even chloroquine resistant field isolates showed high degree of susceptibility to inhibition of proteasome raising possibility of targeting drug resistant strains of this parasite [66]. These studies together highlight the effectiveness of proteasome inhibition in combating malaria.

Besides the proteasome, other components of the UPS also show the promise of serving as effective drug targets as these are sufficiently different from the host machinery. Recent report shows that three enzymes of *Plasmodium*, HRD1 (E3), UBC (E2) and UBA1 (E1), are integral components of the parasite's ERAD pathway wherein misfolded proteins from ER are transported back to the cytosol for degradation by the proteasome [67]. While the E1 and E2 enzymes have been found to localize to cytosol, the E3 localized to ER membrane. *Plasmodium* HRD1 has shown to be very different in sequence from host's E3s and thus is likely to be a drug target [67]. Comparative genomics also shows that the ERAD pathway of pathogenic protists, such as Plasmodia, Trypanosomes, Leishmania and Toxoplasma species have less redundancy compared to that of the higher eukaryotes [68]. This simplified system is thus likely to be more susceptible to chemical inhibitors. This has already been demonstrated for *P. falciparum* p97 protein, which is involved in the extraction of misfolded proteins from the ER. The parasite p97 is approximately ten times more sensitive to the inhibitor DBeQ compared to the orthologous enzyme of the host [68, 69]. Apart from enzymes involved in ubiquitination, a DUB of *Plasmodium*, PfUCHL3, has been shown to bind to ubiquitin differently compared to its human counterpart. PfUCHL3 is essential for survival of parasite and thus is likely to be a potential therapeutic target [70]. Another significant observation is that besides the 26S proteasome, *Plasmodium* contains an ATP-dependent threonine protease that is an orthologue of the HslV protease of bacteria [71]. Since this protein is not present in higher eukaryotes, it is also a potential therapeutic target.

### 6.2. Entamoeba

*Entamoeba* is a unicellular protozoan, which is the causative agent for the diarrheal disease amoebiasis. The parasite infects approximately 10% of the global population, causing amoebic dysentery [72]. In some cases infection results in hepatic abscesses and the disease can sometimes be fatal. There are many species of amoeba like *Entamoeba histolytica, Entamoeba invadens, Entamoeba dispar* etc. The life cycle of this parasite switches between two morphological states, an active, motile, trophozoite stage, which can survive only within the host and a non-motile cyst form that is encased by a thick cyst wall coating around it and is able to survive outside the host for months [73]. The treatment for amoebiasis includes administration of drugs like metronidazole, emetine, chloroquine, diloxanide and iodoquinol [74]. Although these drugs are still in use and in most case the diarrhea is cured, there are reports of the emergence of drug resistant strains, thus creating a need for finding new drugs [74, 75].

Presence of 20S catalytic core was first reported in *Entamoeba* as far back as in the nineties. The authors of this study also reported the presence of a unique 11S protease complex in *Entamoeba* [76]. This 11S protease was found to have a homohexameric structure, which appears to be unique to *Entamoeba*. Later on the 19S cap subunits were found to be present along with 20S catalytic core [77]. The distribution of both 19S cap as well as 20S catalytic core is unique in *Entamoeba*. Whereas in mammalian and yeast cells the proteasome is found in the nucleus as well as in cytosol, immunolocalization and subcellular fractionation studies of *Entamoeba* trophozoites show that the proteasome is absent from the nucleus [77]. The proteasome is documented to play a role in the morphological stage transition of amoeba as the proteasome inhibitor lactacystin prevented cyst formation in *Entamoeba* cultures. Fifty per cent inhibition of encystation was achieved using lactacystin concentrations between 1.25 and 2.5 μM, and complete inhibition was achieved with 10 μM concentration [78]. Later on comparative studies with diverse proteasome inhibitors were performed in *Entamoeba.* Among inhibitors like lactacystin, clasto-lactacystin, beta-lactone, and MG-132, beta-lactone

showed best inhibitory effect on encystation of *E. histolytica*. Amongst the different *Entamoeba* species, *E. histolytica* was found to be more susceptible to inhibition of proteasome than *E. invadens* [79]. Multinucleation was prominent in *Entamoeba* trophozoites when treated with proteasome inhibitors.

For a long period of time UPS research in *Entamoeba* was limited to characterization of proteasomes and inhibition of this proteolytic machine. But recent study of Bosch et al. identified components of the ubiquitinating machinery of this parasite. The study provided mechanistic insight into the ubiquitination process through the sequential action of E1, E2 and E3 enzymes [80]. *Entamoeba* Uba1 (E1) exhibit greater maximal velocity of pyrophosphate:ATP exchange than its human ortholog. Ubc5 (E2) interacts with Uba1 via the ubiquitin-fold domain of Uba1 and this interaction is 10-fold stronger when Uba1 is ubiquitin conjugated rather than when it is in its free form. This study also reports the crystal structure of *Entamoeba* ubiquitin (EhUbiquitin). Although the sequence of ubiquitin is extremely conserved in all domains of eukaryotes, the EhUbiquitin appears to have an additional lysine residue (K54) that is usually occupied by an arginine residue in ubiquitin from other organisms. In addition, there are other unique residues of the EhUbiquitin that cluster together at the helix α1, close to the K54 [80]. This raises the possibility of the existence of unique K54-linked polyubiquitin chains and thus the corresponding E3 ligase may serve as a potential therapeutic target.

The unique 11S protease, mechanistic alterations of the ubiquitination machinery compared to that of the host's and the possible existence of unique polyuibiquitin conjugates may serve as important areas where novel inhibitors can be designed to control the parasite [76].

### 6.3. Trypanosoma

*Trypanosoma*, a flagellated unicellular protozoan of the order Kinetoplastida, restricts its life cycle between blood-feeding reduviid bug vector and mammalian hosts. Trypanosomes have been associated with diseases like Chagas disease (*T. cruzi*) and sleeping sickness (*T. brucei*). After the trypomastigote-carrying reduviid bug vector feeds blood from a healthy individual, the metacyclic trypomastigotes are introduced in mucosal membranes. This non-dividing metacyclic form can invade a wide range of cells. Once inside a cell, the metacyclic form begins to differentiate into the amastigote form. The amastigotes continue dividing until the cell is filled with several amastigotes. Then the amastigotes differentiate into flagellated trypomastigote form by intermediate intracellular epimastigode formation. This slender trypomastigote form is capable of extracellular differentiation and can enter the lymph, blood or other cell types [81]. The treatment of African trypanosomiasis includes treatment with drugs like difluoromethylornithine (DFMO; Ornidyl®), suramin, melasoprol etc., while the Chagas disease is treated with drugs like benznidazole and nifurtimox [82, 83]. Although these drugs are still in use to treat trypanosome diseases but drug resistance cases are becoming increasingly prevalent [84].

It is hypothesized that non-lysosomal protein degradation plays a crucial role in *Trypanosoma*. Apart from containing a canonical 20S catalytic proteasome, *Trypanosoma* contain threonine protease HslVU complex [85]. While the 20S proteasome was found to localize in the nucleus, cytosol and kinetoplast, the HslVU complex has been shown to localize only to the kinetoplast of epimasgotes [86]. In addition, although both HslVU and 20S proteasome were found to be present in epimastigotes, they are differently expressed within the cell [86]. Li et al. have documented possibly novel functions of this HslVU complex. They have shown that this complex is associated with *Trypanosoma* kinetoplast DNA replication and absence of it leads to the accumulation of un-segregated kinetoplast DNA after replication [86]. This HslVU complex is absent in human hosts and hence can be a target for selective therapeutic intervention. The 20S catalytic core of *Trypanosoma* proteasome can assemble with either the 19S regulatory particle or with an 11S activator complex [87, 88]. The 11S activator complex is different in *Trypanosoma* as compared to that of the human host. The human orthologue, PA28 complex, is a heptameric or hexameric ring composed of PA28α and PA28β subunits. But in *Trypanosoma*, there is a single subunit that assembles together to form 11S activator complex [87]. This PA26 shares very little sequence homology with either PA28α or PA28β sequences of other eukaryotes and its molecular mass is also less as compared with others (26 kDa versus 28 kDa)[87]. Considering these uniqueness *Trypanosoma* UPS can be considered for targeting selectively in drug development.

It has been shown that proteasomal proteolysis plays crucial part during differentiation of *T. cruzi* replicative epimastigotes to non-replicative trypomastogotes [89]. Mutomba et al. showed interesting functions of proteasomal complex in cell cycle progression in procyclic as well as blood stream forms of the parasite. Proteasome inhibitor lactacystin inhibits the cell cycle of bloodstream form of the parasite at both G1 and G2 phase, thereby inhibiting G1→S or G2→M phase transition [90]. However it inhibits only at the G2 stage of the procyclic form [90]. Apart from lactacystin, methyl vinyl sulfones and β-lactone-γ-lactam class of proteasome inhibitors showed significant trypanocidal activity, even at the nanomolar range [91, 92]. Proteasome inhibitor bortezomib, which has been found to be effective in treating multiple myolema, showed promise by inhibiting blood stages of trypanosoma thereby suggesting the usage of proteasome inhibitor in treatment of trypanosome associated diseases [93].

 

### 6.4. Leishmania

Like *Trypanosoma*, *Leishmania* also is a parasite of order kinetoplastida and associated with the disease leishmaniasis. The infection can be either visceral leishmaniasis (caused by *L. donovanii*) or cutaneous leishmaniasis (caused by *L. tropica, L. mexicana, L. braziliensis*). The life cycle of *Leishmania* is very similar like *Trypanosoma* and involves stages within the sand fly and vertebrate host. The infection cycle starts when the infected sandfly bites the host. Promastigotes are injected into the skin and are phagocytosed rapidly by neutrophils. Upon release from neutrophils, these parasites are consumed by macrophages. Inside macrophage promastigotes transform into amastigotes and these amastigotes keep on increasing in number and infect other tissues. These amastigotes are taken up by the sandfly, where the rest of the lifecycle is completed [94]. The treatment of either form of leishmaniasis includes administration of antimony-containing compounds, glucantime, amphotericin or pentamidine drugs [95]. These drugs are useful in treating majority of cases, although drug resistance cases have been reported in numerous occasions [96]. These drug resistances develops from multiple reasons like altered drug metabolism, decreased drug uptake, increased drug efflux and alteration of drug target.

The presence of the proteasome is well documented in these parasites. Robertson et al. first showed the presence of 20S proteasome in *L. Mexicana* [97]. Later on 20S proteasome was shown in *L. donovanii* [98]. Apart from that HslV protease, that is present in other primordial eukaryotes have been shown to be present in *Leishmania* [99]. Methionine adenosyltransferase (MAT) is an important enzyme of *Leishmania*. Its product S-adenosylmetheomine (SAM) is involved in trans-methylation, trans-sulphuration and polyamine production reactions. Modulation of MAT levels play important role during transition of life cycle phases in *Leishmania*. Recent studies have shown an important role of proteasome in the turnover of MAT and hence can influence survival of this protist [100]. UPS also plays crucial part in cell-cycle regulation in *Leishmania*. Kin-13 kinesin was found to be abundant during G2 and M phases but its level decreased after mitosis. This degradation is essential for next cycle to start and the turnover has been found to be mediated by UPS [101]. All the above examples show the essential function played by UPS in the survival and pathogenesis of *Leishmania*. Selective targeting of unique HslV or inhibition of the proteasome may prove to be effective therapeutic strategies.

### 6.5. Giardia

*Giardia lamblia* (also known as *G. intestinalis* or *G. duodinalis*) is the causative agent of the diarrheal disease giardiasis. It accounts for 20% of all diarrheal cases reported worldwide [102, 103]. Like *Entamoeba*, this parasite also has two morphological states in its lifecycle, the trophozoites and cysts. While the trophozoites are binucleated, the cysts are tetranucleated. The trophozoite is the vegetative form of the parasite that resides in the intestine of vertebrate hosts. Upon coming in contact with high bile concentration in the lower intestine, the trophozoites are transformed to cysts. The cysts are released with the stool of infected hosts and can survive in harsh environmental condition for months. Cysts enter a new host through contaminated food and water. They undergo excystation in the acidic environment of the stomach, thus completing the cycle of infection [104]. The treatment of infection includes administration of acridine, benzimidazole, nitroimidazole and thiazolide drugs [105]. These drugs target diverse cellular processes; for example, some bind DNA and cleave it; some interfere with anaerobic metabolism, while others interact with the cytoskeleton. Although these drugs are clinically proven to eradicate the infection, they are known to cause side effects. Hence there is a demand for new drug targets in this parasite.

An attractive strategy for therapeutic intervention is to target the altered cellular machinery of this protist. *Giardia* appears to have undergone several reductive evolutionary changes. For example, it has a simplified repertoire of cellular organelles, characterized by the lack of classical mitochondria, stacked Golgi compartments and peroxisomes [106]. The UPS is also likely to be simplified compared to that of its vertebrate host and this notion is supported by the presence of only one gene encoding ubiquitin in the *Giardia* genome [107], which is in contrast to most organisms that have multiple ubiquitin genes. The genes of the ubiquitinating machinery are also likely to be different. For example, the ubiquitin activating enzyme (E1) of this parasite is novel as it is proteolyticaly processed into two halves, a 68 kDa N-terminal half and a 47 kDa C-terminal half. This kind of cleavage is unique and has not been documented in any other eukaryote [108]. The E1 enzyme has been found to modulate the encystation of *Giardia*. Gene silencing experiments revealed absence of E1 hampers the encystation efficiency of the parasite and overexpression of this protein increases the encystation efficiency of the parasite. The presence of 20S catalytic proteasome in *Giardia* was first reported by Emmerlich et al. [109]. Jerlström-Hultqvist et al. documented the presence of the 19S lid subunit components [110]. Although majority of 19S components are conserved in this parasite, the complex itself appears to be simpler as genes encoding Rpn13 and Rpn12 could not be detected in the *Giardia* genome. In a very recent study Niño et al reported the cellular distribution of ubiquitin during encystation. Although ubiquitin was found to be present in both cytosol and nucleus in trophozoites, it was found to be attached with the walls of cysts [111]. Encystation appears to be proteasome-dependent as inhibition of proteasome with lactacystin at 10 μmol/litre concentration results in decreased cyst count, while not affecting trophozoite viability [111]. Taken together, the possibly altered structure of the proteasome, the divergent ubiquitination machinery and the requirement for the proteasome during encystation indicates that it may be possible to find inhibitors that selectively act upon the UPS of this protist.

## 7. Future of UPS research in parasites

Besides the above-mentioned parasites, therapeutic approaches targeting the UPS may be possible for other parasites as well. For example, the intracellular parasite *Toxoplasma* has been found to be susceptible to proteasomal inhibition as their growth, daughter cell budding and DNA synthesis are compromised [112, 113]. In the parasites like *Trichomonas* and *Microsporidia*, although the 20S core particle genes are annotated in the genomes, no information is available regarding their intracellular distribution or function [114]. Although knowledge of parasitic proteasome is still rudimentary, the recent spate of studies in this area is very encouraging. These studies have largely been confined to only those parasites that are causative agents of diseases with high mortality and morbidity rates. However, more attention needs to be focused on this important parasite machinery that is involved in several cellular processes. Thus far there is only documentation of the presence of the UPS system and its importance in parasite survival. However, questions regarding the cellular processes controlled by the UPS, as well as its own regulation and assembly of the proteasome remain unanswered. Structural studies of parasitic proteasomes are also needed as these are likely to be considerably different from that of the hosts'. As mentioned previously, parasite genomes have been found to lack conventional subunits of proteasome complexes. Thus knowledge of the structural differences of the parasitic proteasomes will not only uncover potential therapeutic targets but also add to our knowledge of how structurally different proteasomes function. These studies are also likely to help us understand how the proteasome has evolved from its simplified state in the prokaryotes, to the complex form that is present in higher eukaryotes.

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