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Ubiquitin and ubiquitin-like conjugation systems in trypanosomatids

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In eukaryotic cells, reversible attachment of ubiquitin and ubiquitin-like modifiers (Ubls) to specific target proteins is conducted by multicomponent systems whose collective actions control protein fate and cell behaviour in precise but complex ways. In trypanosomatids, attachment of ubiquitin and Ubls to target proteins regulates the cell cycle, endocytosis, protein sorting and degradation, autophagy and various aspects of infection and stress responses. The extent of these systems in trypanosomatids has been surveyed in recent reports, while in *Leishmania mexicana*, essential roles have been defined for many ubiquitin-system genes in deletion mutagenesis and life-cycle phenotyping campaigns. The first steps to elucidate the pathways of ubiquitin transfer among the ubiquitination components and to define the acceptor substrates and the downstream deubiquitinases are now being taken.

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Introduction

Ubiquitin is a single-domain protein of 76 residues with a β -grasp fold. Its sequence is highly conserved with just 2–4 residue differences between the ubiquitins of *Trypanosoma* species and their mammalian hosts [1]. Its attachment to target proteins is a widespread post-translational modification controlling molecular fate and cellular processes.

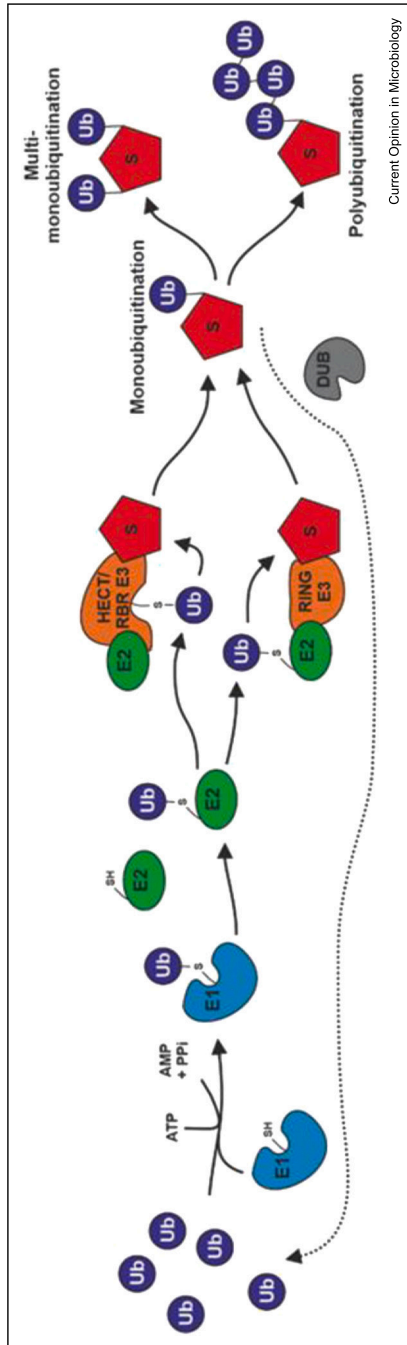
Ubiquitination is mediated by tripartite enzyme systems comprising ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligation (E3) components (Figure 1). Cellular homeostasis is ensured by deubiquitinases (DUBs), which return the ubiquitin to the cellular pool (Figure 1). Ubiquitin can be attached in multiple copies and in different linkages to target proteins, giving rise to enormous complexity in what has been termed the Ubiquitin Code [2]. For example, Lys48-linked ubiquitination is associated with proteasomal protein degradation, while Lys-63-linked ubiquitination plays a role in protein recruitment to sites of DNA damage. This complexity is augmented by an array of structurally similar, but sequence-divergent, ubiquitin-like modifiers (Ubls), which have cognate E1, E2 and E3 components and Ubl-specific proteases. In trypanosomatids, hundreds of proteins are predicted to be involved in the attachment and removal of ubiquitin and Ubls [3]. Here, we review recent progress in defining and functionally characterising the ubiquitination systems of trypanosomatids to complement an excellent review of the ubiquitination system as a drug target in trypanosomatid diseases [4].

Ubiquitination and deubiquitination components in trypanosomatids

A survey of ubiquitination-system genes in *Leishmania mexicana* has been presented in recent papers [5–7]. These analyses identified 2 ubiquitin-activating (E1), 13 ubiquitin-conjugating (E2) and 79 ubiquitin-ligating (E3) enzymes together with 20 family C12, C19 and C65 cysteine protease deubiquitinases, 5 C97 PPPDE1-containing proteins that may have deubiquitinase activity and 3 JAMM (JAB1/MPN/MOV34) metallopeptidase DUBs. Deletion mutagenesis was used to generate a set of 58 null mutant parasites, each possessing a unique barcode for subsequent life-cycle phenotyping experiments. Null mutants of the E1, UBA1a, the E2s, UBC3, UBC7, UBC12 and UBC13, and the DUBs, DUB1, DUB2, DUB12 and DUB16, were not obtained, implying essential promastigote roles for these factors [5,6]. Twenty-three null mutants were pooled as procyclic promastigotes and grown to stationary phase before differentiation was induced and the survival of the parasites as axenic or intra-macrophage amastigotes, and in mouse footpads, was determined [5,6].

Among the ubiquitination components, the $\Delta ubc2$ and $\Delta uev1$ mutants exhibited the most severe loss-of-fitness

Figure 1



Current Opinion in Microbiology

Ubiquitination and deubiquitination. Ubiquitination is brought about by the sequential action of E1, E2, and E3 enzymes. An E1 ubiquitin-activating enzyme catalyses the reaction of the C-terminal carboxylate of ubiquitin (Ub) and ATP to form a ubiquitin-adenylate intermediate. This reacts with a conserved cysteine to form an E1-Ub thioester. The ubiquitin is next transferred to an E2 ubiquitin-conjugating enzyme in a transthioesterification step. Finally, an E3 ubiquitin ligase transfers Ub from the E2 onto (typically) a lysine residue of an acceptor substrate (S) with formation of an isopeptide bond. For HECT and RBR-type E3s (upper pathway), a covalent E3-Ub thioester intermediate is formed. For the RING E3 ligases (lower pathway), the E3 facilitates direct transfer of the Ub from the E2 to the substrate. Additional Ubs may be added to other sites on S (multimonoubiquitination) or to an already-attached Ub (polyubiquitination). A DUB cleaves S-Ub or Ub-Ub bonds to return Ub to the cellular pool.

during differentiation [6]. Knockdown of the UBC2 orthologue in *T. brucei* using RNA-interference (RNAi) also leads to a severe reduction in viability [8]. The crystal structure of a complex of UBC2 and UEV1, a ubiquitin E2 variant lacking the conserved cysteine required for catalytic activity, revealed a heterodimeric structure and an interface that is conserved in human and yeast orthologues. Ubiquitination assays showed that in the presence of *Leishmania* UBA1a and the human E3s RNF8 or BIRC2, UBC2 promoted the formation of polyubiquitin chains on proteins present in the assay. The inclusion of UEV1 restricted the products to K63-linked ubiquitin dimers [6].

The life-cycle phenotyping assay emphasised the important role played by DUBs in *L. mexicana* with three of the deubiquitinases required for transformation to amastigotes and seven more required for proliferation in mice [5]. These findings are consistent with the extensive protein turnover that accompanies the changes in cell structure during differentiation. DUB2, which was further characterised, exhibits broad linkage specificity in cleaving diubiquitin substrates [5]. This suggests that a broad repertoire of ubiquitin linkages may be formed in trypanosomatids, but so far, direct evidence is lacking.

Ubiquitination in the cell cycle of trypanosomatids

The levels of many cell-cycle regulators oscillate during the eukaryotic cell cycle because of periodic ubiquitination and proteolysis. Two key multisubunit E3 complexes important for cell-cycle progression are the anaphase-promoting complex or cyclosome (APC/C) and the SKP1-CULLIN1-F-box (SCF) complex. APC/C controls chromosome segregation and exit from mitosis by triggering ubiquitination and degradation of cell-cycle regulators. Depletion of key components of the APC/C leads to metaphase arrest in *T. brucei* procyclic forms and anaphase arrest in bloodstream forms [9,10], suggesting that mitosis is regulated in a stage-specific manner. Depletion of another APC/C component, AP2, results in mitotic arrest of procyclic forms and stabilisation of a potential substrate, the mitotic cyclin CycB2/cyc6 [11]. Interestingly, deletion of only three of the 10 identified core APC/C components produces a phenotype, showing that the *T. brucei* APC/C has a smaller group of core components than the corresponding complex in *S. cerevisiae*. A further four non-core APC/C-associated proteins were later shown to play essential roles in regulating cell-cycle progression [12]. All are annotated as regulatory subunits of the proteasome, effectively coupling the ubiquitination of cell-cycle regulators in *T. brucei* to their proteolytic degradation [12].

Meanwhile, depletion of components of a putative *T. brucei* SCF complex revealed a conserved role for SKP1 in the G1/S transition, a possible role for RBX1 in kinetoplast DNA replication and a role for the E2 ubiquitin-conjugating enzyme CDC34 in cytokinesis [13]. CDC34 is essential for infection progression in mice, consistent with the rapid growth arrest observed in cells in which CDC34 is depleted. Interestingly, no phenotype was observed following depletion of CULLIN1, suggesting redundancy in cullin function in *T. brucei* or incomplete knockdown [13].

Roles for ubiquitin in trypanosomatid endocytosis

In eukaryotes, ubiquitination plays an important role in receptor endocytosis and the sorting of proteins through the endosomal system [14]. In *T. brucei*, the glycosomal matrix receptor PEX5 is ubiquitinated by the E2 ubiquitin-conjugating enzyme PEX4, which also localises to the glycosome. This function of PEX4 is at least partially redundant, however, since PEX5 remains ubiquitinated in a $\Delta pex4$ background [15]. Furthermore, two ubiquitin-associated (UBA) domains in *Leishmania* myosin XXI are required for normal endocytic trafficking, although the underlying mechanism is not known [16].

A high-throughput RNAi screen implicated genes encoding the DUBs, Usp7 and Vdu1 in the susceptibility of *T. brucei* to suramin [17]. Usp7 regulates expression of the invariant-surface glycoprotein ISG75, which is involved in suramin uptake and sensitivity, and ubiquitination of its cytoplasmic domain leads to its internalisation and degradation [18,19]. Cycloheximide-chase experiments showed that knockdown of Usp7 and Vdu1 destabilised ISG75 by increasing the rate of its turnover, presumably by blocking ISG75 deubiquitination [20]. It was proposed that silencing of the DUBs diverts ISG proteins from the endosome-recycling pathway into its degradative arm [20]. Aquaglyceroporin 2, which contributes to the sensitivity of *T. brucei* to pentamidine and melarsoprol, is also directed to the lysosomal-degradation pathway following its ubiquitination [21].

Ubiquitination and infection

During the acute phase of *T. cruzi* human infection, the host E3 ubiquitin ligase GRAIL (gene related to anergy in lymphocytes), a negative regulator of CD4 T-cell responsiveness, is upregulated. This is achieved through disruption of the Akt-mTOR pathway, resulting in the downregulation of otubain-1, a human DUB that negatively regulates GRAIL function [22,23]. During later stages of infection, GRAIL expression is downregulated as otubain-1 expression is upregulated [23]. Elsewhere, recombinant *L. infantum* otubain was reported to stimulate lipid-droplet biogenesis and cytokine secretion in macrophages [24]. While this hints at a role for this

DUB in the pro-inflammatory response of macrophages during infection, secretion of the parasite otubain into host cells is yet to be demonstrated.

A clearer example of parasite ubiquitination-system components modifying host-cell targets is the SPRING (secretory protein with a RING finger domain) E3 ligase of *T. cruzi* [25]. Amastigotes secrete SPRING into host cells where it becomes localised to the nucleus. A possible target is breast cancer-associated protein 3 (BCA3), which coimmunoprecipitates with SPRING following their coexpression in wheat germ lysates, and which is ubiquitinated by SPRING *in vitro* [25].

A screen of a bloodstream-form *T. brucei* RNAi library for sensitivity to recombinant apolipoprotein-L1 (apoL1), a component of the TLF1 and TLF2 trypanolytic complexes found in human serum, identified six putative ubiquitin-system components among 63 hits [26]. These included two RING E3 ligases (Tb927.10.12940 and Tb927.11.4860), an E2 ubiquitin-conjugating enzyme (Tb927.9.8000), two DUBs (Tb.927.9.5520 and Tb.927.9.14470) and a p97/cdc48 cofactor (Tb927.11.6340). The most prominent hit was Tb927.10.12940, a putative lysosomal RING E3 ubiquitin ligase, whose knockdown led to a dramatic reduction in parasite sensitivity. Contrastingly, knockdown of Tb927.10.12940 enhanced parasite sensitivity to human serum in which TLF1 is the dominant trypanolytic complex, suggesting that other components shape the response to apoL1. Follow-up analysis suggested that the activity of Tb927.10.12940 was dependent on the function of Tb927.9.8000, the E2 ubiquitin-conjugating enzyme identified in the screen, though the target(s) of ubiquitination have yet to be identified. These observations may help to explain the susceptibility of different African trypanosome species to lysis by human serum [26].

Ubiquitination in translation and protein quality control

The RNA helicase DDX3 is important for optimal elongation of translating ribosomes and for stimulating the dissociation and recycling of ribosomes that have arrested. Prolonged ribosome stalling in *L. infantum* cells lacking DDX3 induces quality-control responses, including recruitment of ubiquitination and proteasome components that degrade nascent polypeptides [27]. Among the components found to be enriched at stalled ribosomes are a HECT (homologous to E6AP C terminus) family E3 ubiquitin ligase (LINF_350029800), subunits APC3, APC6 and APC10 of the APC/C complex, the E2 factor, UBC2, the ubiquitin C-terminal hydrolase DUB15 and the proteasomal ubiquitin receptor Rpn13. Interestingly, the deubiquitinase DUB10 is upregulated in cells depleted of DDX3 [27].

A quality-control system has recently been described in *T. brucei* that removes mistargeted aggregation-prone mitochondrial proteins that accumulate in the cytosol [28]. Its components include an E3 ubiquitin ligase (TbE3HECT1), TbUbl1, which harbours a ubiquitin-like protein domain, a mitochondrial protein of unknown function (Tb927.9.7200) and the proteasome. The system is triggered by ablation of ATOM69, a component of the atypical outer membrane translocase and the mitochondrial import receptor for hydrophobic proteins [28]. Upon triggering of the pathway, TbUbl1, a normally nuclear protein, is released into the cytosol, where it binds to mislocalised mitochondrial proteins and, by mechanisms yet to be elucidated, facilitates their ubiquitination and degradation by the proteasome.

Ubiquitin-like modifiers in trypanosomatids

Excellent reviews of ubiquitin-like modifiers in protozoan parasites have been published [29,30]. In *Leishmania*, the best-characterised UbIs are Atg8 and Atg12, which play roles in parasite autophagy [31,32]. In *Leishmania*, conjugation of ATG12–ATG5 is promoted by the E1-like enzyme ATG7 and the E2-like enzyme ATG10. ATG12–ATG5 is required for phagophore development, including the attachment of ATG8-phosphatidylethanolamine to autophagic membranes [32]. In *L. donovani*, where Atg8 was observed to accumulate around the damaged mitochondrion, deletion of *Atg8* compromised parasite differentiation and infectivity [33]. A functional ATG8-conjugation system has also been found in *T. brucei* and *T. cruzi* [34,35].

A SUMO (small ubiquitin-like modifier) -conjugation system has been characterised in *T. brucei*. Aos1/Uba2 (heterodimeric SUMO E1), Ubc9 (SUMO E2) and SIZ1 (SUMO E3 ligase) play a role in SUMO conjugation. A SUMO-specific protease, sentrin-specific protease (SENP), has also been described [36–38]. SUMO, which has a nuclear localisation, is required for cell-cycle regulation, expression of variant-surface glycoprotein (VSG) genes and chromatin organisation [38–40]. Additionally, the RNA pol-I complex, which is responsible for VSG transcription, is SUMOylated in bloodstream forms by SIZ1, demonstrating one way in which SUMOylation could contribute towards VSG expression [38]. SUMOylation of Centrin, which plays an important role in cell motility, has also been observed *in vitro* [36,41]. These studies point towards nuclear and non-nuclear SUMO functions. In *L. mexicana*, a SUMO orthologue has been identified together with a putative E1 catalytic subunit, UBA2 and an E2 enzyme, UBC9 [6]. SUMO and an associated SENP are constitutively expressed [42]. SENP null mutants are viable albeit with reduced vitality, while SUMO null mutants were not obtained,

pointing to an essential role. In the SENP null mutants, C-terminal processing of SUMO precursors is disrupted and covalent SUMO attachment to proteins is prevented, as is the translocation of SUMO to the nucleus. Curiously, *in vitro* infectivity is not affected by loss of SENP-mediated SUMO processing, leading these authors to conclude that functions of unprocessed SUMO are critical for viability [42].

In *T. brucei*, Nedd8 and its putative E2-conjugating enzyme, Ubc12, are enriched in the nucleus and flagellum. Depletion of Nedd8 by RNAi led to reduced levels of protein ubiquitination and caused DNA rereplication in post-mitotic cells [43]. It also impaired spindle assembly and compromised the flagellum-attachment zone filament, leading to flagellum detachment. Six cullins, TbCUL1–TbCUL6, were identified as substrates of TbNedd8 [43]; neddylation is known to positively regulate the activity of Cullin-RING ubiquitin ligases [44]. In *L. mexicana*, E1 catalytic (UBA3) and E2 (UBC12) neddylation components have been proposed based on orthology [6].

The structures of *T. brucei* URM1 and UFM1 have been determined using NMR spectroscopy [45,46]. In *L. donovani*, UFM1 conjugation involves the action of UBA5, an E1 enzyme, and UFC1, an E2 enzyme. UFM1, UBA5 and UFC1 localise to the mitochondrion and ufmylation is important for β -oxidation and amastigote growth in macrophages [47,48]. *L. donovani* Urm1, which is associated with early endosome proteins, and its E1, LdUba4, have also been identified [49].

Conclusions

A current challenge is to elucidate the pathways of ubiquitin transfer among the E1s, E2s and E3s of trypanosomatids and link these to the acceptor substrate proteins and downstream DUBs. This in turn will illuminate cellular function and regulation in these parasites. Many of these functions will be common to model organisms, but given their ancient evolutionary character and often-divergent biochemistry, insights into parasite-specific processes can be anticipated.

Structural biology will contribute to this understanding with accurate models of individual components predicted on deep-learning platforms such as AlphaFold2 [50] and RoseTTAFold [51]. Structure predictions for proteins from trypanosomatids have to date tended to be of lower confidence. The cause is the poor sampling of this branch of life in the protein-sequence databases that underpin structure prediction. By gathering openly available protein-sequence data for species from this lineage, significant improvements to protein- structure

prediction have been demonstrated [52]. This will enable description of the structural context of ubiquitination on a proteome-wide scale [53].

The proteasome is a validated therapeutic target in the treatment of leishmaniasis, Chagas disease and sleeping sickness, and potent and selective inhibitors of high efficacy have been developed [54,55]. Since many components of the trypanosomatid ubiquitin and Ubl-modification systems belong to the ubiquitin-proteasome pathway, they also have great potential as drug targets in neglected tropical diseases.

CRedit authorship contribution statement

Rebecca Burge: Writing – original draft, **Jeremy Mottram:** Writing – review & editing, **Tony Wilkinson:** Writing – review & editing.

Conflict of interest statement

The authors declare no competing interests.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Zuin A, Isasa M, Crosas B: **Ubiquitin signaling: extreme conservation as a source of diversity.** *Cells* 2014, **3**:690-701.
2. Komander D, Rape M: **The ubiquitin code.** *Annu Rev Biochem* 2012, **81**:203-229.
3. Gupta I, Aggarwal S, Singh K, Yadav A, Khan S: **Ubiquitin Proteasome pathway proteins as potential drug targets in parasite *Trypanosoma cruzi*.** *Sci Rep* 2018, **8**:8399.
4. Bijlmakers MJ: **Ubiquitination and the proteasome as drug targets in trypanosomatid diseases.** *Front Chem* 2020, **8**:630888.
5. Damianou A, et al.: **Essential roles for deubiquitination in**
 - ***Leishmania* life cycle progression.** *PLoS Pathog* 2020, **16**:e1008455.

Detailed bioinformatic survey of 20 cysteine peptidase-type deubiquitinases of *L. mexicana* revealing their importance in the life cycle of the parasite. Deletion mutagenesis revealed 4 DUBs to be essential in promastigotes with 10 more needed for differentiation and infectivity in mice. Detailed studies of DUB2 showed broad linkage specificity.
6. Burge RJ, Damianou A, Wilkinson AJ, Rodenko B, Mottram JC:
 - ***Leishmania* differentiation requires ubiquitin conjugation mediated by a UBC2-UEV1 E2 complex.** *PLoS Pathog* 2020, **16**:e1008784.

Important paper giving insight into the scope and importance of ubiquitination in *L. mexicana*. It presents a survey of the ubiquitination components with follow-up deletion mutagenesis and life-cycle profiling studies. Genes essential in promastigotes and others required for differentiation and infectivity are identified. Biochemical assays of ubiquitination are established for the UBC2-UEV1 pair whose crystal structure was also determined.
7. Grewal JS, Catta-Preta CMC, Brown E, Anand J, Mottram JC: **Evaluation of clan CD C11 peptidase PNT1 and other *Leishmania mexicana* cysteine peptidases as potential drug targets.** *Biochimie* 2019, **166**:150-160.
8. Alsford S, et al.: **High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome.** *Genome Res* 2011, **21**:915-924.
9. Zhou Z, He M, Shah AA, Wan Y: **Insights into APC/C: from cellular function to diseases and therapeutics.** *Cell Div* 2016, **11**:9.
10. Kumar P, Wang CC: **Depletion of anaphase-promoting complex or cyclosome (APC/C) subunit homolog APC1 or CDC27 of *Trypanosoma brucei* arrests the procyclic form in metaphase but the bloodstream form in anaphase.** *J Biol Chem* 2005, **280**:31783-31791.
11. Bessat M, Knudsen G, Burlingame AL, Wang CC: **A minimal anaphase promoting complex/cyclosome (APC/C) in *Trypanosoma brucei*.** *PLoS One* 2013, **8**:e59258.
12. Bessat M: **Knockdown of APC/C-associated genes and its effect on viability and cell cycle of protozoan parasite of *Trypanosoma brucei*.** *Parasitol Res* 2014, **113**:1555-1562.
13. Rojas F, et al.: **The ubiquitin-conjugating enzyme CDC34 is essential for cytokinesis in contrast to putative subunits of a SCF complex in *Trypanosoma brucei*.** *PLoS Negl Trop Dis* 2017, **11**:e0005626.
14. Haglund K, Dikic I: **The role of ubiquitylation in receptor endocytosis and endosomal sorting.** *J Cell Sci* 2012, **125**:265-275.
15. Gualdrón-López M, et al.: **Ubiquitination of the glycosomal matrix protein receptor PEX5 in *Trypanosoma brucei* by PEX4 displays novel features.** *Biochim Biophys Acta* 2013, **1833**:3076-3092.
16. Bajaj R, Ambaru B, Gupta CM: **Deciphering the role of UBA-like domains in intraflagellar distribution and functions of myosin XXI in *Leishmania*.** *PLoS One* 2020, **15**:e0232116.
17. Alsford S, et al.: **High-throughput decoding of antitrypanosomal drug efficacy and resistance.** *Nature* 2012, **482**:232-236.
18. Chung WL, Leung KF, Carrington M, Field MC: **Ubiquitylation is required for degradation of transmembrane surface proteins in trypanosomes.** *Traffic* 2008, **9**:1681-1697.
19. Leung KF, Riley FS, Carrington M, Field MC: **Ubiquitylation and developmental regulation of invariant surface protein expression in trypanosomes.** *Eukaryot Cell* 2011, **10**:916-931.
20. Zoltner M, Leung KF, Alsford S, Horn D, Field MC: **Modulation of the surface proteome through multiple ubiquitylation pathways in African trypanosomes.** *PLoS Pathog* 2015, **11**:e1005236.
21. Quintana JF, et al.: **Instability of aquaglyceroporin (AQP) 2 contributes to drug resistance in *Trypanosoma brucei*.** *PLoS Negl Trop Dis* 2020, **14**:e0008458.
22. Whiting CC, Su LL, Lin JT, Fathman CG: **GRAIL: a unique mediator of CD4 T-lymphocyte unresponsiveness.** *FEBS J* 2011, **278**:47-58.
23. Stempin CC, Rojas Marquez JD, Ana Y, Cerban FM: **GRAIL and Otubain-1 are related to T Cell hyporesponsiveness during *Trypanosoma cruzi* infection.** *PLoS Negl Trop Dis* 2017, **11**:e0005307.
24. Azevedo CS, et al.: **Revealing a novel Otubain-like enzyme from *Leishmania infantum* with deubiquitinating activity toward K48-linked substrate.** *Front Chem* 2017, **5**:13.
25. Hashimoto M, Murata E, Aoki T: **Secretory protein with RING finger domain (SPRING) specific to *Trypanosoma cruzi* is directed, as a ubiquitin ligase related protein, to the nucleus of host cells.** *Cell Microbiol* 2010, **12**:19-30.
26. Currier RB, Cooper A, Burrell-Saward H, MacLeod A, Alsford S: **Decoding the network of *Trypanosoma brucei* proteins that determines sensitivity to apolipoprotein-L1.** *PLoS Pathog* 2018, **14**:e1006855.
27. Padmanabhan PK, et al.: **Genetic depletion of the RNA helicase DDX3 leads to impaired elongation of translating ribosomes triggering co-translational quality control of newly synthesized polypeptides.** *Nucleic Acids Res* 2021, **49**:9459-9478.

Interesting paper that expands the repertoire of roles for ubiquitination in trypanosomatids. It demonstrates that depletion of an RNA helicase in *Leishmania* slows down ribosome movement and increases ribosome stalling. In response, E3 ligases and proteasome components are recruited to the ribosomes accompanied by ubiquitination of nascent polypeptides.

28. Dewar CE, *et al.*: **Mistargeting of aggregation prone mitochondrial proteins activates a nucleus-mediated posttranscriptional quality control pathway in trypanosomes.** *Nat Commun* 2022, **13**:3084.
- Insightful study of protein translocation that led to the discovery of a quality-control system in *T. brucei* that removes mistargeted and aggregation-prone mitochondrial proteins that accumulate in the cytosol. The authors identified new ubiquitin-system factors in their wide-ranging study and they interpret their findings in terms of a plausible model.
29. Karpiyevich M, Artavanis-Tsakonas K: **Ubiquitin-like modifiers: emerging regulators of protozoan parasites.** *Biomolecules* 2020, **10**:1403.
30. Ponder EL, Bogoy M: **Ubiquitin-like modifiers and their deconjugating enzymes in medically important parasitic protozoa.** *Eukaryot Cell* 2007, **6**:1943-1952.
31. Williams RA, Woods KL, Juliano L, Mottram JC, Coombs GH: **Characterization of unusual families of ATG8-like proteins and ATG12 in the protozoan parasite *Leishmania major*.** *Autophagy* 2009, **5**:159-172.
32. Williams RA, Smith TK, Cull B, Mottram JC, Coombs GH: **ATG5 is essential for ATG8-dependent autophagy and mitochondrial homeostasis in *Leishmania major*.** *PLoS Pathog* 2012, **8**:e1002695.
33. Giri S, Shaha C: ***Leishmania donovani* parasite requires Atg8 protein for infectivity and survival under stress.** *Cell Death Dis* 2019, **10**:808.
34. Alvarez VE, *et al.*: **Autophagy is involved in nutritional stress response and differentiation in *Trypanosoma cruzi*.** *J Biol Chem* 2008, **283**:3454-3464.
35. Proto WR, Jones NG, Coombs GH, Mottram JC: **Tracking autophagy during proliferation and differentiation of *Trypanosoma brucei*.** *Microb Cell* 2014, **1**:9-20.
36. Ye K, Zhang X, Ni J, Liao S, Tu X: **Identification of enzymes involved in SUMOylation in *Trypanosoma brucei*.** *Sci Rep* 2015, **5**:10097.
37. Klein CA, Droll D, Clayton C: **SUMOylation in *Trypanosoma brucei*.** *PeerJ* 2013, **1**:e180.
38. Lopez-Farfan D, Bart JM, Rojas-Barros DI, Navarro M: **SUMOylation by the E3 ligase TbSIZ1/PIAS1 positively regulates VSG expression in *Trypanosoma brucei*.** *PLoS Pathog* 2014, **10**:e1004545.
39. Liao S, Wang T, Fan K, Tu X: **The small ubiquitin-like modifier (SUMO) is essential in cell cycle regulation in *Trypanosoma brucei*.** *Exp Cell Res* 2010, **316**:704-715.
40. Iribarren PA, Di Marzio LA, Berazategui MA, De Gaudenzi JG, Alvarez VE: **SUMO polymeric chains are involved in nuclear foci formation and chromatin organization in *Trypanosoma brucei* procyclic forms.** *PLoS One* 2018, **13**:e0193528.
41. Wei Y, Hu H, Lun ZR, Li Z: **Centrin3 in trypanosomes maintains the stability of a flagellar inner-arm dynein for cell motility.** *Nat Commun* 2014, **5**:4060.
42. Bea A, Krober-Boncardo C, Sandhu M, Brinker C, Clos J: **The *Leishmania donovani* SENP protease is required for SUMO processing but not for viability.** *Genes* 2020, **11**:1198.
43. Liao S, Hu H, Wang T, Tu X, Li Z: **The protein neddylation pathway in *Trypanosoma brucei*: functional characterization and substrate identification.** *J Biol Chem* 2017, **292**:1081-1091.
44. Deshaies RJ, Joazeiro CA: **RING domain E3 ubiquitin ligases.** *Annu Rev Biochem* 2009, **78**:399-434.
45. Zhang W, *et al.*: **Solution structure of Urm1 from *Trypanosoma brucei*.** *Proteins* 2009, **75**:781-785.
46. Diwu Y, *et al.*: **Solution structure of TbUfm1 from *Trypanosoma brucei* and its binding to TbUba5.** *J Struct Biol* 2020, **212**:107580.
47. Gannavaram S, Sharma P, Duncan RC, Salotra P, Nakhasi HL: **Mitochondrial associated ubiquitin fold modifier-1 mediated protein conjugation in *Leishmania donovani*.** *PLoS One* 2011, **6**:e16156.
48. Gannavaram S, *et al.*: **Deletion of mitochondrial associated ubiquitin fold modifier protein Ufm1 in *Leishmania donovani* results in loss of β -oxidation of fatty acids and blocks cell division in the amastigote stage.** *Mol Microbiol* 2012, **86**:187-198.
49. Sharma V, Sharma P, Selvapandiyam A, Salotra P: ***Leishmania donovani*-specific Ub-related modifier-1: an early endosome-associated ubiquitin-like conjugation in *Leishmania donovani*.** *Mol Microbiol* 2016, **99**:597-610.
50. Jumper J, *et al.*: **Highly accurate protein structure prediction with AlphaFold.** *Nature* 2021, **596**:583-589.
- The first of a pair of transformative papers in the field of protein-structure prediction that is now producing high-confidence models of trypanosomatid proteins. It describes the design of a deep-learning algorithm that incorporates physical and biological knowledge of protein structure and leverages multiple sequence alignments.
51. Baek M, *et al.*: **Accurate prediction of protein structures and interactions using a three-track neural network.** *Science* 2021, **373**:871-876.
- The second of a pair of transformative papers in the field of protein-structure prediction. It describes algorithms able to produce high-confidence models of trypanosomatid proteins. It successively transforms and integrates information at the 1-D sequence level, the 2D distance-map level and the 3D coordinate level contributing to a 3-track network.
52. Wheeler RJ: **A resource for improved predictions of *Trypanosoma* and *Leishmania* protein three-dimensional structure.** *PLoS ONE* 2021, **16**:e0259871, <https://doi.org/10.1101/2021.09.02.458674>.
- Machine-learning-generated structure predictions for trypanosomatid proteins are currently of lower confidence than those of proteins from model organisms. This is due to the under-representation of their sequences in key databases. This important paper reports the collation of openly available sequence data from trypanosomatid parasites to create a resource to support improved protein-structure prediction.
53. Bludau I, *et al.*: **The structural context of posttranslational modifications at a proteome-wide scale.** *PLoS Biol* 2022, **20**:e3001636.
54. Khare S, *et al.*: **Proteasome inhibition for treatment of leishmaniasis, chagas disease and sleeping sickness.** *Nature* 2016, **537**:229-233.
55. Wyllie S, *et al.*: **Preclinical candidate for the treatment of visceral leishmaniasis that acts through proteasome inhibition.** *Proc Natl Acad Sci USA* 2019, **116**:9318-9323.