Review Article

Update on the biological relevance of lysine acetylation as a novel drug target in trypanosomatids

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Abstract: The number of acetylated proteins identified from bacteria to mammals has grown exponentially in the last ten years and it is now accepted that acetylation is a key component in most eukaryotic signaling pathways, as important as phosphorylation. The enzymes involved in this process are well described in mammals; acetyltransferases and deacetylases are found inside and outside the nuclear compartment and have different regulatory functions. In trypanosomatids several of these enzymes have been described and are postulated to be novel antiparasitic targets for the rational design of drugs. In this review article we present an update of the most important known acetylated proteins in trypanosomatids analyzing the acetylomes available. Also, we summarize the information available regarding acetyltransferases and deacetylases in trypanosomes and their potential use as chemotherapeutic targets.

Keywords: Acetylation, Acetyltransferases, Deacetylases, Acetylome, Trypanosomatids, Cytoskeleton

1. INTRODUCTION

Lysine (K) acetylation is a reversible and highly regulated posttranslational modification (PTM) involved in a wide range of cellular processes in eukaryotes that was also recently found in prokaryotes. It was originally described many years ago, but in the last years, a huge number of acetylated proteins have been identified using high-resolution mass spectrometry, enhancing the interest about this PTM and making emerge the idea that acetylation might have the same regulatory relevance as phosphorylation. The major difference between acetylation and phosphorylation is that acetylation is not commonly transferred from one protein to another, in contrast, this is very frequent in phosphorylation-regulated pathways. Furthermore, the number of acetylases/deacetylases in any cell type is significantly lower than the number of kinases/phosphatases, which suggest a more general or basal level of regulation[1]. However, autoacetylation is frequently associated to activation of the acetyltransferase activity.

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During the past four decades lysine acetylation was associated with important roles in the regulation of nuclear transcription in different organisms. Proteome-wide analyses revealed many acetylated proteins in the cytoplasm, such as α- and β-tubulin, and the mitochondria, including most of the enzymes involved in the intermediate metabolism. These findings suggest a central role for an acetylation related regulatory mechanism in cell development inside and outside the nucleus[2]. Currently, acetylation research associated with both normal and pathological situations are a very active field of work.

Lysine acetylation is catalyzed by acetyltransferases, which transfer the acetyl group of acetyl CoA to the *ε*-amino group of an internal lysine residue. The reverse reaction is catalyzed by deacetylases. Both types of enzymes are usually part of protein complexes and were named histone acetyltransferases (HATs) and histone deacetylases (HDACs) because they were initially found to target histones, but later they were shown to target also non-histone proteins. It was proposed several years ago, to start calling them lysine acetyltransferases (KATs) and lysine deacetylases (KDACs), names that better correlate with the broader substrate specificity of these enzymes[3]. In the specific case of histones, when the acetyl group is transferred, the positive charges of lysines are neutralized, which

decreases the interactions between histones and DNA. KDAC enzymes generate an antagonistic effect to that of KATs, which restores the positive charge to lysines. This action stabilizes the chromatin structure, which is why most HDACs are transcriptional repressors[4]. In this review we use the KAT and KDAC nomenclature because we consider it better describes the activity of these enzymes. However, we also had to take into account that many proteins have been already named in literature and database as HATs and HDACs, in these cases we respected previous nomenclature for enzymes from other species different from Tritryps.

The trypanosomatid parasites *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania* spp. are collectively known as the Tritryps (class Kinetoplastea, order Tripanosomatida, family Tripanosomatidae). They cause different disease and lead to the death of millions of people in tropical and subtropical regions of the world[5]. Molecular evolutionary analysis indicates that kinetoplastids are one of the earlier diverging eukaryote lineages after acquisition of the mitochondria. This group shares many atypical characteristics like polycistronic transcription, trans-splicing and the involvement of RNA polymerase I in the transcription of certain protein coding genes. There is plenty of evidence showing that they do not have the canonical transcriptional control of gene expression as other eukaryotes. The only well stablished regulatory control was described at the initiation of the transcription of polycistronic transcription units (PTUs). This mechanism is associated to changes in chromatin produced by modifications in histones, including acetylation[6].

T. cruzi is the parasite that causes Chagas disease. It infects through a complex zoonosis, transmitted by more than 100 species of hematophagous insects, triatomines (Order: Hemiptera; Family: Reduviidae) and affects near 70 genera of mammalian hosts (including humans). Once in the mammalian host, it multiplies intracellularly (amastigote stage) and differentiates then into the non-replicative infective form present in the bloodstream (trypomastigote stage). These trypomastigotes are ingested by the insect vectors and transform in the lumen of the digestive tract into the replicative non-infective epimastigote stage[7].

T. brucei (*T. brucei gambiense* and *T. brucei rhodesiense*) is the causal protozoa of human African trypanosomiasis. This disease is spread in sub-Saharan Africa, where it threatens the lives of 65 million people each year. The parasite enters the human body through the bite of *Glossina* spp. flies (tsetse flies). The trypanosomes present in the digestive tract of the tsetse fly are in the procyclic stage. They then migrate to the salivary glands and transform into the metacyclic stage, the infectious form that can be transmitted to new mammalian hosts. In the mammalian host they change to the bloodstream trypomastigote stage that has a replicative slender and a no-replicative stumpy form. These trypomastigotes survive free in the bloodstream because they are able to evade the host immune response through a complex process of antigenic variation. *T. brucei*, a closely related subspecies to the human-infective ones, causes animal trypanosomiasis (Nagana) through a similar life cycle[8].

Leishmaniasis is an infectious disease caused by parasites of the genus *Leishmania* that affect humans and other animals and can cause different clinical presentations, from cutaneous and mucosal lesions (tegumentary leishmaniasis) up to visceral infection with tropism for the lymphohematopoietic system (visceral leishmaniasis). *Leishmania* spp. is transmitted by the bite of sand flies (Phlebotomies) and has two main life cycle morphologies: the intracellular amastigote in the mammalian host and the promastigote in the fly[9].

Despite the efforts made in the last decades, there is no spectation for the approval of a human-use vaccine against *T. cruzi* in the short time. Many surface proteins have proven to rise antibodies after injection in animal, but none of them gives complete protection against experimental infection presumably due to the complex life cycle of this parasite that include an intracellular replicative and a different no replicative blood form in mammalian hots. Conversely there are animal-use vaccines against *Leishmania* currently available, most of them produced from attenuated or dead parasites. There is also a few second and third generation vaccines for human-use under trial, but most of them are still in Fase I and II. Due to their well-known surface antigen variation mechanism, development of a vaccine against *T. brucei* is difficult to imagine. Associated to the lack of vaccines, only a few drugs, which are inadequate because of toxicity and resistance, are available to be used against trypanosmatids. Benznidazol and Nifurtimox, the sole alternatives for Chagas´disease treatment, are only effective during the acute face of the disease and pentavalent antimonials, the most affective drug against leishmaniasis have severe use limitations due to the development of resistance. Only the treatment of African Trypanosomiasis has a broad range of options that has recently increased with the inclusion of new drugs[10]. Under this situation, it is important to continue investigating new targets and possible compounds with trypanocidal activity. This article will review the current knowledge of lysine acetylation, a post-translational modification that is becoming more and more relevant with the years. We have previously reviewed this topic in 2012 and now we will revisit it with the current advances in the field and an analysis of the acetylome of trypanosomatids. We will focus on trypanosomatids, reviewing the enzymes involved, acetyltransferases and deacetylases, and a series of inhibitors described to date that could function as possible chemotherapeutic agents.

2. ACETYLATION: A UBIQUITOUS POSTTRANSLATIONAL MODIFICATION

A large number of PTMs have been evolutionarily conserved in both prokaryotic and eukaryotic organisms. Proteome-wide studies carried out in the last decade have shown the importance of acetylation in different organisms such as *E. coli*, *S. cerevisiae* or humans. Several authors using high-resolution mass spectrometry solved complete cell acetylomes. The acetylated proteins found in these studies were found to be involved in transcription, translational regulation, DNA repair, chromatin remodeling, cell cycle, splicing, metabolism, stress response, cytoskeletal dynamics, apoptosis, nuclear import, protein folding, and cellular signaling, among others. It is interesting to note that only a few years ago it was believed that the number of identified acetylation sites increased with the complexity of the organisms. However, the explosion of data produced by mass spectrometry is challenging this concept. For example, in 2009 only 138 acetylated lysines had been identified for *E. coli*[11], but a recent publication reported up to 3,840 acetylated lysines[12]. This number is not too different from other more complex organisms such as *S. cerevisiae*, which has around 4,000

acetylated lysines[13]. It is also worth mentioning that all acetylomes available are partial and the number of acetylated proteins reported will increase in the future.

2.1. Acetylation of Histone Proteins

The first publication about acetylation of histone proteins dates from 1964[14]. There are other PMTs such as phosphorylation, methylation, ubiquitination, and sumoylation that also occur over the histones and most of them are localized on the N-terminal tails of these proteins. Histone modifications occur at specific residues generating various patterns that can be associated with different biological events, for example, acetylation and transcription are closely linked. These patterns, which can be read by particular "reader" proteins, is known as the Histone Code. This model explains how the histones' PTMs alter their interaction with DNA, allowing a greater or lesser condensation of chromatin, and also recruiting other proteins, such as transcription factors[15].

The nucleosome structure, formed by histones H2A, H2B, H3 and H4, and their variants, presents a conformation such that the N-terminal tail is exposed and allows easy access to a series of histone-related protein families. These can be classified into three categories: writers, erasers, and readers. The writers are in charge of carrying out modifications of histones, for example HATs; on the other hand, we have the erasers that conversely, remove the histone marks, for example HDACs. And finally, the readers, which are proteins that have domains that allow them to recognize specific modifications, like the bromodomains that recognized acetylation marks. Bromodomains are conserved structures found primarily in nuclear proteins that recognize acetylated lysines (Kac) on histones and non-histones proteins. To date, the bromodomain are the only known protein domains that specifically bind to lysine residues. Many histone acetyltransferases that are associated with transcriptional cofactors also contain bromodomains[16].

Trypanosomatids possess the four canonical histones and the linker histone (H2A, H2B, H3, H4 and H1), as well as several variants (H2A.Z, H2B.V, H3.V and H4.V). Their sequences diverge from those of other eukaryotes, particularly in the histone tails, which are subject to post-translation modification. Histones H2A.Z and H2B.V are essential for cell viability, unlike H3.V and H4.V, which appear to be unique to trypanosomatids. Of these two exclusive histones, in *T. brucei*, the sequence identity between H3.V and H3 is only 45%, while between H4.V and H4 is 85%[17]. Despite this last large percentage of similarity, there is no data for the orthologous histone H4.V in *T. cruzi* or *Leishmania*. H2A.Z and H2B.V, usually associated to less stable nucleosomes, were found to be enriched at the putative RNA Polymerase II transcription initiation sites, while histone variants H3V and H4V were found enriched at the end of the polycistronic transcription units, suggesting a role in transcription termination. This data suggests that histone modifications and histone variants play crucial roles both in transcription initiation and termination in trypanosomatids. Another interesting characteristic of many histone genes in these organisms is that they are clustered in tandem arrays, but each gene type is found in a distinct cluster, often on separate chromosomes. This arrangement is shared by most canonical core histones, with some exceptions in *Leishmania*. Furthermore, histone variants are similar in sequence to canonical histones but they are encoded by discrete genes[18]. To date, ten types of histone PTMs have been reported in trypanosomatids: acetylation, crotonylation, 2-hydroxyisobutyrylation, malonylation, succinylation, phosphorylation, trimethylation, ubiquitination, N-glycosylation and O-GlcNAcylation. For *T. cruzi*, around 50 acetylation, methylation and phosphorylation sites were described, mostly located in canonical histones, some of which seem to be regulated through the different stages of the parasite[19]. For African trypanosomatids, histone modifications are highly conserved in canonical histones. A total of 162 histone marks were identified in *T. brucei*, 135 of them recently reported for the first time, as well as 134 marks in *Trypanosoma evansi*. In addition, 119 histone marks were shared by the two parasites[20]. It is interesting to note that despite the fact that variant histones are less conserved than canonical histones, the modified sites of the histone variants are very similar between the two parasites. Acetylation sites on histone proteins are summarized in Supplementary Table SI.

Acetylation of histones is very important in trypanosomatids, for example H4K4ac, H4K10ac and H4K14ac participate in the assembling and remodeling of chromatin during replication and transcription. H4K4ac is cell cycle regulated and its acetylation is mediated by DAC3 in *T. brucei*. H4K10 is likely acetylated in all nucleosomes at transcription start sites[21,22]. Also, it was described that deacetylation of H4K14ac and replacing H4K10ac for arginine delayed DNA replication and reduced transcription in *T. cruzi*[23]. Furthermore, the acetylation of histone H4 would play an important role in the transcription of *Leishmania donovani*. It has been shown that *Ld*HAT2 participates in chromatin compaction. *Ld*HAT2 acetylates the K4 of histone H4 and neutralizes the positive charge on the tail protruding from the amino terminal, generating less packed nucleosomes[24]. In *T. brucei* and *T. evansi* several hyperacetylated regions have been also identified, which lead to a less compact chromatin. These regions are thought to be important in the establishment of euchromatin and the regulation of particular gene clusters under specific circumstances. Not only canonical histones are targets for acetylation, but also histone variants (Supplementary Table SI). H2A.Z was found to be predominantly regulated by hyperacetylation, with K49 in the N-terminus exhibiting greater levels of acetylation in *Tb*H2A.Z. Conversely, *T. evansi* shows greater levels of acetylation at the C terminus of H2A.Z, at the C terminus of histone H2A as well as the N terminus of histone H4[20].

2.2. Acetylation of Non-histone Proteins

As mentioned before, histones are not the only proteins capable of being acetylated. To date a large amount of non-histone proteins have been described, which are involved in many different processes mentioned in the Introduction. Without a doubt acetylation is an important regulatory mechanism during the cell cycle, involving several kinases in conjunction with acetyltransferases and deacetylases. Phosphorylation of acetyltransferases is the proof of the crosstalk between these PTM-based regulatory systems during the cell cycle[25]. For example, the phosphorylation of the human NF-κB p65 subunit by MAPK and IκB kinase (IKK), in two different Serine residues, promotes its

acetylation by KAT p300 and enhances its transcriptional activity.

Several acetylomes have been reported in trypanosomatids. In *T. cruzi*, a total of 235 lysine acetylated non-histone proteins have been identified in epimastigotes, with 389 acetylated sites. While for *T. brucei*, 210 and 285 proteins in procyclic trypomastigote form (PCF) (288 acetylated sites) and bloodstream trypomastigote form (BSF) (380 acetylated sites), respectively[26] (Supplementary Table SII). These results suggest that acetylation is variable not only between species, but also between cell stages in trypanosomes. In *T. brucei*, only 44 proteins were found acetylated in both PCF and BSF, which means that the number of acetylable proteins in this specie rise to 451. In fact, since the experimental approach used in this report explored only around 30% of the proteome of these parasites, the global acetylome should be even higher. Most acetylated proteins contain a single acetylation site in both *T. brucei* and *T. cruzi*. For the former, more than 80% (417 proteins) had one acetylation sites considering the two stages that were studied. For *T. cruzi* the number of single acetylated proteins is 67% (158 proteins). But there are other proteins that have multiple acetylation sites, for example in the case of *T. brucei*, glyceraldehyde-3-phosphate dehydrogenase (9 sites), fructose-bisphosphate aldolase (7 sites), hexokinase 1 (6 sites), paraflagellar rod protein 1 (10 sites), α-tubulin (7 sites) and β-tubulin (5 sites). High levels of acetylation for *T. cruzi* were also found in proteins such as heat shock protein 70 (12 sites) and pyruvate phosphate dikinase (9 sites). In *T. brucei* many of the proteins involved in the Embden-Meyerh pathway (glycolysis) are acetylated: Hexokinase 1 and 2 (HK 1/2); glucose phosphate isomerase (IGP); phosphofructokinase (PFK); fructose-1,6-bisphosphate aldolase (ALD); glycerol-3-phosphate dehydrogenase (GPDH); triosephosphate isomerase (TIM); glycerol kinase (GK); glyceraldehyde-3- phosphate dehydrogenase (GAPDH) (Non-histone acetylated proteins are summarized in Supplemertary Table SII). Interestingly, glycolytic enzymes were found more heavily acetylated in the PCF, which replicates in the amino acid-rich insect gut and generates ATP by oxidative phosphorylation, in contrast to the BSF that replicates in the blood and relies on glycolysis to generate ATP. Also, fructose-1,6-bisphosphate aldolase from *T. brucei* shows an acetylation site placed on the active site of the enzyme and this modification causes a reduction in its activity[27]. These observations suggest that acetylation could act as a global modulator of the main function of the glycosome between PCF and BSF. In the case of *T. cruzi*, some glycolytic enzymes capable of being acetylated were also found: glucose-phosphate isomerase, fructose-1,6-bisphosphate-aldolase, triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase. Many of these acetylation sites were not found in *T. brucei*, thus indicating that the regulation between the two species follows different paths. In contrast, many antioxidant enzymes were found acetylated only in *T. cruzi*. Among them, *Fe*SOD isoform B. The structure of this enzyme has been solved showing that lysine-acetylation occurs at a position close to that responsible for the regulation of acetylation-mediated activity previously described in the human homologue[28].

2.3 Acetylated proteins in the cytoskeleton

We performed an analysis of the acetylomes available in TritrypsDB and the proteomic analysis performed recently by Zhang and coworkers[20] and found that many cytoskeletal and flagellar proteins are acetylated. We summarized them in Supplementary Table SIII and Figure 1. Besides the flagellar axoneme, which is heavily acetylated, the paraflagellar rod and the flagellum attachment zone contain proteins with acetylated lysines. Also, acetylation was detected in basal body proteins and in the Tripartite Attachment Complex (TAC) that links the basal body to the kinetoplast. Furthermore, several Microtubules Associated Proteins (MAPs) were present in the acetylome of *T. brucei* and *T. cruzi*, like WCB and CAP51/51V. WBC is involved in the interaction of the membrane with the sub-pellicular microtubule corset, being essential for cell morphogenesis[29]. On the other hand, CAP51 and CAP51V are located in the sub-pellicular microtubule corset and are essential in the organization of the cytoskeleton and cytokinesis[30]. Also, several dyneins and kinesins are acetylated, suggesting a role for this PTM in these motor proteins and intraflagellar transport. K1 and K134 of the inner arm dynein IAD5-1 was found acetylated exclusively in *T. brucei* in comparison with *T. evansi*. Zhang and coworkers proposed that the differential PTMs associated with flagellar proteins could explain the more flexible appearance of the *T. evansi* flagellum[20]. Finally, is very interesting to note that both α-tubulin and β-tubulin has 11 sites reported between *T. cruzi* and *T. brucei*. However, the only acetylated site well characterized is α-tubulin K40, for which *Tc*ATAT[31] is the only acetyltransferase and *Li*SIR2RP1 the only deacetylase described[32].

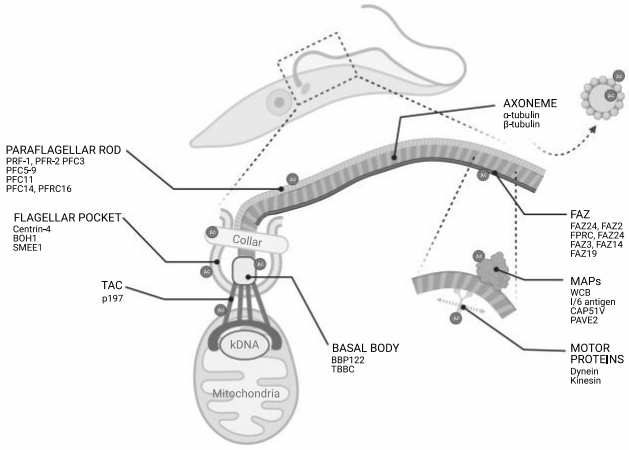

Figure 1: Summary of acetylated proteins related to the cytoskeleton of T. brucei and T. cruzi identified by mass spectrometry obtained from TritrypsDB. Ac, acetylated; TAC, tripartite attachment complex; MAPs, microtubule associated proteins; FAZ, flagellum attachment zone. Created with BioRender.com

3. LYSINE ACETYLTRANSFERASES: THE WRITERS

Most KAT enzymes can be classified into three main families based on their homology to yeast orthologs, and also on their catalysis mechanism: the p300/CREB-binding proteins (p300/CBP); the GCN5-related N-acetyltransferases (GNAT), and the MOZ, Ybf2, Sas2, and Tip60 (MYST) family. In mammals, the p300/CBP family comprises two paralog proteins: p300 (KAT3B) and CBP (KAT3A). They have a high sequence homology and similar structures[33]. The GNAT family includes the GCN5 (General Control Nonderepressible 5, also known as KAT2A), PCAF (p300/ CBP associated factor, also known as KAT2B), the ELP3 (Elongator complex protein 3, also known as KAT9), Hpa2 (KAT10), Hpa3, the mediator complex subunit Nut1, and α-tubulin acetyltransferase 1 (ATAT1). Lastly, the MYST family is composed by the following enzymes: MOZ (monocytic leukemia zinc finger protein, KAT6A), MORF (MOZ related factor, KAT6B), MOF (males absent on the first, KAT8), Tip60 (Tat-interacting protein, KAT5) and HBO1 (HAT bound to ORC1, KAT7). GNAT and MYST families are quite different in structure and catalyze the acetyltransferase reaction by different mechanisms: the GNAT family was shown to use a ternary complex mechanism, the MYST family use a ping-pong mechanism or a ternary complex mechanism and p300/use a catalytic "hit and run" mechanism, also known as Theorell-Chance[34].

As reviewed previously by Alonso & Serra[2] in trypanosomatids there are fewer acetyltransferases compared to other eukaryotes. The Trityps genomes contain eleven histone acetyltransferases that are very divergent. Four of them are related to the MYST family and the rest belong to the GNAT family (three of them containing a ELP3 domain). When compared to other species, the Tritryps enzymes are only slightly conserved and not all of them have the exact same genes. The fact that KATs from trypanosomatids do not contain any other recognizable domains, highlights their differences with those from mammals or yeast. Additional domains like a second acetyltransferase domain or a bromodomain are very common un mammals and yeast.

We summarize in Table I the KATs present in Tritryps and their known functions, which are detailed next. It is important to highlight that, as can be observed in Table I, the numbers in the nomenclature of KATs/HATs from Tritryps do not reflect any similarity, neither phylogenetic relationship, with HATs from mammals or any other species. Therefor, we will name the Tritryps enzymes as KATs but mantaining the number that was previously used when they were refered as HATs (see Table I). *T. brucei* contains three KATs that belong to the MYST family. *Tb*KAT3 has been shown to be a nuclear protein that is responsible for acetylation of H4K4, a modification considered more structural than regulatory[35].

Table I: Lysine Acetyltransferases from Tritryps

Acetyltransferase	*T. brucei*	*L. major*	*T. cruzi*	Family	Subcellular localization	Function
KAT1 (HAT1)	Tb927.7.4560	LmjF14.0140	TcCLB.506605.160 TcCLB.511239.150	MYST	Nuclear (*T. brucei)*	Essential for growth, required for telomeric silencing. required for DNA replication and RNA transcription.
KAT2 (HAT2)	Tb927.11.11530	LmjF28.2270	TcCLB.511017.69 TcCLB.509203.60	MYST	Nuclear (*T. brucei)*	Essential for growth, responsible for H4K10 acetylation at RNAPII TSRs; required for H2A.Z deposition.
KAT3 (HAT3)	Tb927.10.8310	LmjF36.6990	TcCLB.507611.290 TcCLB.507723.110	MYST	Nuclear (*T. brucei)*	Not essential for growth, cell cycle regulated, responsible for H4K4 acetylation
KAT4 (HAT4)	-	LmjF13.0170	TcCLB.506227.160	MYST	Cytoplasmic in all life cycle stages, nuclear in postmitotic cells (*L. donovani*)	Acetylates histone H4, modulates navigation across G2/M and re-entry into G1.
ELP3a	Tb927.8.5770	LmjF16.0240	TcCLB.506743.120	GNAT	Nuclear periphery (*T. brucei*)	
ELP3b	Tb927.8.3310	LmjF23.1350	TcCLB.509769.110	GNAT	Nucleolar (*T. brucei*)	Controls rDNA transcription
ELP3	Tb11.v5.0520	-	-	GNAT		
ATAT/MEC17	Tb927.3.1400	LmjF.25.1150	TcCLB.509233.160	GNAT	Cytoplasmatic	Acetylates α-tubulin in *T. cruzi*
NatA	Tb927.11.4530	LmjF.13.0260	TcCLB.506227.230	GNAT	Perinuclear/Cytoplasmic (*T. cruzi*)	
NatC	Tb927.7.2360	LmjF.22.0450	TcCLB.511809.120 TcCLB.511811.30	GNAT	Cytoplasmic (*T. cruzi*)	RNAi reduce parasite growth (*T. brucei*)
RimI	Tb927.10.12830 Tb927.1.4490	LmjF.18.1310	TcCLB.509805.200	GNAT	-	-

Another report demonstrated that *Tb*KAT3 is dispensable, while *Tb*KAT1 and 2 are required for parasite growth. *Tb*KAT1 knock-down cells have telomeric silencing specifically compromised, and this enzyme might be required for DNA replication. *Tb*KAT2 displays *in vitro* acetyltransferase activity towards H4K10[21]. Also, *Tb*KAT3 is involved in DNA repair promoting ssDNA formation and recombination at chromosome-internal sites but it has the opposite effect at a subtelomeric VSG sites, antagonizing with *Tb*Sir2RP1[36]. Kraus and coworkers found that H4 and H2A.Z acetylation in transcription starting sites are mediated by two different histone acetyltransferases, KAT2 and KAT1 in *T. brucei*, respectively[37]. Apparently, trypanosomes use specific MYST acetyltransferases to acetylate H4K4 and H4K10, which further supports the idea of a nonredundant histone code present in these parasites[38].

T. cruzi and *Leishmania* have a fourth acetyltransferases from the MYST family: KAT4, that is absent in *T. brucei*[39]. *L. donovani* KAT1 acetylates H4K10 *in vitro* and its phosphorylation by an S-phase kinase inhibits its acetylation activity. This in an important mechanism of periodic regulation of histone acetylation during cell cycle progression[40]. *Ld*KAT2 is essential and acetylates histone H4K10 *in vitro* and *in vivo*. *Ld*KAT2-depleted cells display growth and cell cycle defects and poor survival in host cells[41]. Another report indicates that KAT2 over-expression in *L. donovani* resulted in highly accessible chromatin suggesting chromatin decondensation and significantly increases acetylation of H4K4[24]. *Ld*KAT3 depletion causes a decreased in cell viability and an aberrant cell cycle progression pattern. *Ld*KAT3 associates with proliferating cell nuclear antigen (PCNA), helping load PCNA onto chromatin in proliferating cells. Also, HAT3-nulls show heightened sensitivity to UV radiation[42]. *Ld*KAT4 has a cytoplasmatic localization throughout the parasite life cycle and a nuclear localization in postmitotic cells. *Ld*KAT4 acetylates histone H4 at K4 residue almost exclusively and K2 in a minor proportion. These results suggest that H4 modification might happen in the cytoplasm prior to histone transport to the nucleus or soon after mitosis inside the nuclear compartment[43]. The function of *Ld*KAT4 has been studied *in vivo*, and it was found that it plays a role in the modulation of *Leishmania* G2/M events and stimulates the transition to the G1 phase[44]. In *T. cruzi*, no KATs have been experimentally characterized. All this evidence clearly illustrates that KATs seem to be equivalent among the three genera but are involved in distinct cellular processes and play quite different roles in each organism.

The GNAT is characterized by 4 homologous motifs (A-D) of 15–33 amino acids each. The central core domain is structurally conserved in different organisms and interacts with coenzyme A (CoA) through motif A which plays an important role in catalysis[45]. The two members of this family are present in trypanosomatids show similarity to Elp3 (Elongator protein 3) and are named Elp3a and Elp3b (a third gene coding for an Elp3 ortholog, can be found only in *T. brucei*). Despite the high divergence with orthologues from other organisms, these proteins have the conserved amino acids that are necessary for substrate binding and both *Tb*Elp3a and b were demonstrated to be enzymatically active. It should be noted that only Tritryps have two Elp3 orthologues, a unique feature that may reflect distinct roles for these organism's enzymes. *Tb*Elp3a is localized at the nuclear periphery, while *Tb*Elp3b is concentrated in the inner part of the nucleus both in the bloodstream and in insect-stage cells[46]. In the case of *T. cruzi*, using CFP fusions, it was possible to show that Elp3a and Elp3b are not nuclear proteins[2]. Another KAT belonging to the GNAT family, called MEC-17/ATAT, exhibits K40-specific acetyltransferase activity for α-tubulin in *Tetrahymena* and *Caenorhabditis elegans*. MEC-17 homologues are present in most eukaryotes with the exception of fungi and plants[47]. We have recently characterized *Tc*ATAT in *T. cruzi* as detailed below. Finally, at least three other members of the GNAT superfamily can be found in the Tritryps genomes, however they do not encode for ε–lysine acetyltransferases. For example, NatA and NatC are the catalytic subunits of N-terminal acetyl-transferase complexes and one of them has been characterized in *T. cruzi*[48]. On the other hand, a putative otholog of RimI, an enzyme that acetylates N-terminal alanines in specific ribosomal proteins and is found exclusively in bacteria and protists appears to be in Tritryps (Table I).

It is interesting to highlight that all KATs of *T. brucei* were found exclusively in the nucleus and mainly related to transcription. This suggests that at least one of these enzymes should have a dual cytoplasmic/nuclear localization not yet described. Alternatively, an unknown cytoplasmic enzyme should be responsible for the acetylation of cytoplasmic proteins, as occurs in other organisms.

3.1. ATAT is the primary α-tubulin acetyltransferase

Tubulin acetylation plays a fundamental role in the organization of the cytoskeleton that is formed by polymerized subunits of α and β-tubulin. α-tubulin is acetylated at K40 by the cytoplasmic acetyltransferase αTAT1, encoded by ATAT1 in mammals and MEC-17 in *C. elegans* and deacetylated by the cytoplasmic deacetylase HDAC6 and NAD-dependent deacetylases. Homologs of MEC-17/ATAT1 are present in animals and many protists, but absent in fungi and plants[49].

The mechanism of catalysis of protozoan α-tubulin acetyltransferase has not yet been determined, but previous studies have proposed a mechanism for the action of human ATAT1. The aspartic acid (D) 157 of ATAT1 plays a fundamental role in the tubulin-enzyme interaction, coordinating the peptide backbone of tubulin through bidentate hydrogen bond with the amides of the backbone of D39 and K40 of α-tubulin. To be acetylated, the Ɛ group of K40 needs to be deprotonated, which is followed by nucleophilic attack by the amine on the carbonyl carbon of acetyl coenzyme A. The conserved D157 of ATAT1 is well positioned to deprotonate the Ɛ-amino of K40, suggesting that D157 could play a role not only in substrate binding, but also in catalysis (a mutation in ATAT1 D157 reduces microtubule acetylation by 92%)[50]. Studies were also carried out in mice, these showed that in the absence of HDAC6 almost 100% of α-tubulin is acetylated, while in the absence of ATAT1 acetylated tubulin is practically not detected[51].

Table II: KDACs present in Tritryps

Deacetylase	*T. brucei*	*L. major*	*T. cruzi*	Class	Subcellular localization	Comments
DAC1	Tb927.10.1680	LmjF21.0680	TcCLB.511911.159 TcCLB.508637.114	I	Predominantly nuclear proteins in bloodstream form and cytoplasmatic in the insect stage (*T. brucei)*	Antagonizes SIR2RP1-dependent telomeric silencing, essential for viability.
DAC2	Tb927.11.15600		TcCLB.504159.80 TcCLB.506821.140	I	Cytoplasmatic *(T. brucei*)	Not essential for viability
DAC3	Tb927.2.2190	LmjF21.1870	TcCLB.509395.120 TcCLB.503653.50	II	Nuclear in all life cycle stages *(T. brucei)*	Specifically required for silencing at VSG ES promoters in both bloodstream and insect-stage cells essential for viability
DAC4	Tb927.5.2900	LmjF08.1090	TcCLB.507063.270	II	Cytoplasmatic *(T. brucei*)	Not essential for viability, knock out results in a cell cycle delay.
SIRRP1	Tb927.7.1690	LmjF26.0210	TcCLB.507519.60 TcCLB.508207.150	III	Cytoplasmatic (*T. cruzi*). Promastigote y amastigote stages (*L. major, L. amazonensis, and L. infantum*). Citosolic Nuclear in all life cycle stages *(T. brucei)*	Deacetylates *α*-tubulin and is partially associated with the microtubule network. Important for proliferation and differentiation in *T. cruzi*. Glycosylated in *Leishmania*. Catalyzes ADP ribosylation and deacetylation of histones and is involved in DNA repair in *T. brucei.*
SIR2RP2	Tb927.8.3140	LmjF23.1210	-	III	Mitocondrial (*T. brucei, L. donovani*)	NAD+-dependent ADP-ribosyltransferase activity, essencial for growth and cell cycle progression in *Leishmania.*
SIR2RP3	Tb927.4.2520	LmjF34.2140	TcCLB.447255.20 TcCLB.506559.80	III	Mitocondrial (*T. brucei, T. cruzi*)	Important for proliferation and differentiation in *T. cruzi*.

HDAC6 and MEC-17/ATAT1 are two fundamental regulators of α-tubulin K40 acetylation in different eukaryotic organisms. Interestingly MEC-17/ATAT1 was not found to be essential, for example, abolition of αTAT activity in *C. elegans* gives viable worms with reduced touch response and mouse knock-out for ATAT1 develop normally but shows altered sperm motility and male fertility[47,52,53]. Recently, we have reported that *T. cruzi* ATAT acetylates α-tubulin *in vivo* and is also capable of auto-acetylate itself[31]. *Tc*ATAT is located in the cytoskeleton and flagella of epimastigotes and colocalizes with acetylated α-tubulin in these structures. The regulated over-expression of *Tc*ATAT causes an increase of acetylated α-tubulin, as expected, that was associated to growth defects related to a cell cycle arrest and impairment of kinetoplast division. Tubulin hyperacetylation also induces morphological and ultrastructural changes, especially in the mitochondrial branches and in kinetoplast DNA topology. The over-expressing parasites also became more resistant to microtubule depolymerizing drugs. These evidence supports the idea that a-tubulin acetylation is tightly regulated in *T. cruzi* and indicates that although the cytoskeleton arrangement is considered stable in trypanosomatids, a dynamic instability of microtubules is required for replication and cell cycle progression.

4. LYSINE DEACETYLASES: THE ERASERS

Mammalian and yeast KDACs can be divided into four classes: Class I, II, III, and IV corresponding to two families: zinc dependent and NAD^+ dependent (Figure 2). Those of class I, II and IV belong to the zinc-dependent group, while those of class III are the NAD+ dependent ones, also called Sirtuins (SIRTs)[2]. In Table II we summarize the KDACs present in Tritryps and their known functions. Again, the number of enzymes is small in comparison to mammals. The number of KDACs is reduced to only four zinc dependent enzymes belonging to class I and II, and three Sirtuins, named SIR2-related proteins (SIRT2RPs) because of they were first found related to yeast SIRT2. Similar to HATs, numbers in the nomenclature of Tritrps KDACs/HDACs do not reflec any relationship with the corresponding HDACs other species. This situation made more relevant to us to name/mention Tritryp´s ezymes as KDACs to avoid confussion with mammal enzymes, that we named as HDACs. Tritryps class I KDACs (KDAC1 and KDAC2) have 350–500 amino acid residues (~50 kDa), a deacetylase domain and a small C-terminal region that can suffer several PTMs. The two members of class II, KDAC3 and KDAC4 are very different between them: KDAC4 is short with only 80 amino acids in protein has a highly acidic segment in the C-terminal domain. This region could play a role in the interactions with other proteins and/or substrates, and also be involved in its subcellular localization. KDAC3 is longer and have an insertion of around 180 amino acids in the deacetylase domain. In *T. brucei* the predicted KDAC2 has a highly acidic segment in the C-terminal domain. This region could also play a role in the interactions with other proteins and/or substrates, and also be involved in its subcellular localization.

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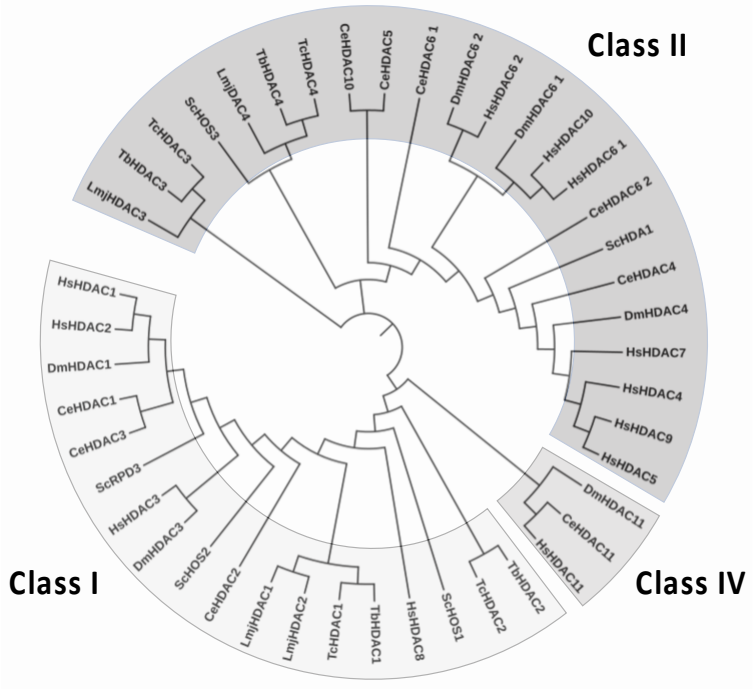


Figure 2: Unrooted phylogenetic tree of KDACs sequences from Tritryp and different taxa. The sequences corresponding to the catalytic sites of the KDACs (obtained through Pfam) were used to carry out the alignment by Clustal Omega and the tree was built by maximum likelihood, using the package MEGA V. Final presentation was generated with the iTOL online tool for phylogenetic tree display. Nomenclature: sequences are named by abbreviations of genus and species and accompanied by their respective accession numbers in parentheses. Homo sapiens: HsHDAC1(NP_004955.2), HsHDAC2 (NP_001518.3), HsHDAC3 (AAC52038.1), HsHDAC8 (NP_060956.1), HsHDAC4 (AAH39904.1), HsHDAC5 (NP_005465.2), HsHDAC7 (AAH64840.1), HsHDAC9 (NP_478056.1), HsHDAC6 1 (AAH69243.1), HsHDAC6 2 (AAH69243.1), HsHDAC10 (NP_114408.3), HsHDAC11 (NP_079103.2); Caenorhabditis elegans: CeHDAC1 (NP_506599.1), CeHDAC2 (NP_495678.1), CeHDAC3 (NP_493026.1), CeHDAC4 (NP_001257279.1), CeHDAC5 (NP_741051.1), CeHDAC6_1 (NP_500788.1), CeHDAC6_2 (NP_500788.1), CeHDAC10 (NP_496910.1), CeHDAC11 (NP_505699.3); Drosophila melanogaster: DmHDAC1 (NP_647918.2), DmHDAC3 (NP_651978.2), DmHDAC4 (NP_572868.3), DmHDAC6 1 (AFI26268.1), DmHDAC6 2 (AFI26268.1), DmHDAC11 (NP_001247296.1); Saccharomyces cerevisiae: ScRPD3 (NP_014069.1), ScHOS1 (Q12214.1), ScHOS2 (P53096.1), ScHOS3 (Q02959.1), ScHDA1 (P53973.1); Trypanosoma cruzi: TcHDAC1 (PWU88134.1), TcHDAC2 (PWU99330.1), TcHDAC3 (PBJ71521.1), TcHDAC4 (PWU93520.1); Trypanosoma brucei: TbHDAC1 (XP_822424.1), TbHDAC2 (XP_011780894.1), TbHDAC3 (XP_951529.1), TbHDAC4 (XP_844962.1); Leishmania spp.: LmjHDAC1 (CAC5429807.1), LmjHDAC2 (XP_001683001.1), LmjHDAC3 (XP_001683153.1), LmjDAC4 (CAJ0228

This is an unusual protein because it lacks several pocket residues, which are predicted to be required for deacetylase activity[54].

Through the disruption of deacetylase genes in bloodstream *T. brucei* cells, it was determined that DAC1 is essential, while DAC2 is expendable. On the other hand, *Tb*DAC3 is essential unlike *Tb*DAC4, although mutants for the latter show a delay in the G2/M cell cycle phase[54]. The two essential deacetylases *Tb*DAC1 and *Tb*DAC3 have a nuclear location and are necessary for growth in the bloodstream forms of *T. brucei*[54]. *Tb*KDAC1 antagonizes telomeric silencing and *Tb*KDAC3 is required for variant surface glycoprotein expression sites (VSG ESs) silencing in bloodstream and insect stage cells[55]. In this last work it was also reported that the other two deacetylases *Tb*KDAC2 and *Tb*KDAC4 are predominantly found in the cytoplasm.

Among mammalian HDACs, two of them are the most atypical: HDAC6 and HDAC11. HDAC6 is a Class II structurally unique isoenzyme. It harbors two functional deacetylase domains and, unlike any other KDACs, it is confined to cytoplasm[56]. HDAC6 does not modify histones but controls the acetylation status of many non-histone substrates and is considered responsible for deacetylating α-tubulin. Since it is well established that HDAC6 exerts functions in various disease processes, specific inhibitors against different pathologies, including some types of cancer, have been described. No KDACs from Tritryps or from any other protist show characteristics that could be associated with HDAC6, even though as we describe in section 5.2, some HDAC6 specific inhibitors have interesting antiparasitic effects. In 2002, Gao and coworkers identified and characterized human HDAC11, the only class IV histone deacetylase known to date. The sequence homology of

HDAC11 shared with HDAC class I and II is very small. HDAC11 is highly conserved, even in invertebrates and plants but, like HDAC6, is absent in protists.

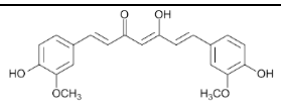
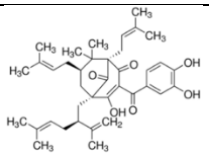
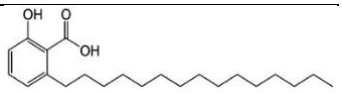
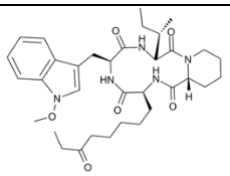
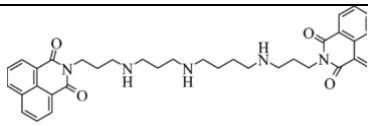
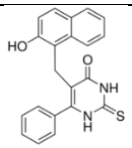
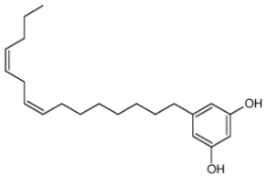
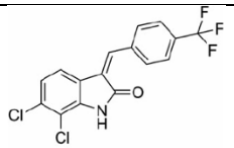
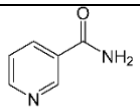
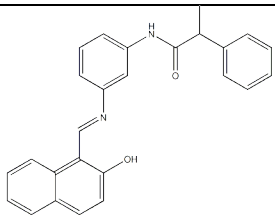
Class III KDACs or Sirtuins (SIRTs) have different subcellular locations and have functions in a wide range of processes such as: transcriptional silencing, DNA repair, cell cycle progression, chromosome segregation and life span. SIRTs remove acetyl groups in nuclear, cytoplasmatic and mitochondrial substrates. During the deacetylation reaction, acetyl-lysine and NAD^+ are converted into lysine, nicotinamide and O-acetyl-ADPribose65. SIRTs are involved in various biological processes and orthologs can be found in different eukaryotic organisms. In humans there are seven Sirtuins (SIRT1-7), in *T. brucei* and *Leishmania* spp. there are three (SIR2RP1-3), while in *T. cruzi* there are two, *Tc*SIR2RP1 and *Tc*SIR2RP3 (no SIR2RP2 ortholog). The functions of the Sirtuins and the locations of each of them varies between the orthologs of the different trypanosomatids as summarized in Table II. SIR2RP2 from Tritryps are phylogenetically closer to bacterial SIRTs, while SIR2RP1 and SIR2RP3 are more related to yeast and human SIRTs, respectively. We have previously analyzed the phylogeny of Tritryps Sirtuins[58]. When compared with human Sirtuins, SIR2RP1 is found related to the human proteins SIRT2 (29% of amino acid identity) and SIRT3 (24% of amino acid identity), while SIR2RP3 is more related to SIRT4 (23% of amino acid identity) and SIRT5 (28% of amino acid identity. The first experimental characterization of the *T. cruzi* Sirtuins showed that overexpression of *Tc*SIR2RP1 causes no alteration of epimastigote growth, but it increases the number of trypomastigotes obtained by *in vitro* metacyclogenesis and their infectivity rate. In contrast, overexpression of *Tc*SIR2RP3 slightly decreases epimastigote growth and the infectivity rate, but it does not affect the *in vitro* differentiation to metacyclic trypomastigotes, in turn it increases the proliferation rate of intracellular amastigotes. Also, overexpression of either of these Sirtuins protects the parasite from the effect of Sirtuin inhibitors. Apparently, Sirtuin activity is important for the proliferation of *T. cruzi* replicative forms, for the host cell-parasite interplay and for differentiation among life-cycle stages. But each one performs different roles in these processes. *Tc*SIR2RP1 is strongly expressed in epimastigotes and amastigotes, but at lower levels in trypomastigotes. The expression levels of *Tc*SIR2RP3 are higher in epimastigotes than in amastigotes, and it seems that it would not be expressed in trypomastigotes[58]. In another report from the same year, Moretti and coworkers using specific antibodies and cell lines overexpressing the tagged versions of these enzymes, also described that *Tc*SIR2RP1 is localized in the cytosol and *Tc*SIR2RP3 in the mitochondrion. *Tc*SIR2RP1 overexpression acts to impair parasite growth and differentiation, whereas the wild-type version of *Tc*SIR2RP3 and not an enzyme mutated in the active site improves both. The effects observed with TcSIR2RP3 were fully reverted by adding salermide, which inhibited recombinant *Tc*SIR2RP3[59]. *T. brucei Tb*SIR2RP1 is a nuclear deacetylase not essential for the viability of the parasite or for antigenic variation, but it is necessary for the silencing of some telomeric genes and is also involved with the acetyltransferase HAT3 in RAD51-dependent DNA double-strandbreak (DSB) repair at a chromosome-internal locus[36]. It was also reported that *Tb*SIR2RP1 displays both deacetylase and ADP-ribosyltransferase activities[60] involved in DNA repair[61].

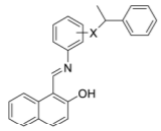
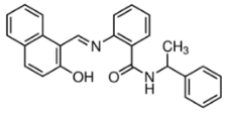
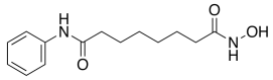
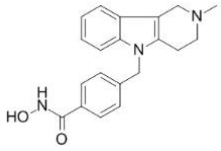
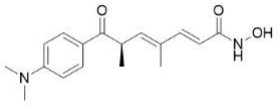
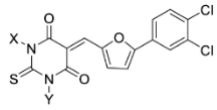
Before the Tritryps genomes were available, a cDNA from *L. major* encoding for a a protein homologous to the *Saccharomyces cerevisiae* silent information regulator 2 (SIR2) proteins was identified[62]. Currently, there is plenty of information regarding *Leishmania* SIR2RP1-3 which will be detailed next. The Sirtuin orthologue to SIR2RP1 in *Leishmania* is located in the cytosol, and in the particular case of *L. infantum* it functions as an α-tubulin deacetylase that is essential for the survival of the parasites. *Li*SIR2RP1 also exhibits ADP-ribosyltrasferase activity and is capable of deacetylating tubulin, either in dimers or, when present, in taxol-stabilized microtubules or in promastigote and amastigote extracts[32]. In *Leishmania mexicana*, SIR2RP1 is also located in the cytosol and overexpression leds to the accumulation of the protein in the parasite cytoplasm of both promastigote and amastigote forms and a striking increase in the survival of amastigotes, suggesting a role in the lifespan of this parasite[63] as observed for *T. cruzi* epimastigotes[58]. Later it was shown that *L. donovani* SIR2RP1 is involved in in parasite survival and apoptosis[64]. *L. amazonensis* SIR2RP1 homologue was also characterized, and it was immunodetected in total protein extracts, in cytoplasmic granules and in the secreted material of both promastigotes and lesion-derived amastigotes[65]. The first report of a *Leishmania* SIR2RP2 was in *L. donovani* where this enzyme possesses NAD+-dependent ADP-ribosyltransferase activity and localizes to the mitochondria. Null mutants had a restrictive growth phenotype associated with accumulation of cells in the G2/M phase and a compromised mitochondrial function. The null mutants also had attenuated infectivity and increased sensitivity of the parasites to the known SIR2 inhibitors[64].

5. KATS AND KDACS INHIBITORS AS CHEMOTHERAPEUTIC AGENTS

There are many KAT and KDAC inhibitors (iKATs and iKDACs) described in literature that were developed and tested against many types of cancer where acetylase and deacetylase enzymes are deregulated (generally overexpressed)[66]. iKATs are less studied than iKDACs and until a few years ago only a small number of them were known, such as curcumin, anacardic acid, garcinol, and isothiazolones.

Table III: KAT and KDAC inhibitors

Name	Type	Organism	IC_{50} / EC_{50}	Selectivity	Structure
Curcumin	iKAT	*T. brucei* and *T. b. rhodesiense*	3.2 µM (Tb) and 7.75 µM (Tbr)	13.7 (*Tb*)	
Curcumin + RK-52	iKAT	*T. brucei* and *T. b. rhodesiense*	4.64 µM (Tb) and 3.05 µM (Tbr)	n.d.	
Garcinol	iKAT	*L. donovani*	0.82 µM (amastigotes)	n.d.	
Anarcardic acid	iKDAC	*T. cruzi*	41.67 µM (Amastigote)	>2	
Apicidin analogs	iKDAC	*T. brucei*	2 to 12 mg/ml	65-12	
Bisnaphthalimidopropyl (BNIP)	iKDAC	*L. infantum*	1.30 µM (promastigotes) and 1.68 µM (amastigotes)	n.d.	
Cambinol analog	iKDAC	*T.cruzi*	19.35 µM (Amastigotes)	2	
Cardol derivatives	iKDAC	*T.cruzi*	12.25 µM (Trypomastigote) and 14.70 µM (Amastigote)	2-4	
KH-TFMDI	iKDAC	*T. cruzi and L. amazonensis*	0.5 µM (*Tc* amastigote), 8.8 µM (*Tc* trypomastigote) and 2 µM (*La* promastigote), 1.2 µM (*La* amastigote)	162-10 depending on the life cycle stage	
Nicotinamide	iKDAC	*Leishmania spp.* and *T. cruzi*	25 mM (Tb epimastigotes), 13.9 mM (Li promastigotes), 5.5 mM (Li amastigotes)	n.d.	
Salermide	iKDAC	*T. cruzi* and *L. donovani*	2 µM (Tc amastigote) and 25.7 µg/ml (Ld promastigote)	n.d. but effective against infective forms of T. cruzi by reducing the parasitemia of infected BALB/c mice by	

				approximately 40% after treatment with 100 µM	
Salermide analogue	iKDAC	*T.cruzi*	40.6 µM (Amastigotes)	2	
Sirtinol	iKDAC	*T.cruzi* and *L. infantum*	30 µM (Li amastigotes)	n.d.	
Suberoylanilide hydroxamic acid (SAHA)	iKDAC	*Leishmania* ssp.	29.7 µg/ml (Ld)	4.8 (Ld)	
Tubastatin A (TBA)	iKDAC	*T. cruzi* and *L. donovani*	4.4µg/mL (Ld promatigotes), 8.8µg/mL (Ld amastigotes), 0.5 µM (Tc amastigote), 7 µM (Tc epimastigote) and 0.8-2.5 µM (Tc trypomastigote)	n.d.	
Trichostatin A (TSA)	iKDAC	*T. cruzi*, *T. brucei and L. donovani*	60 µM (Tc), 63 µM (Tb BSF) and 1.4 µg/ml (Ld)	2.6 (Ld)	
Thiobarbiturates	iKDAC	*T. cruzi*	30-32 µM (Amastigotes)	2	

5.1. KATs Inhibitors

Nowadays many others iKATs are under study in addition to these ones, but only a few new compounds have been tested as antiparasitic drugs. The compounds described below are iKATs that have been obtained from natural sources or are synthetic derivatives of plant extracts: Polyphenols, Quinones, Anacardic Acid and derivatives, Alkaloids, Prostaglandins and Peptides. Although many of these naturally derived iKAT have a poorly understood mechanism of action and some unwanted effects, these compounds can serve as leaders to optimize inhibitory activity and selectivity. In the case of polyphenols, compounds such as garcinol (*Garcinia indica*)[67] and isogarcinol (*Garcinia mangostana*)[68] have been described. Both have non-specific iKAT activity towards human p300 and PCAF in the same micromolar range (p300 $IC_{50} \simeq 7$ µM and PCAF $IC_{50} \simeq 5$ µM *in vivo* and *in vitro*). From isogarcinol, other derivatives were synthesized in order to improve selectivity and potency, and also to reduce toxicity like LTK-13, LTK-14 and LTK-19, all with specificity for human p300[69]. For garcinol, *in vitro* studies showed that the use of this drug leads to an inhibition of essential *Toxoplasma gondii* KAT *Tg*GCN5b, required for tachyzoite replication, and through *in vivo* studies a decrease in acetylation was detected in Histone H3, which is the preferred substrate of GCN5 family KATs[70]. The benzylidenebarbituric acid derivative called EML425 is another compound with potent and selective inhibition of CBP/p300[71]. Epigallocatechin-3-gallate (EGCG), is a non-selective iKAT that inhibits human p300, CBP, PCAF and Tip60, with different intensity[72]. In contrast, Procyanidin B3 (Pro-B3) is a specific inhibitor that showed KAT activity inhibition in a dose dependent fashion in all enzymes tested. Pro-B3 was found to be an effective inhibitor of p300 acetyltransferase activity, showing 60% inhibition. While for Tip60, PCAF and CBP an inhibition of 40%, 23% and 20% respectively was detected[73]. Delphinidin, is an anthocyanidin extracted from *Punica granatum*, with specific inhibitory activity for CBP/p300[74].

Another compound worth mentioning is curcumin, it is a natural product extracted from *Curcuma longa* and is a non-competitive p300 inhibitor[75]. Different synthetic derivatives have been developed from curcumin, which successfully improved potency and selectivity, which makes them promising iKAT[76]. Curiously, the combination of curcumin with the synthetic inhibitor RK-52 showed synergistic effect

for the reduction of rhodesain activity. Rhodesain is a cysteine protease, an enzyme essential for the life of *T. brucei rhodesiense*. This allows the parasite to cross the blood-brain barrier reaching the central nervous system, thus inducing the second stage of Human African Trypanosomiasis. RK52 has been shown to be a competitive inhibitor as it binds to the active site of the enzyme, while curcumin is a reversible, non-competitive inhibitor as it binds to an allosteric site[77,78].

Among the quinones described with iKAT activity we can mention plumbagin, a hydroxynaphthoquine obtained from *Plumbago rosea* that inhibits histone acetyltransferase p300 activity through a non-competitive mechanism. This compound specifically inhibits the p300-mediated acetylation of p53 but not the acetylation by PCAF *in vivo*. Within this category is also embelin, a derivative of hydroxybenzoquinone obtained from *Embelia ribes* with inhibitory activity for PCAF[79].

Anacardic acid is a 6-pentadecylsalicylic acid extracted of cashew nuts and has been described to act as an inhibitor of KAT enzymes with a wide range of IC_{50} values, acting on p300, PCAF and MYST (in particular Tip60 and MOF)[80–83]. Studies carried out in *Plasmodium falciparum* showed that anacardic acid has a reversible and non-competitive inhibition mechanism on the HAT activity of recombinant *Pf*GCN5[84]. A disadvantage of this compound is that it inhibits multiple proteins and has low cell permeability[85]. Due to this, different derivatives have been synthesized to improve IC_{50} and selectivity[81,82].

Other plant-derived compounds such as alkanoids have also been identified, with inhibitory activity against p300 working at very low concentrations. One of these compounds is palmatine, from which B-homo palmatine and B-homo berberine derivatives were designed. These were shown to be effective for p300, with a ten times higher potency compared to its parent compound[86]. Some cyclopentenone prostaglandins were shown to possess p300 inhibitory activity[87].

Finally, even though they have low cellular absorption and metabolic instability, some peptide metabolites of *Penicillium* species NK13650A and NK13650B are potent and selective p300 inhibitors[88].

Although this list of compounds seems large, the number of known iKATs is significantly less than that of iKDACs. Until now, there are no published reports in which iKATs were assayed succesfully against trypanosomes or leishmania. The only exception that could be mention is garcinol and curcumin, that show trypanocidal and leishmanicidal activity (Table III). However, these activities were associated to other mechanisms of action, different to KAT inhibition. Very few studies exist to date on iKATs for tritryps, which makes it an interesting line of research to explore.

5.2. KDACs Inhibitors

Some inhibitors of KDACs, like sodium butyrate, trichostatin A (TSA) and valproic acid were known before the discovery of their target enzymes[89–91]. These compounds were described to induce histone acetylation in mammals many years ago. TSA, a class I/II inhibitor, is a hydroxamic acid derivative isolated from *Streptomyces hygroscopicus* and characterized for the first time as an antifungal agent[92]. Studies were performed with the inhibitors TSA and suberoylanilide hydroxamic acid (SAHA) with the deacetylase HDLP (histone deacetylase-like protein) from *Aquifex aeolicus*, a thermophilic bacterium, which has a 35.2% identity with human HDAC1. These compounds are capable of binding to the active site of KDAC, avoiding their interaction with lysines and resulting in an increase in the global level of acetylation. In the crystal structure of the HDLP-Zn^{2+}-TSA complex, TSA binds by inserting its long aliphatic chain into the HDLP pocket, make multiple contacts to the tube-like hydrophobic portion of the pocket[93]. In a recent report, the effect of this drug was specifically studied on *T. cruzi*, seeing an increase in acetylation in histones and α-tubulin. TSA does not have trypanocidal activity since it does not kill the parasite but rather inhibits its proliferation (Table III). Contrary to expectations, the chromatin organization was not massively affected after TSA treatment. TSA produced α-tubulin hyperacetylation, this led to a reorganization of the microtubules (MTs) of the cytoskeleton, abnormalities in terms of division and size of the parasite[94]. Also, in *T. cruzi*, TSA modulates the expression of genes required for division and differentiation, besides inhibiting metacyclogenesis, which is essential for parasite infectivity. In this last work, in addition to TSA, tests were done with a class III inhibitor called Sirtinol. For both inhibitors, studies were carried out with the proliferative and infectious forms of this parasite. The two histone deacetylase inhibitors tested led to hyperacetylation of histones, resulting in life stage specific effects. These had a low effect on the growth of parasites in the epimastigote stage and blocked the differentiation to metacyclic trypomastigotes. On the other hand, a slight increase in the number of trypomastigote infections was seen in *in vitro* studies. Finally, these inhibitors led to modifications at the transcriptional level, with Sirtinol showing the greatest changes[95].

Tubastatin A (TST) is a rationaly designed inhibitor of HDAC6 at nanomolar concentrations, with a selective index (SI) with the other KDACs of 1000 except for HDAC8 (SI=60)[96]. Tubastatin A has been tested against *P. falciparum*, *T. gondii* and *L. donovani* (Table III)[97,98]. In *Plasmodium* and *Toxoplasma* TST showed a broad effect that include cytoskeleton alterations and the hyperacetylation of histones. However, the histone hyperacetylation pattern observed was different to that found with all other iKDACs tested. Tubastatin A showed a high activity over *Leishmania* (IC_{50} of 4.4μg/mL for promatigotes and 8.8μg/mL for axenic amastigotes) but the pan iKDAC SAHA and the HDAC8 selective inhibitor PCI-34051 did not showed cytotoxicity. In this report the authors compare the activity of many other HDAC6 and HADC8 inhibitors against *Leishmania* and conclude that antiparasitic activity is associated to a HDAC6 iKDAC profile, proposing that it should exist a HDAC6-like activity essential for the viability of the amastigote and promastigote of *L. donovani*, but until now no such enzyme was identified.

Antimonial drugs have been used for several decades for leishmaniasis, due to their antiparasitic action and relatively low toxicity. Although the mode of action of these compounds

is not yet fully understood, evidence suggests that *in vivo* Sb (III) interferes with trypanothione/trypanothione reductase system, which protects against oxidative stress. This system is found in protozoa of the genera *Leishmania* and *Trypanosoma* and is vital for the survival of the parasites. As mentioned above, hydroxamic acids possess iKDAC activity. From the reaction of benzohydroxamic acid (Bha), 2-pyridinehydroxamic acid (2-pyha), 2-amino-phenylhydroxamic acid (2-NH2-pha) and salicylhydroxamic acid (Sha) with SbCl3, a novel Sb hydroxamate complexes were obtained: [Sb (Bha-1H) 2Cl], [SbCl2 (2-pyha-1H)], [Sb (2-NH2-pha-1H) (2-NH3-pha-1H)] Cl2 and [SbCl (Sha-1H) 2], respectively. Also, it has been found that the use of SAHA, combined with $Sb(OEt)_3$ resulted in the Sb(III) hydroxamato/hydroximato complex, [Sb(SAHA-1H)(SAHA-2H)]. These complexes were tested as inhibitors of *L. amazonensis* and *L. infantum* in the promastigote stage. This type of compounds generates changes in the morphology of the parasites, such as smaller size and loss of flagellum as well as reduction in the permeability of the plasma membrane and alterations in mitochondrial metabolism[99].

Trypanosomatid Sirtuins have been the target of many drug-discovery projects over the last years. Many of them involved the isolation of molecules from natural products (Table III). For example, the Sirtuin inhibitory activity of 6 isolated compounds of *Anacardium occidentale* (cashew nut) was tested on the two Sirtuins of *T. cruzi* SIR2RP1 and RP3. Those compounds were two derivates of cardol, two derivates of cardanol, and anacardic acid. Among the compounds studied one cardol inhibited *Tc*SIR2RP1 and anacardic acids inhibited both *T. cruzi* Sirtuins[100]. In a recent report, human Sirtuin inhibitors were assayed against *T. cruzi*. Two compounds with inhibitory activity were found for *Tc*SIR2RP1, both cambinol analogs, while for *Tc*SIR2RP3 five compounds were found (one analog of salermide, two analogs of cambinol and two thiobarbiturates). The seven compounds showed trypanocidal activity and for some of them a synergistic effect was observed when they were combined with benznidazole[101]. It was also reported that treatment with salermide during *T. cruzi* infection prevented growth and initial multiplication after mammalian cell invasion at concentrations that did not affect host cell viability. In addition, *in vivo* infection was partially controlled upon administration of salermide[59]. To date, the most widely reported antitrypanosidal drugs with high efficacy are Sirtuin inhibitors (Table III). For example, compounds such as bisnaphthalimidopropyl (BNIP) that act by inhibiting the parasite sirtuin *Li*SIR2RP1. Due to the toxicity that these compounds have on macrophages, recently the inhibitory activity of BNIP derivatives on *L. infantum* parasites has been tested, although the results of these studies have not yet been published[102]. Another Sirtuin inhibitor is KH-TFMDI, a 3-arylideneindolin-2-one, which showed *in vitro* activity against mammalian SIRT1-3[103]. This compound turned out to be a very potent inhibitor for the growth of *T. cruzi*. KH-TFMDI has been shown to alter the proliferation of epimastigotes and amastigotes causing drastic alterations in the ultrastructure of *T. cruzi* that lead to the death of the parasite through the induction of mechanisms such as apoptosis and autophagy. Trypanocidal activity was also reported for the BSF stage[104]. In the case of *Leishmania* a significant antiproliferative effect in intracellular promastigotes and amastigotes could be observed (Table III). This was due to morphological changes accompanied by an increase in acetylated α-tubulin, different size and shape of promastigotes, alteration of mitochondrial function, among others, which together led to apoptosis.

6. CONCLUSIONS

Acetylation is a widespread post-translational modification identified both in eukaryotic and prokaryotic cells. The acetylation status of a protein can modulate its function, through different mechanisms such as changes in the intracellular location, modification of the electrostatic charge at sites of interaction with other proteins or modification of lysines involved in active sites, among others. Although the way in which this modification is regulated is not fully understood, each year the evidence on the participation of different acetyltransferases and deacetylases in diverse cellular processes increases. As this information accumulates, new opportunities appear for the therapeutic use of inhibitors of these enzymes.

In trypanosomatids, the number of acetylases and deacetylases is small compared to mammals, making the function/functions of each one even more important. Although the existing information is partial and, to a certain extent, more descriptive than functional, the importance of acetylation has been clearly evidenced in some situations. For instance, in *T. cruzi*, overexpression of acetyltransferase *Tc*ATAT is lethal to parasites, presumably due to hyperacetylation of tubulin. Furthermore, in *T. brucei* acetylation seems to control the energy metabolism of the parasite by inhibiting glycolytic enzymes in the bloodstream form. Also in *T. brucei* it has been shown that two acetyltransferases (KAT1 and KAT2) and two deacetylases (DAC1 and DAC3) are essential, however the precise function of these enzymes is far from being understood. The fact that not all the enzymes of the different trypanosomatids appear to fulfill the same functions is another point to be taken into account. For example, the genomic data available shows differences in the number and cellular localization of Sirtuins, in the number of KDACs and in the number of KATs present in the different Tritryps. Taking into account the number of genomes resolved for each of these species and the current quality of the assemblies, it is difficult to think that these differences are not real.

On the other hand, even with the partial information available, the differences between these enzymes in trypanosomatids compared to mammals are notable. For example, so far only one cytoplasmic KAT has been described, the MEC17/ATAT1 orthologue of *T. cruzi* (*Tc*ATAT), responsible for α-tubulin acetylation. At the same time in *T. brucei* all other KATs have been reported as nuclear. In general, most class I KDACs are subunits of multiprotein nuclear complexes that participate in epigenetics, while class II KDACs regulate cytoplasmic processes or act as signal transducers in the traffic between the cytoplasm and the nucleus. On the contrary, the existing information to date indicates that in *T. brucei*, there is one cytoplasmic and one nuclear KDAC within each class I and class II group. It is also yet undetermined which is the KDAC responsible for a-tubulin deacetylation. It has been reported that the cytoplasmic Sirtuins of *Leishmania* and *T. cruzi* could fulfill this role, but no functional homolog of HDAC6 has been identified. None of the trypanosomatid KDACs has any noticeable similarity to HDA6. In *T. brucei*, the only cytoplasmic

class II DAC is DAC4, a small deacetylase that does not possess any of the additional domains that HDAC6 possesses, nor is it large enough to harbour alternative functionally equivalent domains. Despite this, there is evidence that indicates that it would exist, at least in *Leishmania*, a HDA6-like enzyme in terms of recognition by specific inhibitors. This information clearly indicates that much research is still needed to understand exactly the function of each of these enzymes in trypanosomatids.

It is currently accepted that the enzymes involved in the acetylation and deacetylation of proteins are a potential target for the development of new drugs against cancer, inflamatory deseases, among others. So far, numerous iKATs and iKDACs compounds of natural and synthetic origin have been tested in trypanosomatids with varying results. The use of molecules that have been developed against mammalian enzymes presents a problem of their "original sin": having activity on mammalian cells. However, they can be used as lead compounds for generating synthetic new ones, more specific. Alternatively, compounds synthetized in the frame of the search of new human KATs or KDACs that result inactive, could be an interesting source for the search of specifics inhibitors against trypanosomatids as well as for other protist parasites. A third way to produce specific inhibitors is through the rational design as was recently done with some human enzymes such as HDAC5 and HDAC6.

Advances in *ab initio* prediction of protein structures opens a new road to explore this approach without the limitations due to the need of producing enough well folded recombinant proteins to be crystalized or used in MNR. Recently, DeepMind in partnership with EMBL's European Bioinformatics Institute (EMBL-EBI), have generated, and made available to the community through UniProt, the predicted structures of the proteins from twenty organisms (https://alphafold.ebi.ac.uk/). Among them are the whole predicted proteome from *T. cruzi* (CLBrener strain) and *L. infantum*, including most of the acetylases and deacetylses discussed in this review (Supplementary Tables SIV-SVII). The quality of these structures is high enough to be used in *in silico* docking screenings against a number of available compounds databases like PubChem (https://pubchem.ncbi.nlm.nih.gov/), ZINC (https://zinc.docking.org/), which is a free database that contains over 230 million commercially available compounds in ready-to-dock 3D formats or DrugBank that includes only approved drugs and extensive biochemical and pharmacological information about them (http://www.drugbank.ca). This explosion of information will probably lead to a new age into the development of new pharmaceutical drugs, specially for neglected tropical diseases.

CONFLICT OF INTEREST

The authors declare there is not conflict of interest. This work was supported by Agencia Nacional de Promoción Científica y Tecnológica, Ministerio de Ciencia, Tecnología e Innovación Productiva, Argentina (PICT 2017–1978 and PICT2019-0526) and Universidad Nacional de Rosario (PIP 1BIO490).

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SUPPLEMENTARY MATERIAL

Supplementary material includes Table SI: Acetylation sites on histone proteins in Tritryps, Table SII: Acetylated sites on non-histone proteins in Tritryps and Table SIII: Acetylated cytoskeleton-related proteins in Tritryps. Table SIV-SVII: Acetyltransferases and Deacetylases from *T. cruzi* CL strain and *Leishmania infantum* with structures predicted by AlphaFold deposited in UniProt.

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