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Review

Histone-modifying proteins and DNA-methyltransferases in the epigenetic regulation of protozoa parasites

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ABSTRACT

Diseases produced by protozoa parasites are still a public health problem in the entire world. These parasites should have a severe control on the expression of genes involved in their development and pathogenicity. Epigenetic mechanisms through chromatin modifications performed by histone-modifying enzymes as well as DNA methylation could be involved in the expression regulation of those genes. The identification and characterization of the enzymes that achieve the chromatin modifications could help to develop novel therapeutic strategies against protozoa parasites. Here, we review the current understanding of the histone-modifying enzymes and DNA-methyltransferases in different protozoa parasites.

KEYWORDS: protozoa parasites, histone-modifying enzymes, histone acetyltransferases, histone deacetylases, histone methyltransferases, histone demethylases, DNA methyltransferases

INTRODUCTION

Epigenetic changes are defined as the heritable changes that affect gene expression without altering the DNA sequence. Epigenetic regulation of gene expression primarily works through modifying the structure of chromatin, which makes it more or less accessible to transcription. In all eukaryotic organisms chromatin is organized into basic units called nucleosomes. Each nucleosome consists of an octamer of two molecules of each of the histones H2A, H2B, H3 and H4, around which 147 base pairs of DNA are wrapped [1]. Nucleosomes are connected by linker DNA which is associated with linker histones, usually H1 and H5. Nucleosomes together with the linker DNA are progressively folded and compacted into structures of higher-order.

Histones are globular basic proteins that are subject to various covalent modifications that occur primarily on the N-terminal tail and they appear to act sequentially or in combination to form a recognizable code that is identified by specific proteins to regulate distinct downstream events such as transcriptional activation or repression [2]. Examples of such modifications are acetylation, methylation, phosphorylation, and ubiquitylation. Histone acetylation is, in general, associated with gene activation. In contrast, methylation on a specific lysine (K) or arginine (R) residue is correlated with either an active or a silent state of gene expression, depending on the residues being methylated.

Histone acetylation is achieved by enzymes called histone acetyltransferases (HATs), whereas acetyl groups are removed by histone deacetylases (HDACs). Deacetylation is generally associated with loss of gene expression or silencing [2]. On the other hand, histone methylation is catalyzed

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by histone methyltransferases (HMTs). Lysine residues can be mono-, di- and tri-methylated, whereas arginine residues can carry one or two methyl groups on their guanidinyl group. The di-methyl arginine state is further defined by whether the modification exists in the symmetric or the asymmetric configuration [2]. Arginine methylation is transcription-activating, and lysine methylation can cause either transcriptional activation or repression, depending on the lysine residue methylated. In general, methylation on histone H3 lysine 4 (H3K4), H3K36, and H3K79 is linked to active gene expression, whereas di- and tri-methylation on H3K9, H3K27, and H4K20 are associated with gene silencing. Thus, a balance in histone acetylation and methylation may be important in determining chromatin architecture and gene silencing or activation [2].

In addition, methylation of cytosine bases at the C5 position of CpG islands leads to transcriptional silencing due to chromatin condensation, increased recruitment of transcriptional repressors, and inhibition of transcriptional activators [3]. The enzymes that are involved in this modification are named DNA methyltransferases (DNMT). DNMT1 maintains methylation status, whereas the function of DNMT2 is not yet clear and it has weak methyltransferase activity [3].

Protozoan parasites have remarkable negative impact on human health. Infection with these microorganisms causes high mortality and morbidity, mainly in developing countries. As yet, there are no safe vaccines for any of these parasites, leaving drug treatments as the major strategy for control. However, available drugs are compromised by low efficacy, high toxicity, and wide spread resistance. Therefore, it is important to identify parasite-specific targets and develop novel inhibitors against them. Growth and development of any organism depend upon precise and accurate control of gene expression. Thus, the identification and characterization of the molecules that regulate the expression of parasitic genes involved in transmission, pathogenicity, immune evasion, and drug resistance may help to develop novel therapeutic agents against these microorganisms. Here, we review the general concepts that have emerged with regards to epigenetic regulation of gene expression in *Toxoplasma gondii*, an intracellular protozoan that can cause significant morbidity and mortality in humans and animals [4], *Plasmodium falciparum*, which produces human malaria [5], *Trypanosoma brucei*, parasite responsible for African sleeping sickness, a remarkable public health problem in Africa [6], and the enteric parasites *Giardia intestinalis* and *Entamoeba histolytica*, microorganisms that infect millions of persons around the world [7, 8].

Histones in protozoa parasites

In general, protozoa parasites show a peculiar absence of DNA-binding transcription factors. However, these primitive eukaryotes present the four canonical histones of the nucleosome core and some variants of them [9]. It has been documented that the exchange of canonical histones by their variants may influence the nucleosome stability and the chromatin pattern [10, 11]. Possibly due to this reason the histone variants have a different profile of expression in the different stages of protozoa parasites, affecting the expression of genes involved in virulence and differentiation. For example, *T. gondii* contains two H2B lineages, one containing the genes TgH2Ba and TgH2Bb and other represented by TgH2Bv1 [12]. TgH2Ba is highly expressed in tachyzoites, but TgH2Bv1 is not differentially regulated [12]. In *P. falciparum*, the variant PfCenH3 showed a five- to ten-fold decreasing expression during the transition from mid- to late-trophozoite stages [13], while H3.3 exhibited a reduction in their expression in trophozoites that correlates with the telomeric silencing of the *var* genes, involved in antigenic variation [14, 15]. In contrast, the histone PfH2A.Z is enriched at the transcription start sites of *var* genes only during active transcription in the ring stage of the parasite [16]. In addition, the level of expression of histones appeared to influence the gene expression in *P. falciparum*. Transcription of histones increases during the phase transition from ring to trophozoite and during the transition from early- to mid- trophozoites [17] that lead to the accumulation of histones in the late trophozoite and schizont stages [13].

Interestingly, histones of some protozoa parasites show marked differences with the canonical

histones of other eukaryotes. Histones from trypanosomes could be distinguished from those of higher eukaryotes by differences in charge and/or size [18], and the amino-terminal domains of histones of *E. histolytica* are divergent from metazoan sequences, although they are highly basic with several lysine and arginine residues that are potential targets for modification [19].

It has been reported that protozoa parasites also contain a large repertoire of histone modifications, sustaining the hypothesis that epigenetic events could be involved in the expression of genes that participate in virulence and differentiation of these organisms. Histone modifications in protozoa parasites will be reviewed in the next sections.

Histone acetylation and deacetylation

The genome packaging into a highly compacted chromatin prevents the gene transcription. To deal with this impediment, histone acetylation allows the accessibility of the transcriptional machinery. Histone acetylation is a reversible modification of lysine residues in histone "tails". Acetylation is carried out by histone acetyltransferases (HAT), whereas deacetylation is catalyzed by histone deacetylases (HDACs). There are three major groups of HATs: GCN5-related N-acetyltransferases (GNATs or GCN5 family); E1A-associated protein of 300 kDa (p300; KAT3A) and CBP (KAT3B); and MYST proteins [20]. On the other hand, HDACs are divided into classes I, II and IV and the sirtuin family (also known as class III HDACs) [21]. In general, histone acetylation in protozoa parasites seems to have a role in the epigenetic control of gene expression and the HAT activity in these cells is carried out mainly by members of GCN5 and MYST families (Table 1).

In *T. gondii*, Saksouk *et al.* [22] showed that histones at gene promoter regions of tachyzoite-specific genes are hyperacetylated in the tachyzoite stage, but they are hypoacetylated during the bradyzoite phase. Conversely, histones at bradyzoite-specific gene promoters become hyperacetylated in the bradyzoite stage, but are hypoacetylated in tachyzoites. These results confirm that histone acetylation is a mark of gene activation in this parasite. The analysis of the *T. gondii* genome database allowed the identification of four HATs in this parasite (Table 1): i) two GCN5 members, TgGCN5-A that acetylates only H3K18, and TgGCN5-B, which acetylates H3K9, H3K14, and H3K18 [23]; and ii) two MYST members (MYST A and MYST B) [24]. In addition, *T. gondii* also contains two genes encoding for putative ADA2 homologues (TgADA2-A and -B) [23], proteins that potentiate the GCN5 catalytic activity. This parasite also has seven HDACs, including two Sir2-related proteins (Table 2). It is known that TgHDAC3 removes the acetyl groups from H4K5, H4K8, and H4K12, whereas TgSir2 removes the acetyl group from H3K56 [22, 25].

P. falciparum has six HATs of the GCN5 family and one MYST member (Table 1). One member of the GCN5 family, named PfGCN5, mainly acetylates H2B and H3 [26]. Specifically, this enzyme catalyzes the acetylation of H3K9 and H3K14, modifications that have essential roles in the activation of gene expression in trophozoites and schizonts [13]. In concordance, PfGCN5 is expressed during the erythrocyte stages, correlating with high levels of H3K9ac [27]. In addition, a homologue of ADA2 forms a complex with PfGCN5 [28]. The only member of the MYST family in *P. falciparum* (PfMYST) showed specificity to acetylate H4K5, H4K8, H4K12, and H4K16 [29]. On the other hand, five homologues of HDACs distributed in three classes have been identified in *P. falciparum* (Table 2): PfHDAC1, belonged to the class I, which is mainly located in nuclei and has an important role during the erythrocytic stages [30]; two putative HDACs that were classified into the class II due to their large size and the location of their HDAC domain in the C-terminus [31]; and two HDACs of the sirtuin family named PfSir2A and PfSir2B [32]. These sirtuins are NAD^+-dependent enzymes and display two activities: deacetylation and the transference of ADP-ribosyl to several cellular proteins [33, 34]. The knockout silencing of PfSir2A demonstrated that this protein participates in the chromatin stabilization of subtelomeric regions and in the maintenance of the telomeres length due to the deacetylation of the N-terminus of the H3 y H4 histones, regulating the expression of the *var* genes [16, 34-36].

Several lines of evidence showed that chromatin modification has an important role in gene expression, cell cycle control and differentiation

Table 1. HATs in protozoa parasites.

Gene family	*P. falciparum*	*T. gondii*	*T. cruzi*	*T. brucei*	*L. major*	*G. intestinalis*	*T. vaginalis*	*E. histolytica*
GCN5	PF14 0350 PF08 0034 PF11 0192 PFL1345c PFD0795 PFA0465c	TGGT_004130 TGME49_043440	NI	NI	NI	GL50803_10666	TVAG_091650 TVAG_340910 TVAG_308330 TVAG_319980 TVAG_324780 TVAG_497120 TVAG_473850 TVAG_059320	EHI_140660 EHI_139360
MYST	PF11_0192	TGGT1_122910 TGME49 007080	Tc00.1047053506605.160 Tc00.1047053511239.150 Tc00.1047053511017.69 Tc00.1047053509203.60 Tc00.1047053507611.290 Tc00.1047053507723.110	Tb07.26A24.750 Tb11.01.3380 Tb10.6k15.2190	LmjF14.0140 LmjF28.2270 LmjF36.6990	GL50803_17263 GL50803_2851	TVAG_345100 TVAG_216320 TVAG_131130 TVAG_296330 TVAG_296750 TVAG_359840 TVAG_233420 TVAG_395439 TVAG_028440 TVAG_394510 TVAG_408380 TVAG_285090	EHI_142000

Accession number for each putative HAT is given.
NI: not identified.

Table 2. HDACs in protozoa parasites.

Gene family	*P. falciparum*	*T. gondii*	*T. cruzi*	*T. brucei*	*L. major*	*G. intestinalis*	*T. vaginalis*	*E. histolytica*
HDAC	PFI1260c PF14_0690 PF10_0078	TGGT1_006030 TGME49_049620 TGME49_02729 TGME49_057790 TGME49_002230	Tc00.1047053511911.159 Tc00.1047053508637.114 Tc00.1047053504159.80 Tc00.1047053506821.140 Tc00.1047053509395.120 Tc00.1047053503653.50 Tc00.1047053507803.49 Tc00.1047053507063.270 Tc00.1047053507805.9	Tb10.70.6220 Tb11.01.7240 Tb927.2.2190 Tb05.26K5.290	LmjF21.0680 LmjF24.1370 LmjF08.1090 LmjF21.1870	GL50803-3281	TVAG_182970 TVAG_185870 TVAG_226910 TVAG_166720 TVAG_426760 TVAG_059170 TVAG_464210 TVAG_390760 TVAG_178760	EHI_119320
Sirt2	PF13_0152 Pf14_0489	TGGT1_082510 TGME49_067360	Tc00.1047053507519.60 Tc00.1047053508207.150 Tc00.1047053506559.80 Tc00.1047053506559.80	Tb07.43M14.60 Tb08.28L1.80 Tb04.1H19.1040	LmjF26.0210 LmjF23.1210 LmjF34.2140	GL50803_10707 GL50803_10708 GL50803_16569 GL50803_6942 GL50803_11676	TVAG_190210 TVAG_319320 TVAG_549940 TVAG_026260 TVAG_480900 TVAG_362260 TVAG_413390 TVAG_409810 TVAG_016210 TVAG_256040 TVAG_146810	EHI_151300 EHI_201210 EHI_120280 EHI_007500

Accession number for each putative HDAC is given.

in trypanosomes [37-39]. The *in silico* analysis of the genome database of *T. brucei* suggested that HATs of the GCN5 family are absent in this organism as well as in the trypanosomatides *Trypanosoma cruzi* and *Leishmania major* (Table 1), but they have three distinct members of the MYST family (Table 1). Non-redundant roles for each of these HATs were described in bloodstream-forms of *T. brucei* [40]. HAT1 modulates telomeric silencing and is required for growth, and possibly for DNA replication; HAT2 is required for H4K10 acetylation and growth; and HAT3 is required for H4K4 acetylation and is dispensable for growth [40]. The non-redundant functions for *T. brucei* HAT1-3 appear to reflect unique substrates for each acetyltransferase and support the idea of a simplified, non-redundant histone code in this parasite. The genome database of *T. brucei* reveals the presence of seven HDACs (Table 2). Four of these proteins belong to HDACs classes I (HDAC1 and HDAC2) and II (HDAC3 and HDAC4) [41], and three of them correspond to members of the Sir2 family (Table 2). HDAC1 and HDAC3 display histone deacetylase activity and they appear to be essential for growth in the bloodstream form and have an important role in the silencing of *VSG* genes [41, 42]. In addition, it has been demonstrated that Sir2rp1, the only nuclear protein of this class in *T. brucei*, is required for basal telomeric silencing, but not for *VSG* silencing, whereas the other two Sir2-related enzymes of *T. brucei* were found in mitochondria [43].

Analysis of the *G. intestinalis* genome database revealed the presence of: i) three putative HATs, one of the GCN5 family and two MYST-related proteins (Table 1); and ii) six putative HDACs (Table 2), one of them is homologue of the classical HDAC family, and the five additional are predicted to belong to the Sir2 family [44]. The recombinant protein of the typical HDAC fused to an HA-tag was located in nuclei, suggesting a deacetylase activity on histones [44].

In *E. histolytica*, Western blot assays revealed that trophozoites contain nuclear proteins of 16- to 23-kDa that can be acetylated, suggesting that histones of this parasite are susceptible to this modification. In concordance, three genes encoding for HATs were identified in the genome of *E. histolytica*, two GCN5-related enzymes and one member of the MYST family (EhMYST) [19] (Table 1). The expression of these proteins in trophozoites was confirmed by RT-PCR and the characterization of EhMYST showed that this protein has HAT activity on H4 [19]. In addition, the genome of *E. histolytica* revealed the presence of one HDAC of the class I (EhHDAC) (Table 2) and RT-PCR assays demonstrated the expression of this protein in trophozoites [19].

Histone methylation and demethylation

Histone methyltransferases (HMTs) add methyl groups to lysine (histone lysine methyltransferases, HKMTs) or arginine (protein arginine methyl-transferases, PRMTs) residues.

Most HKMTs that confer either transcriptional silencing or activation contain a conserved domain named SET (SUV39 [suppressor of variegation 3-9], enhancer or Zeste, Trithorax). These enzymes have very defined residue substrates and modification state specificity. Proteins containing SET domains can be classified into five subfamilies; SET1, SET2, SUV39, RIZ (retinoblastoma protein-interacting zinc-finger) and SMYD3 (SET- and MYND-domain containing protein 3) [45]. In addition, methylation of H3K79 is mediated by a protein without SET domain named DOT1 [46].

On the other hand, PRMTs can be divided into four types (I to IV) depending on the type of methylarginine that they generate [47]. In contrast to HKMTs, PRMTs are typically more promiscuous and often target multiple arginine residues on the N-terminal tails of histones H3 and H4. Furthermore, PRMT enzymes also target a broad range of other cellular proteins, suggesting that they contribute to regulation of additional non-chromatin based processes [48]. In humans, nine PRMTs have so far been identified [47]. These enzymes are classified in two groups, type I (PRMT1, PRMT3, PRMT4, PRMT6, and PRMT8) and type II (PRMT5, PRMT7, and PRMT9). An overview of the evolutionary conservation of each human PRMT in unicellular eukaryotes, including yeasts, molds, amoebae, and protozoa reveals that PRMT1, PRMT3, and PRMT5 are the arginine methyltransferases most strictly conserved throughout eukaryotic evolution [47].

In silico analysis showed that protozoa parasites, with exception of *T. brucei*, have not HKMTs similar to DOT1, but they contain several genes encoding for putative HKMTs of the SET family (Table 3), although most of these proteins have not been characterized so far. In addition, protozoa parasites, except *G. intestinalis*, have several genes that encode for putative PRMTs (Table 3), but due that these enzymes also target other non-histone proteins, their role in epigenetic control need to be proved. On the other hand, histone demethylases were only found in Apicomplexa.

T. gondii has several putative HKMTs of the SET family (Table 3), and it has been reported that TgSET1, TgSET2, TgSET3 methylate H3K4, H3K36 and H3K9, respectively [49]. In addition, TgSET8-related proteins can mono-, di-, and tri-methylate H4K20, but unlike other eukaryotes this modification does not seem to correlate with promoters [50]. Another HKMT, named KMTox/ SET13, methylates lysines on histones H4 and H2A [51]. On the other hand, concerning to PRMTs, *T. gondii* has six of these enzymes (Table 3). TgPRMT1 and TgPRMT5 methylate H4R3 and H3R2 respectively, while TgCARM1 methylates H3R17 [49].

In silico analysis of the *P. falciparum* genome demonstrated the presence of nine HKMTs containing SET domains (Table 3). These enzymes are named PfSET1–9 [31, 52]. PfSET1, -2, -3, and -8 are homologous to the well characterized HKMTs that methylate H3K4, K36, K9 y H4K20, respectively [52]. Recombinant proteins of PfSET2 and PfSET8 showed enzymatic activity and the last one is able to add one, two and three methyl groups to H4K20 [52]. Additionally, three putative PRMTs have been identified in this parasite (Table 3), they were called PfPRMT1, PfPRMT4 (or PfCARM1) and PfPRMT5 [53]. PfPRMT1 methylates H4R3, H2A and several conserved substrates as fibrillarin, poly(A)-binding protein II, ribosomal protein S2 and a putative splicing factor [54].

T. brucei has three putative HKMTs of the SET family and two homologues of DOT1 (DOT1A and DOT1B) (Table 3). DOT1A and DOT1B are responsible for dimethylation and trimethylation of H3K76 (synonymous to K79 in other organisms) [55]. DOT1B seems to play an important role in regulate the antigenic variation, because the transcriptionally silent genes encoding VSGs located in telomeric regions become partially derepressed when DOT1B is deleted, whereas non-telomeric loci are unaffected [56]. Additionally, the transcriptional switch is so slow that cells expressing two VSGs persist for several weeks in parasites that has deleted the DOT1B gene, indicating that monoallelic transcription is compromised [56]. In contrast, silencing of DOT1A by RNAi resulted in a premature progression through mitosis without DNA replication generating a proportion of cells with a haploid DNA content [55]. Thus, DOT1A and DOT1B appear to influence the antigenic variation and the cell cycle of *T. brucei* by regulating the degree of H3K76 methylation. On the other hand, five putative PMRTs were found in the *T. brucei* genome database (Table 3), including: i) canonical homologues of the human PRMT1 and PMRT5; ii) a protein that appears to be homologue to the human PRMT3, iii) an enzyme similar to human PRMT6 (TbPRMT6); and iv) a unique type III enzyme named TbPRMT7 [57]. TbPRMT6 utilizes bovine histones as substrate and among the TbPRMT6-associated proteins identified by mass spectrometry were found the parasite histones [57]. Knockdown of TbPRMT6 produced aberrant morphologies in both procyclic and bloodstream forms, indicating defects in cell division [57].

In silico analysis of the genome of *G. intestinalis* showed that this parasite has six putative histone methyltransferases (Table 3) [44], but their role in the epigenetic regulation is unknown. HKMTs of the DOT1 family and typical PMRTs seem to be absent in *G. intestinalis* (Table 3) [44].

Epigenetic silencing of specific genes of *E. histolytica* was achieved by the transfection of plasmids containing the respective encoding gene and a DNA segment of the 5' upstream region of the amebapore-a encoding gene (*ap-a*) [58-61]. Specific antibodies against methylated H3 evidenced that *E. histolytica* genes were silenced by methylation in H3K4 [59], suggesting that gene expression in this parasite could be epigenetically regulated and that methylation in H3K4 probably depends of histone methyltransferases. However, no HKMTs have been reported in *E. histolytica*. Our *in silico* analysis of the genome database

Table 3. HMTs in protozoa parasites.

Gene family	*P. falciparum*	*T. gondii*	*T. cruzi*	*T. brucei*	*L. major*	*G. intestinalis*	*T. vaginalis*	*E. histolytica*
SET	PFF1440w MAL13P1.122 PF08 0012 PFI0485c PFL0690c PF13 0293 PF11 0160 PFD0190w PFE0400w	TGME49_026810 TGME49_057770 TGME49_055970 TGME49_088330 TGME49_092170 TGME49_094610 TGME49_095610 TGME49_001250 TGME49_002490 TGME49_011730 TGME49_016080 TGME49_046910 TGME49_076120 TGME49_111660 TGME49_119660 TGME49 _042870 TGME49 _062750 TGME49_081900 TGME49_018230 TGME49_084160	Tc00.1047053509551.140 Tc00.1047053508153.220 Tc00.1047053508169.50	Tb09.211.1620 Tb03.3K10.450 Tb10.70.2620	LmjF35.4550 LmjF21.1750 LmjF36.0210	GL50803_8921 GL50803_13838 GL50803_13790 GL50803_9130 GL50803_17036 GL50803_221691	TVAG_120120 TVAG_162900 TVAG_185780 TVAG_433010 TVAG_440830 TVAG_302280 TVAG_177920 TVAG_087990 TVAG_127920	EHI_080240 EHI_069080 EHI_092690 EHI_031960
DOT1	NI	NI	NI	Tb08.26N11.380 Tb927.1.570	NI	NI	NI	NI
PRMT	PF14 0242 PF08 0092 PF13 0323	TGGT1_030400 TGGT1_073730 TGME49_052420 TGME49_094270 TGME49_015560 TGME49_019520	Tc00.1047053506529.50 Tc00.1047053508593.110 Tc00.1047053508153.1110 Tc00.1047053510311.140 Tc00.1047053506947.80 Tc00.1047053507057.30 Tc00.1047053509153.100 Tc00.1047053510303.310	Tb927.1.4690 Tb10.70.3860 Tb05.6E7.1000 Tb07.10C21.54	LmjF12.1270 LmjF03.0600 LmjF16.0030 LmjF06.0870	NI	TVAG_433490 TVAG_199700 TVAG_028100 TVAG_045760 TVAG_225950 TVAG_048280 TVAG_096150 TVAG_254540	EHI_152460 EHI_105780 EHI_159180 EHI_158560 EHI_152400

Accession number for each putative HMT is given.
NI: not identified.

revealed that this microorganism has four putative proteins that contain a SET domain (Table 3) and five putative PRMTs (Table 3) [53]. However, there are no experimental studies demonstrating their activity.

Just as there are HMTs to methylate histones, there exist enzymes responsible for methyl group removal from histones as well (histone demethylases). Histone demethylases are of two general classes: i) the class enclosed amine oxidase enzymes, characterized by the mammalian lysine specific demethylase 1 (KDM1/LSD1), which uses FAD as a co-factor and removes the mono- and di-methylated modification states [62]; and ii) the class belonged to a large family of proteins that contain a *Jumonji-C* (JmjC) domain as their catalytic core [63]. The JmjC domain-containing proteins are iron and alpha-ketoglutarate dependent oxygenases that target the removal of all three histone lysine methylation states.

In protozoa parasites, histone demethylases only have been identified in Apicomplexa. *T. gondii* has two LSD1-like proteins and eight JmjC-containing proteins [49], suggesting that lysine demethylation has a role in the regulation of gene expression in this parasite. On other hand, the genome of *P. falciparum* encodes one LSD1-like protein and two putative JmjC-like proteins [52]. Both JmjC-like proteins are expressed in the erythrocytic stages of the parasite [52].

In silico analysis on the genome databases of *T. brucei*, *T. cruzi*, *L. major*, *G. intestinalis*, and *E. histolytica* indicate that canonical histone demethylases are absent in these parasites, suggesting that other unidentified enzymes are implicated in the histone demethylation.

DNA methylation

The enzymes responsible for DNA methylation are referred to as DNA methyltransferases (DNMTs), which catalyze the reaction through the transfer of the methyl group from S-adenosylmethionine to cytosine. In mammals, five members of the DNMT protein family have been discovered (Dnmt1, Dnmt2, Dnmt3a, Dnmt3b, and Dnmt3L).

Two putative DNMT proteins have been identified in *T. gondii*, and one in *P. falciparum*, *E. histolytica*, and *T. cruzi*. Interestingly, methylated cytosine were not detected in the genomic DNA of *Toxoplasma*, *Cryptosporidium* and *Plasmodium*, suggesting that DNA methylation is unlikely to be a major mechanism for regulation of gene expression in Apicomplexa during their asexual phases [64].

A monoclonal antibody against 5-methylcytosine was used to demonstrate the presence of methylated DNA in *T. brucei* [65]. In addition, a DNMT enzyme of this parasite (TbDMT) with a greater homology to prokaryotic than to eukaryotic DNMTs was identified [65]. TbDMT is expressed in both bloodstream- and procyclic-forms of the parasite, but there is a little stage-specific regulation [65].

In *E. histolytica*, the 5' region of the gene encoding for the heat shock protein 100 (hsp100) and a reverse transcriptase of a LINE retroposon (RT-LINE) were isolated by affinity chromatography using antibodies against 5-methylcytosine as ligand [66, 67], indicating the presence of methylated DNA in this parasite. However, a genome-wide analysis of methylated DNA in *E. histolytica* showed that only 2.1% of the genes are transcriptionally modulated by DNA methylation, suggesting that this modification has limited effects on gene expression in this parasite [68]. Nevertheless, a DNA methyltransferase homologous to the human Dnmt2 (Ehmeth) was identified in *E. histolytica* [69]. Ehmeth has a dual DNA/tRNA (Asp) methyltransferase activity [70] and the over expression of this enzyme in trophozoites produced the accumulation of multinucleated cells, upregulation of the heat shock protein 70 and resistance to oxidative stress [69]. These results suggested that this enzyme could have an important role in the expression of some genes [69]. Interestingly, the enolase enzyme of *E. histolytica* acts as an inhibitor of Ehmeth [71]. Its inhibitory activity is antagonized by 2-phosphpglycerate, suggesting that glucose metabolism controls the function of enolase as Ehmeth inhibitor [71].

Histone-modifying enzymes in other protozoa parasites

Little data are available with respect to histone-modifying enzymes and DNA methyltransferases

in different protozoa parasites to those described here, but their genome databases showed that these microorganisms contain most, but not all, of the enzymes implicated in the epigenetic control in higher eukaryotes (Tables 1-3). For instance, *Trichomonas vaginalis* has several HATs of the GCN5 and MYST families, as well as numerous HDACs, PRMTs and HKMTs of the SET family, but it does not contain DOT1-like HKMTs or typical DNA methyltransferases.

Inhibitors of DNA-modifying enzymes on protozoa parasites

Several studies have focused on the anti-parasitic activity of HDAC inhibitors of various structural classes, highlighting the potential of these drugs for anti-parasitic intervention. HDAC inhibitors like tetrapeptides (apicidin or synthetic analogues and FR235222) [44, 49, 72-74], short-chain fatty acids (Valproic acid, sodium butyrate) [75], hydroxamates (trichostatin A and synthetic compounds including suberoylanilidie hydroxamic acid (SAHA)) [76, 77], as well as Sir2 inhibitors [78, 79] produced significant alterations in the development of protozoa parasites. However, the poor selectivity of some of these compounds for parasites versus mammalian cells, and their poor bioavailability, means that it is not considered clinically suitable. To try to address the issue of selectivity, apicidin analogues with indole modifications, and tryptophan- or quinolone - replacements have been tested against *P. falciparum* parasites. Some quinolone derivatives, but not N-substituted indole derivatives, were found to have increased selectivity (up to 200-fold) for *P. falciparum* versus mammalian cells at the whole-cell level [80-82]. In addition, several hydroxamic acid-based HDAC inhibitors analogues to SAHA have been described with better potency against *P. falciparum* parasites *in vitro*, and in some cases much better selectivity [83-85].

To explore HAT enzymes as potential targets for controlling malaria, some studies have been focused on the inhibition of PfGNC5. Curcumin, a potent drug against chloroquine-resistant *P. falciparum* strains, induced hypoacetylation of histone H3 at K9 and K14, suggesting that this drug caused specific inhibition of the PfGCN5 HAT [86]. In concordance, curcumin inhibited the HAT activity of the recombinant PfGCN5 [86]. In addition, Cui *et al.* [87] showed that the anacardic acid (AA), an inhibitor for both p300 and PCAF HAT families, inhibits *P. falciparum* growth *in vitro*. Consistent with the role of PfGCN5 in regulating global gene expression, AA treatment inhibits PGCN5 activity, resulting in histone hypoacetylation and downregulation of a panel of developmentally regulated genes in the parasite [87].

On the other hand, the search for inhibitors of histone methyltransferases and histone demethylases is still in its infancy. Only a few inhibitors of these enzymes have been discovered and tested in higher eukaryotes [88], but they have not been used as potential anti-parasitic drugs. Nevertheless, the development of specific inhibitors of HMTs from parasites is an important task that will certainly be taken up in the years to come.

CONCLUDING REMARKS

Protozoa parasites posses in their genomes a wide repertoire of histone-modifying enzymes that play important roles in the expression of proteins involved in their development and pathogenicity. However, methylated DNA as well DNA methyltransferases appear to have a modest influence in gene expression regulation in most of these microorganisms. On the other hand, some histone modifying-enzymes are parasite-specific and that could help to develop new therapeutic strategies against them. Interestingly, *in silico* analysis on the genome databases showing that some canonical histone-modifying enzymes seem to be absent in some of the protozoa parasites, suggest that their respective histone modifications are not important in epigenetic control in these organisms, or that these modifications could be performed by other enzymes that need to be identified in further studies.

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ABBREVIATIONS

DNMT, DNA methyltransferase; HAT, histone methyltransferase; HDAC, histone deacetylase;

HKMT, histone-lysine methyltransferase; HMT, histone methyl transferase; PRMT protein arginine methyl transferase; SET, SUV39 (suppressor of variegation 3-9), Enhancer and Trithorax domain; VSG, variant surface glycoprotein

REFERENCES

1. Hayes, J. and Hansen, J. 2001, Curr. Opin. Genetics Dev., 11, 124.
2. Strahl, B. and Allis, C. 2000, Nature, 403, 41.
3. Jones, P. and Liang, G. 2009, Nat. Rev. Genet., 10, 805.
4. Elmore, S., Jones, J., Conrad, P., Patton, S., Lindsay, D. and Dubey, J. 2010, Trends Parasitol., 26, 190.
5. Sachs, J. and Malaney, P. 2002, Nature, 415, 680.
6. Simarro, P., Jannin, J. and Cattand, P. 2008, PLoS Med., 5, e55.
7. Ankarklev, J., Jerlström-Hultqvist, J., Ringqvist, E., Troell, K. and Svärd, S. 2010, Nat. Rev. Microbiol., 8, 413.
8. Ximénez, C., Morán, P., Rojas, L., Valadez, A. and Gómez, A. 2009, Infect. Genet. Evol., 9, 1023.
9. Gomez, C., Esther Ramirez, M., Calixto-Galvez, M., Medel, O. and Rodríguez, M. A. 2010, J. Biomed. Biotechnol., 2010, 726045.
10. Lieb, J. and Clarke, N. 2005, Cell, 123, 1187.
11. Raisner, R. and Madhani, H. 2006, Curr. Opin. Genet. Dev., 16, 119.
12. Dalmasso, M., Echeverria, P., Zappia, M., Hellman, U., Dubremetz, J. and Angel, S. 2006, Mol. Biochem. Parasitol., 148, 103.
13. Miao, J., Fan, Q., Cui, L., Li, J., Li, J. and Cui, L. 2006, Gene, 369, 53.
14. Duraisingh, M., Voss, T., Marty, A., Duffy, M., Good, R., Thompson, J., Freitas-Junior, L., Scherf, A., Crabb, B. and Cowman, A. 2005, Cell, 121, 13.
15. Freitas-Junior, L., Hernandez-Rivas, R., Ralph, S., Montiel-Condado, D., Ruvalcaba-Salazar, O., Rojas-Meza, A., Mâncio-Silva, L., Leal-Silvestre, R., Gontijo, A., Shorte, S. and Scherf, A. 2005, Cell, 121, 25.
16. Petter, M., Lee, C., Byrne, T., Boysen, K., Volz, J., Ralph, S., Cowman, A., Brown, G. and Duffy, M. 2011, PLoS Pathog., 7, e1001292.
17. Inselburg, J. and Banyal, H. 1984, Mol. Biochem. Parasitol., 10, 79.
18. Bender, K., Betschart, B., Schaller, J., Kämpfer, U. and Hecker, H. 1991, Acta Trop., 50, 169.
19. Ramakrishnan, G., Gilchrist, C., Musa, H., Torok, M., Grant, P., Mann, B. and Petri, W. 2004, Mol. Biochem. Parasitol., 138, 205.
20. Cheung, P., Allis, C. and Sassone-Corsi, P. 2000, Cell, 103, 263.
21. Yang, X. and Seto, E. 2008, Nat. Rev. Mol. Cell Biol., 9, 206.
22. Saksouk, N., Bhatti, M., Kieffer, S., Smith, A., Musset, K., Garin, J., Sullivan, W., Cesbron-Delauw, M. and Hakimi, M. 2005, Mol. Cell Biol., 25, 10301.
23. Bhatti, M., Livingston, M., Mullapudi, N. and Sullivan, W. 2006, Eukaryot. Cell, 5, 62.
24. Smith, A., Tucker-Samaras, S., Fairlamb, A. and Sullivan, W. 2005, Eukaryot. Cell, 4, 2057.
25. Bougdour, A., Maubon, D., Baldacci, P., Ortet, P., Bastien, O., Bouillon, A., Barale, J., Pelloux, H., Ménard, R. and Hakimi, M. 2009, J. Exp. Med., 206, 953.
26. Fan, Q., An, L. and Cui, L. 2004, Eukaryot. Cell, 3, 264.
27. Cui, L., Miao, J., Furuya, T., Li, X., Su, X.-zhuan and Cui, L. 2007, Eukaryot. Cell, 6, 1219.
28. Fan, Q., An, L. and Cui, L. 2004, Gene, 336, 251.
29. Miao, J., Fan, Q., Cui, L., Li, X., Wang, H., Ning, G., Reese, J. and Cui, L. 2010, Mol. Microbiol., 78, 883.
30. Joshi, M., Lin, D., Chiang, P., Goldman, N., Fujioka, H., Aikawa, M. and Syin, C. 1999, Mol. Biochem. Parasitol., 99, 11.
31. Horrocks, P., Wong, E., Russell, K. and Emes, R. 2009, Mol. Biochem. Parasitol., 164, 9.
32. Cui, L. and Miao, J. 2010, Eukaryot. Cell, 9, 1138.
33. French, J., Cen, Y. and Sauve, A. 2008, Biochemistry, 47, 10227.
34. Merrick, C. and Duraisingh, M. 2007, Eukaryot. Cell, 6, 2081.
35. Merrick, C., Dzikowski, R., Imamura, H., Chuang, J., Deitsch, K. and Duraisingh, M. 2010, Int. J. Parasitol., 40, 35.

36. Tonkin, C., Carret, C., Duraisingh, M., Voss, T., Ralph, S., Hommel, M., Duffy, M., Silva, L. da, Scherf, A., Ivens, A., Speed, T., Beeson, J. and Cowman, A. 2009, PLoS Biol., 7, e84.
37. Ersfeld, K., Melville, S. and Gull, K. 1999, Parasitol. Today, 15, 58.
38. Belli, S. 2000, Int. J. Parasitol., 30, 679.
39. Horn, D. 2001, Int. J. Parasitol., 31, 1157.
40. Kawahara, T., Siegel, T., Ingram, A., Alsford, S., Cross, G. and Horn, D. 2008, Mol. Microbiol., 69, 1054.
41. Ingram, A. and Horn, D. 2002, Mol. Microbiol., 45, 89.
42. Wang, Q., Kawahara, T. and Horn, D. 2010, Mol. Microbiol., 77, 1237.
43. Alsford, S., Kawahara, T., Isamah, C. and Horn, D. 2007, Mol. Microbiol., 63, 724.
44. Sonda, S., Morf, L., Bottova, I., Baetschmann, H., Rehrauer, H., Caflisch, A., Hakimi, M. and Hehl, A. 2010, Mol. Microbiol., 76, 48.
45. Keppler, B. and Archer, T. 2008, Expert Opin. Ther. Targets, 12, 1301.
46. Feng, Q., Wang, H., Ng, H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and Zhang, Y. 2002, Curr. Biol., 12, 1052.
47. Bachand, F. 2007, Eukaryot. Cell, 6, 889.
48. Bedford, M. 2007, J. Cell Sci., 120, 4243.
49. Bougdour, A., Braun, L., Cannella, D. and Hakimi, M. 2010, Cell Microbiol., 12, 413.
50. Sautel, C., Cannella, D., Bastien, O., Kieffer, S., Aldebert, D., Garin, J., Tardieux, I., Belrhali, H. and Hakimi, M. 2007, Mol. Cell. Biol., 27, 5711.
51. Sautel, C., Ortet, P., Saksouk, N., Kieffer, S., Garin, J., Bastien, O. and Hakimi, M. 2009, Mol. Microbiol., 71, 212.
52. Cui, L., Fan, Q., Cui, L. and Miao, J. 2008, Int. J. Parasitol., 38, 1083.
53. Fisk, J. and Read, L. 2011, Eukaryot. Cell, 10, 1013.
54. Fan, Q., Miao, J., Cui, L. and Cui, L. 2009, Biochem. J., 421, 107.
55. Janzen, C., Hake, S., Lowell, J. and Cross, G. 2006, Mol. Cell, 23, 497.
56. Figueiredo, L., Janzen, C. and Cross, G. 2008, PLoS Biol., 6, e161.
57. Fisk, J., Zurita-Lopez, C., Sayegh, J., Tomasello, D., Clarke, S. and Read, L. 2010, Eukaryot. Cell, 9, 866.
58. Anbar, M., Bracha, R., Nuchamowitz, Y., Li, Y., Florentin, A. and Mirelman, D. 2005, Eukaryot. Cell, 4, 1775.
59. Mirelman, D., Anbar, M. and Bracha, R. 2008, Parasite, 15, 266.
60. Mirelman, D., Anbar, M. and Bracha, R. 2008, IUBMB life, 60, 598.
61. Tovy, A. and Ankri, S. 2010, Future Microbiol., 5, 1875.
62. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J., Cole, P., Casero, R. and Shi, Y. 2004, Cell, 119, 941.
63. Tsukada, Y., Erdjument-Bromage, H., Warren, M., Borchers, C., Tempst, P. and Zhang, Y. 2006, Nature, 439, 811.
64. Gissot, M., Choi, S., Thompson, R., Greally, J. and Kim, K. 2008, Eukaryot. Cell, 7, 537.
65. Militello, K., Wang, P., Jayakar, S., Pietrasik, R., Dupont, C., Dodd, K., King, A. and Valenti, P. 2008, Eukaryot. Cell, 7, 2012.
66. Bernes, S., Siman-Tov, R. and Ankri, S. 2005, FEBS Lett., 579, 6395.
67. Harony, H., Bernes, S., Siman-Tov, R. and Ankri, S. 2006, Mol. Biochem. Parasitol., 147, 55.
68. Ali, I., Ehrenkaufer, G., Hackney, J. and Singh, U. 2007, BMC Genomics, 8, 7.
69. Fisher, O., Siman-Tov, R. and Ankri, S. 2004, Nucleic Acids Res., 32, 287.
70. Tovy, A., Hofmann, B., Helm, M. and Ankri, S. 2010, J. Vis. Exp., 44, e2390.
71. Tovy, A., Siman Tov, R., Gaentzsch, R., Helm, M. and Ankri, S. 2010, PLoS Pathog., 6, e1000775.
72. Darkin-Rattray, S., Gurnett, A., Myers, R., Dulski, P., Crumley, T., Allocco, J., Cannova, C., Meinke, P., Colletti, S., Bednarek, M., Singh, S., Goetz, M., Dombrowski, A., Polishook, J. and Schmatz, D. 1996, Proc. Nat. Acad. Sci. USA, 93, 13143.
73. Chaal, B., Gupta, A., Wastuwidyaningtyas, B., Luah, Y. and Bozdech, Z. 2010, PLoS Pathog., 6, e1000737.
74. Murray, P., Kranz, M., Ladlow, M., Taylor, S., Berst, F., Holmes, A., Keavey, K., Jaxa-Chamiec, A., Seale, P., Stead, P., Upton, R., Croft, S., Clegg, W. and Elsegood, M. 2001, Bioorg. Med. Chem. Lett., 11, 773.

75. Strobl, J., Cassell, M., Mitchell, S., Reilly, C. and Lindsay, D. 2007, J. Parasitol., 93, 694.
76. Andrews, K., Walduck, A., Kelso, M., Fairlie, D., Saul, A. and Parsons, P. 2000, Int. J. Parasitol., 30, 761.
77. Ehrenkaufer, G., Eichinger, D. and Singh, U. 2007, BMC Genomics, 8, 216.
78. Prusty, D., Mehra, P., Srivastava, S., Shivange, A., Gupta, A., Roy, N. and Dhar, S. 2008, FEMS Microbiol. Lett., 282, 266.
79. Vergnes, B., Vanhille, L., Ouaissi, A. and Sereno, D. 2005, Acta Trop., 94, 107.
80. Meinke, P., Colletti, S., Doss, G., Myers, R., Gurnett, A., Dulski, P., Darkin-Rattray, S., Allocco, J., Galuska, S., Schmatz, D., Wyvratt, M. and Fisher, M. 2000, J. Med. Chem., 43, 4919.
81. Colletti, S., Myers, R., Darkin-Rattray, S., Gurnett, A., Dulski, P., Galuska, S., Allocco, J., Ayer, M., Li, C., Lim, J., Crumley, T., Cannova, C., Schmatz, D., Wyvratt, M., Fisher, M. and Meinke, P. 2001, Bioorg. Med. Chem. Lett., 11, 107.
82. Colletti, S., Myers, R., Darkin-Rattray, S., Gurnett, A., Dulski, P., Galuska, S., Allocco, J., Ayer, M., Li, C., Lim, J., Crumley, T., Cannova, C., Schmatz, D., Wyvratt, M., Fisher, M. and Meinke, P. 2001, Bioorg. Med. Chem. Lett., 11, 113.
83. Dow, G., Chen, Y., Andrews, K., Caridha, D., Gerena, L., Gettayacamin, M., Johnson, J., Li, Q., Melendez, V., Obaldia, N., Tran, T. and Kozikowski, A. 2008, Antimicrob. Agents Chemother., 52, 3467.
84. Chen, Y., Lopez-Sanchez, M., Savoy, D., Billadeau, D., Dow, G. and Kozikowski, A. 2008, J. Med. Chem., 51, 3437.
85. Wheatley, N., Andrews, K., Tran, T., Lucke, A., Reid, R. and Fairlie, D. 2010, Bioorg. Med. Chem. Lett., 20, 7080.
86. Cui, L., Miao, J. and Cui, L. 2007, Antimicrob. Agents Chemother., 51, 488.
87. Cui, L., Miao, J., Furuya, T., Fan, Q., Li, X., Rathod, P., Su, X. and Cui, L. 2008, Eukaryot. Cell, 7, 1200.
88. Spannhoff, A., Hauser, A., Heinke, R., Sippl, W. and Jung, M. 2009, ChemMedChem., 4, 1568.