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Canonical and variant histones of protozoan parasites

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TABLE OF CONTENTS

1. ABSTRACT

Protozoan parasites have tremendously diverse lifestyles that require adaptation to a remarkable assortment of different environmental conditions. In order to complete their life cycles, protozoan parasites rely on fine-tuning gene expression. In general, protozoa use novel regulatory elements, transcription factors, and epigenetic mechanisms to regulate their transcriptomes. One of the most surprising findings includes the nature of their histones – these primitive eukaryotes lack some histones yet harbor novel histone variants of unknown function. In this review, we describe the histone components of different protozoan parasites based on literature and database searching. We summarize the key discoveries regarding histones and histone variants and their impact on chromatin regulation in protozoan parasites. In addition, we list histone genes IDs, sequences, and genomic localization of several protozoan parasites and Microsporidia histones, obtained from a thorough search of genome databases. We then compare these findings with those observed in higher eukaryotes, allowing us to highlight some novel aspects of epigenetic regulation in protists and to propose questions to be addressed in the upcoming years.

2. INTRODUCTION

Protozoan parasites include a number of pathogenic eukaryotes that infect humans and animals of economic importance, thereby having a profound impact on the health and the socioeconomic landscapes for most parts of the globe. Protozoans are unicellular organisms that represent unique cell models with an extraordinary range of adaptations. Almost all of the protozoan parasites have complex life cycles requiring both intra- and extracellular stages. In addition, some parasites need to adapt to life in multiple different hosts, or have to devise strategies to survive in the environment for extended periods of time. One of the most intriguing aspects of parasite biology is how these primitive eukaryotes modulate gene expression throughout their life cycles. While the basal mechanisms for the regulation of gene expression are generally conserved in protozoa, important nuances exist in genome organization, transcription factors, and chromatin remodelers, some of which have been previously reviewed (1-5).

Epigenetic mechanisms are a key component of gene regulation. Chromatin-mediated epigenetic

mechanisms rely on nucleosome-DNA interactions that can be modulated by DNA methylation, addition/removal of chemical moieties on histones, or by the energy of ATP hydrolysis, histone codes, replacement of canonical histones with variants, and subnuclear localization, all of which are present in protozoan parasites (1). Several studies have shown the presence of canonical and histone variants in the different protozoa. Interestingly, some protozoa appear to lack some histone components and/or possess novel histone variants. In this review, we provide an in-depth analysis and discussion of recent articles regarding the contribution of histone variants in protozoa gene regulation.

3. PROTOZOAN PARASITE HISTONES AND THEIR ROLE IN PARASITE BIOLOGY

Cellular development, differentiation, growth and survival would not be possible without tight control of gene expression, replication, and DNA repair. Histones are basic structural proteins that compact the genome and regulate access to DNA. Histones are classified into five main types: the core histones H2A, H2B, H3 and H4, and the linker histone H1. Core histones fold together in pairs: H3 with H4, and H2A with H2B, via their *histone fold domain*, which contains three alpha-helices separated by two loops. These histone dimers build the nucleosome core particle that is the fundamental unit of chromatin organization (6). Two dimers H2A-H2B bind to each side of a tetramer H3-H4 forming the nucleosome octamer that wraps ~146 bp of genomic DNA. Amino-terminal sequences of the core histones extend from the base unit and are referred to as "histone tails". Canonical and non-canonical histone variants are present in nearly all eukaryotes. Canonical histones are transcribed during S-phase and their function is primarily associated with genome packaging and gene expression. In metazoans, genes encoding these histones are present in highly similar multiple copies. Variant histones have been identified for every histone type, and they show significant differences in primary sequence and expression profiles (independent of the cell-cycle) relative to their canonical counterparts. They are involved in a wide range of processes including DNA repair, recombination, transcription, gene silencing, and chromosome condensation.

The presence of histone variants together with post-translational modification (PTM) of histone tails constitute the first level of the histone code (7), which specifies that unique downstream activities are associated with particular nucleosomal configurations and modifications. PTMs are generated by histone-modifying enzymes (code writers) and are recognized by diverse nuclear factors (code readers), resulting in a specific response. More than one PTM can be observed in the same histone tail; in addition, more than one histone can be modified in the same nucleosome and these modifications are interdependent, producing nucleosomes with different PTM combinations. Finally, the concentration of differentially modified nucleosomes is in part responsible for higher order chromatin, such as euchromatin and heterochromatin. Histone variants, PTMs, and chromatin remodeling complexes are present in all eukaryotes, suggesting that epigenetic regulation is an ancient and conserved means of gene expression control. In protist parasites, epigenetic mechanisms become particularly important because it has been reported that they participate in the regulation of differentiation between infective and non-infective forms. Notably in Apicomplexa*, Giardia lamblia,* and *Entamoeba* spp., histone deacetylases (HDAC) inhibitors disrupt normal growth and differentiation, and in many cases have been proposed as a potential drug therapy (8-13). Trypanosomatids and Apicomplexa PTMs, histone-modifying enzymes, and chromatin remodeling complexes have been reviewed recently (3, 14-16). In this section, we will focus on the histones found in parasitic protozoa, discussing and highlighting the presence of unusual variants, the way they interact, and their localization in the genome.

3.1. Canonical histones

Core histones are the building blocks of the nucleosome. They have been classified into two groups, canonical histones and histone variants. It is widely accepted that canonical histones are present within cells in equimolar amounts (17-19) and they are characterized by a transcription tightly coupled to DNA replication. The high demand for canonical histones during S phase is met through the presence of multiple copies of the histone genes in metazoans. These genes are clustered together in the genome and these clusters typically contain multiple copies of the genes that encode the four core histone and the linker histone proteins (20). Protist parasites exhibit some differences in the number and organization of histone genes compared to mammals. In every protist parasite analyzed so far, the four core histones have been identified, but not the linker histone H1 (see section 3.2). In addition, the number of genes encoding for canonical histones and their organization across the genome is not the same. A large number of core histone gene copies are only observed in Trypanosomatids and *Trichomonas vaginalis* (Table 1). However, none of the parasites analyzed here have a gene cluster containing all of the core histones. *Trypanosoma* spp. have one cluster for each core histone localized in different chromosomes, whereas *Leishmania* spp. generally have one or two clusters each and many other copies distributed in diverse chromosomes (www.EuPathDB.org, Supplemental table 1, (21)). *T. vaginalis* contains a large number of histone genes, most of them organized as gene pairs in a head-to-head manner. *T. vaginalis* contains 17 copies of H2A, 14 of H2B, 21 of H3, and 22 of H4. Interestingly, 11 copies of H2A/H2B and 19 of H3/H4 are organized in pairs. At the protein level, *T. vaginalis* H2As can be divided into three isoforms with only one amino acid difference, all H2Bs have identical sequences, only one H4 has one amino acid different from the rest, and there are two isoforms of H3 that differ in six amino acids (22). In contrast, apicomplexan parasites and the pathogenic fungi Microsporidia generally have only one copy of each canonical histone gene, and they are not localized in gene clusters or on a single chromosome (Supplemental table 1). Some apicomplexan parasites, however, such as

Table 1. Number and type of histones present in protozoan parasites and parasitic fungi

	Organism	H2A	H2AZ	H2AX	H2B	H2Bv	H3	H3.3	cenH3	H3V	H4	H4V
Parasitic fungi	*E. cuniculi*	1			1		2 [3]				1	
	E. intestinalis	1			1		2 [3]				1	
Amebae	*E. dispar*	2 [3]			2		6 [3]				2	
	E. histolytica	2 [3]			2		6 [3]				1	
	E. invadens	4 [4]			2 [4]		6 [3]				3 [2]	
Apicomplexa	*T. gondii*	1	1	1	2	1	1	1	1		1	
	N. caninum	1	1	1	2	1	1	1	1		1	
	C. hominis		1	1	1	1	1	1	1		1	
	C. muris		1	1	1	1	1	1	1		1	
	C. parvum		1	1	1	1	1	1	1		1	
	P. berghei	1	1		1	1	1	1	1		1	
	P. chabaudi chabaudi	1	1		1	1	1	1	1		1	
	P. falciparum	1	1		1	1	1	1	1		1	
	P. knowlesi	1	1		1	1	1	1	1		1	
	P. vivax SaI-1	1	1		1	1	1	1	1		1	
	P. yoelii yoelii.	1	1		1	1	1	1	1		1	
Metamonada	*G. lamblia*			2	2		2		1	1	3	
	T. vaginalis	17 [1]			14		21				22	
Trypanosomatid (Euglenozoa)	*L. braziliensis*	7 [4]	1		4 [3]	1	4			1	14 [2]	
	L. infantum	5 [2]	1		4 [3]	1	6 [2]			1	7 [2]	
	L. major	6 [3]	1		5 [3]	1	7			1	7 [3]	
	L. mexicana mexicana	3	1		4 [3]	1	7			1	7 [3]	
	T. brucei	13	1		14	1	7			1	10	1
	T. brucei gambiense	5	1		10	1	8			1	6	1
	T. cruzi Brener E-L	7	1			1	4			1	11	1
	T. cruzi Brener N-E-L	9	1		2	1	5			1	8	1
	T. vivax	18	1		7	1	3			1	8	1

[1] 1 aa difference. [2] 2-5 aa difference. [3] 5-10 aa difference. [4] more than 10 aa difference.

Toxoplasma gondii and *Neospora caninum,* have two canonical *h2ba* and *h2bb* genes.

Disruption of histone synthesis and deposition during S phase leads to DNA replication arrest, and inhibition of DNA synthesis leads to a reduction in histone synthesis, indicating that the two processes are tightly linked. S-phase cells need to rapidly assemble nascent DNA into nucleosomes while avoiding the generation of excess histones (for review see (19)). In metazoans, this is regulated by a 3' stem-loop present in their mRNAs instead of a polyadenylation signal. The interaction of this 3' end with the stem–loop binding protein (SLBP) provokes an increase in the mRNA half-life (23). In *Drosophila melanogaster*, the disruption of *SLBP* blocked normal S phase-associated histone mRNA biosynthesis and altered chromatin assembly, resulting in genomic instability, cell cycle abnormalities, and interrupted development (24). Consequently, a specific coordinated regulation of replication-dependent histone production is functionally important. Notably, in lower eukaryotes and plants, histone mRNAs are polyadenylated and their enrichment is also restricted to S phase. In yeast, expression of histone genes is controlled mainly at the transcription level (19). In Trypanosomatids, histone gene transcription seems to be constitutive like most other genes, but histone biosynthesis is coupled with DNA synthesis (21). The mechanism regulating the cell cycle expression of histones differs among Trypanosomatids. *Trypanosoma* spp. has different levels of histone mRNAs through the cell cycle (25-28), whereas *Leishmania* spp. use translational regulation in a cell cycle-dependent manner mainly directed by their 3' UTR (21, 29). In *Plasmodium* spp. and *T. gondii*, canonical histone transcript levels are associated with the cell cycle, however, the mechanism that coordinates this process has not been described (30-32). Interestingly, homologues of SLBP can be observed in several protozoa, including *Trichomonas* and Euglenozoa (33). In addition, careful examination of the canonical histone genes in these organisms revealed a potential stem–loop structure in their 3′ UTR (33). Authors propose that the specialized histone transcription regulation involving the 3'UTR originated early in the evolution of eukaryotes but was completely lost in several lineages, including plants, fungi and most protozoa (19, 33).

An interesting case is *Giardia*, which has only 2 identical copies of H2A, H2B and H3, and 3 copies of the H4 gene ((34) and www.EuPathDB.org). These histones are constitutively expressed at approximately equivalent levels and their mRNAs are polyadenylated. This replication-independent expression is a hallmark feature of histone variants. The H2A protein sequences contain the characteristic H2AX motif (see section 3.2), indicating that the unique H2A present in the parasite could be a variant. Since *Giardia* represents one the earliest diverging lineage of eukaryotes (35), it would be very interesting to determine if all histones in these parasites are variants or not. Defining the *Giardia* histones as variants or canonical would illuminate the evolution of histones and transcription regulation.

In summary, the number and arrangement of core histone genes, as well as their transcriptional regulation, differ among eukaryotes. Nevertheless, in all of them canonical histone biosynthesis must be coupled to the S-phase. It would be of interest to determine if the number of histone gene copies and their organization in clusters is dependent on the complexity of the genome and/or transcriptional regulation.

3.2 Linker histone

Successive nucleosomes are joined by linker DNA, the length of which varies among species. Histone H1 binds to the linker DNA, connecting nucleosomes to form the 30 nm chromatin fiber. A large number of extremely divergent H1 variants have been described (reviewed in (36) and (37)). The H1 variants are classified into three groups (i) possessing a tripartite structure, which contains a globular domain flanked by basic unstructured N- and C-terminal domains (ii) possessing only the C-terminal domain, and (iii) the *Saccharomyces cerevisiae* H1 that has a second globular domain (for review, see (38)). As a general rule, single domain H1s are found in lower eukaryotes and the tripartite H1s are found in higher eukaryotes (39). In parasites such as Entamoebida, Kinetoplastids, Ciliates and Dinoflagellates, the H1 histone lacks the globular domain, which is in accordance with the general rule. Even though *Trypanosoma cruzi* H1 bears only 43.6% similarity to human H1, it is able to induce a 30 nm fiber-like structure when added to rat nucleosomes (40). By contrast, no histone H1 has been identified in Apicomplexa or Microsporidia parasites to date (1, 34). In *Giardia,* findings have been contradictory. Triana *et al* (41) reported the isolation of a basic protein using protocols based on the biochemical properties of histone H1, which was subsequently identified by mass spectrometry to be similar to chicken H1. On the other hand, recent studies have reported that no H1 could be found in the *Giardia* genome (34, 42).

The role of histone H1 in parasites as well as in higher eukaryotes is poorly understood. Since it is involved in the formation of higher order chromatin structure, it was postulated to act as a chromatin repressor. However, deletion of H1 in lower eukaryotes, like yeast and fungi, does not show a significantly different phenotype, but an increased accessibility of the chromatin to nuclease (MNase) digestion as well as a decrease in their life span (reviewed by (37)). Recently, Flourescence Recovery After Photobleaching (FRAP) assays show that H1 binding to linker DNA is more dynamic than previously thought (reviewed in (43)). The function of H1 in protist parasites has yet to be extensively examined. *Leishmania* H1 (LeishH1 or LNP18) is differentially expressed during its life cycle, accumulating in non-dividing metacyclic promastigotes and in amastigotes that have more compacted chromatin than other stages (44). Parasites overexpressing LeishH1 display a reduction in their virulence *in vitro* and *in vivo*. No difference in the rate of macrophage invasion in control and LeishH1-overexpressing parasites was observed. However, mutants exhibited a delay in parasite cell cycle progression and differentiation (45, 46). Additionally, in the presence of excess histone H1, parasites display resistance to MNase treatment and accumulate clusters of condensed chromatin (47). In *T. cruzi*, histone H1 (TcH1) is phosphorylated in the infective forms (trypomastigotes) but not in proliferating forms (epimastigotes) (48). Moreover, TcH1 is phosphorylated at a cyclin-dependent kinase (CDK) consensus site when cells progress from S to M phase, reaching a maximal level at G_2-M (49, 50). In addition, multiple experiments indicate that the phosphorylated TcH1is more weakly associated with chromatin than the non-phosphorylated form (48, 50). In *Tetrahymena thermophila,* H1 is phosphorylated during the cell cycle as well as outside mitosis, and phosphorylation of specific sites by CDKs is required in order to recruit other kinases that phosphorylate the remaining sites (51). While it has been reported that some genes are up-regulated in protozoa expressing forms of H1 mutated to mimic constitutive phosphorylation (52), H1 phosphorylation is not essential for *T. thermophila* viability (53). These results show that phosphorylation of parasite H1 is important for the cell cycle, but its role in chromatin compaction is less clear. As described above, TcH1 is able to form the 30 nm fiber in mammalian cells and LeishH1 over-expression produces resistance to MNase digestion, suggesting that protozoan H1 may have role in chromatin condensation. However, these early protists do not form the typical 30 nm fiber and do not condense chromosomes during mitosis. Recently, it was observed that the variable C-terminal domain is responsible for the various mammalian H1 subtypes for being weak, intermediate, or strong condensers of the chromatin (54). Protozoan H1is comprised of only the C-terminal domain, making it possible that this histone might be compacting chromatin sufficiently for mitosis without generating the 30 nm fiber.

3.3. Histone variants

Histone variants are paralogs of the canonical histones, transcribed in a replication-independent manner, which assemble into nucleosomes with specialized functions (36). H4 and H2B lineages are practically invariant, whereas H2A and H3 have extensively specialized variants for many roles in transcription, DNA organization, and repair (55). Recent studies have revealed that the histone variants in protozoan parasites possess many unique and interesting features.

3.3.1. Histone H2A: hypervariant family

Among the core histones, H2A has the largest number of variants. Some of them are unique to vertebrates: such as H2A.Bbd (for Barr body deficient) and macroH2A (for macro-domain-containing H2A) (see (55)). H2A.Bbd has a shorter C-terminal domain and shows only 48% identity with canonical H2A. It has been associated with active chromatin since it is absent in the inactive X chromosome (Xi), co-localizes with acetylated histone H4 (56), and increases instability of nucleosomes in comparison with canonic H2A (57). MacroH2A has a large C-terminal non-histone domain (macro domain) and is enriched on the Xi chromosome, suggesting a role in chromatin silencing (58, 59). Recently, human variants macroH2A1 and macroH2A2 were detected in autosomal repressed chromatin, at many genes encoding key regulators of development (60). The expression of some of these genes is positively regulated by macroH2A1 (61), leading to the proposal that macroH2A may be an epigenetic regulator of developmental genes.

Other H2A variants, such as H2AZ and H2AX, are highly conserved among all eukaryotes (see (55)), including protozoan parasites (Table 1). The H2AX core

region is nearly identical to that of the canonical H2A, but has a characteristic C-terminal SQ-motif: SQ(E/D)Fi, where Fi indicates a hydrophobic residue. The serine residue of this motif becomes rapidly phosphorylated in response to DNA double-stranded breaks (62) by phospho-inositide 3-kinase-like kinases (PIKK) (63). The phosphorylated H2AX (known as gamma-H2AX) recruits repair proteins and chromatin remodeling complexes, facilitating non-homologous end joining (NHEJ) (64) or homologue recombination (HR) (65). Both pathways are well described and discussed by van Attikum H. & Gasser S. M. (66) and Morrison A. J. & Shen X. (67). The study of H2AX knock out (KO) mice and stem cells revealed an essential role of this histone in DNA repair and genome integrity in mammals (68, 69). This variant represents 5–25% total H2A in mammals, but is the main histone in yeast (62, 70). Notably, in protist parasites this variant can be present or absent. Among Apicomplexa, *Cryptosporidum* spp. have H2AX and do not have canonical H2A, *T. gondii* and *N. caninum* have both of them, and *Theileria* spp., *Plasmodium* spp. and *Babesia bovis* have only canonical H2A (Table 1, Supplemental table 1). The closely related *T. thermophila* has H2A as well as H2AX. Phylums grouped in Excavatas also cluster parasites with and without H2AX: Trypanosomatids and *T. vaginalis* have multiple copies of canonical H2A, but do not have H2AX ; in contrast, *Giardia* has only H2AX. None of the *Entamoeba* analyzed (*E. histolitica* and *dispar*) have H2AX (Table 1, Supplemental table 1). Interestingly, almost all of the Apicomplexa and *Entamoeba* canonical H2As contain a C-terminal sequence that shows partial similarity to the SQ- motif (1) (Figure 1), which may mimic H2AX function in the parasites lacking this variant. *T. gondii* and *T. thermophila* H2AX have been characterized and their function in DNA repair seems to be conserved. In *T. gondii*, H2AX is phosphorylated during normal tachyzoites growth and gamma-H2AX levels increase with DNA damage generated by H_2O_2 in a dose-dependent manner (31). In *Tetrahymena,* H2AX is phosphorylated in both the mitotic micronucleus and the amitotic macronucleus in response to double-stranded breaks induced by chemical agents (71). Experiments with dominant-negative mutants showed that the SQ-motif is required for normal micronuclear meiosis and mitosis and, to a lesser extent, for normal amitotic macronuclear division (71). Based on the conserved role of this variant, together with the presence of a truncated or similar SQ-motif in these protist canonical H2As, it appears possible that an ancestral H2AX existed that lost this motif to generate the canonical H2A. This idea has also been proposed recently by Talbet and Henikoff (72). The accepted theory postulates multiple evolutionary origins of this variant (55, 73, 74). In this case, H2AX could be associated with a special characteristic of each species. *Plasmodium* spp., *Trypanosoma* spp. and *Leishmania* spp. do not have H2AX and they exhibit chromatin fragility as a distinctive feature, characterized by DNA breaks and chromosomal rearrangements during cell proliferation. Moreover, in *Plasmodium falciparum*, chromosome breakage occurs frequently in subtelomeric regions where genes responsible for parasite virulence are located; this allows rapid generation of antigenic diversity (75-77). On the other hand, *Toxoplasma* has H2AX and no genome instability has been observed in this parasite (78). A possible hypothesis could be that the presence of H2AX is related to the plasticity of the genome in protist parasites. Further studies need to be performed to shed light on the evolutionary origin of the H2AX variant. A novel finding regarding H2AX was observed in *T. gondii*, namely expression levels of H2AX are higher in latent bradyzoites than proliferating tachyzoites (31), suggesting a role for this variant in parasite differentiation.

Mammalian H2AX contains the SQEY motif, in which the hydrophobic amino acid is tyrosine. In the absence of DNA damage, H2AX is constitutively phosphorylated on this residue by WSTF (Williams–Beuren syndrome transcription factor) – a component of the WICH complex (an ATP-dependent chromatin-remodelling complex) (79). After DNA damage, Y142 becomes dephosphorylated by the EYA1/3 phosphatase, allowing the recruitment of ATM and MDC1 to generate and maintain γ-H2AX for DNA repair. In the absence of Y142 phosphorylation, the kinetics of the phosphorylation/dephosphorylation cycle of gamma-H2AX occur much more rapidly (79-82). *Xenopus laevis* has two H2AX histones, one with the SQEY motif (H2AX-Y, like mammalian H2AX) and the other with the SQEF motif (H2AX-F). H2AX-Y is the main H2AX histone in adults whereas H2AX-F is the main H2AX in late-staged oocytes, eggs, and early embryos, and it is not present in somatic cells. H2AX-F is phosphorylated in actively replicating cells, in the absence of DNA damage and/or checkpoint activating signal (83). H2AX-F phosphorylation does not appear to be associated with DNA repair and may be involved in modulating the cellular response during early development. Interestingly, the SQEY motif seems to be restricted to metazoans. Protist parasites analyzed here possess SQEF (*Toxoplasma*, *Neospora* and *Cryptosporidium* spp.) or SQDL (*Giardia*) motifs (Figure 1). None have a hydrophobic amino acid at the end that can be phosphorylated. In addition, *Toxoplasma* has a basal level of gamma-H2AX, with phosphorylation apparently independent of exogenous DNA damage (31). Protozoan H2AX might functionally resemble *X. laevis* H2AX-F rather than H2AX-Y. Whether H2AX containing either one or the other motifs can be considered different variants remains to be elucidated and protozoan parasites may prove to be interesting models to shed light on this question.

H2AZ is a family of H2A variants that are highly conserved across species and substantially divergent from S-phase-H2A in any given species (73). The largest region where these two H2As diverge considerably is the 'docking domain', a sequence at the C-terminus that corresponds to their interaction surface with the $(H3–H4)_2$ tetramer (84). The H2AZ docking domain gives distinctive features to the nucleosomes containing this variant. A functional replacement study in *Drosophila* indicated an

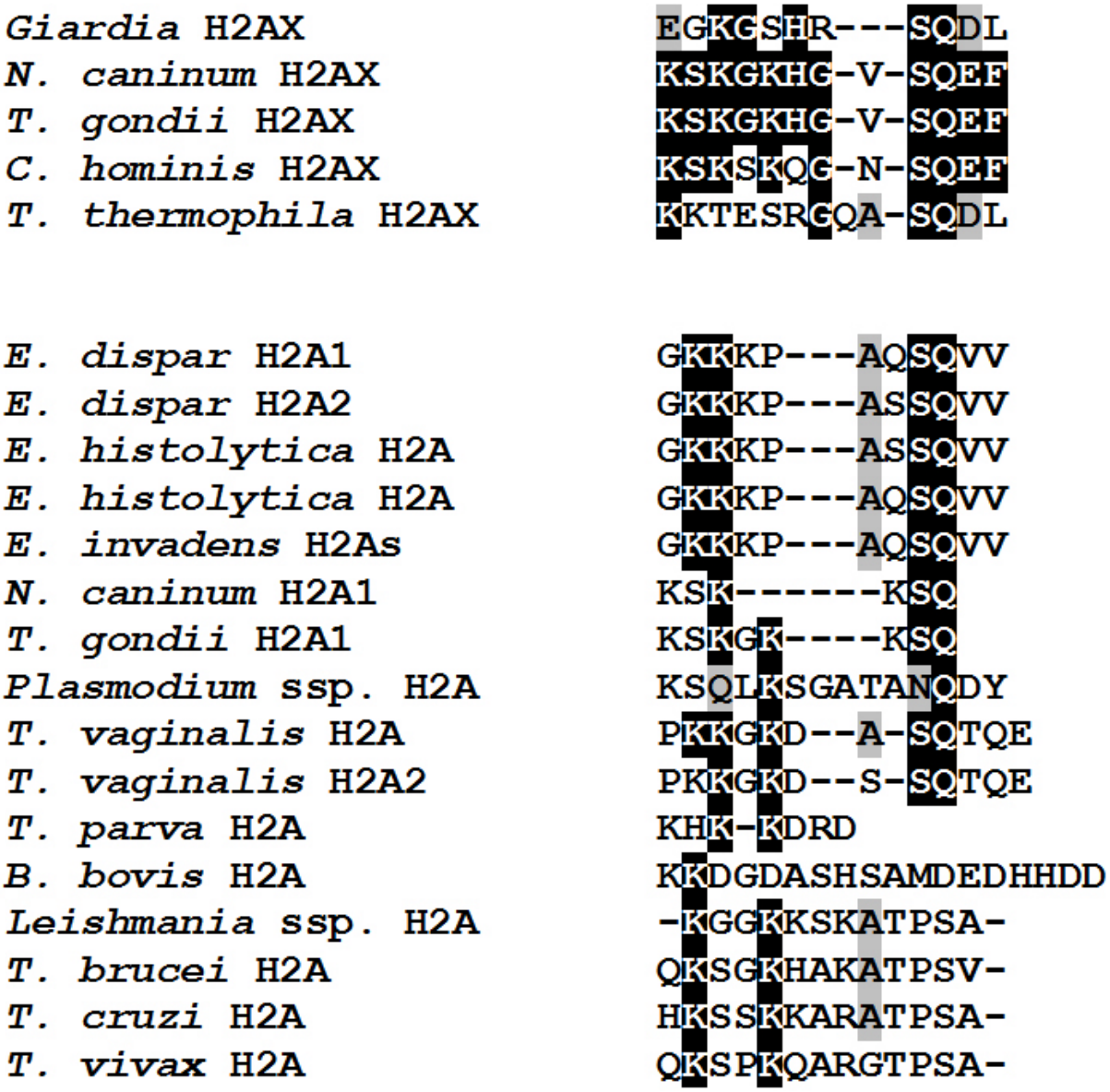

Figure 1. Sequence aligment of protist parasites histones H2A. Comparison by multiple alignment (clustal W) of the carboxi-terminal deduced amino acid sequences of histones H2A (Bioedit). Identical amino acids are denoted by a black shadow and similar amino acids are denoted by grey shadow. Sequences were obtained from: EuPathDB (www.EuPathDB.org), GeneDB (www.GeneDB.org) and NCBI (http://www.ncbi.nlm.nih.gov).

essential role of this domain in H2AZ function (85)). This histone variant is essential from *Tetrahymena* to mammals (86, 87), even though it is the minor histone H2A (5 to 10%). The only exception described so far are yeasts, where H2AZ deletion makes cells grow slowly (88). The function of H2AZ is not very clear. First insights about H2AZ functions were developed in the protist parasite *T. thermophila,* which has the unique arrangement of a transcriptionally active macronucleus and a transcriptionally inert micronucleus as well (89). H2AZ is present in macronuclei but not in micronuclei during growth or starvation. Strikingly, H2AZ appears in micronuclei during early stages of conjugation, when it becomes transcriptionally active, leading to the hypothesis that H2AZ is involved in transcription (89). Subsequent studies reported that it was necessary for RNA polymerase II recruitment (90, 91) and protection of euchromatin from ectopic spread of heterochromatin (92). On the other hand, H2AZ was observed to interact directly with heterochromatin proteins, and it is important for heterochromatin formation and correct chromosome segregation (93-96). Besides its role in transcription regulation, H2AZ has also been shown to be involved in DNA repair (97). These multiple and sometimes contradictory functions could be explained by the ability of this variant to interact with diverse chromatin remodeling

proteins and/or the presence of different post-translational modifications. *Tetrahymena* H2AZ is highly acetylated on its N-terminal tail and this protozoan cannot survive if every acetylation site is replaced with an arginine residue (98). Hyperacetylation of H2AZ has also been observed in *P. falciparum* (32, 99) and every acetylated lysine is conserved in *Toxoplasma* and *Neospora* (Supplemental table 1), indicating that this may also occur in these other apicomplexan parasites. *P. falciparum* H2AZ is reduced in trophozoites, which correlates with the telomeric silencing of *var* gene expression (32). *T. gondii* H2AZ is the minor histone H2A and its transcription is not affected by stress or bradyzoite induction (31). Studies on *Trypanosoma brucei* H2AZ showed that this H2A variant is detected as bright, punctuate spots within the nucleus at all stages of cell cycle (100). Functional studies have not been done in any other parasite; however, genome localization experiments provide evidence about their function (see section 3.5). This ancient histone variant, known to be essential in almost every eukaryote, is not present in *Giardia*, *Trichomonas* and *Entamoeba* spp. The essential role of H2AZ in virtually every eukaryote raises the question of how these parasites replace its function. In addition, it is intriguing how these parasites can regulate gene expression with only one H2A (see Table 1). The study of chromatin regulation in these parasites would help to elucidate H2AZ function.

3.3.2. Histone H3

Histone H3, like H2A, has different variants with specialized functions. Most eukaryotes have three H3 types: canonical H3, H3.3 and CenH3, which are present in almost all protozoan parasites (Table 1). H3.3 is highly similar to canonical H3, with only a few amino acids difference. In most animals they differ in four amino acid substitutions: residue 31 in the N-terminal tail, which is an alanine in H3, and 87, 89 and 90 at the beginning of the alpha 2 helix (see (101)). H3.3 residues 87 to 90 have been shown to be important for incorporation into chromatin in a replication independent fashion, while canonical H3 is incorporated strictly during DNA replication (102). The ciliate *T. thermophila* has two very similar H3 variants: H3.3 and H3.4 (*HHT3* and *HHT4* genes). Both contain the same residues at position 87-90 (QAIL) and they differ from the canonical H3 in 16 amino acids (103). In this protozoan, H3 deposition is coupled to DNA synthesis during replication and repair, whereas most H3.3/4 are deposited in association with transcription and independent of replication, although some of them can be assembled in a replication-coupled manner as well (103). Mutational analysis of *T. thermophila* H3.3 showed that it is not essential for transcription but is required in germ line micronuclei (103). The apicomplexans *P. falciparum* and *T. gondii* contain both H3 and H3.3 histones, with only H3 transcripts accompanying DNA synthesis (32, 104). In both cases, amino acids 87 and 90 are different in H3.3 with respect to H3, generating the motif QA(I/V)L. Another peculiar feature that appears to be exclusive to Apicomplexa H3.3 is the substitution of KF for the conserved RY at position 54-55 (104). *T. vaginalis* also has a single H3 variant with 6 amino acid substitutions in comparison to the multiple identical H3s; however, none of them are localized at positions 87-90 (22). The evolutionary implications and functional role of substitutions in the protozoan H3.3 deposition, as well as how they may be post-translationally modified, remain a challenge for future research.

CenH3, the centromere-specific H3, is essential for kinetochore assembly. They are not as well conserved as canonical H3s. Their N-terminal regions are divergent and can vary from 20 to 200 amino acids in different lineages, and their loop 1 region is not conserved. Phylogenetic analyses were unable to determine the evolutionary origin of this variant (42, 55). CenH3 can be detected in Apicomplexa, including *T. gondii*, *Plasmodium spp.* and *N. caninum* ((32), www.EuPathDB.org), however little is known about its functional properties in these parasites. Interestingly, *T. gondii* centromere sequences have been identified based on the localization of CenH3 across the genome (Gissot M., Brooks C.F., Striepen B., Kim K. Manuscript in preparation, www.ToxoDB.org). *Giardia* has two H3 variants, with longer N-terminal sequences compared to canonical H3. One of them has been classified as CenH3 because it has the conserved centromere localization. The other one (H3B) has a punctate distribution in the nuclei that does not colocalize with an epigenetic marker of transcription (42) and is not related to H3.3. A centromeric histone H3 is also present in *T. thermophila* (named Cna1) localized at centromeres in the micronucleus. Cna1 was also detected in some developing macronuclei and is required for formation of DNA elimination structures (105). Trypanosomatids have only one H3 variant (H3V) that contains an unusual lengthy and divergent N-terminal tail resembling CenH3 (106). However, neither contains an insertion at Loop 1, which has been considered a hallmark of CenH3 (55). H3V sequences from different Trypanosomatids are fairly dissimilar relative to one another. For example, *T. brucei* and *L. major* H3Vs share ~66% and ~50% sequence identity respectively with *T. brucei*. Moreover, *T. brucei* H3V (TbH3V) was localized at telomeres (not at centromeres) and null mutants do not show any particular phenotype. The data lead the authors to propose Trypanosomatid H3V as a new H3 variant (106). New insights about TbH3V function are deduced from its genome localization (see section 3.5). How the Trypanosomatid parasites form kinetochores without an obvious CenH3 histone remains unclear.

3.3.3. Histone H2B and H4: linage specific variants

H2B and H4 histones are markedly deficient in variants. It has been proposed that each histone dimer is composed of a variant histone (H2A and H3) and an invariant histone (H2B and H4) (55). Some protist parasites are unusual in possessing variants of these two histones (Table 1). Trypanosomatids and Apicomplexan parasites contain two lineages of H2B, one of them resembling canonical H2B and the other harboring characteristics of variant histones (21, 30, 32, 100). *P. falciparum* and *T. gondii* H2Bv are constitutively transcribed, in contrast to canonical H2Bs that have replication-associated transcription (30, 32). These variants differ from canonical H2B mainly in their N-

terminal sequence. Recently, it has been determined that this longer N-tail is hyperacetylated in *P. falciparum* and every site is conserved in its *T.gondii* ortholog (99). *T. brucei* H2BV is essential for parasite viability. Deletion of the first 23 amino acids did not affect parasite survival, but residues 128–142 could not be deleted, illustrating their critical role (100). *T. brucei* H2BV also has a longer N-terminal tail relative to H2B that is acetylated (107). In all cases, H2B variants form dimers with H2AZ and no other H2A (see section 3.4), suggesting an important role in chromatin organization. Nonetheless, Apicomplexa and Trypanosomatids H2B variants do not exhibit the same evolutionary origin and are thus considered linage specific. These observations raise the question of why these protists need H2B variants and are they playing the same role in both phyla. Only a few H2B variants have been described in other eukaryotes, all of them from gametes. In humans, two testes-specific variants have been reported so far: hTSH2B (108), which destabilizes the nucleosome, and H2BFWT (109), which is related to the formation of the telomere binding-complex in human sperm. In *Bos taurus*, SubH2Bv is important for sperm head development related to acrosome formation (110). Mice have H2BL1 and H2BL2, which are involved in pericentric heterochromatin reprogramming during mouse spermiogenesis (111) similar to SubH2Bv and H2BFWT, respectively. Finally, one H2B variant has been reported in plants, gH2B from *Lilium longiflorum*, which is involved in packaging of chromatin in pollen (112). In a recent study, the evolution of histone H2B variants has been analyzed (113). Authors propose a birth-and-death long term evolution: new gene copies are generated by gene duplication ("birth" process) that are under a strong selective pressure acting at the protein level that can inactivate a gene ("death" process), which then remains as a pseudogene for long periods of time in the genome. This evolutionary process would eventually lead to the functional differentiation of a new gene copy (113).

Most eukaryotic histone H4s differ at only a few amino acid positions (114). Exceptions to this conservative pattern of evolution can be seen among some protist parasites (115-117). An interesting and evolutionary study proposes that histone H4 protein diversifies faster in ciliates than in other eukaryotes. Paralogs of H4s within ciliate genomes can differ up to 25% (118). *Entamoeba* spp. H4 acetylation is selectively sensitive to different HDAC inhibitors (12). Trophozoites of this parasite become hyperacetylated in response to trichostatin A or HC-toxin, but not butyrate. In general, these inhibitors are short chain fatty acids that parasites can encounter at high levels in the host colon. *In vitro*, these compounds prevent trophozoite differentiation into infectious cysts. It seems that *Entamoeba* spp. have acquired a histone acetylation mechanism that may allow for its growth and differentiation in response to its environment (12). This could be a possible explanation for the high diversity in the H4s found in ciliates. Further analysis must be done to determine if they are novel H4 variants. The unique histone H4 variant described so far is *T. brucei* H4V (119), which shares 85% sequence identity with canonical counterpart. H4V does not exhibit cell cycle regulation, and a knock out parasite did not show any particular phenotype as observed for H3V (119). Nevertheless, their genomic localization suggests a role in transcription regulation (see section 3.5).

3.4. Nucleosome composition

The presence or absence of histone variants and/or modified histones at nucleosomes is very important because they are regulating the chromatin state. In fact, the different *in vitro* salt-dependent stability of nucleosomes containing histone variants suggests a role in changing the chromatin structure for a more permissive or repressive state (120-124). It is accepted that interactions among histones and histone variants are not random. For example, the exchange of H2A.Bbd-H2B for H2A-H2B dimers *in vitro* is more efficient in H3.3 than H3-containing nucleosomes (125). Recently, it has been reported that the nucleosome-free regions (NFRs) in human cells are in fact occupied by nucleosomes containing both H3.3 and H2AZ variants that are highly destabilized by salt (126) (Figure 2). This finding is relevant because NFRs are located at active transcription start sites (127), flanked by nucleosomes (positioned -1 and +1 respect the NFR) containing the variant H2AZ (see section 3.6). Therefore, the existence (or absence) of several histone variants in protist parasites generates the question whether nucleosomal composition follows the same rules characterized in higher eukaryotes. The best studied cases are *T. gondii* and *T. brucei*, which clearly display specificity in dimer formation, leading to distinctive nucleosomal arrangements involved in transcription regulation.

T. brucei H2AZ forms dimers with H2BV, but it does not with canonical H2B. TbH2AZ does not co-immumnoprecipitate with H3V, indicating that these two variants are not present in the same nucleosome (100). In addition, histone H3 trimethylated at K4 and K76 is preferentially associated with H2BV-containing nucleosomes (128). On the contrary, canonical histones are able to interact among them and they can be present in the same nucleosomes (100). In *T. gondii*, similar results were observed; H2Bv forms dimers mainly with H2AZ, but not with H2AX. Moreover, H2AZ and H2AX are not present in the same nucleosome. Interestingly, acetylated H3 can be present in the same nucleosome with H2Bv, H2AX and H2AZ, but in varying degrees (31). These findings reveal that nuclesosmal arrangements are not random in protozoa, highlighting their relevance in chromatin composition and regulation.

Purified H2AZ histones from chicken can assemble *in vitro* into either homo- or hetero-nucleosomes that contain one H2AZ and one H2A, with the H2AZ homo-nucleosome being the most stable (120). Hetero-nucleosomes are the standard in human HeLa cells (129) and macroH2A preferentially assembles hybrid nucleosomes (130). In *T. brucei*, H2AZ does not co-immunoprecipitate with canonical H2A, and H3V does not interact with H3, indicating that hetero-nucleosomes are not formed in this parasite (100). In *T. gondii*, however, H2AZ and H2AX seem to interact with canonical H2A,

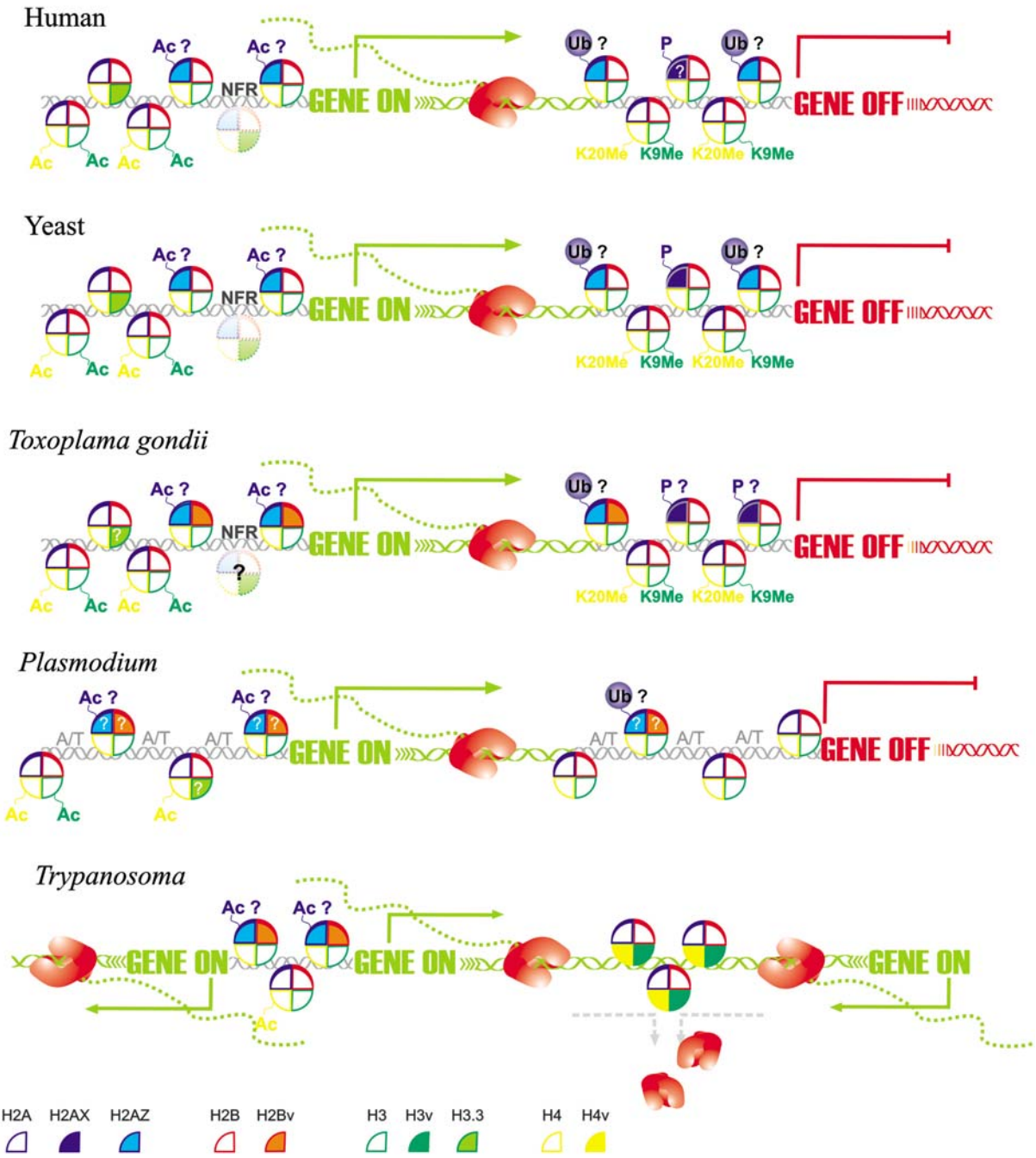

Figure 2. Schematic representation of chromatin features at active and silenced promoters. Post-translational modifications are: Ac, acetylated; P, phosphorylated; Ub, monoubiquitinated; K20me, lysine 20 methylated; and K9me, lysine 9 methylated. RNAPII is represented as a red protein on DNA strands and green dotted-lines correspond to nascent mRNA. A/T on DNA strands symbolizes the A/T rich genome of *Plasmodium* spp. The transparent nucleosome represents the H2AZ-H3.3 containing nucleosome present in the nucleosome free region (NFR) *in vivo*.

generating hetero-nucleosomes (31). The differences in nucleosome composition between *Trypanosoma* and *Toxoplasma* suggest that each protozoan species has adapted their histone variant repertoire in a specialized manner. Given their unique histone complements, it will be important to elucidate nucleosome configurations for additional protozoan species.

3.5. Nucleosome occupancy

Histones are constantly being shifted, modified, evicted, and re-deposited as chromatin is continually remodeled (131). Chromatin can be reconstituted from purified histones and DNA spontaneously *in vitro*. Several studies have shown that nucleosomes have preference for distinct DNA-sequences (132-135). It was found that

exons have augmented nucleosome-occupancy levels with respect to introns, which seems to be based on the GC content between them (136). DNA sequences rich in A/T dinucleotides intrinsically destabilize nucleosome formation *in vitro* and they are present in the NFR upstream coding sequences facilitating promoter accessibility *in vivo* (134, 137). A correlation between genome-wide positioning of nucleosomes assembled with yeast genomic DNA *in vitro* (based only in the intrinsic sequence preferences of nucleosomes) and the nucleosome occupancy *in vivo* has been observed. Since nucleosomes tend to be absent at the beginning and end of genes both *in vivo* and *in vitro*, the DNA sequence has been suggested to specify nuclesosome assembly (134). However, Zhang *et al* (135) proposed that even when the DNA sequence contributes to the nucleosome assembly, the correct positioning of the +1 nucleosome is due to the transcription machinery and/or chromatin remodeling complexes. Thus, the DNA sequence directs the formation of nuclesome-rich or deficient arrangements, which facilitates the association of the transcription machinery and chromatin remodeling complexes, and completes this process by positioning the +1 nucleosome for subsequent initiation events.

A genome-wide nuclesome mapping was performed in *P. falciparum* in the intra-erythrocytic cycle (138). In general, intergenic regions displayed a low density of nucleosomes whereas coding regions were highly occupied with nucleosomes. Only several constitutive genes presented low nucleosomal occupancy throughout their coding regions, like tRNAs, ribosomal proteins, and basal transcription machinery. The most remarkable changes were observed at telomeric and subtelomeric regions. At telomeric regions closest to the chromosome end, the nucleosome enrichment was moderate in rings, low in trophozoites, and high in schizonts. In late schizonts, DNA is replicated and assembled into daughter cells. Nucleosome enrichment at this stage could be due to condensation of telomeric sequences in response to DNA replication (138). In contrast, nucleosome enrichment in subtelomeric genes was low in rings, and high in trophozoites and schizonts. The subtelomeric region contains multigene families of antigenic surface proteins, the expression of which is highest in rings, therefore possibly explaining the low nucleosomal occupancy. Nucleosomes located at intergenic regions are enriched with H3 acetylated at K9 (H3K9ac) (Figure 2). However, neither the nucleosome occupancy nor H3K9ac enrichment correlate with changes in mRNA levels, indicating the importance of other regulatory mechanisms. An exception was observed for *var* genes, the expression of which is inversely correlated with nucleosomal occupancy. These findings show that these parasites have a different nucleosome arrangement in comparison with model eukaryotes, where nucleosomes are usually absent only at promoters. The low density of nucleosomes in *Plasmodium* may be the result of its AT-rich genome, where the AT content of intergenic regions may destabilize nucleosome formation (138) (Figure 2). Information about the occupancy of nucleosomes helps us to understand transcription regulation and chromosome structure. Observations in *P. falciparum* demonstrate that parasites do not have the exact same chromosome structure as seen in other eukaryotes (Figure 2). Chromatin structure needs to be analyzed in other protist parasites, with an eye towards the preference of variant-containing nucleosomes for distinctive DNA sequences.

3.6 Histones Genome localization

New technologies (ChIP-on-chip, ChIP-seq, etc.) have led to an explosion of studies revealing genome localization of histones, modified histones, and histones variants. These studies revealed that diverse types of chromatin are enriched with specific nucleosomes. For example, H3 trimethylated at K9 (H3K9me3) is localized mainly at heterochromatin, whereas acetylated forms of H3 are enriched at promoters (7). Interestingly, these histone modifications are highly conserved among eukaryotes, much more than transcription factors and *cis*-elements, suggesting that they arose very early in evolution.

Trypanosomatids use polycistronic transcription, in which hundreds of genes are transcribed in polycistronic transcription units (PTUs). Convergent or divergent PTUs are separated by strand switch regions (SSRs). RNA Polymerase II (RNAPII) transcription initiates at divergent SSRs and terminates at convergent SSRs. Distinctive nuclesosme arrays were detected at both sites (Figure 2). In *T. brucei,* divergent SSR nucleosomes are enriched with H2AZ, H2BV and H4 acetylated at K10 (H4K10ac). In contrast, convergent SSR nucleosomes contain H3V and H4V and canonical H2A and H2B (119). Recently, it was observed that H3 trimethylation at K4 (H3K4me3) co-localizes with H4K10ac, with a bias towards the upstream side of the H4K10ac peak, probably a characteristic nucleosome composition at transcription initation regions (139). In the *T. cruzi* genome, divergent SSRs are enriched with acetylated H3 and H4 (at K9 and K14, and K5, K8, K12 and K16, respectively), and H3 methylated at K4 (140). In *Leishmania major* as well as in *T. cruzi*, the enrichment of acetylated H3 at K9 and K14 was detected at SSRs (141). Therefore, in every Trypanosomatid studied, post-translational histone modifications and histone variants mark initiation and termination of transcription sites, possibly generating an open chromatin structure that facilitates transcription. In these parasites, no transcription factors and/or regulatory elements were identified so far. It has therefore been proposed that these parasites might be using this mechanism to transcribe PTUs, suggesting an ancestral mode of transcriptional regulation that might have preceded transcription factors (72).

T. gondii and *P. falciparum*, which use monocistronic transcription, show a histone pattern similar to that observed in mammals and yeast (Figure 2). However, the parasites appear to have more activation than silencing marks when compared to human cells, which may be associated to the proportion of the genome silenced in both cases (3). Regions upstream of active genes are enriched in H3K9ac, acetylated H4 (H4ac), and H3K4me3; whereas only some of them are enriched in H3 di-

methylated at R17 (9, 142, 143). The *T. gondii* H3K4me3 peak is skewed toward the initiation codon with respect to the H4ac peak, which might be predictive of promoter direction (143). Mono- and di-methylated H4 at K20 and H3K9me3 are marks of heterochromatin in both parasites (144-146). Despite these similarities, some differences between *Toxoplasma* and *Plamodium* chromatin structure and regulation have been observed. In *P. falciparum*, the enrichment of the repressive histone modification H3K9me3 is restricted to subtelomeric loci and internal regions on chromosomes 4, 6, 7, 8, and 12 (145, 146). This mark is not present in other intergenic regions and is localized at the nuclear periphery. Authors propose the existence of perinuclear repressive centers that control the expression of genes involved in pathogenesis and phenotypic variation (145). Another unique feature observed in *P. falciparum* is that intergenic regions are deficient in nucleosomes (see section 3.5), but nucleosomes present in these areas are rich in H3K9ac and H3K4me3 (146). Interestingly, when parasite culture was synchronized and localization of these histone marks was analyzed in rings and schizonts separately, a stage-specific distribution was observed (146). In rings, H3K9ac and H3K4me3 are homogenous across the genome, whereas in schizonts, they are enriched at intergenic regions. Moreover, a detailed comparison of the most active and inactive genes revealed that H3K4me3 and H3K9ac are enriched toward the 5' end of highly active genes in schizonts, but are absent in the least active genes (146).

Besides Trypanosomatids, genome localization of histone variants has only been studied in *T. gondii* to date (31). H2AZ and H2Bv are preferentially located at active gene promoters and co-localize with acetylated H3 (Figure 2), similar to what is observed in Trypanosomatids and in correlation with their specificity to form dimers. By contrast, H2AX is predominantly at repressed promoters (Figure 2) and silenced chromatin (31). This was the first report of genome localization of this histone variant. Surprisingly, H2AX did not display a homogeneous and nonspecific distribution across the genome, as expected for a histone localized only at DNA repair sites. H2AX levels are increased in bradyzoites, which have a higher number of silenced genes compared to tachyzoites. It could be proposed that H2AX has a novel role in gene silencing in this apicomplexan parasite. Recently, similar results were observed in yeast (both copies of H2A have the SQ motif) when gamma-H2A localization was analyzed (147). About half the sites enriched for gamma-H2A match with repressed protein-coding genes. These loci have little RNAPII and gamma-H2A formation is dependent on the presence of HDACs. They propose that this chromatin structure might cause a problem for replisome progression or stability (147). In addition, we have observed that bradyzoites contain higher levels of H2AX transcripts, indicating a possible role for this histone in parasite differentiation. With exception of bradyzoite-specific genes, gene expression in general is expected to be decreased in bradyzoites since they are virtually dormant. It is tempting to speculate that the increase of H2AX is necessary to spread chromatin repression during the latent bradyzoite stage. Recently, it was proposed that H2AX in *X. laevis* has a role in early development; specifically, phosphorylation of H2AX at T16 is necessary for anterior neural formation (148). These observations suggest that H2AX may have other functions beside DNA repair, possinly mediated by novel PTMs that have yet to be described.

Many contradictory results are reported for H2AZ genome localization and function. It has been associated with promoters, enhancers, pericentric heterochromatin and facultative heterochromatin (reviewed in (149)). However, other results are worthy of special attention. The localization of H2AZ-containing nucleosomes at mononucleosome resolution has been demonstrated at flanking NFR in yeast, human cells, and *Drosophila* (91, 150, 151) (Figure 2). In yeast, H2AZ is present at active and inactive promoters (121, 150, 152), whereas in human cells H2AZ co-localizes with RNAPII at transcribed genes and genes poised for transcription (91). Time course experiments on mammalian cells revealed that H2AZ is recruited to the promoter just prior to RNAPII and leaves the promoter as the polymerase is recruited; H2AZ then reappears after RNAPII has left the promoter. In spite of these differences in yeast and human, H2AZ helps with the recruitment of RNAPII (90, 91). In accordance with these findings, H2AZ-containing nucleosomes co-localize with active transcription in protist parasites (31, 119) (Fig.2). Additionally, H2AZ has been reported to be associated with heterochromatin (149). Hardy S. *et al* (91) proposed that H2AZ is randomly incorporated into the genome at low levels and transcription induces the depletion of these H2AZ from active genes, in turn leading to an accumulation of this varint in heterochromatic regions. They also detected that the H2AZ variants present in heterochromatin are hypoacetylated relative to H2AZ in euchromatin. There are only a few reports describing H2AZ modifications; however it seems plausible that the location of H2AZ could be dependent on the type of modifications. The acetylated form of H2AZ is enriched at the 5' end of active genes in comparison with the non-acetylated form (153, 154). In human and mouse, H2AZ can be mono-ubiquitylated, and this modified histone variant is localized at Xi (155). These seemingly contradictory results could also be explained with the model that specific PTMs dictate different positions and/or functions of the same histone (156) (Figure 2).

3.7. Nuclear compartmentalization

It has been demonstrated in human cells that the nucleus in interphase is compartmentalized into chromosome territories, and chromosome homologues do not share the same territory (157, 158). In addition, nuclear events, including transcription, replication and silencing, do not occur ubiquitously all over the nucleus; rather, these events are limited to specific and spatially defined sites, functionally organized with discrete subcompartments enriched in factors and machineries involved in each process (159). Condensed heterochromatin typically contains transcriptionally silent genomic regions and localizes primarily to the nuclear periphery. The less condensed euchromatin is composed of poised and

transcriptionally active genomic regions and localizes towards the nuclear interior (158). This localization is accompanied by epigenetic marks, such as H3K9me3 and H4K20me3 that mark chromocentres, clusters of centromeric chromatin that tend to be localized at the nuclear periphery, whereas the euchromatic marker H3K4me3 labels the entire nuclei except the chromocenters (for review see (160)). An inverted nuclear organization has been observed in rod cells of nocturnal mammals, showing a functional nuclear genome reorganization adapted for light transmission (161). Consequently, nuclear architecture is important for cell function.

Several studies have demonstrated that the *P. falciparum* nucleus is also structurally and functionally divided into compartments (145, 162, 163). Electron microscopy reveals that the parasite nuclear periphery consists of an electron-dense zone reminiscent of heterochromatin interspersed by electron-translucent space, presumably of noncondensed chromatin. Telomeres form 4 to 7 clusters at nuclear periphery. In addition, repressive marks, like H3K9me3, and HP1, are localized at the electron-dense nuclear periphery whereas active marks, like H4ac and H3K9ac, are restricted to the interior of the nucleus. H3K9me3 forms loci at the nuclear periphery outside of the DAPI-stained area that co-localize with the telomere clusters, and does not overlap with CenH3 as was observed by ChIP experiments (145, 162). Although the active marks H3K4me2 and H3K4me3 co-localize with nuclear DAPI stain, H3K4me2 has a punctate pattern, whereas H3K4me3 labels the periphery of the DAPI stained area. The repressive mark H4K20me3 labels the outside periphery of the DAPI stain (163). In *Toxoplasma*, H4K20me3 and its modifying enzyme SET8 display a peripheral localization as well (144). In *T. brucei,* H4K10ac is found at the center of the nucleus, clearly excluding the nucleolus (119). While there is currently limited information about the nuclear architecture in protozoan parasites, the evidence to date suggests a compartmentalization of heterochromatin at the periphery of the nuclei and euchromatin in the nuclear interior.

4. SUMMARY AND PERSPECTIVES

Epigenetic-mediated events are proving to be critically important in the viability and development of many parasitic protozoa. Histones and histone variants are the building blocks of epigenetic mechanisms and the potential for a histone code seems to be present in every eukaryote analyzed so far. In protozoa, we and others have found intriguing and unusual differences in the histone repertoire that are characteristic of each species (Figure 2). For instance, Trypanosomatids and apicomplexans have a histone H2B variant that is able to dimerize with H2AZ but no other H2A. In *T. brucei*, these two variants mark the RNAPII start sites; in addition, *T. brucei* contains one variant each of H3 and H4 that mark RNAPII end sites. In *Toxoplasma*, H2Bv and H2AZ are enriched at active promoters whereas H2AX is associated to silenced region. Aside from these differences, specific localization of H2AZ at active promoters can be observed in both cases. The specific role that H2AZ is playing transcription activation points in protozoan parasites has not been described to date. It would be very interesting to elucidate its contribution to transcription in each protozoal species.

Toxoplasma is one of the few parasites that has H2AX, and this variant is enriched at silenced genes. Similar results observed in yeast hint that this variant may be involved in a conserved mechanism beyond DNA repair. Only *Giardia*, *T. thermophila*, *N. caninum,* and *Cryptosporidium* spp. have this histone variant. How parasites without H2AX repair DNA or block the replisome is a mystery. In addition, not all protists have the same histone variants, and some protists have only one type of core histone. *Giardia* has only one type of H2A, the histone variant H2AX. *T. vaginalis* has many copies of histone-coding genes, yet none of them encodes for a histone variant. In both cases, the parasite has only one kind of H2A. How these parasites regulate many diverse processes, like DNA transcription, replication, and repair, without the well conserved H2A variants is an intriguing question.

P. falciparum possesses intergenic regions deficient in nucleosomes that facilitate the access of transcription machinery, based mainly on its A/T rich genome. In addition, the repressive mark H3K9me3 is localized only at telomeres and some restricted chromosome regions, forming repressive centers on the nuclear periphery.

In addition to H2B, other linage-specific variants have been described in protists, such as variants of H3 and H4 in *T. brucei* and H3 in *Giardia*, and it is possible that there might be additional linage-specific histone variants. *Entamoeba* spp. have fairly divergent histones and virtually none of them have been examined in detail. *Entamoeba* have several histone coding genes with differences in size and sequence among the same core histone. The most representative are H2A and H3 (see Table 1). Interestingly, these parasites have acquired an H4 acetylation mechanism that may allow for its growth and differentiation in response to its environment. The characterization of these histones will shed light on their role in parasite adaptation to the environment.

Further studies on histones and histone variants and their modifying enzymes will help us understand the epigenetic mechanisms used by these important parasites to replicate, differentiate, and survive in varying environmental conditions.

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6. REFERENCES

1. W. J. Sullivan, Jr., A. Naguleswaran and S. O. Angel: Histones and histone modifications in protozoan parasites. *Cell Microbiol*, 8(12), 1850-61 (2006)

2. L. M. Iyer, V. Anantharaman, M. Y. Wolf and L. Aravind: Comparative genomics of transcription factors and chromatin proteins in parasitic protists and other eukaryotes. *Int J Parasitol*, 38(1), 1-31 (2008)

3. A. Bougdour, L. Braun, D. Cannella and M. A. Hakimi: Chromatin modifications: implications in the regulation of gene expression in *Toxoplasma gondii*. *Cell Microbiol*, 12(4), 413-23 (2010)

4. A. M. Gopalakrishnan and C. Lopez-Estrano: Comparative analysis of stage specific gene regulation of apicomplexan parasites: *Plasmodium falciparum* and *Toxoplasma gondii*. *Infect Disord Drug Targets*, 10(4), 303-11 (2010)

5. C. Gomez, M. Esther Ramirez, M. Calixto-Galvez, O. Medel and M. A. Rodriguez: Regulation of gene expression in protozoa parasites. *J Biomed Biotechnol*, 2010, 726045

6. K. Luger, A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond: Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, 389(6648), 251-60 (1997)

7. B. D. Strahl and C. D. Allis: The language of covalent histone modifications. *Nature*, 403(6765), 41-5 (2000)

8. S. J. Darkin-Rattray, A. M. Gurnett, R. W. Myers, P. M. Dulski, T. M. Crumley, J. J. Allocco, C. Cannova, P. T. Meinke, S. L. Colletti, M. A. Bednarek, S. B. Singh, M. A. Goetz, A. W. Dombrowski, J. D. Polishook and D. M. Schmatz: Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proc Natl Acad Sci U S A*, 93(23), 13143-7 (1996)

9. N. Saksouk, M. M. Bhatti, S. Kieffer, A. T. Smith, K. Musset, J. Garin, W. J. Sullivan, Jr., M. F. Cesbron-Delauw and M. A. Hakimi: Histone-modifying complexes regulate gene expression pertinent to the differentiation of the protozoan parasite *Toxoplasma gondii*. *Mol Cell Biol*, 25(23), 10301-14 (2005)

10. S. Sonda, L. Morf, I. Bottova, H. Baetschmann, H. Rehrauer, A. Caflisch, M. A. Hakimi and A. B. Hehl: Epigenetic mechanisms regulate stage differentiation in the minimized protozoan *Giardia lamblia*. *Mol Microbiol*, 76(1), 48-67 (2010)

11. A. Bougdour, D. Maubon, P. Baldacci, P. Ortet, O. Bastien, A. Bouillon, J. C. Barale, H. Pelloux, R. Menard and M. A. Hakimi: Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. *J Exp Med*, 206(4), 953-66 (2009)

12. J. Byers and D. Eichinger: Acetylation of the *Entamoeba* histone H4 N-terminal domain is influenced by short-chain fatty acids that enter trophozoites in a pH-dependent manner. *Int J Parasitol*, 38(1), 57-64 (2008)

13. K. T. Andrews, T. N. Tran, N. C. Wheatley and D. P. Fairlie: Targeting histone deacetylase inhibitors for anti-malarial therapy. *Curr Top Med Chem*, 9(3), 292-308 (2009)

14. S. E. Dixon, K. L. Stilger, E. V. Elias, A. Naguleswaran and W. J. Sullivan, Jr.: A decade of epigenetic research in *Toxoplasma gondii*. *Mol Biochem Parasitol*, 173(1), 1-9 (2010)

15. S. Martinez-Calvillo, J. C. Vizuet-de-Rueda, L. E. Florencio-Martinez, R. G. Manning-Cela and E. E. Figueroa-Angulo: Gene expression in trypanosomatid parasites. *J Biomed Biotechnol*, 2010, 525241

16. L. Cui and J. Miao: Chromatin-mediated epigenetic regulation in the malaria parasite *Plasmodium falciparum*. *Eukaryot Cell*, 9(8), 1138-49 (2010)

17. S. C. Albright, P. P. Nelson and W. T. Garrard: Histone molar ratios among different electrophoretic forms of mono- and dinucleosomes. *J Biol Chem*, 254(4), 1065-73 (1979)

18. D. Meeks-Wagner and L. H. Hartwell: Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. *Cell*, 44(1), 43-52 (1986)

19. W. F. Marzluff, E. J. Wagner and R. J. Duronio: Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. *Nat Rev Genet*, 9(11), 843-54 (2008)

20. W. F. Marzluff, P. Gongidi, K. R. Woods, J. Jin and L. J. Maltais: The human and mouse replication-dependent histone genes. *Genomics*, 80(5), 487-98 (2002)

21. S. Alsford and D. Horn: *Trypanosomatid* histones. *Mol Microbiol*, 53(2), 365-72 (2004)

22. P. Cong, Y. Luo, W. Bao and S. Hu: Genomic organization and promoter analysis of the *Trichomonas vaginalis* core histone gene families. *Parasitol Int*, 59(1), 29-34 (2010)

23. W. F. Marzluff and R. J. Duronio: Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. *Curr Opin Cell Biol*, 14(6), 692-9 (2002)

24. H. R. Salzler, J. M. Davidson, N. D. Montgomery and R. J. Duronio: Loss of the histone pre-mRNA processing factor stem-loop binding protein in *Drosophila* causes genomic instability and impaired cellular proliferation. *PLoS One*, 4(12), e8168 (2009)

25. K. Ersfeld, R. Docherty, S. Alsford and K. Gull: A fluorescence in situ hybridisation study of the regulation of histone mRNA levels during the cell cycle of Trypanosoma brucei. *Mol Biochem Parasitol*, 81(2), 201-9 (1996)

26. J. A. Garcia-Salcedo, P. Gijon and E. Pays: Regulated transcription of the histone H2B genes of *Trypanosoma brucei*. *Eur J Biochem*, 264(3), 717-23 (1999)

27. R. F. Recinos, L. V. Kirchhoff and J. E. Donelson: Cell cycle expression of histone genes in Trypanosoma cruzi. *Mol Biochem Parasitol*, 113(2), 215-22 (2001)

28. V. Sabaj, L. Aslund, U. Pettersson and N. Galanti: Histone genes expression during the cell cycle in Trypanosoma cruzi. *J Cell Biochem*, 80(4), 617-24 (2001)

29. D. R. Abanades, L. Ramirez, S. Iborra, K. Soteriadou, V. M. Gonzalez, P. Bonay, C. Alonso and M. Soto: Key role of the 3' untranslated region in the cell cycle regulated expression of the *Leishmania infantum* histone H2A genes: minor synergistic effect of the 5' untranslated region. *BMC Mol Biol*, 10, 48 (2009)

30. M. C. Dalmasso, P. C. Echeverria, M. P. Zappia, U. Hellman, J. F. Dubremetz and S. O. Angel: *Toxoplasma gondii* has two lineages of histones 2b (H2B) with different expression profiles. *Mol Biochem Parasitol*, 148(1), 103-7 (2006)

31. M. C. Dalmasso, D. O. Onyango, A. Naguleswaran, W. J. Sullivan, Jr. and S. O. Angel: *Toxoplasma* H2A Variants Reveal Novel Insights into Nucleosome Composition and Functions for this Histone Family. *J Mol Biol* 392(1), 33-47 (2009)

32. J. Miao, Q. Fan, L. Cui and J. Li: The malaria parasite *Plasmodium falciparum* histones: organization, expression, and acetylation. *Gene*, 369, 53-65 (2006)

33. M. Davila Lopez and T. Samuelsson: Early evolution of histone mRNA 3' end processing. *RNA*, 14(1), 1-10 (2008)

34. J. Yee, A. Tang, W. L. Lau, H. Ritter, D. Delport, M. Page, R. D. Adam, M. Muller and G. Wu: Core histone genes of *Giardia intestinalis*: genomic organization, promoter structure, and expression. *BMC Mol Biol*, 8, 26 (2007)

35. F. D. Ciccarelli, T. Doerks, C. von Mering, C. J. Creevey, B. Snel and P. Bork: Toward automatic reconstruction of a highly resolved tree of life. *Science*, 311(5765), 1283-7 (2006)

36. R. T. Kamakaka and S. Biggins: Histone variants: deviants? *Genes Dev*, 19(3), 295-310 (2005)

37. J. S. Godde and K. Ura: Cracking the enigmatic linker histone code. *J Biochem*, 143(3), 287-93 (2008)

38. H. E. Kasinsky, J. D. Lewis, J. B. Dacks and J. Ausio: Origin of H1 linker histones. *FASEB J*, 15(1), 34-42 (2001)

39. A. C. Harvey and J. A. Downs: What functions do linker histones provide? *Mol Microbiol*, 53(3), 771-5 (2004)

40. W. Schlimme, M. Burri, B. Betschart and H. Hecker: Properties of the histones and functional aspects of the soluble chromatin of epimastigote *Trypanosoma cruzi*. *Acta Trop*, 60(3), 141-54 (1995)

41. O. Triana, N. Galanti, N. Olea, U. Hellman, C. Wernstedt, H. Lujan, C. Medina and G. C. Toro: Chromatin and histones from *Giardia lamblia*: a new puzzle in primitive eukaryotes. *J Cell Biochem*, 82(4), 573-82 (2001)

42. S. C. Dawson, M. S. Sagolla and W. Z. Cande: The cenH3 histone variant defines centromeres in *Giardia intestinalis*. *Chromosoma*, 116(2), 175-84 (2007)

43. N. Happel and D. Doenecke: Histone H1 and its isoforms: contribution to chromatin structure and function. *Gene*, 431(1-2), 1-12 (2009)

44. T. M. Noll, C. Desponds, S. I. Belli, T. A. Glaser and N. J. Fasel: Histone H1 expression varies during the *Leishmania major* life cycle. *Mol Biochem Parasitol*, 84(2), 215-27 (1997)

45. F. T. Papageorgiou and K. P. Soteriadou: Expression of a novel *Leishmania* gene encoding a histone H1-like protein in *Leishmania major* modulates parasite infectivity in vitro. *Infect Immun*, 70(12), 6976-86 (2002)

46. D. Smirlis, S. N. Bisti, E. Xingi, G. Konidou, M. Thiakaki and K. P. Soteriadou: *Leishmania* histone H1 overexpression delays parasite cell-cycle progression, parasite differentiation and reduces *Leishmania* infectivity in vivo. *Mol Microbiol*, 60(6), 1457-73 (2006)

47. S. Masina, H. Zangger, D. Rivier and N. Fasel: Histone H1 regulates chromatin condensation in *Leishmania* parasites. *Exp Parasitol*, 116(1), 83-7 (2007)

48. R. Marques Porto, R. Amino, M. C. Elias, M. Faria and S. Schenkman: Histone H1 is phosphorylated in non-

replicating and infective forms of Trypanosoma cruzi. *Mol Biochem Parasitol*, 119(2), 265-71 (2002)

49. J. P. da Cunha, E. S. Nakayasu, M. C. Elias, D. C. Pimenta, M. T. Tellez-Inon, F. Rojas, M. J. Munoz, I. C. Almeida and S. Schenkman: *Trypanosoma cruzi* histone H1 is phosphorylated in a typical cyclin dependent kinase site accordingly to the cell cycle. *Mol Biochem Parasitol*, 140(1), 75-86 (2005)

50. L. M. Gutiyama, J. P. da Cunha and S. Schenkman: Histone H1 of *Trypanosoma cruzi* is concentrated in the nucleolus region and disperses upon phosphorylation during progression to mitosis. *Eukaryot Cell*, 7(4), 560-8 (2008)

51. B. A. Garcia, S. Joshi, C. E. Thomas, R. K. Chitta, R. L. Diaz, S. A. Busby, P. C. Andrews, R. R. Ogorzalek Loo, J. Shabanowitz, N. L. Kelleher, C. A. Mizzen, C. D. Allis and D. F. Hunt: Comprehensive phosphoprotein analysis of linker histone H1 from *Tetrahymena thermophila*. *Mol Cell Proteomics*, 5(9), 1593-609 (2006)

52. Y. Dou, C. A. Mizzen, M. Abrams, C. D. Allis and M. A. Gorovsky: Phosphorylation of linker histone H1 regulates gene expression in vivo by mimicking H1 removal. *Mol Cell*, 4(4), 641-7 (1999)

53. C. A. Mizzen, Y. Dou, Y. Liu, R. G. Cook, M. A. Gorovsky and C. D. Allis: Identification and mutation of phosphorylation sites in a linker histone. Phosphorylation of macronuclear H1 is not essential for viability in *Tetrahymena*. *J Biol Chem*, 274(21), 14533-6 (1999)

54. J. Clausell, N. Happel, T. K. Hale, D. Doenecke and M. Beato: Histone H1 subtypes differentially modulate chromatin condensation without preventing ATP-dependent remodeling by SWI/SNF or NURF. *PLoS One*, 4(10), e0007243 (2009)

55. H. S. Malik and S. Henikoff: Phylogenomics of the nucleosome. *Nat Struct Biol*, 10(11), 882-91 (2003)

56. B. P. Chadwick and H. F. Willard: A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. *J Cell Biol*, 152(2), 375-84 (2001)

57. T. Gautier, D. W. Abbott, A. Molla, A. Verdel, J. Ausio and S. Dimitrov: Histone variant H2ABbd confers lower stability to the nucleosome. *EMBO Rep*, 5(7), 715-20 (2004)

58. B. P. Chadwick and H. F. Willard: Histone H2A variants and the inactive X chromosome: identification of a second macroH2A variant. *Hum Mol Genet*, 10(10), 1101-13 (2001)

59. C. Costanzi and J. R. Pehrson: MACROH2A2, a new member of the MARCOH2A core histone family. *J Biol Chem*, 276(24), 21776-84 (2001)

60. M. Buschbeck, I. Uribesalgo, I. Wibowo, P. Rue, D. Martin, A. Gutierrez, L. Morey, R. Guigo, H. Lopez-Schier and L. Di Croce: The histone variant macroH2A is an epigenetic regulator of key developmental genes. *Nat Struct Mol Biol*, 16(10), 1074-9 (2009)

61. M. J. Gamble, K. M. Frizzell, C. Yang, R. Krishnakumar and W. L. Kraus: The histone variant macroH2A1 marks repressed autosomal chromatin, but protects a subset of its target genes from silencing. *Genes Dev*, 24(1), 21-32 (2010)

62. E. P. Rogakou, D. R. Pilch, A. H. Orr, V. S. Ivanova and W. M. Bonner: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem*, 273(10), 5858-68 (1998)

63. T. Stiff, M. O'Driscoll, N. Rief, K. Iwabuchi, M. Lobrich and P. A. Jeggo: ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res*, 64(7), 2390-6 (2004)

64. J. A. Downs, N. F. Lowndes and S. P. Jackson: A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature*, 408(6815), 1001-4 (2000)

65. S. K. Mahadevaiah, J. M. Turner, F. Baudat, E. P. Rogakou, P. de Boer, J. Blanco-Rodriguez, M. Jasin, S. Keeney, W. M. Bonner and P. S. Burgoyne: Recombinational DNA double-strand breaks in mice precede synapsis. *Nat Genet*, 27(3), 271-6 (2001)

66. H. van Attikum and S. M. Gasser: Crosstalk between histone modifications during the DNA damage response. *Trends Cell Biol*, 19(5), 207-17 (2009)

67. A. J. Morrison and X. Shen: Chromatin remodelling beyond transcription: the INO80 and SWR1 complexes. *Nat Rev Mol Cell Biol*, 10(6), 373-84 (2009)

68. C. H. Bassing, K. F. Chua, J. Sekiguchi, H. Suh, S. R. Whitlow, J. C. Fleming, B. C. Monroe, D. N. Ciccone, C. Yan, K. Vlasakova, D. M. Livingston, D. O. Ferguson, R. Scully and F. W. Alt: Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc Natl Acad Sci U S A*, 99(12), 8173-8 (2002)

69. A. Celeste, S. Petersen, P. J. Romanienko, O. Fernandez-Capetillo, H. T. Chen, O. A. Sedelnikova, B. Reina-San-Martin, V. Coppola, E. Meffre, M. J. Difilippantonio, C. Redon, D. R. Pilch, A. Olaru, M. Eckhaus, R. D. Camerini-Otero, L. Tessarollo, F. Livak, K. Manova, W. M. Bonner, M. C. Nussenzweig and A. Nussenzweig: Genomic instability in mice lacking histone H2AX. *Science*, 296(5569), 922-7 (2002)

70. C. Redon, D. Pilch, E. Rogakou, O. Sedelnikova, K. Newrock and W. Bonner: Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev*, 12(2), 162-9 (2002)

71. X. Song, E. Gjoneska, Q. Ren, S. D. Taverna, C. D. Allis and M. A. Gorovsky: Phosphorylation of the SQ H2A.X motif is required for proper meiosis and mitosis in *Tetrahymena thermophila*. *Mol Cell Biol*, 27(7), 2648-60 (2007)

72. P. B. Talbert and S. Henikoff: Histone variants--ancient wrap artists of the epigenome. *Nat Rev Mol Cell Biol*, 11(4), 264-75 (2010)

73. T. H. Thatcher and M. A. Gorovsky: Phylogenetic analysis of the core histones H2A, H2B, H3, and H4. *Nucleic Acids Res*, 22(2), 174-9 (1994)

74. A. Li, A. H. Maffey, W. D. Abbott, N. Conde e Silva, A. Prunell, J. Siino, D. Churikov, A. O. Zalensky and J. Ausio: Characterization of nucleosomes consisting of the human testis/sperm-specific histone H2B variant (hTSH2B). *Biochemistry*, 44(7), 2529-35 (2005)

75. R. Hernandez-Rivas, K. Hinterberg and A. Scherf: Compartmentalization of genes coding for immunodominant antigens to fragile chromosome ends leads to dispersed subtelomeric gene families and rapid gene evolution in *Plasmodium falciparum*. *Mol Biochem Parasitol*, 78(1-2), 137-48 (1996)

76. J. Henriksson, B. Porcel, M. Rydaker, A. Ruiz, V. Sabaj, N. Galanti, J. J. Cazzulo, A. C. Frasch and U. Pettersson: Chromosome specific markers reveal conserved linkage groups in spite of extensive chromosomal size variation in *Trypanosoma cruzi*. *Mol Biochem Parasitol*, 73(1-2), 63-74 (1995)

77. A. K. Cruz, R. Titus and S. M. Beverley: Plasticity in chromosome number and testing of essential genes in *Leishmania* by targeting. *Proc Natl Acad Sci U S A*, 90(4), 1599-603 (1993)

78. J. K. Frenkel and P. Ambroise-Thomas: Genomic drift of *Toxoplasma gondii*. *Parasitol Res*, 83(1), 1-5 (1997)

79. A. Xiao, H. Li, D. Shechter, S. H. Ahn, L. A. Fabrizio, H. Erdjument-Bromage, S. Ishibe-Murakami, B. Wang, P. Tempst, K. Hofmann, D. J. Patel, S. J. Elledge and C. D. Allis: WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature*, 457(7225), 57-62 (2009)

80. P. J. Cook, B. G. Ju, F. Telese, X. Wang, C. K. Glass and M. G. Rosenfeld: Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature*, 458(7238), 591-6 (2009)

81. J. S. Dickey, C. E. Redon, A. J. Nakamura, B. J. Baird, O. A. Sedelnikova and W. M. Bonner: H2AX: functional roles and potential applications. *Chromosoma*, 118(6), 683-92 (2009)

82. J. Yuan, R. Adamski and J. Chen: Focus on histone variant H2AX: To be or not to be. *FEBS Lett* 584(17), 3717-24 (2010)

83. D. Shechter, R. K. Chitta, A. Xiao, J. Shabanowitz, D. F. Hunt and C. D. Allis: A distinct H2A.X isoform is enriched in *Xenopus laevis* eggs and early embryos and is phosphorylated in the absence of a checkpoint. *Proc Natl Acad Sci U S A*, 106(3), 749-54 (2009)

84. R. K. Suto, M. J. Clarkson, D. J. Tremethick and K. Luger: Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat Struct Biol*, 7(12), 1121-4 (2000)

85. M. J. Clarkson, J. R. Wells, F. Gibson, R. Saint and D. J. Tremethick: Regions of variant histone His2AvD required for *Drosophila* development. *Nature*, 399(6737), 694-7 (1999)

86. X. Liu and M. A. Gorovsky: Cloning and characterization of the major histone H2A genes completes the cloning and sequencing of known histone genes of *Tetrahymena thermophila*. *Nucleic Acids Res*, 24(15), 3023-30 (1996)

87. R. Faast, V. Thonglairoam, T. C. Schulz, J. Beall, J. R. Wells, H. Taylor, K. Matthaei, P. D. Rathjen, D. J. Tremethick and I. Lyons: Histone variant H2A.Z is required for early mammalian development. *Curr Biol*, 11(15), 1183-7 (2001)

88. A. M. Carr, S. M. Dorrington, J. Hindley, G. A. Phear, S. J. Aves and P. Nurse: Analysis of a histone H2A variant from fission yeast: evidence for a role in chromosome stability. *Mol Gen Genet*, 245(5), 628-35 (1994)

89. L. A. Stargell, J. Bowen, C. A. Dadd, P. C. Dedon, M. Davis, R. G. Cook, C. D. Allis and M. A. Gorovsky: Temporal and spatial association of histone H2A variant hv1 with transcriptionally competent chromatin during nuclear development in *Tetrahymena thermophila*. *Genes Dev*, 7(12B), 2641-51 (1993)

90. M. Adam, F. Robert, M. Larochelle and L. Gaudreau: H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol Cell Biol*, 21(18), 6270-9 (2001)

91. S. Hardy, P. E. Jacques, N. Gevry, A. Forest, M. E. Fortin, L. Laflamme, L. Gaudreau and F. Robert: The euchromatic and heterochromatic landscapes are shaped by antagonizing effects of transcription on H2A.Z deposition. *PLoS Genet*, 5(10), e1000687 (2009)

92. M. D. Meneghini, M. Wu and H. D. Madhani: Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell*, 112(5), 725-36 (2003)

93. D. Rangasamy, L. Berven, P. Ridgway and D. J. Tremethick: Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. *EMBO J*, 22(7), 1599-607 (2003)

94. D. Rangasamy, I. Greaves and D. J. Tremethick: RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. *Nat Struct Mol Biol*, 11(7), 650-5 (2004)

95. J. Y. Fan, D. Rangasamy, K. Luger and D. J. Tremethick: H2A.Z alters the nucleosome surface to promote HP1alpha-mediated chromatin fiber folding. *Mol Cell*, 16(4), 655-61 (2004)

96. H. Hou, Y. Wang, S. P. Kallgren, J. Thompson, J. R. Yates, 3rd and S. Jia: Histone variant H2A.Z regulates centromere silencing and chromosome segregation in fission yeast. *J Biol Chem*, 285(3), 1909-18 (2010)

97. M. Kalocsay, N. J. Hiller and S. Jentsch: Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol Cell*, 33(3), 335-43 (2009)

98. Q. Ren and M. A. Gorovsky: Histone H2A.Z acetylation modulates an essential charge patch. *Mol Cell*, 7(6), 1329-35 (2001)

99. M. B. Trelle, A. M. Salcedo-Amaya, A. M. Cohen, H. G. Stunnenberg and O. N. Jensen: Global Histone Analysis by Mass Spectrometry Reveals a High Content of Acetylated Lysine Residues in the Malaria Parasite *Plasmodium falciparum*. *J Proteome Res*, 8(7), 3439-3450 (2009)

100. J. E. Lowell, F. Kaiser, C. J. Janzen and G. A. Cross: Histone H2AZ dimerizes with a novel variant H2B and is enriched at repetitive DNA in *Trypanosoma brucei*. *J Cell Sci*, 118(Pt 24), 5721-30 (2005)

101. S. B. Hake and C. D. Allis: Histone H3 variants and their potential role in indexing mammalian genomes: the "H3 barcode hypothesis". *Proc Natl Acad Sci U S A*, 103(17), 6428-35 (2006)

102. K. Ahmad and S. Henikoff: The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol Cell*, 9(6), 1191-200 (2002)

103. B. Cui, Y. Liu and M. A. Gorovsky: Deposition and function of histone H3 variants in *Tetrahymena thermophila*. *Mol Cell Biol*, 26(20), 7719-30 (2006)

104. W. J. Sullivan, Jr.: Histone H3 and H3.3 variants in the protozoan pathogens *Plasmodium falciparum* and *Toxoplasma gondii*. *DNA Seq*, 14(3), 227-31 (2003)

105. B. Cui and M. A. Gorovsky: Centromeric histone H3 is essential for vegetative cell division and for DNA elimination during conjugation in Tetrahymena thermophila. *Mol Cell Biol*, 26(12), 4499-510 (2006)

106. J. E. Lowell and G. A. Cross: A variant histone H3 is enriched at telomeres in *Trypanosoma brucei*. *J Cell Sci*, 117(Pt 24), 5937-47 (2004)

107. V. Mandava, J. P. Fernandez, H. Deng, C. J. Janzen, S. B. Hake and G. A. Cross: Histone modifications in *Trypanosoma brucei*. *Mol Biochem Parasitol*, 156(1), 41-50 (2007)

108. A. O. Zalensky, J. S. Siino, A. A. Gineitis, I. A. Zalenskaya, N. V. Tomilin, P. Yau and E. M. Bradbury: Human testis/sperm-specific histone H2B (hTSH2B). Molecular cloning and characterization. *J Biol Chem*, 277(45), 43474-80 (2002)

109. D. Churikov, J. Siino, M. Svetlova, K. Zhang, A. Gineitis, E. Morton Bradbury and A. Zalensky: Novel human testis-specific histone H2B encoded by the interrupted gene on the X chromosome. *Genomics*, 84(4), 745-56 (2004)

110. R. B. Aul and R. J. Oko: The major subacrosomal occupant of bull spermatozoa is a novel histone H2B. *Dev Biol*, 242(2), 376-87 (2002)

111. J. Govin, E. Escoffier, S. Rousseaux, L. Kuhn, M. Ferro, J. Thevenon, R. Catena, I. Davidson, J. Garin, S. Khochbin and C. Caron: Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. *J Cell Biol*, 176(3), 283-94 (2007)

112. K. Ueda and I. Tanaka: The appearance of male gamete-specific histones gH2B and gH3 during pollen development in Lilium longiflorum. *Dev Biol*, 169(1), 210-7 (1995)

113. R. Gonzalez-Romero, C. Rivera-Casas, J. Ausio, J. Mendez and J. M. Eirin-Lopez: Birth-and-death long-term evolution promotes histone H2B Variant diversification in the male germinal cell line. *Mol Biol Evol*, 27(8), 1802-12 (2010)

114. D. Wells and C. McBride: A comprehensive compilation and alignment of histones and histone genes. *Nucleic Acids Res*, 17 Suppl, r311-46 (1989)

115. G. Wu, A. G. McArthur, A. Fiser, A. Sali, M. L. Sogin and M. Mllerm: Core histones of the amitochondriate protist, *Giardia lamblia*. *Mol Biol Evol*, 17(8), 1156-63 (2000)

116. J. Lukes and D. A. Maslov: Unexpectedly high variability of the histone H4 gene in *Leishmania*. *Parasitol Res*, 86(3), 259-61 (2000)

117. M. Binder, S. Ortner, B. Plaimauer, M. Fodinger, G. Wiedermann, O. Scheiner and M. Duchene: Sequence and organization of an unusual histone H4 gene in the human parasite *Entamoeba histolytica*. *Mol Biochem Parasitol*, 71(2), 243-7 (1995)

118. L. A. Katz, J. G. Bornstein, E. Lasek-Nesselquist and S. V. Muse: Dramatic diversity of ciliate histone H4 genes revealed by comparisons of patterns of substitutions and paralog divergences among eukaryotes. *Mol Biol Evol*, 21(3), 555-62 (2004)

119. T. N. Siegel, D. R. Hekstra, L. E. Kemp, L. M. Figueiredo, J. E. Lowell, D. Fenyo, X. Wang, S. Dewell and G. A. Cross: Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei*. *Genes Dev*, 23(9), 1063-76 (2009)

120. T. Ishibashi, D. Dryhurst, K. L. Rose, J. Shabanowitz, D. F. Hunt and J. Ausio: Acetylation of vertebrate H2A.Z and its effect on the structure of the nucleosome. *Biochemistry*, 48(22), 5007-17 (2009)

121. H. Zhang, D. N. Roberts and B. R. Cairns: Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell*, 123(2), 219-31 (2005)

122. C. Jin and G. Felsenfeld: Nucleosome stability mediated by histone variants H3.3 and H2A.Z. *Genes Dev*, 21(12), 1519-29 (2007)

123. A. Thakar, P. Gupta, T. Ishibashi, R. Finn, B. Silva-Moreno, S. Uchiyama, K. Fukui, M. Tomschik, J. Ausio and J. Zlatanova: H2A.Z and H3.3 histone variants affect nucleosome structure: biochemical and biophysical studies. *Biochemistry*, 48(46), 10852-7 (2009)

124. J. Y. Fan, F. Gordon, K. Luger, J. C. Hansen and D. J. Tremethick: The essential histone variant H2A.Z regulates the equilibrium between different chromatin conformational states. *Nat Struct Biol*, 9(3), 172-6 (2002)

125. M. Okuwaki, K. Kato, H. Shimahara, S. Tate and K. Nagata: Assembly and disassembly of nucleosome core particles containing histone variants by human nucleosome assembly protein I. *Mol Cell Biol*, 25(23), 10639-51 (2005)

126. C. Jin, C. Zang, G. Wei, K. Cui, W. Peng, K. Zhao and G. Felsenfeld: H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. *Nat Genet*, 41(8), 941-5 (2009)

127. G. C. Yuan, Y. J. Liu, M. F. Dion, M. D. Slack, L. F. Wu, S. J. Altschuler and O. J. Rando: Genome-scale identification of nucleosome positions in *S. cerevisiae*. *Science*, 309(5734), 626-30 (2005)

128. V. Mandava, C. J. Janzen and G. A. Cross: Trypanosome H2Bv replaces H2B in nucleosomes enriched for H3 K4 and K76 trimethylation. *Biochem Biophys Res Commun*, 368(4), 846-51 (2008)

129. A. Viens, U. Mechold, F. Brouillard, C. Gilbert, P. Leclerc and V. Ogryzko: Analysis of human histone H2AZ deposition in vivo argues against its direct role in epigenetic templating mechanisms. *Mol Cell Biol*, 26(14), 5325-35 (2006)

130. S. Chakravarthy and K. Luger: The histone variant macro-H2A preferentially forms "hybrid nucleosomes". *J Biol Chem*, 281(35), 25522-31 (2006)

131. S. Henikoff and K. Ahmad: Assembly of variant histones into chromatin. *Annu Rev Cell Dev Biol*, 21, 133-53 (2005)

132. A. Thastrom, L. M. Bingham and J. Widom: Nucleosomal locations of dominant DNA sequence motifs for histone-DNA interactions and nucleosome positioning. *J Mol Biol*, 338(4), 695-709 (2004)

133. S. Henikoff: Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat Rev Genet*, 9(1), 15-26 (2008)

134. N. Kaplan, I. K. Moore, Y. Fondufe-Mittendorf, A. J. Gossett, D. Tillo, Y. Field, E. M. LeProust, T. R. Hughes, J. D. Lieb, J. Widom and E. Segal: The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, 458(7236), 362-6 (2009)

135. Y. Zhang, Z. Moqtaderi, B. P. Rattner, G. Euskirchen, M. Snyder, J. T. Kadonaga, X. S. Liu and K. Struhl: Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions *in vivo*. *Nat Struct Mol Biol*, 16(8), 847-52 (2009)

136. S. Schwartz, E. Meshorer and G. Ast: Chromatin organization marks exon-intron structure. *Nat Struct Mol Biol*, 16(9), 990-5 (2009)

137. V. Iyer and K. Struhl: Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. *EMBO J*, 14(11), 2570-9 (1995)

138. S. J. Westenberger, L. Cui, N. Dharia and E. Winzeler: Genome-wide nucleosome mapping of *Plasmodium falciparum* reveals histone-rich coding and histone-poor intergenic regions and chromatin remodeling of core and subtelomeric genes. *BMC Genomics*, 10, 610 (2009)

139. J. R. Wright, T. N. Siegel and G. A. Cross: Histone H3 trimethylated at lysine 4 is enriched at probable transcription start sites in *Trypanosoma brucei*. *Mol Biochem Parasitol*, 172(2), 141-4 (2010)

140. P. Respuela, M. Ferella, A. Rada-Iglesias and L. Aslund: Histone acetylation and methylation at sites initiating divergent polycistronic transcription in *Trypanosoma cruzi*. *J Biol Chem*, 283(23), 15884-92 (2008)

141. S. Thomas, A. Green, N. R. Sturm, D. A. Campbell and P. J. Myler: Histone acetylations mark origins of polycistronic transcription in *Leishmania major*. *BMC Genomics*, 10, 152 (2009)

142. L. Cui, J. Miao, T. Furuya, X. Li and X. Z. Su: PfGCN5-mediated histone H3 acetylation plays a key role in gene expression in *Plasmodium falciparum*. *Eukaryot Cell*, 6(7), 1219-27 (2007)

143. M. Gissot, K. A. Kelly, J. W. Ajioka, J. M. Greally and K. Kim: Epigenomic modifications predict active promoters and gene structure in *Toxoplasma gondii*. *PLoS Pathog*, 3(6), e77 (2007)

144. C. F. Sautel, D. Cannella, O. Bastien, S. Kieffer, D. Aldebert, J. Garin, I. Tardieux, H. Belrhali and M. A. Hakimi: SET8-mediated methylations of histone H4 lysine 20 mark silent heterochromatic domains in apicomplexan genomes. *Mol Cell Biol*, 27(16), 5711-24 (2007)

145. J. J. Lopez-Rubio, L. Mancio-Silva and A. Scherf: Genome-wide analysis of heterochromatin associates clonally variant gene regulation with perinuclear repressive centers in malaria parasites. *Cell Host Microbe*, 5(2), 179-90 (2009)

146. A. M. Salcedo-Amaya, M. A. van Driel, B. T. Alako, M. B. Trelle, A. M. van den Elzen, A. M. Cohen, E. M. Janssen-Megens, M. van de Vegte-Bolmer, R. R. Selzer, A. L. Iniguez, R. D. Green, R. W. Sauerwein, O. N. Jensen and H. G. Stunnenberg: Dynamic histone H3 epigenome marking during the intraerythrocytic cycle of *Plasmodium falciparum*. *Proc Natl Acad Sci U S A*, 106(24), 9655-60 (2009)

147. R. K. Szilard, P. E. Jacques, L. Laramee, B. Cheng, S. Galicia, A. R. Bataille, M. Yeung, M. Mendez, M. Bergeron, F. Robert and D. Durocher: Systematic identification of fragile sites via genome-wide location analysis of gamma-H2AX. *Nat Struct Mol Biol*, 17(3), 299-305 (2010)

148. S. Y. Lee, A. T. Lau, C. H. Jeong, J. H. Shim, H. G. Kim, J. Kim, A. M. Bode and Z. Dong: Histone XH2AX is required for *xenopus* anterior neural development: the critical role of threonine 16 phosphorylation. *J Biol Chem* 285(38), 29525-34 (2010)

149. J. Zlatanova and A. Thakar: H2A.Z: view from the top. *Structure*, 16(2), 166-79 (2008)

150. R. M. Raisner, P. D. Hartley, M. D. Meneghini, M. Z. Bao, C. L. Liu, S. L. Schreiber, O. J. Rando and H. D. Madhani: Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell*, 123(2), 233-48 (2005)

151. T. N. Mavrich, C. Jiang, I. P. Ioshikhes, X. Li, B. J. Venters, S. J. Zanton, L. P. Tomsho, J. Qi, R. L. Glaser, S. C. Schuster, D. S. Gilmour, I. Albert and B. F. Pugh: Nucleosome organization in the *Drosophila* genome. *Nature*, 453(7193), 358-62 (2008)

152. B. Guillemette, A. R. Bataille, N. Gevry, M. Adam, M. Blanchette, F. Robert and L. Gaudreau: Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biol*, 3(12), e384 (2005)

153. C. B. Millar, F. Xu, K. Zhang and M. Grunstein: Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. *Genes Dev*, 20(6), 711-22 (2006)

154. K. Bruce, F. A. Myers, E. Mantouvalou, P. Lefevre, I. Greaves, C. Bonifer, D. J. Tremethick, A. W. Thorne and C. Crane-Robinson: The replacement histone H2A.Z in a hyperacetylated form is a feature of active genes in the chicken. *Nucleic Acids Res*, 33(17), 5633-9 (2005)

155. E. Sarcinella, P. C. Zuzarte, P. N. Lau, R. Draker and P. Cheung: Monoubiquitylation of H2A.Z distinguishes its association with euchromatin or facultative heterochromatin. *Mol Cell Biol*, 27(18), 6457-68 (2007)

156. A. A. Thambirajah, A. Li, T. Ishibashi and J. Ausio: New developments in post-translational modifications and functions of histone H2A variants. *Biochem Cell Biol*, 87(1), 7-17 (2009)

157. A. Bolzer, G. Kreth, I. Solovei, D. Koehler, K. Saracoglu, C. Fauth, S. Muller, R. Eils, C. Cremer, M. R. Speicher and T. Cremer: Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLoS Biol*, 3(5), e157 (2005)

158. T. Cremer and C. Cremer: Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat Rev Genet*, 2(4), 292-301 (2001)

159. T. Misteli: Beyond the sequence: cellular organization of genome function. *Cell*, 128(4), 787-800 (2007)

160. E. Bartova, J. Krejci, A. Harnicarova, G. Galiova and S. Kozubek: Histone modifications and nuclear architecture: a review. *J Histochem Cytochem*, 56(8), 711-21 (2008)

161. I. Solovei, M. Kreysing, C. Lanctot, S. Kosem, L. Peichl, T. Cremer, J. Guck and B. Joffe: Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. *Cell*, 137(2), 356-68 (2009)

162. C. Flueck, R. Bartfai, J. Volz, I. Niederwieser, A. M. Salcedo-Amaya, B. T. Alako, F. Ehlgen, S. A. Ralph, A. F. Cowman, Z. Bozdech, H. G. Stunnenberg and T. S. Voss: *Plasmodium falciparum* heterochromatin protein 1 marks genomic loci linked to phenotypic variation of exported virulence factors. *PLoS Pathog*, 5(9), e1000569 (2009)

163. N. Issar, S. A. Ralph, L. Mancio-Silva, C. Keeling and A. Scherf: Differential sub-nuclear localisation of repressive and activating histone methyl modifications in *P. falciparum*. *Microbes Infect*, 11(3), 403-7 (2009)

Abbreviations: HDAC: Histone deacetylase; PTM: post-translational modifications; CDK: cyclin-dependent kinase; Xi: inactive X chromosome; TSS: transcriptional start site; NFR: nucleosome free region; PTU: polycistronic transcription units; SSR: strand switch regions; RNAPII: RNA polymerase II; H3K9ac: H3 acetylated at lysine 9; H3K9me3: H3 trimethylated at lysine 9; H4K10ac: H4 acetylated at lysine 10; H3K4me3: H3 trimethylated at lysine 4; H4ac: acetylated H4.

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