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Introducing histone modification in trypanosomes

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Abstract

Nuclear DNA is wrapped with histones and vigorous research over the past decade has established a central role for histone post-translational modification in controlling the DNA-protein interactions required for successful growth and propagation. Recent work now provides a description of acetylated and methylated residues in the divergent trypanosome core histones. Future studies should provide insights into the genomic distribution of each modification and their roles in growth and pathogenesis.

Histone modification

Eukaryotic nuclear DNA is wound around core nucleosomal histone octamers containing two units of each histone, H2A, H2B, H3 and H4. The histones are conserved and abundant, small, basic proteins and the chromatin complex is the natural substrate for all the regulatory factors that interact with nuclear DNA. Over the past decade, a huge volume of research has established the central role for covalent post-translational modification (PTM) of core histones in modulating these interactions. In particular, acetylation (Ac) on ϵ -amino groups of lysine (K) and methylation (Me) of lysine or arginine (R) have a conserved role in regulating gene expression at the level of transcription initiation and/or elongation. Addition of such small chemical groups also regulates DNA replication, recombination and repair. In trypanosomatids, packaging in chromatin allows 20cm of DNA to reside in a 2 μ m diameter nucleus but these organisms branched early from the eukaryal lineage and, largely because the epitopes recognised by the panels of PTM-specific antibodies available for other eukaryotes are not present [1], progress in this area has been delayed. This means that trypanosome PTM must be characterised *de novo*. This survey focuses on two recent publications that report such characterisation in *Trypanosoma brucei* [2] and *Trypanosoma cruzi* [3] although the discussion is also relevant to other trypanosomatids.

Most histone PTMs generate binding-platforms for regulatory factors [4], while at least one (H4-K16-Ac) alters higher-order chromatin compaction [5]. In chromatin, H3 and H4 N-termini and the H2A C-terminus are exposed and it is here that PTMs are concentrated: Ac and Me of the N-terminal tails of H3 and H4 has been particularly well-characterised. Other, more centrally located, PTMs have also been described. Together, they appear to form a combinatorial epigenetic histone code interpreted by regulatory factors [4] which allows propagation of information through generations. Me groups add significant additional complexity since they exist in three states; mono-, di- and tri-Me (Me1, Me2, Me3, respectively). Ac and Me are reversible, allowing changes in a short time-frame and widely conserved enzymes have been characterised that are responsible for their addition and removal. Many groups are currently engaged in investigating the function of orthologous factors in trypanosomatids and other parasites [6].

Histone modification in trypanosomes

The recent reports by Janzen, Cross and colleagues [2] and da Cunha, Schenkman and colleagues [3] represent crucial steps towards defining the consequences of histone PTM in trypanosomes. Mass spectrometry [7] and other methods are outlined in Box 1 and a summary of the findings is illustrated in Figure 1. Mature histones lack the initial methionine residue and the *T. brucei* studies [2] indicate the presence of Me1 alanine at the N-terminus of H2A, H2B and H4, a novel histone PTM. The H2A C-terminal is also unusual in that it is hyper-acetylated. There is a relative paucity of PTM in the N-termini of *T. brucei* core histones, but two Ac and two Me groups are found towards the N-terminal of H4. Little data are reported for H3 because the N-terminus is blocked by Ac or Me3, but another recent report from the same group provides a characterisation of H3-K76-Me2/3 [8].

The *T. brucei* and *T. cruzi* histones are closely related: only H2A differs in length by one amino acid and H2A, H2B, H3 and H4 share 86, 81, 92 and 96% identity, respectively, so common PTMs might be expected. The *T. cruzi* studies [3] focus on H4 which has an N-terminal Me1 alanine similar to the *T. brucei* protein. H4-K4-Ac, H4-K10-Ac and H4-K18-Me1 are also common, but H4-K17-Me1/2 and H4-K18-Me3 were not seen in *T. cruzi*. PTMs seen only in *T. cruzi* were H4-K14-Ac, H4-K57-Ac and H4-R53-Me2, possibly reflecting a low proportion of histones with each of these modifications. Data on the proportion of histones with certain PTMs is provided using Edman sequencing data and new Ac-specific antisera. The data confirm acetylation at three positions and show that H4-K4-Ac is abundant relative to H4-K10-Ac and H4-K14-Ac. Further application of PTM-specific antibodies may reveal differential abundance at different cell- or life-cycle stages and may provide further clues to function.

With knowledge regarding the location of Ac and Me groups and PTM-specific antibodies, it will now be possible to link PTMs to the modifiers. Genome sequencing has revealed a similar complement of candidates in all three trypanosomatids [see Table S4 in reference 9], including six acetylases, at least three Suvar-E(z)-Trithorax (SET)-type and two Disruptor-Of-Telomeric silencing (DOT)-type methylases, some potential arginine methylases, five deacetylases and, more recently, two JmjC-type demethylases. In other organisms, regulators often contain domains that selectively bind PTMs. Bromodomains bind Ac groups and are often found in transcription factors, while chromodomains, tudor domains and plant homeodomain (PHD) fingers bind Me groups. Genes encoding all four interaction-modules are represented within the trypanosomatid genome sequences. Indeed, some proteins contain modifier domains and interaction modules.

Characterisation of several putative modifiers has already been published and the process of linking modifiers to the corresponding PTM has also entered the literature recently. *T. brucei* deacetylases function in cell cycle control [10] and DNA repair [11] and the *T. brucei* methylases, DOT1A and DOT1B, are responsible for H3-K76-Me2 and Me3, respectively [8]. Perturbation of the latter factors interferes with DNA replication, cell-cycle progression and differentiation [8]. In addition, telomeric gene silencing, demonstrated recently in *T. brucei* [12], is dependent upon a sirutin-type deacetylase [13].

The significance of histone modification in trypanosomes

The potential impact of histone PTM in trypanosomes merits further consideration. Crucially, PTMs are expected to play a central role in transcription, DNA replication, recombination and repair. Certain chromosomal regions, such as centromeres and telomeres, require functional specialisation, usually mediated through chromatin. In addition, precedents exist in yeast and *Plasmodium falciparum* for chromatin-mediated control of virulence gene expression [14] and this is also predicted in trypanosomes.

Transcription in trypanosomes displays unusual features [15], in particular, it is almost exclusively constitutive and polycistronic. Although RNA polymerase II promoters have not been identified for protein-coding genes, transcription is predominantly in the 'sense' direction and certain non-coding regions are not-transcribed. In addition, *T. brucei* virulence genes (encoding Variant Surface Glycoproteins) are regulated at transcription/elongation [16] and transcription can be down-regulated at certain stages of the cell- or life-cycle [17]. DNA homologous recombination and repair are important for immune evasion [18] and homologous recombination has also been exploited to facilitate genetic manipulation. Histone PTM is likely to be involved in all of these processes and is likely to be important to coordinate transcription with DNA replication, recombination and repair.

Some PTMs may be synonymous with those found in other organisms. Examples include trypanosome H3-K76-Me with H3-K79-Me which is involved in transcription repression [8]; H4-K4 and 10 with the histone deposition-related PTMs, H4-K5 and 12; H4-K14-Ac with H4-K16-Ac which alters chromatin structure [5] and H4-K18-Me with H4-K20-Me which is specifically involved in DNA repair in yeast [19]. Other features of histone PTM may act as a guide for studies in trypanosomes. Ac generally determines transcriptional activity (open chromatin), while Me is generally more stable, with the effect more dependent upon the modified site. One particular histone acetylase, Elp3p, is the catalytic component of the transcription elongator complex and directly links Ac to transcription. Interestingly, trypanosomatids appear to be the only organisms with two Elp3p orthologues.

It will also be important to consider how histone PTM distribution intersects with DNA modification [20], linker histones [21] and histone variants (which can also be modified) and chromatin remodelling factors. *T. brucei* histone variants, for example, are enriched within repetitive DNA and are largely absent from sites of transcription [22]. Histone PTM and the RNA interference (RNAi) machinery intersect to repress gene expression in some organisms but RNAi does not operate in all trypanosomatids and initial experiments did not reveal such an association in *T. brucei* [23].

Finally, studies on histone PTM under way in various parasites may reveal opportunities for chemotherapy. Histone deacetylase inhibitors are progressing well in phase II clinical trials for cancer chemotherapy, related compounds are active against trypanosomatids [24] and potential targets have been genetically validated in *T. brucei* [10].

Future prospects

To determine the role of each PTM it will be crucial to link the various (putative) histone modifiers and PTM-binding factors to specific PTM(s). This process will require the application of, and generation of new, PTM-specific antibodies to examine the regions of the genome targeted by each modifier and the abundance and distribution of each PTM under various conditions. Also, the current work does not represent an exhaustive survey of PTM, so other modifications remain to be revealed. H3-K4-Me might be expected for example; Me3 at this residue determines gene activity in yeast [25]; and phosphorylation, ADP-ribosylation and other PTMs may also be important.

Most of the work to date has been carried out in *T. brucei* and similar approaches will continue to be important. However, functional-consequence assays are a weakness. New assays should be developed to assess the effect of specifically altering PTM by perturbing modifiers using gene knockout, or knockdown using RNAi or chemical probes, for example. Chromatin immunoprecipitation (ChIP) allows the detection of protein-DNA interactions and has been used in *T. brucei* to show that histone variants exhibit non-random distribution [22]. Regions of modified chromatin should be identified using ChIP and another option may be genome-wide ChIP in combination with microarrays [26]. Vast amounts of data are

available on the roles of histone PTM in other eukaryotes, particularly in yeast and humans. This will facilitate the identification of conserved chromatin control mechanisms in trypanosomatids, although it is the novelties in these divergent organisms that may be particularly interesting. Taking the approaches outlined above, mechanisms of genome regulation that impact on important aspects of trypanosomatid growth and pathogenesis should be revealed in the near future.

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Box 1**Methods used to characterise histone post-translational modifications****Histone purification and digestion**

- Acid-soluble histones were extracted from nuclei and purified by HPLC.
- Endoproteinases; Glu-C, Arg-C and trypsin were used to generate peptides.

Mass Spectrometry (MS)

- PTM changes the molecular weight of the protein or peptide and the resulting mass increment (Δm) can be detected relative to the nascent unmodified species.
- Tandem MS (MS/MS) involves mass analysis, ion manipulation and peptide sequencing. Briefly, an MS survey scan allows selection of peptide ions for isolation by virtue of their mass to charge ratio (m/z). The selected ion is then fragmented (the most labile peptide backbone bonds break preferentially) for the second round of m/z analysis. The mass intervals in the resulting b-ion (from N-terminus) and y-ion (from C-terminus) series reveal the amino acid sequence. Where the sequence is already known, deviation from the expected mass reveals PTM.
- Δm values: Removal of the leader Met, -131 Da; Ac, $+42$ Da; Me1, $+14$ Da; Me2, $+28$ Da; Me3, $+42$ Da (more labile relative to Ac).
- MALDI-TOF: Matrix-assisted laser desorption/ionisation time-of-flight MS.
- ESI: Electrospray ionisation MS.

Other methods: that also distinguish between Ac and Me3

- Acid-urea (AU)-PAGE: reveals the charge difference associated with Ac.
- PTM-specific antibodies.

Figure 1. Overview of histone PTM in trypanosomes

The figure was compiled from published data [2, 3, 8]. PTM in *T. brucei* and *T. cruzi* are indicated by triangles above and below the black bars respectively. The grey boxes indicate the approximate extent of the globular domains. Open triangles represent conserved residues where PTM is expected but has yet to be reported/defined. Where abundance estimates are available; bold text, >80%; grey text, 10% or less. Numbers below the bars represent mono (1), di (2) or tri (3) Me. Only Me1 was detected at *T. cruzi* H4-K18.