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Trypanosoma cruzi histone H1 is phosphorylated in a typical cyclin dependent kinase site accordingly to the cell cycle

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Abstract

Histone H1 of most eukaryotes is phosphorylated during the cell cycle progression and seems to play a role in the regulation of chromatin structure, affecting replication and chromosome condensation. In trypanosomatids, histone H1 lacks the globular domain and is shorter when compared with the histone of other eukaryotes. We have previously shown that in *Trypanosoma cruzi*, the agent of Chagas' disease, histone H1 is phosphorylated and this increases its dissociation from chromatin. Here, we demonstrate using mass spectrometry analysis that *T. cruzi* histone H1 is only phosphorylated at the serine 12 in the sequence SPKK, a typical cyclin-dependent kinase site. We also found a correlation between the phosphorylation state of histone H1 and the cell cycle. Hydroxyurea and lactacystin, which, respectively, arrest parasites at the G1/S and G2/M stages of the cell cycle, increased the level of histone H1 phosphorylation. Cyclin-dependent kinase-related enzymes TzCRK3, and less intensely the TzCRK1 were able to phosphorylate histone H1 in vitro. Histone H1 dephosphorylation was prevented by treating the parasites with okadaic acid but not with calyculin A. These findings suggest that *T. cruzi* histone H1 phosphorylation is promoted by cyclin dependent kinases, present during S through G2 phase of the cell cycle, and its dephosphorylation is promoted by specific phosphatases.

Keywords: Histone H1; Phosphorylation; Cell cycle; *Trypanosoma cruzi*; Phosphatase; CDK

1. Introduction

Histone H1, also known as linker histone, consists of a conserved central globular domain flanked by a relatively short amino- and a long carboxy-terminal tail. The globular domain seems to interact with linker DNA outside the nucleosome core, and the tails with the linker DNA and with the amino-terminal tails of core histones [1]. Histone H1 affects many features of chromatin structure and function. It stabilizes the high-order structure of chromatin [2,3] and affects nucleosome position and spacing [4,5]. It is involved in chromatin assembly during replication [6], chromatin remodeling [7] and condensation [8], gene transcription [9] and cell apop-

Abbreviations: a.m.u., atomic mass unit; ESI-TOF-MS, electrospray ionization-time of flight-mass spectrometry; ESI-IT-MS, electrospray ionization-ion trap-mass spectrometry; *m*/*z*, mass to charge ratio; CDKs, cyclin dependent kinases; HU, hydroxyurea; OA, okadaic acid; AUT-PAGE, polyacrylamyde gel electrophoresis containing acetic acid urea and Triton-DF-16; TzCRK1, *T. cruzi* cyclin related kinase 1; TzCRK3, *T. cruzi* cyclin related kinase 3.

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tosis [10]. However, the exact mechanism of interaction of this protein with the nucleosome remains unknown [11,12]. In addition, the essentiality of histone H1 is also a matter of controversy, as histone H1 knockouts are viable [13].

Different variants of the protein and post-translational modifications, mainly phosphorylation, seem to be involved in these histone H1 functions. It is well known that histone H1 phosphorylation increases as the cell progresses into the cell cycle, with specific phosphorylation events occurring at interphase, before mitosis. These phosphorylation events are catalyzed by cyclin-dependent protein kinases (CDKs) [1], mainly CDC2/cdc28 (yeasts CDK1), which are active from the S to M phase of the cell cycle [14–18], and are important for chromosome condensation. Many histone H1 phosphorylation events occur at S/TPXK sites along the tails, but, as reviewed earlier, there is no obvious correlation between the sites and a particular stage of the cell cycle [19]. More recently, molecular modeling studies have shown that the phosphorylation of histone H1 at CDC2 sites of the tail domains modifies the interaction of the protein with the DNA [20]. Indeed, replacement of threonines and serines by alanine in these phosphorylation sites in mammalian histone H1 decreases the protein mobility in the nucleus [18]. The phosphorylation at these sites seems to be required to promote chromatin replication in vitro [6]. As histone H1 becomes hyperphosphorylated before mitosis, when the chromosomes condense [8], and the localization of histone H1 is uncertain in the chromosome structure [21], it has been proposed that histone H1 phosphorylation might allow the binding of accessory factors to promote chromosome condensation [22].

Histone H1 of early protests, such as the Entamoebida, Kinetoplastids, Ciliates and Dinoflagellates, lacks the globular domain, presenting just the regions corresponding to the C-terminal domains of most eukaryotic histones [1]. Accordingly, there is no formation of the typical 30 nm fibers and no condensation of chromosomes during mitosis. In Kinetoplastids, a group containing several protozoan parasites (e.g., *Trypanosoma cruzi*, *Trypanosoma brucei* and several species of *Leishmania*), the presence of histone H1 has been extensively described [23–31], but the function of this protein is poorly understood. Histone H1 mRNA is mainly expressed in replicating forms during the S phase, but is also found in non-dividing cells [32–34]. We have recently shown that histone H1 of *T. cruzi*, the parasite that causes Chagas' disease, is differentially phosphorylated in proliferating versus non-proliferating forms of the parasite [35]. Also, we provided evidence that phosphorylated histone H1 is released from chromatin more easily than the non-phosphorylated form. Such histone H1 phosphorylation may not be related to the regulation of transcriptional activity, as this group of organisms show a primitive control of gene expression, with most of their genes being regulated at the post-transcriptional level [36]. As proteins similar to CDKs are present in the S phase of trypanosomatid extracts that can phosphorylate mammalian histone H1 in vitro [37–40], it is possible that the histone H1 phosphorylation could be promoted by these CDKs and be related to the cell cycle control in these organisms. These findings and the fact that trypanosomatids are disease-promoting agents, prompted us to study in more detail the localization of the phosphorylation sites of *T. cruzi* histone H1, the cell cycle regulation of this event, and the enzymes involved in the phosphorylation and dephosphorylation steps. We found that histone H1 is phosphorylated when cells progress from the S to M phase of the cell cycle at a typical CDK consensus site in an organism that does not condense chromosomes in mitosis and does not have transcriptional control.

2. Material and methods

2.1. Parasites, cell cycle synchronization and FACs analysis

T. cruzi (Y strain) epimastigote forms were cultured in liver infusion-tryptose medium, supplemented with 10% FBS at 28 °C [41]. Tissue culture derived trypomastigotes were obtained from infected LLCMK2 cells as described [42]. The medium containing parasites was collected and centrifuged at 1000 × *g*, the pellets were washed with phosphate-buffered saline (PBS) and immediately used, or stored at −70 °C. Hydroxyurea treatment was done as described [43]. For G2 phase blockage, 1.5×10^8 parasites at the exponential growth phase were incubated 24 h at 28 °C with 10 M lactacystin pre-dissolved in DMSO. Controls were performed with an equivalent volume of dimethyl sulfoxide. For flow cytometry analysis, 5×10^6 parasites were washed twice in PBS and fixed with 1 ml of 50% methanol in PBS at 4 °C for 10 min. The fixed parasites were washed once with PBS and incubated for 20 min at 37 °C with 10 μg ml^{-1} of DNAse-free RNase A, (Roche-Diagnostics). Samples were washed in PBS and resuspended in 500 μl of PBS containing 20 μg ml^{-1} propidium iodide and analyzed using a flow cytometer using the CELLQuest software (Becton–Dickinson Excalibur). The data corresponding to 10,000 events was analyzed using the WinMid 2.8 software. When indicated, parasites were washed in PBS and attached to glass slides coated with 0.1% poly-L-lysine in PBS. Attached parasites were fixed with 4% *p*-formaldehyde in PBS for 20 min, washed, stained with 10 μg DAPI/ml, and observed with a 100×/1.4 Plan-Apochromatic lens in a Nikon E600 fluorescence microscope.

2.2. Histones and histone H1 extraction and AUT gel electrophoresis

Frozen epimastigotes or trypomastigotes (5×10^8 parasites) were resuspended in 1 ml of 10 mM potassium glutamate, 250 mM sucrose, 2.5 mM $CaCl_2$ and lysed by the addition of 0.1% Triton X100. The lysate was centrifuged, washed once with the same buffer without Triton X100 and twice with the buffer lacking sucrose. All solutions contained 0.1 mM phenyl–methyl–sulfonyl fluoride, 0.2 mM benzami-