Biochemical and Biophysical Research Communications 370 (2008) 53–56

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Protein acetylation sites mediated by *Schistosoma mansoni* GCN5

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ARTICLE INFO

Article history:
Received 27 February 2008
Available online 17 March 2008

Keywords:
Schistosoma mansoni
SmGCN5
Histone acetyltransferase
Transcription activation

ABSTRACT

The transcriptional co-activator GCN5, a histone acetyltransferase (HAT), is part of large multimeric complexes that are required for chromatin remodeling and transcription activation. As in other eukaryotes, the DNA from the parasite *Schistosome mansoni* is organized into nucleosomes and the genome encodes components of chromatin-remodeling complexes. Using a series of synthetic peptides we determined that Lys-14 of histone H3 was acetylated by the recombinant SmGCN5-HAT domain. SmGCN5 was also able to acetylate schistosome non-histone proteins, such as the nuclear receptors SmRXR1 and SmNR1, and the co-activator SmNCoA-62. Electron microscopy revealed the presence of SmGCN5 protein in the nuclei of vitelline cells. Within the nucleus, SmGCN5 was found to be located in interchromatin granule clusters (IGCs), which are transcriptionally active structures. The data suggest that SmGCN5 is involved in transcription activation.

Gene expression in eukaryotes involves the participation of nucleosomes and the packaging of DNA into higher order chromatin structures [1]. A compact chromatin represents a physical barrier to transcription factors hindering their access to DNA. Biochemical mechanisms that circumvent this problem must then be invoked.

Post-translational modifications of nucleosomal histones have been associated to transcription activation or repression events in the chromatin [2,3]. One of the most extensively studied modifications is the acetylation of the highly conserved N-terminal histone tails. The basic repeating unit of chromatin is the nucleosome consisting of 146 bp of DNA wrapped around a histone octamer containing two copies of each histones H2A, H2B, H3, and H4. The steady-state acetylation level of histone proteins is accomplished by action of histone acetyltransferases (HATs) and histone deacetylases (HDACs) [4]. The modifications carried out by the different HATs take place on the ε-amino group of lysine (K) residues using acetyl coenzyme A as a coenzyme. Acetylation affects higher-order folding of chromatin fibers, loosening of the contacts between the DNA and the histones and/or non-histone proteins [5]. Thus, HATs are thought to increase the loosening of chromatin, which in turn may increase the accessibility of factors that promote transcription [5].

Several studies have provided a direct molecular link between histone acetylation and transcriptional activation [6,7]. In these reports, it has been shown that several previously identified co-activators/adaptors of transcription possess intrinsic HAT activity. Interestingly, many of these chromatin-modifying activities have been found within large multiprotein complexes. Importantly, these HAT-activity containing complexes can also function as simple acetyl transferases and catalyse the acetylation of non-histone substrates leading to changes in their activity or stability [8,9].

The yeast adaptor GCN5 (General Control Nonderepressible-5) histone acetyltransferase from *Saccharomyces cerevisiae* was the first transcription factor identified as a *bona fide* HAT [6]. The yeast GCN5 has been characterized as the HAT subunit of at least two large macromolecular complexes, SAGA (Spt-Ada-GCN5 acetyltransferase) and ADA [10]. The specific biological role of ADA is not clear. However, SAGA has been shown to stimulate transcription in a subset of yeast genes and requires HAT activity of the GCN5 subunit [10]. Both SAGA and ADA acetylate histone H3 and H2B on nucleosome targets [10]. Studies using recombinant GCN5 indicated that it displays a non-random specificity for acetylation of lysines, predominantly acetylating H3 at lysine 14 [10]. *In vivo*, acetylation of H3 can occur at lysines 9, 14, 18, and 23. In this regard, acetylation of lysine 9 of H3 is associated with histone deposition, whereas acetylation of lysine 14 is associated with transcription [11].

We have previously cloned the GCN5 from the human parasite *Schistosoma mansoni* (SmGCN5) [12]. We have shown that SmGCN5 was able to mono-acetylate histones H3 and H2A [12]. Moreover, we have recently demonstrated the involvement of SmGCN5 with the nuclear receptor signaling pathway in *S. mansoni* [13]. In this context, we have shown that SmGCN5 physically interacted with schistosome nuclear receptors SmRXR1 and SmNR1, as well as with the schistosome co-activators SmNCoA-62 and SmCBP-1 [13].

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doi:10.1016/j.bbrc.2008.03.022

In the present study, we provided evidence that may link SmGCN5 to transcription regulation in *S. mansoni*.

Materials and methods

Histone acetyltransferase (HAT) assay. Expression and purification of the SmGCN5/HAT enzyme were carried out as previously described [12]. HAT assays were performed using the following substrates: recombinant GST (glutathione-*S*-transferase), GST-SmNCoA-62, MBP (maltose binding protein), MBP-SmRXR1, and MBP-SmNR1, all bound to the beads; commercial histone H3 (H4380-SIGMA) and histone H3 and H4 N-terminal synthetic peptides (kindly synthesized by Dr. Luis Juliano Neto, Escola Paulista de Medicina, São Paulo, Brazil). Acetylation reactions were carried out with 0.5 μg of recombinant GST-SmGCN5/HAT and 0.25 μCi [^{3}H]acetyl-CoA (GE HealthCare) in reaction buffer containing 50 mM Tris, pH 8.0, 5% glycerol, 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 1 mM PMSF, at 30 °C in a total volume of 30 μl. The samples with the bead-bound proteins were washed three times with PBS and resuspended in SDS sample buffer. The histone H3 samples and the peptides were directly resuspended in SDS sample buffer. Gels were stained with Coomassie Blue, destained, then soaked with Amplify (GE HealthCare) for 30 min and vacuum dried. The gels were exposed to Hyperfilm (GE HealthCare) and left at −80 °C for 1–7 days.

Transmission electron microscopy and immunolabeling. Adult worms of *S. mansoni* were obtained by perfusion of Swiss mice and immersed in fixative solution containing 0.7% glutaraldehyde (v/v), 0.1% picric acid, 0.1% sucrose, 2% paraformaldehyde and 5 mM $CaCl_2$ in 0.1 M cacodylate buffer (pH 7.2), dehydrated in ethanol and embedded in Unicryl resin (Ted Pella, Redding, CA). Ultrathin sections were quenched in NH_4Cl for 30 min and incubated in the presence of polyclonal anti-SmGCN5 antibody (1:100), raised against the bromo-domain of the enzyme. Controls consisted of pre-immune serum used in the same concentration as the immune serum. After several washes in PBS with 1% albumin, sections were incubated in the presence of 10 nM gold labeled goat anti-rabbit IgG (BB International, UK), washed and observed in a ZEISS 900 transmission electron microscope.

Results

Acetylation of histone H3–K14 by SmGCN5

The histone H3 N-terminus contains four lysine residues that are acetylation sites for lysine–histone acetyltransferases. We have previously shown that H3 is acetylated by SmGCN5 [12]. In order to identify which of the lysine residues in H3 are acetylated by SmGCN5, we used synthetic peptides as substrates. One was based on the wild type sequence of the mammalian H3 N-terminus and the three remaining peptides carried each a substitution in one of the lysine residues (Fig. 1A). Acetylation assays showed that SmGCN5 specifically acetylated lysine 14 of histone H3 (Fig. 1B). Moreover, we showed that histone H4 was not a substrate for SmGCN5 (Fig. 1B, lane 5).

In vitro acetylation of SmRXR1, SmNR1 and SmNCoA-62 by SmGCN5. The acetylation of lysine groups in transcription factors modulates their activities, both *in vivo* and *in vitro* [14]. We then investigated whether SmGCN5 could acetylate recombinant SmRXR1 and SmNR1 *in vitro*. The results in Fig. 2 showed that SmGCN5 was able to acetylate full length SmRXR1 (Fig. 2, lane 4), but not full length SmNR1 protein (Fig. 2, lane 5). The observation that SmNCoA-62 contains a large number of lysine residues throughout the polypeptide chain, prompted us to test the ability of SmGCN5 to acetylate this protein. As shown in Fig. 2, lane 2, SmGCN5 acetylated SmNCoA-62. Note that the fusion moieties GST and MBP were not acetylated by SmGCN5 (Fig. 2, lanes 1 and 3). The asterisks in SmRXR1 and SmNR1 indicate the full length proteins; the arrows indicate proteolysis of the full length proteins.

SmGCN5 is localized within interchromatin granule clusters of euchromatin

There is increasing evidence showing that gene activity is non-randomly organized within the cell nucleus [15,16]. Of particular interest, a spatial and functional relationship between intranuclear

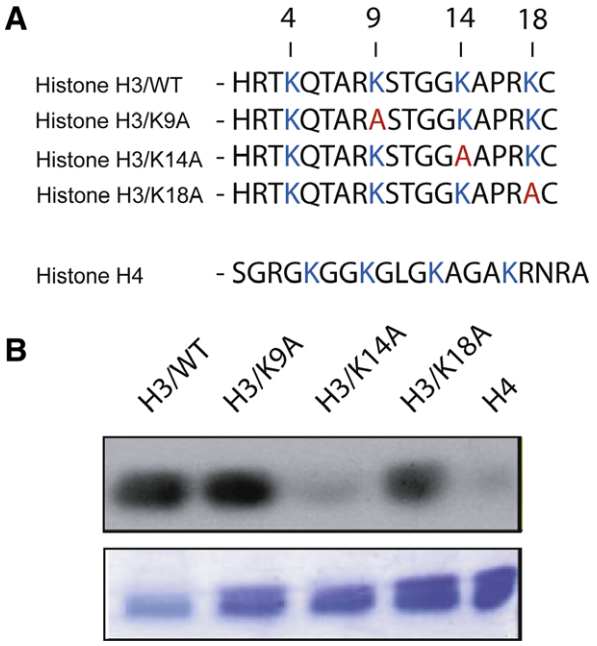

Fig. 1. Lysine 14 of histone H3 is specifically acetylated by SmGCN5. (A) N-terminal peptide of histone H3 (wild-type) and its respective mutated peptides (lysines, in blue, were substituted with alanines, in red). The positions of lysines are marked. Histone H4 N-terminal peptide (wild-type) was used as a control. (B) SmGCN5-mediated *in vitro* acetylation assays were carried out using equal amounts of each peptide (monitored by Coomassie Blue staining). The target acetylated residue was identified by autoradiography. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

structures, termed interchromatin granule clusters (IGCs), and specific transcribed loci has been reported [17]. By using transmission electron microscopy and immunolabelling, we were able to localize SmGCN5 within the nucleus and nucleolus of *S. mansoni* adult parasites (Fig. 3, panel B, arrows). Importantly, SmGCN5 was mainly concentrated within IGCs (Fig. 3, panel B, arrows). In addition, SmGCN5 labeling was found in condensed chromatin (Fig. 3, panel B). When the pre-immune serum was used, no labeling was observed (Fig. 3, panel A). It is worth mentioning that in the nucleus, SmGCN5 was observed mainly in the mature stage (S4) of the vitelline cells of female worm sections. The S4 stage of the vitelline cells could be detected by the presence of vitelline droplets (Fig. 3, panel A). However, SmGCN5 was also found in other tissues of female parasites (data not shown).

Discussion

The SAGA and ADA complexes are essential actors of transcriptional gene regulation in eukaryotes. Their structure and composition have been conserved throughout evolution, from yeast to human, and they exert their function through the catalytic activity of an HAT component. Vertebrate GCN5 have been involved in many biological processes, including the response to steroid/retinoid hormones, cell proliferation and cell differentiation. However, in other organisms, such as the parasite *S. mansoni,* how the function of GCN5 is integrated in the control of development has still to be defined.

The pathology of Schistosomiasis develops through the deposition of eggs in the liver and spleen of infected individuals [18]. Around three hundred eggs are laid everyday by a single sexually mature female worm that is paired to a male.

The *p*14 gene from *S. mansoni* encodes a major eggshell precursor that is expressed only in sexually mature female worms upon

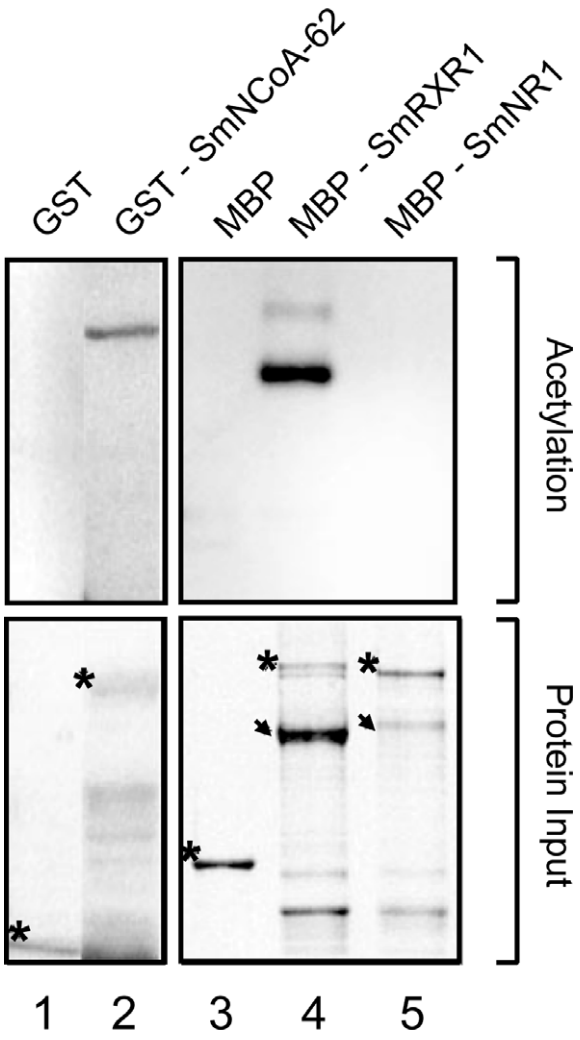

Fig. 2. Acetylation of SmRXR1, SmNR1 and SmNCoA-62 by SmGCN5. Equal amounts of MBP-SmRXR1, MBP-SmNR1 and GST-SmNCoA-62 incubated with recombinant GST-SmGCN5-HAT domain in the presence of [^{3}H]acetyl coenzyme A. The acetylated SmRXR1 and SmNCoA-62 full length proteins were detected by fluorography (lanes 2 and 4). SmNR1 was not acetylated by SmGCN5 (lane 5). No acetylation was observed in GST or MBP alone. The asterisks indicate the full length proteins and the arrows indicate proteolysis of the full length proteins.

mating with male worms [18]. Due to the importance of the *p*14 gene in eggshell development, we (and others) have concentrated our efforts to understand the molecular mechanisms of how the *p*14 gene is regulated. Cumulative data indicate that the *p*14 gene is regulated by nuclear receptors [18–20]. Since it is now well established that GCN5 proteins act as a co-activator in nuclear receptor signaling pathways, we envision that SmGCN5 might be playing a role in the regulation of the *p*14 gene expression. In this respect, we recently showed that, *in vitro*, SmGCN5 was assembled within a high molecular weight complex containing the target DNA, the SmRXR1/SmNR1 heterodimer and the coactivators SmNCoA-62 and SmCBP-1 [13].

In the present work, we report data that support with our hypothesis that SmGCN5 plays a role in transcription activation, and possibly, in transcription activation of the *p*14 gene.

It has been demonstrated that GCN5-dependent complex ADA preferentially acetylates Lys 14 on synthetic H3 peptide substrates and on nucleosomal H3 [1]. Importantly, acetylation of H3 Lys 14 has been linked to transcription activation in both yeast and vertebrate cells [10]. In this respect, we clearly showed that *S. mansoni* GCN5 specifically acetylated lysine 14 of a histone H3 peptide (Fig. 1). Thus, SmGCN5 may affect the cellular transcription profile of this parasite by modifying its higher-order chromatin structure.

The suggestion that SmGCN5 may indeed be involved in transcription activation (possibly of the *p*14 gene) was also based on our results obtained with the electron microscopy. Using antibodies against SmGCN5, we demonstrated the presence of this enzyme in the nuclei of vitelline cells of sexually mature female worms (Fig. 3). SmGCN5 was almost exclusively concentrated in interchromatin granule clusters (IGCs). IGC constituents include, but are not limited, to small nuclear ribonucleoprotein particles (snRNP), splicing factors, and the hyperphosphorylated form of the large subunit of RNA polymerase II [21], and thus, are considered an active transcriptional compartment [22].

It has been shown that histone acetyltransferases, besides histones, also possess a factor acetyltransferase activity, in that they acetylate non-histone substrates, such as specific and general transcription factors or chromatin-related proteins [8]. Transcription factor acetylation can modify protein–DNA and protein–protein interactions [8]. We have recently demonstrated that the DNA binding domain of SmRXR1 and SmNR1 were acetylated by SmGCN5 [13]. Curiously, when we mapped the acetylation sites of both receptors by using synthetic peptides based on putative acetylation sites, we observed acetylation in two peptides from SmNR1 sequence. However, no acetylation was seen with the peptides from SmRXR1 [13]. To try to clarify these discrepancies, we used full length SmRXR1 and SmNR1 proteins as substrates of SmGCN5 (Fig. 2). Our results showed that full length SmRXR1

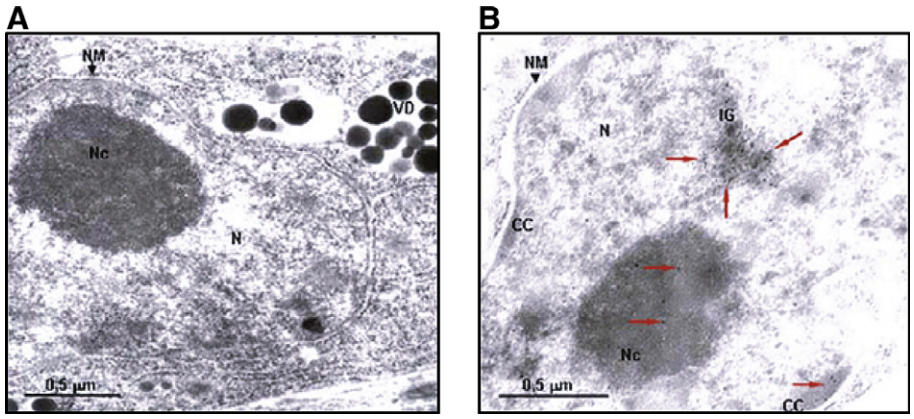

Fig. 3. Electron microscopy and immunolabelling of SmGCN5. Polyclonal antibody was raised against the bromo domain of SmGCN5 to identify the enzyme in *S. mansoni* ultrathin section. (A) Parasite sections were reacted with pre-immune serum. (B) Parasite sections were reacted with SmGCN5 specific antibody. Intense labeling of SmGCN5 was observed in interchromatin granules (arrows). NM, nuclear membrane; VD, vitelline droplet; N, nucleus; CC, condensed chromatin; Nc, nucleolus and IG, interchromatin granules.

protein was highly acetylated by SmGCN5. Alternatively, full length SmNR1 protein was not acetylated by SmGCN5. One explanation for the lack of acetylation of SmRXR1 [13] is that the synthetic peptide is not conducive to acetylation by SmGCN5. In the same context, the lack of acetylation of SmNR1 could be due to the steric hydrance posed by the MBP moiety in SmNR1.

In summary, the data presented here provide insights into the role that SmGCN5 may play in transcription regulation in the parasite *S. mansoni*.

Acknowledgments

We thank Dr. Phil LoVerde and Dr. Wenjie Wu for providing the MBP-SmRXR1 and MBP-SmNR1 clones. This research received grants to MRF, from Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq-Universal 2007 and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, FAPERJ. We wish to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES for providing the fellowship to R.M.M.

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