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Functional connection between histone acetyltransferase Gcn5p and methyltransferase Hmt1p

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ABSTRACT

Histone acetylation and methylation are linked to a variety of nuclear activities, most notably transcriptional regulation. Both synergistic and antagonistic relationships between these two modifications have been reported in different systems. Here we show that the budding yeast histone H4 arginine 3 (R3) methyltransferase Hmt1p binds acetylated histones H3 and H4, and importantly, that acetylated H4 is a significantly better methylation substrate for Hmt1p. Kinetic studies show that acetylation at any of the four acetylatable lysine residues of histone H4 results in more efficient methylation. Among the four, K8 acetylation imposes the strongest effect on reducing K_M, consistent with the observed acetylation-stimulated interaction. In vivo, *hmt1Δ* cells rescue the transcriptional defect caused by *GCN5* deletion, indicating that one of the functions of Gcn5p is to neutralize the negative effect of Hmt1p. Mutating either K8 or R3 to alanine causes similar growth defects in selective histone and *gcn5* mutant background, suggesting that these two residues function in the same pathway for optimal vegetative growth. Together, these results reveal a functional connection between histone acetylation, methylation, and two of the responsible enzymes, Gcn5p and Hmt1p.

1. Introduction

Acetylation is one of the best-characterized post-translational modifications of histones. A variety of nuclear activities including transcriptional regulation, chromatin assembly, DNA repair, recombination, and mitosis, have been linked to histone acetylation (see [1] and references therein). Mechanistically, histone acetylation may reduce the affinity of histone–DNA interactions[2], changes the chromatin structures [3], and recruits or evicts selective histone-binding proteins [4,5]. Furthermore, methylation, phosphorylation, and ubiquitination of histones in many cases are functionally tied to histone acetylation [6]. Both synergism and antagonism have been observed between different histone modifications.

In the budding yeast *Saccharomyces cerevisiae*, Gcn5p is a prototypical histone acetyltransferase (HAT) [7]. Histones H3, H4, and H2B are acetylated by Gcn5p [8–10], however the substrate specificity (histones and the target lysine residues) may vary depending on the assay conditions [11]. Gcn5p is the catalytic subunit of several chromatographically distinct complexes including SAGA [12], SALSA/SLIK [13,14], and ADA [15], that have been linked to transcriptional regulation in yeast (see [16] for review). Recent works also linked Gcn5p and its HAT activity to DNA damage repair [17] and chromosome segregation [18]. In addition to Gcn5p, there are multiple HATs and histone deacetylases (HDACs) that target at different or overlapping histones and lysine residues for a variety of nuclear activities [19]. How these enzymes and selective acetylated histones appropriately perform their biological functions is an important yet largely unanswered question.

A prevailing hypothesis explaining the functional diversity of histone acetylation is that selective proteins are recruited to genomic domains with certain acetylated histones. The notion of modification-dependent recruitment of specific factors has been well-documented in protein phosphorylation [20], and there are an increasing number of examples for other modifications, such as methylation and sumoylation [21]. Similarly, acetylated histones interact with a conserved protein module, the bromodomain [4,22,23], which is found in many transcriptional regulators [24]. Recruitment of bromodomain-containing proteins is important for setting the boundary between euchromatin and heterochromatin [25]. However, the wide spectrum of nuclear functions linked to histone acetylation appears to exceed the number of proteins possessing the bromodomain, suggesting that other protein modules may be capable of binding to the acetylated histones. Using the tethered catalysis/yeast two-hybrid system designed to identify protein-protein interactions stimulated by post-translational modifications [26], several acetylated

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histone binding proteins were found, including Hmt1p (a.k.a. Rmt1p) for protein and histone H4 methylation [27–29], Cac1 (a.k.a. Rlf2) for chromatin assembly [30], and Exo84p that has been implicated in mRNA splicing [31,32]. These results are in excellent agreement with the observations that histone acetylation functions beyond transcriptional regulation. In the current work, we focused on Hmt1p.

Hmt1p is a type I protein arginine methyltransferase that catalyzes asymmetric dimethylation [33,34]. Known substrates for Hmt1p include hnRNP components [28,29], several nucleolar proteins [35], and histone H4 [27]. Arginine 3 (R3) of histone H4 is the methylation target of Hmt1p [27]. Importantly, the Hmt1p methyltransferase activity is critical for rDNA silencing mediated by a histone deacetylase Sir2p [36]. Loss of Hmt1p methyltransferase activity results in hypomethylation of H4 at silent loci, and in impairment of Sir2p recruitment [36]. The transcriptional repression function of Hmt1p differs significantly from its mammalian homologue, PRMT1, in that the latter is important for transcriptional activation of multiple genes [37–39]. PRMT1-mediated histone H4 R3 methylation acts upstream of the p300 histone acetyltransferase in transcriptional activation [37]. How the yeast Hmt1p performs its transcriptional regulatory function remains an open question.

In this work, we present evidence that Hmt1p is an acetylated histone binding protein, and that acetylated H4 is a better substrate for Hmt1p methyltransferase activity. Transcriptional defects caused by the *gcn5* null allele can be suppressed by deleting *HMT1*. Furthermore, histone H4 K8 acetylation and R3 methylation appear to be part of a pathway that becomes essential for optimal mitotic growth in a background devoid of Gcn5p HAT activity and the predominant histone H3 acetylation site. These data reveal novel interactions between histone acetylation and methylation.

2. Materials and methods

2.1. Yeast strains and plasmids

All genetic methods were performed according to standard procedures [40]. Yeast transformation was done using the lithium acetate method [41]. The GST-Hmt1 plasmid was generously provided by S. Clarke (UCLA) [34]. The *dot1Δ* and *hmt1Δ* strains were a gift from J. Côté (Lava University).

To create yeast strains for RNA analyses and 3-AT tolerance tests, BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*), yMK1180 (isogenic to BY4742 except *MATa dot1Δ*), and yMK118 (isogenic to BY4742 except *MATa hmt1Δ*) were first transformed with the 1.75 kb Bam HI/Bam HI fragment from pJJ217 [42] that contained the entire *HIS3* gene, resulting in yMK1185, yMK1186, and yMK1187, respectively. This procedure was to convert these strains to HIS^+ so that the transcriptional status of *HIS3* and cellular sensitivity to 3-AT could be tested. *GCN5* was subsequently deleted from these three strains by using a *gcn5Δ::hisG-URA3-hisG* fragment derived from pMK147 (following Xho I and Xba I digestion and gel-purification of the 4.6 kb fragment) to create yMK1188 (*gcn5Δ*), yMK1189 (*dot1Δ gcn5Δ*), and yMK1190 (*hmt1Δ gcn5Δ*).

For histone mutants, pQQ18 (*ARS1 CEN4 LEU2 HTA1-HTB1-HHT2-HHF2*) [43] was subjected to mutagenesis (see [44] for detailed procedures; oligonucleotide sequences are available upon request) to replace selective lysine or arginine residues (Fig. 4). JHY205 [43] (*MATa his3Δ1 leu2Δ0 ura3Δ0 hta1-htb1Δ::HPH hta2-htb2Δ::NAT hht1-hhf1Δ::KAN hhf2- hht2Δ::NAT* <pJH33 *ARS1 CEN4 URA3 HTA1-HTB1-HHT2-HHF2*>) was transformed with *Ngo* MIV-digested pMK284 F221A to replace the chromosomal copy of *GCN5* with the F221A allele [44], resulting in yDA12. Desired histone mutant plasmids (pQQ18 derivatives) were transformed to either $GCN5^+$ or *gcn5* F221A strains. Leu^+Ura^+ strains were grown overnight in YPD to saturation before plating to 5-FOA medium to assess viability.

2.2. Protein expression, purification, and biochemical assays

Induction and purification of the recombinant Hmt1p were according to Gary et al. [34]. Bacterially expressed yeast histones H3 and H4 were a kind gift of K. Luger (Colorado State University, Fort Collins). Synthetic histone H4 peptides were purchased from the Upstate Biotechnology Inc. ^{3}H-S-adensyl-methionine (SAM) was purchased through Amersham (15 Ci/mmol). Purification of core histones from yeast was based on Edmondson et al. [5]. Methods for recombinant Gcn5p production and in vitro histone acetylation were as previously described [8] except that 1 μg of recombinant histone H3 or H4, or approximately 10 μg of yeast core histones were first treated with 50 ng of His-tagged Gcn5p in 20 μl reactions containing 50 mM Tris–HCl, pH 8.0, 10% glycerol (v/v), 1 mM EDTA and 1 mM unlabelled acetyl coenzyme A. For mock acetylation reaction, 1 mM of coenzyme A was substituted for acetyl coA. The acetylation reactions were conducted at 30 °C for 30 min, immediately followed by GST pulldown or in vitro methylation reactions.

For GST pulldown assays, approximately 1 μg of GST-Hmt1p was added to acetylation or mock acetylation reactions that had been brought to 200 μl with the acetylation buffer without the cofactor. The reactions were gently rocked at 4 °C for overnight, followed by addition of 5 μl of reduced glutathione beads (1:1 slurry). The binding reaction was continued at 4 °C for an additional h. The matrix was pelleted (14,000 rpm for 15 s at room temperature), and washed twice with 500 μl of acetylation buffer. 20 μl of 1× SDS-PAGE loading dye was added to the beads, which were then boiled for 5 min. The supernatant was loaded to 15% SDS-PAGE for resolution and Coomassie Blue staining.

A typical methylation reaction (20 μl) (for non-kinetic studies) contained approximately 3 μM histone or H4 peptide substrates, 3.3 μM of ^{3}H-SAM, and about 50 ng of recombinant GST-Hmt1p in 50 mM Tris–HCl, pH 8.0, 10% glycerol (v/v), and 0.1 mM EDTA. Reactions were carried out at 30 °C for 30 min before P-81 filter assays to assess the incorporation (see below). Alternatively, 15% SDS-PAGE was used to resolve histones for fluorography. Acetylation and methylation reactions were done in the same buffer. Thus, methylation of Gcn5p-acetylated histones was conducted by directly adding radioactive SAM and GST-Hmt1p to the (mock) acetylation reactions after the 30-minute acetylation reaction had been completed. Methylation was extended for 30 min before SDS-PAGE loading dye was added to stop the reaction.

Kinetic studies of H4 peptide methylation was conducted in the following way. Each 10-μl reaction contained 50 mM Tris–HCl, pH 8.0, 10% glycerol, 0.1 mM EDTA, app. 25 ng of GST-Hmt1p, varying concentrations of peptide (ranging from 0.01 to 10 μM), and 3.3 μM ^{3}H-SAM. All ingredients except ^{3}H-SAM were mixed and made into 9-μl aliquots. The mixture was capped and pre-warmed at 30 °C for 30 s. 1 μl of 33 μ_M ^{3}H-SAM was then added to each tube to start the reaction. Reactions were carried out for exactly 3 min. Four 2-μl aliquots from each reaction were then spotted to Whatman P-81 paper discs [45]. After all sample discs were air-dried, they were washed for 10 min in generous amount of 20 mM $NaHCO_3$, pH9.2 under gentle shaking. The wash was repeated twice, followed by brief 95% ethanol wash and air dry. Each disc was then submerged in 2 ml of scintillation fluid and shook gently overnight at room temperature before scintillation counting. The initial rate of methylation from each concentration of the peptide was calculated, and K_M and Vmax were derived from the Hanes/Wolffe $[S]/v$ vs. $[S]$ plot $\{[S]/v = (1/\mathrm{Vmax})\cdot[S] + (K_M/\mathrm{Vmax})\}$. Average numbers of K_M and Vmax are presented in Fig. 2C.

2.3. RNA preparation and RT-PCR

Yeast cells were grown in appropriate selective media till culture density reached 0.5 OD_{600}/ml. Cells were then collected by centrifugation (5000 ×*g*, 5 min, 4 °C) and transferred to synthetic minimal