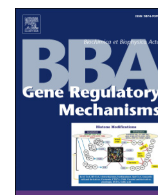
Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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



Histone hypoacetylation-activated genes are repressed by acetyl-CoA- and chromatin-mediated mechanism

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 12 May 2014
Accepted 29 May 2014
Available online xxxx

Keywords:
Histone acetylation
Transcription
Acetyl-CoA
Chromatin
HSP12

ABSTRACT

Transcriptional activation is typically associated with increased acetylation of promoter histones. However, this paradigm does not apply to transcriptional activation of all genes. In this study we have characterized a group of genes that are repressed by histone acetylation. These histone hypoacetylation-activated genes (HHAAG) are normally repressed during exponential growth, when the cellular level of acetyl-CoA is high and global histone acetylation is also high. The HHAAG are induced during diauxic shift, when the levels of acetyl-CoA and global histone acetylation decrease. The histone hypoacetylation-induced activation of HHAAG is independent of Msn2/Msn4. The repression of *HSP12*, one of the HHAAG, is associated with well-defined nucleosomal structure in the promoter region, while histone hypoacetylation-induced activation correlates with delocalization of positioned nucleosomes or with reduced nucleosome occupancy. Correspondingly, unlike the majority of yeast genes, HHAAG are transcriptionally upregulated when expression of histone genes is reduced. Taken together, these results suggest a model in which histone acetylation is required for proper positioning of promoter nucleosomes and repression of HHAAG.

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1. Introduction

The eukaryotic genome is highly organized and compacted into chromatin within the nucleus. The packaging of DNA into chromatin poses a barrier for certain fundamental cellular processes that require access to DNA, and cells rely on complexes that affect structure of chromatin to regulate DNA accessibility [1]. Several different mechanisms contribute to the dynamic properties of chromatin. ATP-dependent remodeling complexes use energy to noncovalently modify the chromatin structure and histone-modifying complexes add or remove covalent modifications from histones. Histone variants and chaperones also contribute to the regulation of chromatin dynamics [2,3]

Histone acetylation is a dynamic modification that occurs on all four core histones; it affects chromatin structure and regulates diverse cellular functions, such as gene expression, DNA replication and repair, and cellular proliferation [4]. Acetylation and deacetylation of chromatin histones, mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, represent the major mechanisms for epigenetic gene regulation. HATs catalyze the acetylation of lysine residues, neutralizing positive charges, relaxing chromatin structure and increasing the accessibility of DNA to the transcription machinery. HDACs remove acetyl groups from histones, thus inducing chromatin condensation and transcriptional repression [5,6]. In addition, the acetylated lysines in histones bind a host of bromodomain-containing proteins that are involved in chromatin remodeling and transcriptional regulation [7,8].

One of the mechanisms that globally regulate acetylation of chromatin histones is cellular concentration of acetyl-CoA that is available to HATs [9–11]. In mammalian cells, the nucleocytosolic enzyme ATP-citrate lyase is the major source of acetyl-CoA for histone acetylation [12]. In yeast, acetyl-CoA synthetase produces acetyl-CoA used by HATs [13]. In both yeast and mammalian cells, the nucleocytosolic acetyl-CoA is the link between cellular energy and carbon metabolism, and histone acetylation and chromatin regulation [9,10,14–16].

Histone acetylation and cellular level of acetyl-CoA are highest during exponential growth of yeast cells, when glucose in the medium is abundant. As the cells enter diauxic shift when glucose is exhausted, and switch their metabolic mode from glycolysis to respiration, the cellular levels of acetyl-CoA and global histone acetylation decrease [16–18]. Transcriptional activation is typically associated with increased acetylation of promoter histones [4,19]. The expression of genes required for rapid yeast growth when glucose is abundant parallels the cellular level of acetyl-CoA and histone acetylation of the corresponding promoters [16,20]. However, this paradigm does not necessarily apply to transcriptional activation of genes induced during diauxic shift and

Abbreviations: HHAAG, histone-hypoacetylation activated genes; RNA pol II, RNA polymerase II; ChIP, chromatin immunoprecipitation; HAT, histone acetyltransferases; HDAC, histone deacetylases; NFR, nucleosome-free region.

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http://dx.doi.org/10.1016/j.bbagrm.2014.05.029
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Please cite this article as: S. Mehrotra, et al., Histone hypoacetylation-activated genes are repressed by acetyl-CoA- and chromatin-mediated mechanism, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagrm.2014.05.029

stationary phase. These genes are induced when glucose is exhausted and cellular level of acetyl-CoA and global histone acetylation are decreased [16–18].

In this study we report that histone hypoacetylation induces expression of a group of genes that are normally induced during diauxic shift. We refer to these genes as histone hypoacetylation-activated genes (HHAAG). During exponential growth, when the nucleocytosolic concentration of acetyl-CoA is high, the HHAAG are repressed by histone acetylation. Since decreased flow of glucose through the glycolytic pathway induces expression of these genes, we propose that the histone acetylation-mediated regulation of these genes depends on the metabolic state of the cell and that histone hypoacetylation may represent a mechanism how cells detect and respond to a metabolic stress and low level of acetyl-CoA. In this study, we report on mechanisms whereby decreased histone acetylation affects chromatin structure and expression of HHAAG.

2. Materials and methods

2.1. Yeast strains and media

All yeast strains used are listed in Table 1 and were derived from previously described strains [21–24]. The strains used are isogenic to the W303 background. Standard genetic manipulation techniques were used to move mutations from the non-W303 strains into the W303 background [25]. Cells were grown at 28 °C in rich medium (YPD, 1% yeast extract, 2% Bacto™ Peptone, 2% glucose) or under selection in synthetic complete medium (SC) containing 2% glucose and, when appropriate, lacking specific nutrients in order to select for a particular genotype.

Table 1
Yeast strains used in this study.

Strain	Genotype	Source/reference
W303-1a	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303-1α	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303	MATa/MATα ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1ura3-1/ura3-1 can1-100/can1-100	R. Rothstein
JHY200	*MATa leu2–3,112 trp1–1 can1–100 ura3-1 ade2-1 his3–11,15 hta1-htb1::Nat hta2-htb2::HPH hht1-hhf1::KAN hht2-hhf2::KAN pJH33* [*URA3 CEN ARS HTA1-HTB1 HHT2-HHF2*]	[21]
LG329	W303-1a *hta1-htb1::Nat hta2-htb2::HPH hht1-hhf1::KAN hht2-hhf2::KAN* pQQ18 [*LEU2 CEN ARS HTA1-HTB1 HHT2 -HHF2*]	This study
LG341	W303-1a *hta1-htb1::Nat hta2-htb2::HPH hht1-hhf1::KAN hht2-hhf2::KAN* pQQ18 [*HTA1-HTB1 HHT2 (K9R, K14R, K18R)-HHF2*]	This study
LG345	W303-1a *hta1-htb1::Nat hta2-htb2::HPH hht1-hhf1::KAN hht2-hhf2::KAN* pQQ18 [*HTA1-HTB1 HHT2-HHF2 (K5R, K8R, K12R)*]	This study
LG548	W303-1a *hta1-htb1::Nat hta2-htb2::HPH hht1-hhf1::KAN hht2-hhf2::KAN* pQQ18 [*HTA1-HTB1 HHT2 (K9R, K14R, K18R)-HHF2 (K5R, K8R, K12R)*]	This study
DY5116	W303-1α *gcn5::HIS3*	[22]
DY4548	W303-1α *rpd3::LEU2*	[22]
DY5068	W303-1α *hda1::URA3*	[22]
YTT2256	W303-1a *yng2:: NatMX*	[23]
AD130	W303-1a *msn2::HIS3 msn4::TRP1*	[24]
KG161	W303-1a *yng2:: NatMX msn2::HIS3 msn4::TRP1*	This study
SM137	W303-1a *yng2:: NatMX rpd3::LEU2*	This study
YJL127C	BY4741 *spt10::Kan*	Open Biosystems
MZ672	W303-1a *spt10::Kan*	This study

2.2. Western blotting

Yeast strains were inoculated at $A_{600nm} = 0.1$ and grown to $A_{600nm} = 0.8$ in YPD medium. Four A_{600nm} units were harvested and boiled immediately in SDS sample buffer. Denatured proteins were separated on a 15% denaturing polyacrylamide gel and western blotting with anti-histone H3 polyclonal antibody (ab1791; Abcam) at a dilution of 1:1000, anti-acetyl histone H3 (Lys14) polyclonal antibody (acH3K14; 07-353, Upstate Biotechnology) at a dilution of 1:500, and anti-hyperacetylated histone H4 polyclonal antibody (acH4K5,8,12,16; 06-946; Upstate Biotechnology) at a dilution of 1:1000 was carried out as described previously [26]. To confirm equivalent amounts of loaded proteins, the membranes were also probed with anti-Pgk1p monoclonal antibody 22C5 (A6457; Invitrogen) at a dilution of 1:3000 and with actin polyclonal antibody (A5060; Sigma) at a dilution of 1:500.

2.3. Real time RT-PCR

Real-time RT-PCR was performed as described [26] using the following primers: *ACT1* (5'-TATGTGTAAAGCCGGTTTTGC-3' and 5'-GACAAT ACCGTGTTCAATTGGG-3'), *HSP12* (5'-AGTCATACGCTGAACAAGGTAA GG-3' and 5'-CGTTATCCTTGCCTTTTTCG-3'), *HSP26* (5'-AAGACGTCAG TTAGCAAACACACC-3' and 5'-CATTGTCGAACCAAT CATCTAAGG-3'), *CTT1* (5'- TCAACCCATACGCTTCTCAATACTC-3' and 5'-TCGAAC TCC AGTCTACAACCACC-3'), *HXT6* (5'-CTATTGCAGAGCAAACTCCTGTG-3' and 5'-TTCAGCCTTGTTTGATGGTGT-3'), *TMA10* (5'-GAACTAGCAAATGG ACAGTCCAC-3' and 5'-CTTTCCCATAGCCTCCTCTCTT-3'), *ACS1* (5'-CTCT GCCGTACAATCATCAA AAC-3' and 5'-CCGAAGTCAAATGTT CATACT CAT-3'), *SER3* (5'-CAAGCA TTGACATTAACAACTTACAA-3' and 5'-CTGT GGAACGGTATTCATGAAAG-3'), *RPS22B* (5'-AGCTGATGCTTTGAATGC CA-3' and 5'-TTCGCCAATGTAACCATGCT-3'), *RPS11B* (5'-AGACCCCAAA GACCGCTATT-3' and 5'-ATCTTGGTGGAGACGACGGTA-3'), *PYK1* (5'-TTGTTGCTGGTTCTGACTTGAG-3'and 5'-CAATGTTCAAACCAGCCTTTCTC-3'), *ADH1* (5'-AATCCCACGGTAAGTTGGAATAC-3' and 5'-AAGCGTGCAA GTCAGTGT GAC-3'), *PFK26* (5'-ACTTCTCTGAAACATCTCCTGTGC-3' and 5'-CTCCGGGATAAA AGATCATAACTG-3').

2.4. ChIP assay

Chromatin was crosslinked and immunoprecipitated as described [26]. The following antibodies were used for immunoprecipitation: anti-Msn2 polyclonal antibody (y-300, sc-33631, Santa Cruz Biotechnology, Inc.), anti-RNA polymerase II monoclonal antibody (8WGI6, Covance), and anti-histone H3 polyclonal antibody (ab1791; Abcam). Total input DNA and coimmunoprecipitated DNA were analyzed by real-time PCR with the Bio-Rad MyIQ single-color real-time PCR detection system (Bio-Rad). Each immunoprecipitation was performed at least three times using different chromatin samples, and the occupancy was calculated using the nucleosome free region (NFR) on chromosome XV (*CHR15*) [27] as a negative control and corrected for the efficiency of the primers. The results were calculated as fold increase in occupancy of the particular protein at the particular locus in comparison with the *CHR15* locus (5'-CAGTCCTTTCCCGCAATTTT-3' and 5'-GAAAATCATTAC CGAGGCATAAA-3'). The primers used for the ChIP assays were described previously [26,28,29].

2.5. Nucleosome-scanning assay

Nucleosome scanning analysis was performed as described [30–32] with minor modifications. Yeast cells were grown in 200 ml YPD to an A_{600} of 1.0 at 28 °C and converted to spheroplasts with yeast lytic enzyme (Sigma). Spheroplasts from each 200 ml culture were resuspended in 500 μl of ice-cold SPC buffer (1 M sorbitol, 20 mM PIPES, 0.1 mM $CaCl_2$, pH 8.3) and stored as 25 μl aliquots. In a 200 μl reaction, each 25 μl aliquot of spheroplasts was resuspended in 166 μl SPC buffer,

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