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

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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.

Keywords

Histone acetylation; transcription; acetyl-CoA; chromatin; *HSP12*

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

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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 (HAT) and histone deacetylases (HDAC), 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 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 nucleocytoplasmic 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 nucleocytoplasmic 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 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 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 nucleocytoplasmic 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.

2.2. Western Blotting

Yeast strains were inoculated at $A_{600\text{nm}} = 0.1$ in and grown to $A_{600\text{nm}} = 0.8$ in YPD medium. Four $A_{600\text{nm}}$ 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'-GACAATACCGTGTTCAATTGGG-3'), *HSP12* (5'-AGTCATACGCTGAACAAGGTAAGG-3' and 5'-CGTTATCCTTGCCTTTTTTCG-3'), *HSP26* (5'-AAGACGTCAGTTAGCAAACACACC-3' and 5'-CATTGTGGAACCAATCATCTAAGG-3'), *CTT1* (5'-TCAACCCATACGCTTCTCAATACTC-3' and 5'-TCGAAC TCC AGTCTACAACCACC-3'), *HXT6* (5'-CTATTGCAGAGCAAACCTCCTGTG-3' and 5'-TTCAGCCTTGTTTGATGGTGT-3'), *TMA10* (5'-GAACTAGCAAATGGACAGTCCAC-3' and 5'-CTTTCCCATAGCCTCCTCTCTT-3'), *ACS1* (5'-CTCTGCCGTACAATCATCAA AAC-3' and 5'-CCGAAGTCAAATGTTTCATACTCAT-3'), *SER3* (5'-CAAGCA TTGACATTAACAACCTTACAA-3' and 5'-CTGTGGAACGGTATTCATGAAAG-3'), *RPS22B* (5'-AGCTGATGCTTTGAATGCCA-3' and 5'-TTCGCCAATGTAACCATGCT-3'), *RPS11B* (5'-AGACCCCAAAGACCGCTATT-3' and 5'-ATCTTGTTGGAGACGACGGTA-3'), *PYK1* (5'-TTGTTGCTGGTTCTGACTTGAG-3' and 5'-CAATGTTCAAACCAGCCTTTCTC-3'), *ADH1* (5'-AATCCCACGGTAAGTTGGAATAC-3' and 5'-AAGCGTGCAAGTCAGTGTGAC-3'),

PFK26 (5'-ACTTCTCTGAAACATCTCCTGTGC-3' and 5'-CTCCGGGATAAAAGATCATAACTG-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'-GAAAATCATTACCGAGGCATAAA-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, and 6 µl of 100 mM CaCl_2 , followed by addition of 3 µl of 10% IGEPAL CA-630 to permeabilize the spheroplasts. Micrococcal nuclease (MNase; Worthington) was added immediately to a final concentration of 0, 1, 2.5, 10, 20, 50 U/ml. The samples were incubated for 5 min at 28°C with occasional gentle tapping of the microfuge tube. The MNase digestion reaction was terminated with 25 µl of stop solution (10% SDS, 100 mM EDTA, pH 7.4) and treated with 11 µl of 20 mg/ml of Proteinase K (Sigma) for 3 hours at 37°C. The samples were extracted with phenol-chloroform, ethanol precipitated and treated with 3 µl of 0.5 mg/ml RNase (Roche) for 2 hours at 37°C. Digested DNA was run on a 2% agarose gel and the reaction that yielded a predominantly mononucleosomal DNA was scaled up and processed as described above. The mononucleosome fraction from the scaled-up reaction was gel purified (Qiagen gel extraction kit) and used for quantitative real-time PCR analysis with a set of overlapping primers. Each primer generates a 100 ± 20 bp product. Primers were located at 30 ± 10 bp intervals and PCR efficiency for each primer pair was normalized with purified genomic DNA. Quantitative PCR analysis was performed using the iQ SYBR Green Supermix and Bio-Rad MyIQ real-time PCR detection system. The nucleosomal DNA enrichment level at a given region of DNA was calculated as a ratio of the PCR product obtained from the purified mononucleosomal DNA and the genomic DNA. The results were normalized for nucleosome free region (NFR) on Chromosome XV [27] as control and nucleosomal DNA enrichment/nucleosome occupancy is presented relative to this standard. Sequences of primers used for the nucleosome-scanning assay of *HSP12* are available as upon request.

2.6. Statistical analysis

The results represent at least three independent experiments. Numerical results are presented as means $\pm$ SE. Data were analyzed by using an InStat software package (GrapPAD, San Diego, CA, USA). Statistical significance was evaluated by one-way Anova analysis, and $p < 0.05$ was considered significant.

3. Results

3.1. Histone acetylation represses transcription of HHAG

Previously we have reported comparison of a transcriptional profile of cells at diauxic shift [33] with transcriptional profile of a strain expressing histone H4 with mutations that change the acetylatable lysine into nonacetylatable arginine residues within the N-terminal tail (H4-K5R/K8R/K12R) [34]. This comparison yielded a significant correlation between the two sets of genes [35] and showed that many of the genes that are induced at diauxic shift are also induced in the non-acetylatable histone H4 mutant (H4-K5R/K8R/K12R). This group of genes includes stress-inducible genes and genes required for metabolic switch from fermentation to oxidative metabolism.

To confirm these *in silico* results experimentally, we analyzed transcriptional regulation of *HSP12*, *HSP26*, *CTT1*, *ACS1*, *HXT6*, *SER3*, and *TMA10*. *HSP12* and *HSP26* are heat shock genes, *CTT1* encodes catalase, *ACS1* encodes acetyl-CoA synthetase, *HXT6* is a glucose transporter, *SER3* encodes an enzyme of serine biosynthesis, and *TMA10* is a putative regulator of ATPase. All these genes display increased transcription in the H4-K5R/K8R/K12R mutant [34], contain TATA box and belong to the group of stress genes [3,36]. While *HSP12*, *HSP26*, and *CTT1* are regulated by Msn2p/Msn4p transcription factors and are repressed during exponential growth by protein kinase A (PKA)-dependent mechanism [37–39], *ACS1*, *HXT6*, *SER3*, and *TMA10* are repressed during exponential growth by glucose in a PKA- and TOR-independent manner [40].

We tested whether acetylation of only histone H4 is specifically required for the repression of these genes or whether the non-acetylatable histone H3 mutant (H3-K9R/K14R/K18R) and double mutant that combines the mutations in both histones H3 and H4 (H4-K5R/K8R/K12R and H3-K9R/K14R/K18R) also display increased expression of these genes [21]. We will refer to these histone mutants as H3-3KR, H4-3KR, and H3/H4-6KR. In wild-type cells growing exponentially on glucose at 28°C, the tested genes are repressed; however, they are derepressed in the three histone mutants (Fig. 1). For all tested genes, the double histone mutant displayed the highest expression, suggesting that it is the overall level of acetylation rather than acetylation of histone H3 or H4 which is responsible for the repression.

To probe the role of histone acetylation in repression of these genes by an alternative approach, we measured the expression in strains harboring mutations in histone acetyltransferases (HATs). In yeast, SAGA and NuA4 complexes are the major HATs [41]. The SAGA complex preferentially acetylates histone H3 and H2B and contains Gcn5p as its catalytic subunit [42–45]. The NuA4 complex acetylates predominantly histone H4, H2A, and histone variant H2A.Z, and contains the essential Esa1p as its catalytic subunit [46,47]. Yng2p is a subunit of the NuA4 complex that regulates its HAT activity; deletion of *YNG2*

significantly reduces NuA4 HAT activity [48,49]. Our results show that *yng2* mutation increases the expression of *HSP12*, *HSP26*, *CTT1*, *SER3*, and *TMA10* and *gcn5* mutation increases the expression of *ACS1*, *HXT6*, *SER3*, and *TMA10* (Fig. 1). These results are consistent with the role of histone acetylation in regulation of expression of these genes. The result that *gcn5* mutation causes derepression of only several of the tested genes can be interpreted by the redundancy of the SAGA complex with other HATs that can acetylate histone H3 or by a requirement of Gcn5p-containing SAGA complex for expression of these genes. The expression of *SER3* is increased only modestly in *yng2*, *gcn5*, and the histone mutants. We interpret this result to mean that the *SER3* promoter has limited responsiveness to histone acetylation. However, it is important to note that transcription of *SER3* is also repressed by an intergenic transcription that occurs within the *SER3* promoter and produces non-protein-coding RNA [50].

To determine how is histone acetylation affected in H3-3KR, H4-3KR, H3/H4-6KR, *yng2*, and *gcn5* mutants, we analyzed the acetylation of bulk histones by western blotting (Fig. 2). To assess acetylation of histones H3 and H4, we used anti-acetyl histone H3 antibody that recognizes histone H3 acetylated at lysine 14 (acH3K14) and anti-acetyl histone H4 antibody that recognizes histone H4 acetylated at lysines 5,8,12,16 (acH4K5,8,12,16), respectively. As expected, the acetylation of histone H3 was reduced in the H3-3KR mutant. In H4-3KR mutant, the acetylation of H3 was only slightly reduced, but the hyperacetylation of H4 was completely absent. In the H3/H4-6KR mutant, the H3 acetylation was significantly reduced and the H4 hyperacetylation was absent. The amount of total histone H3 in H3-3KR and H4-3KR was comparable to wild-type, however the amount of H3 was slightly reduced in H3/H4-6KR mutant. In *gcn5* mutant, the acetylation of both H3 and H4 was reduced. The acetylation of H3 in *yng2* cells was reduced and the hyperacetylation of H4 was absent. The amount of H3 in *gcn5* and *yng2* was comparable to wild-type cells.

To test, whether histone occupancy and acetylation are also affected in the promoters of HHAAG, we used chromatin immunoprecipitation (ChIP) to evaluate the occupancy of histone H3, histone H3 acetylated at lysine 14 (acH3K14), as well as hyperacetylated histone H4 (acH4K5,8,12,16) in the promoters of HHAAG (Fig. 3, 4). Histone H3 occupancy, which represents total nucleosome occupancy, was reduced at most HHAAG loci in H3/H4-6KR, *yng2*, and *gcn5* cells and was less affected in H3-3KR and H4-3KR cells (Fig. 3). We used anti-H3 antibody that recognizes the C-terminal region of H3 that is not posttranslationally modified. The ChIP signal obtained with this antibody thus represents total H3 occupancy and can be used to assess nucleosome content [51–54]. As expected, the ChIP signal obtained with anti-acH3K14 antibody in H3-3KR was reduced to a background level at all tested loci (Fig. 4). The acH4 occupancy in the H3-3KR cells was unchanged at most loci; however, we observed a compensatory H4 acetylation at *HSP26*, *SER3*, and especially *HSP12* loci. The ChIP signal obtained with anti-acH4 antibody in H4-3KR was also reduced to a background level at all tested loci. However, the occupancy of acH3 at all HHAAG promoters was also reduced in the H4-3KR cells. These results suggest that at least at the HHAAG promoters, decreased acetylation of histone H3 in H3-3KR cells does not affect acetylation of histone H4, while decreased acetylation of histone H4 in H4-3KR cells results also in reduced acetylation of histone H3. This result is consistent with the western

blot analysis (Fig. 2). Occupancy of acH3 and acH4 in *gcn5* and *yng2* cells was reduced at most loci, respectively (Fig. 4). Some HHAAG promoters displayed again compensatory acetylation; for example, occupancy of acH3 at *HSP12* was elevated in *yng2* cells and occupancy of acH4 at *TMA10* was elevated in *gcn5* cells.

3.2. Histone hypoacetylation induces expression of HHAAG independently of Msn2p/Msn4p

Cells in the diauxic shift are stressed by the lack of nutrients and by accumulation of toxic metabolites. Some of the changes in the cells undergoing diauxic shift resemble the changes in cells undergoing stress response, such as induction of genes regulated by the transcriptional factors Msn2p/Msn4p [39]. Msn2p together with its partially redundant homolog Msn4p are zinc finger transcription factors that bind to stress response elements (STREs) in the promoters of stress-inducible genes to activate transcriptional program important for response to starvation and other forms of environmental stress [55,56]. Transcriptional activation of Msn2p-dependent genes is very complex. Msn2p is regulated by nuclear translocation [37,38,57,58] by increased binding of Msn2p to the STRE elements in the promoters of stress responsive genes [59] and by other, not so clearly understood mechanisms, including nucleocytoplasmic shuttling [60].

Unlike *ACS1*, *HXT6*, *SER3*, and *TMA10*, the stress-inducible genes *HSP12*, *HSP26*, and *CTT1* are directly regulated by Msn2p/Msn4p [39,40]. Since changes in the chromatin structure of *HSP12*, *HSP26*, and *CTT1* promoters caused by histone hypoacetylation may facilitate recruitment of Msn2p/Msn4p to the corresponding promoters, we wanted to determine whether the observed increase in the transcription of *HSP12*, *HSP26* and *CTT1* is mediated by Msn2p/Msn4p. Deletion of *MSN2/MSN4* results in significantly decreased expression of *HSP12*, *HSP26* and *CTT1*, indicating that even in the absence of stress, Msn2p/Msn4p are important for basal level of expression. Introducing *yng2* mutation in the *msn2 msn4* strain increases the expression of *HSP12*, *HSP26* and *CTT1*, suggesting that the histone hypoacetylation – mediated derepression of these genes occurs independently of Msn2p/Msn4p (Fig. 5A).

To test this conclusion by an independent approach, we measured by chromatin immunoprecipitation (ChIP) occupancy of Msn2p at the *HSP12*, *HSP26* and *CTT1* promoters under non-inducing conditions (28°C) in wild-type, *yng2*, H3-3KR, and H4-3KR strains (Fig. 5B). The results show a very low level of Msn2p at the three promoters in the wild-type strain. This is consistent with a report which shows almost undetectable Msn2p associated with the *HSP12* promoter in the absence of heat-shock [61]. Since the Msn2p occupancy is not increased in the *yng2*, H3-3KR, and H4-3KR strains, we conclude that histone hypoacetylation does not promote recruitment of Msn2p to *HSP12*, *HSP26* and *CTT1* promoters. However, in agreement with the increased expression, the recruitment of RNA Pol II to these three promoters is increased in *yng2* cells, and the recruitment to *HSP12* and *HSP26* is also increased in the H3-3KR and H4-3KR strains (Fig. 5C).

ChIP experiment with anti-H3 antibody showed that histone H3 occupancy at the *HSP12* promoter is significantly reduced in *yng2* cells (Fig. 3 and 5D). This result suggests that

high transcription rate of *HSP12* in *yng2* cells (Fig. 1) results in disruption of promoter nucleosomes. To test this possibility, we determined H3 occupancy also in *yng2 msn2 msn4* cells. Introducing *msn2 msn4* mutations in *yng2* cells results in significantly reduced transcription rate in comparison to *yng2* cells (Fig. 5A) and indeed, results in an increased H3 occupancy in *HSP12* promoter (Fig. 5D). However, this result does not hold true for other genes. Histone occupancy is not significantly reduced at *HSP26* and *CTT1* promoters in *yng2* cells in comparison to wild-type cells (Fig. 3, 5D), even though the transcription of both genes is increased in *yng2* cells (Fig. 5A). In addition, introducing the *msn2 msn4* mutations in *yng2* cells results in reduced transcription of both genes in comparison to *yng2* cells (Fig. 5A), but does not result in a significantly increased H3 occupancy at the corresponding promoters (Fig. 5D). We conclude that high transcription rate at certain promoters, such as *HSP12* in *yng2* cells, may result in disruption of promoter nucleosomes, however, this situation does not occur at all promoters. This conclusion is consistent with the notion that the nucleosome-free region (NFR) becomes more pronounced with higher transcription rates [62,63].

3.3. Histone acetylation affects chromatin structure of the *HSP12* gene

The occupancy of histone H3 in the promoter regions of *HHAAG* as determined by the ChIP analysis represents total occupancy of nucleosomes (Fig. 3); however, it does not distinguish between the occupancy of positioned nucleosomes and the occupancy of delocalized “fuzzy” nucleosomes. To determine whether histone acetylation affects the occupancy of positioned nucleosomes, we performed a nucleosome scanning analysis of the *HSP12* promoter and coding region (Fig. 6). The *HSP12* promoter has two proximal and three distal STRE sequences that are binding sites for transcription factors Msn2p/Msn4p and two heat shock element (HSE) sequences for binding of heat shock factor (HSF). Previous studies have shown that it is the STREs, particularly STRE2, rather than the HSE that are more important for *HSP12* induction upon heat shock as well as at the diauxic shift [61,64]. The nucleosome scanning data suggest that both the STRE2 and TATA box regions are relatively well protected in the wild-type strain. We observe that histone hypoacetylation alters the occupancy of positioned nucleosomes as reflected by the reduced height of the peaks in H3-3KR and H4-3KR cells (Fig. 6). This is particularly evident around the region containing the TATA box, which is three times and four times reduced in the histone H4-3KR and H3-3KR mutants in comparison to the wild-type strain, respectively (Fig. 6B). There is also a significant reduction in the occupancy of positioned nucleosomes in the *yng2* mutant. Introducing *rpd3* mutation in the *yng2* strain results in a partial recovery of occupancy of positioned nucleosomes to the wild-type level (Fig. 6A) and partial suppression of the increased expression of *HSP12* (Fig. 6C). We believe this is because deletion of histone deacetylase *RPD3* increases histone acetylation in the *HSP12* promoter of *yng2* cells.

Perhaps the most significant from these results is the reduced occupancy of positioned nucleosomes in the H3-3KR strain. Since the amount of total cellular H3 as determined by Western blotting (Fig. 2) and the H3 occupancy at the *HSP12* promoter as determined by ChIP analysis (Fig. 3) is comparable between the H3-3KR and the wild-type cells, the lower occupancy of positioned nucleosomes in H3-3KR cells in comparison to wild-type cells can be attributed to delocalization of positioned nucleosomes. The nucleosomes in H3-3KR cells

are hypoacetylated and probably are less efficient substrates for chromatin remodeling complex(es), and consequently are delocalized. These delocalized “fuzzy” nucleosomes do not have consistent positions in all cells and therefore in the nucleosome mapping assays by MNase digestion do not adequately protect the underlying DNA and do not produce peaks of the same height as positioned nucleosomes in the wild-type cells. These results suggest that adequate histone acetylation is required for nucleosome positioning and maintenance of repressive chromatin structure of the *HSP12* promoter.

The decreased occupancy of positioned nucleosomes at the *HSP12* promoter in H4-3KR and *yng2* cells can be interpreted by combination of several mechanisms. Since H3 occupancy at the *HSP12* promoter is decreased in these strains (Fig. 3), delocalization of nucleosomes is probably not the only mechanism responsible for reduction in the occupancy of positioned nucleosomes (Fig. 6). The additional factor responsible for the reduced total occupancy of nucleosomes in H4-3KR and *yng2* strains is likely recruitment of the RNA pol II, since the transcription of the *HSP12* gene is significantly upregulated in these two strains. However, since histone acetylation plays a role in replication-dependent chromatin assembly [65–67], we cannot completely discard the possibility that histone hypoacetylation affects the chromatin structure of the *HSP12* promoter during the replication-coupled nucleosome assembly. This possibility is supported by the finding that mutations in the chromatin assembly factor CAF I result in altered chromatin structure [68]. Moreover, Gcn5p is required for chromatin assembly [69]. The H3 occupancy at the *HSP12* promoter is significantly reduced in the *gcn5* cells, despite the fact that *gcn5* mutation does not upregulate *HSP12* transcription. This result argues against nucleosomes eviction at the *HSP12* promoter in *gcn5* cells caused by the recruitment of RNA pol II.

3.4. Decreased expression of the histone genes in *spt10* cells induces expression of HHAAG

Our results suggest that the increased expression of HHAAG due to histone hypoacetylation occurs by one or combination of the two following mechanisms: (i) loss of nucleosome positioning and/or (ii) decreased cellular abundance of histones, resulting in decreased total occupancy of nucleosomes. Ultimately, each of these mechanisms results in an altered chromatin structure and increased accessibility of the promoter DNA. Despite the fact that decreased transcription of histones does not affect expression of majority of yeast genes [70], we reasoned that decreased histone expression would result in an increased expression of HHAAG. To test this prediction, we used *spt10* strain. Spt10p is a transcription factor specific for histone genes and *spt10* cells display reduced expression of histones [71,72]. Since HHAAG display increased transcription in the H4-K5R/K8R/K12R mutant [34] and contain TATA box [3,36], we compared these two sets of genes with the set of genes upregulated in *spt10* cells [72]. This comparison indicates that the genes that are repressed by histone acetylation represent only a minority of the TATA box-dependent genes (Fig. 7A). Interestingly, significant fraction of genes that are upregulated in the H4-K5R/K8R/K12R mutant and contain TATA box are also upregulated in *spt10* cells. As indicated by the χ^2 test ($P < 0.001$), the correlation of the three data sets is much higher than that expected in an overlap of three data sets of the same size (Fig. 7A, values in parentheses). This result indicates that a subset of TATA box-containing genes is upregulated either by

histone hypoacetylation or by a decreased expression of histone genes. As expected, *spt10* cells display reduced cellular level of histone H3 (Fig. 7B) and significantly increased expression of HHAAG (Fig. 7C). However, expression of TATA box-less growth genes *RPS11B*, *RPS22B*, and *PYK1* did not significantly differ in wild-type and *spt10* cells (Fig. 7D). *ADH1* and *PFK26* contain TATA box, however they are not upregulated in the H4-K5R/K8R/K12R mutant [34]; expression of these two genes also does not differ in wild-type and *spt10* cells (Fig. 7D). Histone occupancy in *spt10* cells was significantly decreased at *HSP12*, *CTT1*, *TMA10*, and *RPS22B* promoters (Fig. 7E and F). This result suggests that even when the cellular abundance of histones is decreased, many loci retain the wild-type level of total occupancy of nucleosomes. However, the increased transcription of *HSP26*, *ACS1*, *HXT6*, and *SER3* in *spt10* cells is probably caused by the delocalization of nucleosomes.

4. Discussion

Yeast genes can be classified into two broad groups. The first group includes genes required for growth, such as ribosomal protein genes and genes encoding glycolytic enzymes. These genes lack a TATA box and their transcription is dominated by TFIID. About 90% of yeast genes belong to this category [36,73]. The expression of the remaining 10% of genes is dominated by the SAGA complex. These genes have TATA box, are regulated by chromatin-remodeling factors, and are transcribed at low levels in glucose-based rich media. Since they are induced by different stress conditions, including diauxic shift, these genes are referred to as stress genes [3]. The growth genes and stress genes differ in their nucleosomal structure. The growth genes usually feature a region depleted of nucleosomes upstream of the coding region, referred to as nucleosome-free region (NFR) [3]. Several mechanisms are responsible for nucleosome depletion at the NFRs. The first mechanism involves poly(dA:dT) sequences that bind nucleosomes with low affinity [30,68,74,75]. The second mechanism is operational at promoters with high transcriptional activity, when the recruitment of RNA pol II causes eviction of promoter nucleosomes [27,76]. The third mechanism involves recruitment of abundant nucleosome-excluding transcription factors such as Rap1p, Abf1p, and Reb1p [68,74,77–79]. The recruitment of sequence-specific transcription factor may also facilitate recruitment of ATP-dependent chromatin-remodeling complex such as RSC, with concomitant nucleosomes eviction [80].

During fermentative growth in the exponential phase, when glucose is abundant, genes required for glucose uptake, utilization, and growth are highly expressed while genes involved in utilization of carbon sources other than glucose are repressed. As glucose becomes limiting, cells enter diauxic shift with concomitant changes in transcription in order to adapt to nutrient limitation and utilization of alternative carbon sources [33,81]. Global untargeted histone acetylation parallels the cellular concentration of acetyl-CoA [13,16,20]; it is high during exponential phase and decreases as cells enter diauxic shift and stationary phase [17,18,82]. It appears that acetyl-CoA is a key metabolite that signals growth and proliferation [16,20]. In response to increased cellular level of acetyl-CoA, histones in the promoters of genes that are important for growth are acetylated, thereby promoting increased transcription and cellular growth. This probably represents another mechanism

that relays the information about the cellular metabolic state to the transcriptional machinery [16,20].

In this study we have shown that histone acetylation represses genes that are induced during diauxic shift when histone acetylation is reduced. *HSP12*, *HSP26*, *CTT1*, *ACS1*, *TMA10*, *HXT6*, and *SER3* belong to the category of TATA box-containing stress genes. However, unlike the majority of TATA box-containing genes, *HSP12*, *HSP26*, *CTT1*, *ACS1*, *TMA10*, *HXT6*, and *SER3* are repressed by histone acetylation and their derepression is triggered by histone hypoacetylation (Fig. 1). This mode of regulation contrasts with the general role of histone acetylation in transcriptional regulation, since on the genome scale, active transcription correlates with increased acetylation of promoter histones [4,19]. The repressive role of histone acetylation endows these genes with the ability to increase their expression upon histone hypoacetylation. Since the acetyl-CoA appears to be a key metabolite that reflects the metabolic state of the cell [16,20] and the defect in synthesis of acetyl-CoA results in global histone hypoacetylation [13], the genes that are repressed by histone acetylation have the ability to detect metabolic stress and reduced level of acetyl-CoA and induce their expression.

How does histone acetylation repress transcription? The nucleosome mapping of the *HSP12* promoter revealed that despite comparable H3 occupancy in the wild-type and H3-3KR cells, the occupancy of positioned nucleosomes in H3-3KR cells is significantly reduced (Fig. 6). This result suggests that the nucleosomes in H3-3KR cells lost their regular positioning seen in the wild-type cells, and are delocalized (Fig. 8). This results in an increased accessibility of the promoter to the transcription machinery and increased transcription. At some promoters, the increased recruitment of RNA pol II may result in nucleosome eviction. It is likely that the same mechanism is responsible for the increased transcription of the HHAAG in the other hypoacetylation strains. However, additional mechanism probably operates in H3/H4-6KR and *spt10* cells, which display reduced cellular level of histones (Fig. 2, 7B) and decreased H3 occupancy in some HHAAG promoters (Fig. 3,7). Similarly to the delocalized nucleosomes, decreased nucleosome occupancy in these strains also increases accessibility of the HHAAG promoters to the transcription machinery (Fig. 8).

These results suggest that a wild-type level of histone acetylation and probably a chromatin remodeling complex are required to establish a repressive chromatin structure over the *HSP12* promoter. Since acetylated histones recruit chromatin remodeling complexes with bromodomains [7,8], it is likely that a bromodomain-containing chromatin-remodeling complex is recruited to maintain repressive chromatin structure at the *HSP12* and other HHAAG promoters. Loss of histone acetylation probably results in a failure to recruit a chromatin-remodeling complex that establishes nucleosome positioning that is required for gene repression. This possibility is supported by the fact that four subunits of the RSC complex, Sth1p, Rsc1p, Rsc2p, and Rsc4p have bromodomains, and the RSC complex binds to acetylated histones within chromatin [83]. RSC complex is a member of the Swi/Snf family of chromatin remodeling complexes in yeast that functions in the regulation of global transcription. Genome-wide binding analyses showed that the RSC complex is targeted to genes regulated by stress, including *HSP12*, *HSP26*, *HXT6*, and the localization changes

upon stress conditions [84]. The involvement of the RSC complex in repression is also indicated by the increased expression of *HSP12* in *rsc* mutants [85].

Acetylation of histones H3 and H4 during exponential phase may facilitate recruitment of bromodomain-containing chromatin remodeling complexes, such as RSC, that establish repressive chromatin structure in the promoters of HHAAG [86]. Reduced histone acetylation would result in a failure to recruit RSC or Swi/Snf, which would relieve repressive chromatin structure and allow transcription. However, the pathways that regulate the chromatin structure and expression of *HSP12* and other HHAAG are likely to be more complex and involve additional chromatin remodeling and histone modifying complexes that are targeted to specific loci to create a local chromatin environment that represses stress inducible genes under non-inducing conditions. For example, chromatin remodeler Isw1p was found to function in parallel with the NuA4 and Swr1 complexes to repress stress-inducible genes [23].

The connection between metabolism and epigenetic mechanisms of transcriptional regulation has been appreciated only recently [9–11]. Intracellular acetyl-CoA reflects the metabolic state of the cell and by promoting histone acetylation activates expression of growth genes [16,20]. This study shows that histone acetylation also acts in an opposite way to repress group of stress genes, here referred to as HHAAG. This mechanism allows transcriptional activation of HHAAG when the cellular concentration of acetyl-CoA decreases, situation that typically occurs during metabolic transitions, such as diauxic shift.

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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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Highlights

- Group of genes are activated by histone hypoacetylation.
- Histone hypoacetylation-activated genes (HHAAG) are induced during diauxic shift.
- Expression of HHAAG requires chromatin remodeling and is independent of Msn2/4.
- Histone hypoacetylation results in delocalization of nucleosomes in promoters of HHAAG.
- Reduced cellular level of histones activates HHAAG.

Figure 1. HHAAG display increased expression in non-acetyltable histone mutants H3-3KR, H4-3KR, H3/H4-6KR and in cells with *gcn5* and *yng2* mutations
Indicated strains were grown in YPD medium to $A_{600} = 1.0$ at 28°C. Total RNA was extracted and assayed for *HSP12*, *HSP26*, *CTT1*, *ACS1*, *HXT6*, *SER3* and *TMA10* transcripts by real-time RT-PCR. The results were normalized to *ACT1* mRNA and expressed relative to the value for the wild-type strain. The experiments were repeated three times, and the results are shown as means $\pm$ SE. Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk.

Figure 2.

H3-3KR, H4-3KR, H3/4-6KR, *yng2*⁻, and *gcn5*⁻ cells display hypoacetylation of histones H3 and/or H4. The indicated strains were grown in YPD medium to $A_{600} = 1.0$ at 28°C and analyzed by Western blotting with antibodies against histone H3 acetylated at lysine 14 (acH3), hyperacetylated histone H4 (acH4), and total histone H3. Even loading of protein samples was confirmed with anti-phosphoglycerate kinase (Pgk1p) and anti-actin (Act1p) antibodies. The experiment was performed three times, and representative results are shown.

Figure 3. Occupancy of histone H3 at the HHAAG promoters

The indicated strains were grown at 28°C in YPD medium to an $A_{600} = 0.8$. ChIP experiments were performed with anti-histone H3 antibody and the histone H3 occupancy was determined at the HHAAG promoters. The results are presented the difference in occupancy of histone H3 at the particular locus in comparison with the *CHR15* locus. The experiments were repeated three times and results are shown as means $\pm$ SE. Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk.

Figure 4. Occupancy of acH3 and acH4 at the HHAAG promoters

The indicated strains were grown at 28°C in YPD medium to an $A_{600} = 0.8$. ChIP experiments were performed with antibodies against total histone H3 (H3), histone H3 acetylated at lysine 14 (acH3), and hyperacetylated histone H4 (acH4) and the occupancies of H3, acH3, and acH4 were determined in the promoters of HHAAG. Acetylation per nucleosome was calculated as ratios of acH3 to total H3 and acH4 to total H3 and the data represent comparison to the values for the wild-type cells. The experiments were repeated three times and results are shown as means $\pm$ SE. Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk.

Figure 5. Increased expression of HHAAG does not depend on Msn2p/Msn4p

(A) Introducing *msn2 msn4* mutations in *yng2* strain results in decreased expression of *HSP12*, *HSP26*, and *CTT1*. The indicated strains were grown in YPD medium to $A_{600} = 1.0$ at 28°C. Total RNA was extracted and assayed for *HSP12*, *HSP26*, *CTT1* transcripts by real-time RT-PCR. The results were normalized to *ACT1* mRNA and expressed relative to the value for the wild-type strain. The experiments were repeated three times, and the results are shown as means $\pm$ SE. (B) Histone hypoacetylation in *yng2*, H3-3KR, and H4-3KR mutants does not result in an increased recruitment of Msn2p to *HSP12*, *HSP26*, and *CTT1* promoters. (C) Increased expression of *HSP12*, *HSP26*, and *CTT1* in *yng2*, H3-3KR, and H4-3KR mutants correlates with increased of RNA pol II occupancy at the corresponding promoters. (D) Histone H3 occupancy at *HSP12*, *HSP26*, and *CTT1* promoters in wild-type, *yng2*, and *yng2 msn2 msn4* cells. (B, C, D) Indicated strains were grown at 28°C in YPD medium to $A_{600} = 0.8$. ChIP experiments were performed with antibodies against Msn2p, RNA pol II and histone H3 and the occupancies were determined in the promoter regions of *HSP12*, *HSP26*, and *CTT1*. The results represent the difference in occupancy of the particular protein at the particular locus in comparison with the *CHR15* locus. The experiments were repeated three times, and the results are shown as means $\pm$ SE. Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk. Values that are statistically different ($p < 0.05$) from each other are indicated by a bracket and asterisk.

Figure 6. Histone hypoacetylation results in reduced occupancy of positioned nucleosomes at the *HSP12* promoter

(A) Chromatin structure of *HSP12* locus in WT, H3-3KR, H4-3KR, *yng2* , and *yng2 rpd3* strains was determined by nucleosome scanning assay. Chromatin in permeabilized spheroplasts was digested with MNase and mononucleosomal DNA was analyzed by real-time PCR quantification. The nucleosomal occupancy was calculated as the ratio between the amounts of PCR products obtained from the purified mononucleosomal DNA and the genomic DNA. The results were normalized for NFR on chromosome XV as control and nucleosomal DNA enrichment/nucleosome occupancy is presented relative to this standard. A schematic of the *HSP12* locus shows relative positions of five stress response elements (STRE) that are binding sites for Msn2/Msn4p, heat-shock elements (HSE) that are binding sites for heat shock factor, and two putative TATA-boxes. (B) Comparison of nucleosome occupancies in WT, H3-3KR, and H4-3KR strains. (C) Deletion of histone deacetylase *RPD3* suppresses increased expression of *HSP12* in the *yng2* strain. Indicated strains were grown in YPD medium to $A_{600} = 1.0$ at 28°C. Total RNA was extracted and assayed for *HSP12* transcripts by real-time RT-PCR. The results were normalized to *ACT1* mRNA and expressed relative to the value for the wild-type strain. The experiments were repeated three times, and the results are shown as means $\pm$ SE. Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk. Values that are statistically different ($p < 0.05$) from each other are indicated by a bracket and asterisk.

Figure 7. HHAAG display increased expression in *spt10* cells

(A) Majority of HHAAG belong to a group of TATA box-dependent genes. The Venn diagram shows the extend of overlap between genes that contain a TATA box in their promoters [38], genes that are upregulated two-fold or more in a strain harboring a non-acetyltable form of histone H4 (H4-K5R/K8R/K12R) [36], and genes that are upregulated in *spt10* cells [74]. (B) reduced cellular level of histone H3 in *spt10* cells. The wild-type and *spt10* cells were grown in YPD medium to $A_{600} = 1.0$ at 28°C and analyzed by Western blotting with antibodies against histone H3 acetylated at lysine 14 (acH3), hyperacetylated histone H4 (acH4), and total histone H3. Even loading of protein samples was confirmed with anti-phosphoglycerate kinase (Pgk1p) and anti-actin (Act1p) antibodies. The experiment was performed three times, and representative results are shown. (C) HHAAG display increased expression in *spt10* cells. (D) Expression of growth genes in wild-type and *spt10* cells. (C, D) Wild-type and *spt10* cells were grown in YPD medium to $A_{600} = 1.0$ at 28°C. Total RNA was extracted and assayed for the indicated transcripts by real-time RT-PCR. The results were normalized to *ACT1* mRNA and expressed relative to the value for the wild-type strain. The experiments were repeated three times, and the results are shown as means $\pm$ SE. (E, F) Histone H3 occupancy in wild-type and *spt10* cells at the indicated promoters. The experiments were repeated three times and results are shown as

means $\pm$ SE. (C, D, E, F) Values that are statistically different ($p < 0.05$) from the wild-type are indicated by an asterisk.

Figure 8.

A model depicting the role of histone acetylation in regulation of HHAAG expression. Histone acetylation represses transcription by maintaining a repressive chromatin structure at the HHAAG promoters. When glucose becomes limiting, reduced level of acetyl-CoA results in global histone hypoacetylation, loss of the repressive chromatin structure, and increased expression. (A) Histone hypoacetylation results in delocalization of positioned nucleosomes and greater accessibility of promoter DNA. (B) Increased recruitment of RNA pol II may result in eviction of nucleosomes. (C) Decreased expression of histones may result in decreased nucleosome occupancy at the promoters of HHAAG and increased transcription.

Table 1

Yeast strains used in this study

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