Deubiquitination of Histone H2B by a Yeast Acetyltransferase Complex Regulates Transcription*

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Jeremy A. Daniel‡, Michael S. Tork‡, Zu-Wen Sun‡‡‡, David Schieltz‡‡, C. David Allis‡‡‡, John R. Yates III, and Patrick A. Grant‡‡‡‡
From the ‡Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, Virginia 22908, the §Diversa Corporation, San Diego, California 92121, and the †Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037

Post-translational modifications of the histone protein components of eukaryotic chromatin play an important role in the regulation of chromatin structure and gene expression (1). Given the requirement of Rad6/Bre1-dependent ubiquitination of histone H2B for H3 dimethylation (at lysines 4 and 79) and gene silencing (2–7), removal of ubiquitin from H2B may have a significant regulatory effect on transcription. Here we show that a putative deubiquitinating enzyme, Ubp8, is a structurally nonessential component of both the Spt-Ada-Gcn5-acetyltransferase (SAGA) and SAGA-like (SLIK) histone acetyltransferase (HAT) complexes in yeast. Disruption of this gene dramatically increases the cellular level of ubiquitinated-H2B, and SAGA and SLIK are shown to have H2B deubiquitinae activity. These findings demonstrate, for the first time, how the ubiquitin moiety can be removed from histone H2B in a regulated fashion. Ubp8 is required for full expression of the SAGA- and SLIK-dependent gene GAL10 and is recruited to the upstream activation sequence (UAS) of this gene under activating conditions, while Rad6 dissociates. Furthermore, trimethylation of H3 at lysine 4 within the UAS increases significantly under activating conditions, and remarkably, Ubp8 is shown to have a role in regulating the methylation status of this residue. Collectively, these data suggest that the SAGA and SLIK HAT complexes are regulated through an integrated set of histone modifications, counteracting repressive effects that alter chromatin and regulate gene expression.

Enzymes responsible for the post-translational modification of histones via acetylation, methylation, and ubiquitination are rapidly emerging. Most of these, thus far, are components of stable multiprotein complexes that act on nucleosomal substrates (8), regulating specific biological processes through a possible histone code that reads distinct sequential and/or combinatorial patterns of modifications (1). The HAT enzyme, Gcn5, is the catalytic subunit of two related high molecular weight (−1.8 MDa) protein coactivator complexes in Saccharomyces cerevisiae called SAGA (Spt-Ada-Gcn5-acetyltransferase) and SLIK (SAGA-like/SALSA (SAGA altered, Sp8 absent) (9–11). SAGA and SLIK have been shown to be required for the expression of a subset of genes as well as being functionally redundant with the general transcription factor, TFIID, in the transcription of most RNA polymerase II-transcribed genes (12). However, distinct and separate functions for the SAGA and SLIK HAT complexes have been established through compositional differences (10, 11, 13). This study demonstrates that the yeast deubiquitinating enzyme, Ubp8, is a component of both SAGA and SLIK and mediates the removal of ubiquitin from histone H2B. The process of H2B monoubiquitination in yeast has recently been shown to regulate H3 methylation and gene silencing in a unidirectional, trans-histone fashion (2–7, 14). Here, we show that Ubp8 is required for full expression of the GAL10 gene and that Ubp8 is present at the promoter of this gene under activating conditions. Furthermore, we find that the removal of ubiquitin from H2B is involved in the regulation of histone H3 (Lys4) trimethylation, a feature of transcriptional activation (15). Our data show a unique attribute of the SAGA coactivator complexes in the dual acetylation of histone H3 and deubiquitination of histone H2B, bridging two histone components of the nucleosome, while regulating the methylation of histone H3. These results have a profound implication for the understanding of how a cascade of post-translational histone modifications is generated in the process of gene expression.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—SAGA and SLIK were isolated for mass spectrometric analysis from yeast strain CY306 (10). GST*-tagged Ubp8 protein was expressed in strain E738 (Research Genetics). Strain YZ236, expressing protein A-His-tagged Rad6 protein, was derived from strain Y131 [hto1-hbt1Δ::LEU2 hto2-hbt2Δ HTA1-HTB1 (2µ UR3)]. BY4741 and derivative ubp8Δ, spt20Δ, and rad6Δ strains were obtained from Research Genetics. Strains MBY1217, YZS276 (FLAG-HTB1) and YZS277 (FLAG-HTB1-K123R) are described previously (2). Strain YZS282 (ubp8Δ::KAN) is derived from YZS276 and

† The abbreviations used are: GST, glutathione S-transferase; HAT, histone acetyltransferase; SAGA, Spt-Ada-Gcn5-acetyltransferase; SLIK, SAGA-like; Ub, ubiquitin-specific protease; ChIP, chromatin immunoprecipitation; UAS, upstream activation sequence; HA, hemagglutinin, SC, synthetic complete; IP, immunoprecipitation.
Fig. 1. Ubp8 is a structurally nonessential component of the SAGA and SLIK HAT complexes. A, silver-stained gels of highly purified SAGA and SLIK. Known components are grouped by class of transcriptional regulator, namely Spts, Adas, and Tafs. The protein band identified by mass spectrometry as Ubp8 is highlighted. Numbers in the center of the panel are molecular masses in kilodaltons. B, Western blots, using GST and Ada2 antisera, of partially purified SAGA and SLIK Mono Q fractions prepared from GST-Ubp8 yeast extract. Through the purification scheme, the SAGA and SLIK fractions were pooled individually. C, Western blots, using Ada2 and Taf9 antisera, and HAT assays of Mono Q fractions prepared from wild type and ubp8Δ strains. A type B H4 HAT complex elutes between SAGA and SLIK from these columns.

Deubiquitination Assay—Plasmid-borne HA-tagged ubiquitin and FLAG-tagged H2B were both expressed in strain YJD1 under galactose induction, and a trichloroacetic acid lysate preparation was immunoprecipitated using Ada2, Taf9, or GST (Santa Cruz Biotechnology) antisera. Western blots, using GST and Ada antisera, and HAT assays of Mono Q fractions prepared from wild type and ubiquitin tagged yeast were also performed as described previously (19). For ChIPs and Northern analyses, yeast were grown in synthetic complete (SC) medium containing 2% raffinose and then reseeded and grown in SC-raffinose medium or SC medium containing 2% galactose for an additional 4 h. Chromatin from whole cell extracts of strains expressing GST-Ubp8 or PrA-Rad6-His6, and respective no tag controls, was then precipitated using glutathione-Sepharose (Amersham Biosciences) or Nε-N-(nitroliotriacetic acid-agarose (Qiagen) and IgG-Sepharose 6 Fast Flow (Amersham Biosciences), respectively. ChIPs were also performed from wild type BY4741, ubp8Δ, and set1Δ strains with H3 (Lys4) mono-, di-, or trimethylated states of lysine 4 (Abcam). Quantitation of relative fold changes was performed using ImageQuant software.

Deubiquitination and HAT Assays—Proteins from column fractions were processed for immunoblotting using Ada2, Taf9, or GST (Santa Cruz Biotechnology) antisera. HAT assays were performed with HeLa free histones or nucleosomes as described previously (17). For H2B studies, whole cell lysates were prepared and FLAG analysis was performed as described (2). Histones from wild type and ubp8Δ yeast were acid extracted and processed for immunoblotting using rabbit H3 antisera specific for the mono-, di-, or trimethylated states of lysine 3 (Abcam). Quantitation of relative fold changes was performed using ImageQuant software.

Chromatin Immunoprecipitation (ChIP) Assays and Northern Blot Analysis—Chromatin preparations and immunoprecipitations were performed as described previously (19). For ChIPs and Northern analyses, yeast were grown in synthetic complete (SC) medium containing 2% raffinose and then reseeded and grown in SC-raffinose medium or SC medium containing 2% galactose for an additional 4 h. Chromatin from whole cell extracts of strains expressing GST-Ubp8 or PrA-Rad6-His6, and respective no tag controls, was then precipitated using glutathione-Sepharose (Amersham Biosciences) or Nε-N-(nitroliotriacetic acid-agarose (Qiagen) and IgG-Sepharose 6 Fast Flow (Amersham Biosciences), respectively. ChIPs were also performed from wild type BY4741, ubp8Δ, and set1Δ strains with H3 (Lys4) mono-, di-, or trimethylated antisera (Abcam). The input and precipitated DNA were amplified by PCR using oligonucleotide primers specific for the UAS within the GAL1–11 or ACT1 promoter regions. Quantitation was performed using an AlphaImager 2000 documentation and analysis system from Alpha Innotech. The ratios of immunoprecipitated to input DNA were calculated and normalized to wild type or tag raffinose values, and these values are shown as relative amounts of IP/input signal. RNA purification and Northern blotting were performed as described previously (10).

RESULTS

Ubp8 Is a Stable Component of the Yeast SAGA and SLIK HAT Complexes—To gain more insight into the transcriptional regulatory function of SLIK, we used mass spectrometric analysis of highly purified complex to identify a putative deubiquitinating enzyme, Ubp8, as a novel component (Fig. 1A). These data are in agreement with large-scale proteomics studies finding Ubp8 in a complex with Gcn5 and Spt7 (20, 21) and also with a study demonstrating that HA-tagged Ubp8 can co-immunoprecipitate certain SAGA/SLIK components (22). To test whether Ubp8 was SLIK-specific, we utilized a strain expressing GST-tagged Ubp8 protein. Immunoblotting of partially purified SAGA and SLIK revealed that GST-Ubp8 pro-
tein co-fractionated with both complexes (Fig. 1B), although existing in higher stoichiometric proportion in SLIK, consistent with the identification of Ubp8 as part of the SLIK complex (Fig. 1A).

The polypeptides Spt20, Spt7, and Ada1 are required for the overall structural integrity of both SAGA and SLIK, because deletions of any of these genes cause severe phenotypes and do not allow normal chromatographic isolation of either complex (9, 10, 23). To test whether the Ubp8 protein is also required for structural integrity, we compared HAT complex fractionation profiles of wild type yeast to those bearing a ubp8 deletion by anion exchange chromatography. We found that SAGA and SLIK complexes lacking Ubp8 are intact and can acetylate free and nucleosomal histone H3 in vitro (Fig. 1C). Further anion exchange and size exclusion chromatography of the ubp8Δ extract indicated that the two mutant complexes have similar charge and size to those of wild type. Therefore, Ubp8 is not necessary for the integrity or HAT activity of either complex, concurring with the lack of any marked phenotype seen with the ubp8Δ yeast (Ref. 24 and data not shown).

Ubp8 Regulates H2B Deubiquitination at Lysine 123—A possible role of the Ubp8 protein within a HAT complex is to deubiquitinate chromatin-associated substrates. A good candidate substrate appeared to be histone H2B, which in yeast is monoubiquitinated at the highly conserved lysine 123 residue (14). Most transcription studies thus far have concentrated on the addition of ubiquitin to proteins (25), even though ubiquitination is an enzymatically reversible process, as evidenced in deubiquitinating activities associated with the proteosome (26). For regulation of rDNA and telomeric silencing, ubiquitination of lysine 123 in H2B by Rad6 has recently been demonstrated to be required for dimethylation of H3 at lysines 4 and 79 by their respective site-specific histone methyltransferase enzymes, Set1 and Dot1 (27, 28). For regulation of rDNA and telomeric silencing, ubiquitination of lysine 123 in H2B by Rad6 has been demonstrated to be required for dimethylation of H3 at lysines 4 and 79 by their respective site-specific histone methyltransferase enzymes, Set1 and Dot1 (2–7, 14). Di-methylation of H3 at these residues and functional telomeric silencing are impaired in the absence of Rad6 or an intact H2B (Lys123) ubiquitination site, indicating that conjugation of ubiquitin to H2B controls these methylation events on H3. Using FLAG-tagged H2B yeast strains, we found that disruption of UBP8 caused a dramatic increase in the monoubiquitinated form of H2B as compared with wild type (Fig. 2A). The disruption of UBP3, another putative ubiquitin-specific protease shown to be involved in transcriptional silencing (27) and to associate with the SAGA/SLIK component Ada3 (28), had no apparent effect on ubH2B (see the Supplemental Figure). This result indicates that Ubp8 specifically regulates the deubiquitination of H2B at lysine 123 in vivo.

Since Ubp8 is a component of SAGA and SLIK, we wanted to test whether these two complexes were capable of directly hydrolyzing the ubiquitin moiety from histone H2B. To this end, we purified ubiquitinated-H2B from a yeast strain coexpressing FLAG-tagged H2B and HA-tagged ubiquitin proteins. Using this substrate, we show that SAGA and SLIK have in vitro H2B deubiquitinae activity (Fig. 2B). The detection of free HA-ubiquitin specifically after the addition of SAGA or SLIK confirms the ubiquitin-specific protease function of these two complexes. This result extends the in vitro finding that Ubp8 deubiquitnates H2B and establishes that this histone modification is also catalyzed, in vitro, in the context of SAGA and SLIK.

Ubp8 Regulates Bulk H3 Methylation at Lysine 4—Given that ubp8Δ yeast have elevated ubH2B and that this modification is required for methylation of H3 (Lys4), we hypothesized that the ubp8Δ strain might have increased H3 methylation at this residue. To test this, we compared H3 (Lys4) methylation of histones from wild type yeast to that of ubp8Δ by Western analysis using antibodies that are specific for the mono-, di-, or trimethylated form of histone H3 at lysine 4. Although we see a slight change in dimethylation, monomethylation of H3 (Lys4) increases significantly in the ubp8Δ mutant, while trimethylation at this residue decreases (Fig. 2C). These results indicate that removal of ubiquitin from H2B regulates the methylation state of lysine 4 on H3, promoting a transition from a mono- to trimethylated state. A recent study using these antibodies in ChIP analyses suggests that trimethylated H3 (Lys4) is present exclusively at active genes, while the dimethylated residue is in chromatin of both repressed and active genes (15). The biological consequence of monomethylation on H3 (Lys4) has not yet been explored. Our results suggest that deubiquitination by Ubp8 affects bulk H3 lysine methylation status in vivo, promoting trimethylation of H3 (Lys4) and as part of HAT/co-activator complexes may promote gene activation.

Ubp8 Is Recruited to a SAGA/SLIK-dependent Promoter during Activation, while Rad6 Dissociates—To utilize galactose as a carbon source, derepression of a number of GAL genes in
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**Fig. 3. Recruitment of Ubp8 to the SAGA/SLIK-dependent gene GAL10 allows for trimethylation of H3 (Lys4) and activated transcription.** A, ChIP assays performed from strains expressing protein A- and His-tagged Rad6 (YZS236) or GST-tagged Ubp8 and two respective isogenic control strains grown in raffinose-noninducing (R) and galactose-inducing (G) conditions. Shown are PCR products using GAL1–10 and ACT1 primers with input and immunoprecipitated DNA templates. Numbers in parentheses show quantitation of relative amounts of (IP/input) signal. B, ChIP assays from wild type, ubp8Δ, and set1Δ strains using H3 (Lys4) antiseras. C, Northern analysis of total RNA prepared from noninduced and galactose-induced H2B-K123R (YZS276) and isogenic wild type (YZS276), ubp8Δ, spt20Δ, rad6Δ, and isogenic wild type (BY4741), and set1Δ (MBY1217) strains hybridized with a GAL10 probe. RNA hybridization with an ACT1 probe is used as the loading control.

**H2B Ubiquitination and H3 Methylation Affect Transcription of the GAL10 Gene**—Since the methylation changes within the GAL1–10 promoter region are regulated by Ubp8, we investigated whether Ubp8 and Set1 had a direct role in the transcription of a gene regulated by this element. To address this, we isolated total RNA from wild type, ubp8Δ, and set1Δ strains grown in raffinose-noninducing or galactose-inducing media and performed Northern analysis with a probe specific for GAL10 expression. We find that the ubp8Δ and more significantly, set1Δ mutants have decreased GAL10 expression compared with wild type, suggesting the Ubp8 and Set1 proteins are required for full transcription of this gene (Fig. 3C, compare lanes 17, 18, and 20). Northern analysis of RNA isolated from an spt20Δ strain, a mutation that disrupts SAGA/SLIK complex integrity, showed a deficiency in GAL10 expression slightly more severe than the ubp8Δ mutant, yet not as drastic as the set1Δ mutant (Fig. 3C). Thus, we demonstrate co-activating functions for the histone H3 methyltransferase, Set1, and the deubiquitinase, Ubp8, in SAGA/SLIK-dependent transcription of the galactose-regulated GAL10 gene.

In contrast, after a 4-hour induction time, transcription of GAL10 is increased in the rad6Δ mutant compared with the isogenic wild type (Fig. 3C, compare lanes 17 and 21). This hyperactivation of GAL10 is visualized more dramatically when comparing two-hour galactose inductions (Fig. 3C, compare lanes 10 and 14). Interestingly, the rad6Δ mutant does not cause induction of GAL10 in noninducing conditions, suggesting that RAD6 is not sufficient to maintain repression of the GAL genes. These studies offer more evidence for the contrasting functional roles of Rad6 and Ubp8 in the transcriptional activation of galactose-regulated genes. Surprisingly, H2B-K123R yeast, bearing a mutation in the H2B ubiquitination site, have decreased GAL10 gene expression (Fig. 3C, compare lanes 15 and 16), demonstrating the need for a modifiable lysine at this position. This result may indicate that H2B ubiquitination, and subsequent deubiquitination, are both required for transcriptional activation. Alternatively, this lysine may be subject to other post-translational modifications such as acetylation or methylation.

**Trimethylation of H3 (Lys4) at the GAL1–10 Promoter Is Dependent on Ubp8 and Set1 during Activation**—We also investigated changes in the methylation state of H3 (Lys4) under activating conditions at the GAL1–10 UAS, compared with the control ACT1 promoter, and whether Ubp8 functions in the regulation of this histone modification. Using H3 (Lys4) antibodies, we performed ChIP analysis from wild type, ubp8Δ, and set1Δ strains. Since Set1 is thought to be the only H3 (Lys4) methylating enzyme in yeast (15), we used the set1Δ mutant as a background control. In wild type yeast, although mono- and dimethylation are relatively unchanged upon activation, trimethylation of H3 (Lys4) at the GAL1–10 UAS increases. However, in the ubp8Δ or set1Δ mutant, trimethylation of H3 (Lys4) is impaired during activation (Fig. 3B). Thus, our data demonstrate, for the first time, that nucleosomal H3 (Lys4) within the GAL1–10 UAS is trimethylated upon activation and that this modification is dependent upon Ubp8.
Our studies call attention to Ubp8, a component of the two yeast HAT complexes SAGA and SLIK. We present evidence that Ubp8 functions in the hydrolysis of ubiquitin from histone H2B and is recruited to the GAL1–10 UAS together with other SAGA and SLIK components during transcriptional activation (10, 30, 31). The association of Ubp8 at this locus coincides with (Lys)\(^{94}\) acetylation (10) and (Lys)\(^{5}\) hypermethylation of H3 allowing for normal expression of the GAL10 gene. In view of studies demonstrating that Rad6-mediated ubiquitination of H2B regulates H3 dimethylation and gene silencing, our data suggest that the SAGA and SLIK HAT complexes may counter Rad6 by influencing multiple modifications on more than one histone. During consideration of this manuscript, Henry et al. (33) also reported Ubp8 as an H2B deubiquitinase within the SAGA complexes and, in keeping with our results, provide evidence of a role for Ubp8 in transcriptional activation of the GAL genes; however, they show that deletion of UBPI8 results in an increase in H3 (Lys)\(^{3}\) trimethylation at the GAL1–10 core promoter (33).

In summary, we propose that repression of some genes through ubiquitinated-H2B is overcome by an activator-targeted HAT complex containing deubiquitinase activity. We favor the view that deubiquitination of H2B occurs in concert or prior to both histone acetyltransferase activity and the intricate regulation of methyltransferase activity. In turn, changes in chromatin structure and/or the epigenetic pattern of modifications within a promoter region allow recruitment of the transcription machinery.

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