The histone H4 basic patch regulates SAGA-mediated H2B deubiquitination and histone acetylation

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Running title: H4 basic patch regulates SAGA histone-modifying activity

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ABSTRACT

Histone H2B monoubiquitylation (H2Bub1) has central functions in multiple DNA-templated processes, including gene transcription, DNA repair, and replication. H2Bub1 also is required for the trans-histone regulation of H3K4 and H3K79 methylation. Although previous studies have elucidated the basic mechanisms that establish and remove H2Bub1, we have only an incomplete understanding of how H2Bub1 is regulated. We report here that the histone H4 basic patch regulates H2Bub1. Yeast cells with arginine-to-alanine mutations in the H4 basic patch (H4²RA) exhibited significant loss of global H2Bub1. H4²RA mutant yeast strains also displayed chemotoxic sensitivities similar to, but less severe than, strains containing a complete loss of H2Bub1. We found that the H4 basic patch regulates H2Bub1 levels independently of interactions with chromatin remodelers and separately from its regulation of H3K79 methylation. To measure H2B ubiquitylation and deubiquitination kinetics in vivo, we used a rapid and reversible optogenetic tool, the light-inducible nuclear exporter (LINX), to control the subcellular location of the H2Bub1 E3-ligase, Bre1. The ability of Bre1 to ubiquitylate H2B was unaffected in the H4²RA mutant. Consistent with a function for the H4 basic patch in regulating SAGA deubiquitinase activity, we also detected increased SAGA-mediated histone acetylation in H4 basic patch mutants. Our findings uncover that the H4 basic patch has a regulatory function in SAGA-mediated histone modifications.

The nucleosome – the fundamental repeating unit of chromatin – is the first level of chromatin organization, and it is essential for the regulation of nearly all DNA-templated processes. Composed of an octamer of histone proteins, two molecules each of histones H2A, H2B, H3 and H4, the nucleosome is a partial barrier to functions such as gene transcription and DNA repair; hence, nucleosomes must be disrupted transiently for these processes to occur. One major mechanism that contributes to the transient disruption of nucleosomes is histone post-translational modifications (PTMs). Histone PTMs are found both in the “tail” domains, which largely influence the recruitment of effector proteins (i.e., readers) and in their globular domains, which largely influence nucleosome-DNA interactions (1).

In addition to histone PTMs being major influencers of chromatin structure and function, histones also contain basic and acidic regions or
“patches” that govern nucleosome-nucleosome interactions or the ability of readers to engage the nucleosome. One such region is the nucleosome acidic patch, a negatively charged cavity formed between histones H2A and H2B that contributes to chromatin function by regulating association of a multiple chromatin-modifying enzymes (2, 3). Another region, the H4 basic patch located in the H4 tail domain (between residues 16 and 20), contains multiple basic residues, i.e., arginine, histidine, and lysine (Figure 1A). This segment of basic residues has important functions in regulating chromatin dynamics and multiple chromatin-modifying enzymes. For example, the H4 basic patch is required for efficient Dot1-mediated histone H3 lysine 79 di- and trimethylation (H3K79me2 and H3K79me3) (4, 5). Additionally, the basic patch has a key function in maintaining the balance of heterochromatin domains in yeast by interacting with the silencing protein Sir3 when H4K16 is unacetylated (5). Acetylation of K16 within the basic patch is critical in chromatin organization (6). Recently, the H4 basic patch has been shown to function as a positive regulator of the ISWI family of chromatin remodeling complexes (7–10) and as a regulator of Snf2 (11, 12). Chd1, another remodeler that organizes nucleosomes across the coding regions of genes, also interacts with the H4 N-terminus (13).

H2Bub1 is a dynamic histone PTM enriched at promoters and across the transcribed regions of genes. This modification is associated with transcription elongation and maintaining chromatin integrity through its conserved trans-histone regulation of H3K79me and H3K4me (14–20). In yeast, H2Bub1 is catalyzed by the E2/E3 ligases Rad6 and Bre1 (21–23) and is removed by the deubiquitinases Ubp8 and Ubp10. As a part of the SAGA coactivator complex, Ubp8 – the catalytic subunit of the SAGA deubiquitinase (DUB) module – deubiquitinates H2Bub1 near the promoters and transcription start sites of virtually all expressed genes in eukaryotic cells to promote transcription by RNA polymerase II (24–27). This cycle of ubiquitylation and deubiquitination is important for the regulation of early elongation and the establishment of serine 2 phosphorylation on the C-terminal domain of RNA polymerase II (28, 29). Monomeric Ubp10 regulates the pattern of H2Bub1 within gene bodies and intergenic regions. Initially identified for its role in maintaining silencing of subtelomeric genes, Ubp10 has been shown to be important in maintaining global H2Bub1 levels (25, 30–32). Nune et al. have described the coordinated activities of Ubp10 and the histone chaperone FACT in H2Bub1 deubiquitination and nucleosome disassembly and reassembly (33).

To further understand the function of the H4 basic patch, we investigated the possibility that there are yet-to-be elucidated aspects of histone PTM crosstalk involving this histone region. In this report, we show that the H4 basic patch is required to maintain proper H2Bub1 levels. Although the basic patch is required for Dot1-mediated H3K79me, we found that the ability of the H4 basic patch to regulate H2Bub1 is independent of Dot1 or H3K79 methylation. Furthermore, we found that the ability of the H4 basic patch to regulate H2Bub1 was also independent of its activity in regulating ATP-dependent chromatin remodelers known to be regulated by H4. We therefore examined the possibility that the H4 basic patch regulates some aspect of the H2Bub1 machinery that installs or removes this mark. Using an optogenetic tool to enable precise and rapid nuclear import or export of Bre1 (LINX-Bre1), we showed that, although Bre1 installed H2Bub1 in wild-type and H4 basic patch mutants at comparable rates, the rate of removal of H2Bub1 by Ubp8 (but not by Ubp10) increased when the H4 basic patch was mutated. Because Ubp8 is a DUB module of the SAGA complex, we further examined and found that the H4 basic patch also contributes to the levels of H3 acetylation by SAGA. Collectively, our findings reveal an unexpected function of the H4 basic patch in negatively regulating the histone-modifying activities of SAGA.

Results

Mutation of H4 basic patch leads to reduced levels of H2B monoubiquitylation

Although studies have defined the relationship between H2Bub1 and H3K4 and H3K79 methylation, less is known about the nature of the histone crosstalk that regulates H2Bub1. In the course of our studies of the regulation of H3K79 methylation by the H4 basic patch (Figure 1A), unexpectedly, we discovered by immunoblotting
that mutation of H4 basic patch residues R17 and R19 to alanine (hereafter H4\textsubscript{2RA}) also resulted in a significant reduction in H2Bub1 (Figure 1B). As controls in these experiments, we included a deletion of \textit{BRE1} (\textit{bre1\Delta}), the H2Bub1 E3 ligase, and a mutation of H3K79 (H3K79R); these mutants confirmed the specificity of the histone modification-specific antibodies and verified that the H4 basic patch regulates H3K79 di- and tri-methylation. We also observed the same reduction in H2Bub1 in a strain that expressed an H4 basic patch mutant lacking the entire basic patch (\textit{\Delta}\textsubscript{16-20}) and in a single H4-R17A mutant (data not shown). Because H4 basic patch regulation of H2Bub1 has not been reported, we sought to verify that this observation was not a property of a specific strain background. As shown in Figure S1A, mutation of the same H4 basic patch residues in another H2A/H2B shuffle strain likewise impaired H2Bub1 levels.

Given the well-established function of the H4 basic patch in regulating Dot1-mediated H3K79 methylation, we next assessed whether the regulation of H2Bub1 by the H4 basic patch might be indirectly due to the ability of this region to regulate Dot1 and H3K79 methylation that, in turn, regulates H2Bub1. This idea was supported by a study of van Welsem \textit{et al.} who found that Dot1 promoted H2Bub1 formation when Dot1 levels were increased (34). However, we found that the deletion of \textit{DOT1} (\textit{dot1\Delta}) or mutation of H3K79 had no discernible effect on the levels of H2Bub1 (Figure 1B, S1A and S1B). Thus, the ability of the H4 basic patch to regulate H2Bub1 is independent of its regulation of Dot1 and H3K79 methylation. In sum, these data defined an important and previously unknown function for the H4 basic patch in regulating H2Bub1.

\textbf{H4 basic patch mutant cells display phenotypes associated with the absence of H2Bub1}

To determine the biological significance of the reduced levels of H2Bub1 in the H4 basic patch mutant, we spotted cells that expressed either H4\textsubscript{WT}, H4\textsubscript{2RA}, H3K79R, or H2BK123R histones onto solid media containing various compounds to identify drug sensitivity phenotypes. As has been previously published, cells lacking all H2Bub1 (e.g., H2BK123R, \textit{rad6\Delta}, \textit{bre1\Delta}), display sensitivity to various toxic agents including caffeine and 6-azauracil (6-AU) (35, 36). Interestingly, the H4\textsubscript{2RA} mutant was also sensitive to these same agents as well as rapamycin (Figure 2). In contrast, the H3K79R mutant, lacking all forms of H3K79 methylation, was not sensitive to these agents, confirming that the mechanism by which the H4 basic patch regulates H2Bub1 is independent of H3K79 methylation.

H2Bub1 has a key function in the timely induction of the yeast \textit{GAL} genes (37). To determine whether the H4 basic patch was also required for the induction of \textit{GAL} genes like H2Bub1, we grew H4\textsubscript{WT} and H4\textsubscript{2RA} cells in raffinose-containing media prior to the addition of galactose (2% final concentration). Total RNA was isolated across the induction time course and RT-qPCR of the \textit{GAL1} locus was performed. Relative to the H4\textsubscript{WT} cells, H4\textsubscript{2RA} cells displayed reduced accumulation of \textit{GAL1} transcripts during the time course (Figure S2), suggesting that the H4 basic patch, like H2Bub1, is important for the proper regulation of \textit{GAL} gene transcription. Collectively, these studies showed that the H4 basic patch is physiologically important and its mutation phenocopies mutations in the H2Bub1 pathway.

\textbf{The H4 basic patch and the ATP-dependent chromatin remodeler Chd1 regulate H2Bub1 by different mechanisms}

Lee \textit{et al.} demonstrated a function for the ATP-dependent remodeler Chd1 in regulation of H2Bub1 (38). Additional studies show that the H4 basic patch regulates the activity of multiple chromatin remodelers including Chd1, Snf2, and Isw1. Therefore, we reasoned that a possible mechanism for how the H4 basic patch regulates H2Bub1 could be by H4 basic patch regulation of the activity of one of these remodelers, which, in turn, would regulate H2Bub1. To test this possibility, we measured global H2Bub1 levels in a panel of strains that contained individual deletions of the aforementioned chromatin remodelers (Figure 3A). Consistent with previous studies, deletion of \textit{CHD1} (\textit{chd1\Delta}) resulted in a reduction of H2Bub1. In contrast to \textit{chd1\Delta}, however, none of the other remodeler deletions exhibited any loss of H2Bub1. To determine whether the loss of H2Bub1 in the H4\textsubscript{2RA} strain was due to Chd1, we deleted \textit{CHD1} in our H4\textsubscript{WT} and H4\textsubscript{2RA} strains and measured H2Bub1 levels in

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all strain combinations. As expected, we observed a decrease in H2Bub1 in the individual chd1Δ and H42RA mutants (Figure 3B). Intriguingly, however, the loss of H2Bub1 in the H42RA chd1Δ double mutant was additive, suggesting that regulation of H2Bub1 by the H4 basic patch and Chd1 occurs by separate (non-epistatic) pathways.

**H4 basic patch regulation of H2B ubiquitylation is independent of the ubiquitylation machinery**

To define how the H4 basic patch regulates H2Bub1, we next considered the possibility that the region is important for Rad6/Bre1 to catalyze H2Bub1. Others have found that, in the absence (e.g., if K123 is a poor substrate) resulted in defect in the ability of Bre1 to ubiquitylate H2B. Specifically, a reduction in H2Bub1 for which absence of a DUB could not compensate. Following this example, we created a deletion of UBP8 (ubp8Δ) in the context of either the H4WT or H42RA mutant and examined the levels of H2Bub1 for which absence of a DUB could not compensate. Upon deletion of UBP8, the levels of H2Bub1 in the H42RA ubp8Δ cells was rescued to a level comparable to that in the H4WT ubp8Δ strain (Figure 4A). These findings suggested that the ubiquitylation machinery in the H42RA mutant was functional and that the H4 basic patch was not essential for H2Bub1 catalysis.

In addition to the above approach, another outcome that we observed for the absence of Bre1 catalysis was reduced Bre1 protein stability (41). To determine if Bre1 protein level was reduced in the H42RA mutant, we expressed a FLAG-BRE1 construct in H4WT bre1Δ and H42RA bre1Δ cells and analyzed Bre1 levels (FLAG) and H2Bub1 levels by immunoblotting. Upon deletion of UBP8, the levels of H2Bub1 in the H42RA ubp8Δ cells was rescued to a level comparable to that in the H4WT ubp8Δ strain (Figure 4A). These findings suggested that the ubiquitylation machinery in the H42RA mutant was functional and that the H4 basic patch was not essential for H2Bub1 catalysis.

That Bre1 catalytic activity, specifically, and the ubiquitylation machinery, generally, were unaffected by the H42RA mutation.

Although the foregoing studies were informative, they were limited by the fact that they captured only final, steady state levels of H2Bub1. Recently, for the study of dynamic epigenetic regulation, optogenetic tools have emerged that enable rapid and reversible nucleo-cytoplasmic shuttling to overcome the steady-state limitation. We used a previously characterized optogenetic tool developed in our lab, LINX (light-inducible nuclear export), that uses blue light to control the cellular localization of Bre1 (42). Briefly, when cells lacking BRE1 and UBP8 are transformed with the LINX-Bre1 construct and grown in blue light, LINX-Bre1 is rapidly sequestered in the cytoplasm and phenocopies a bre1Δ strain. Removal of blue light results in import of LINX-Bre1 into the nucleus, rendering the cells BRE1+, and enabling precise measurement of the in vivo kinetics of ubiquitylation and deubiquitination with unprecedented control and resolution. Thus, we measured the kinetics of LINX-Bre1-mediated ubiquitylation in H4WT and H42RA cells that lacked endogenous BRE1 and UBP8. These cells were grown to log-phase in the presence of blue light before being switched to the dark to release Bre1 into the nucleus. As shown in Figure 4C, H2B ubiquitylation occurred rapidly, consistent with published results (42). Significantly, we did not observe any significant difference in the kinetics of ubiquitylation between the H4WT and H42RA cells, providing strong, in vivo evidence that the ubiquitylation machinery in the H42RA mutant was fully functional and that the H4 basic patch regulates H2Bub1 independent of Bre1.

**The H4 basic patch regulates SAGA-associated Ubp8 deubiquitination**

We next turned our attention to the effect of the H4 basic patch on H2Bub1 deubiquitination. We took advantage of the LINX-Bre1 system to measure the dynamics of deubiquitination. To distinguish between the activities of the two H2Bub1 DUBs, Ubp8 and Ubp10, we created H4WT bre1Δ and H42RA bre1Δ strains that lacked either UBP8 (bre1Δ ubp8Δ) or UBP10 (bre1Δ ubp10Δ). Cells were grown to log phase in the dark to maintain normal H2Bub1 by LINX-Bre1.
Then we exposed the cells to blue light to rapidly remove LINX-Bre1 from the nucleus, preventing any subsequent ubiquitylation and enabling us to measure the kinetics of DUB activity in vivo. In the H4<sub>WT</sub> cells, the half-lives of the H2Bub1 mark in the presence of Ubp8 or Ubp10 were approximately 2.58 and 2 minutes, respectively. The half-life of the H2Bub1 mark in H4<sub>2RA</sub> cells in the presence of Ubp10 was similar to that in H4<sub>WT</sub>, about 1.8 minutes. Strikingly, the half-life of the H2Bub1 mark in H4<sub>2RA</sub> cells in the presence of Ubp8 decreased 3-fold (to less than 1 minute; Figures 5A, 5C). This result suggested that the H4 basic patch regulates Ubp8, and it may limit the activity or accessibility of Ubp8. Given these findings, we also employed an in vitro DUB assay; we used cell extracts from yeast strains doubly deleted for UBP8 and UBP10 to generate high levels of H2bub1 as a substrate for recombinant Ubp8 and Ubp10. We assayed an affinity-purified Upb8-DUB module previously shown to interact with the nucleosome and affinity-purified Ubp10. These in vitro assays did not reveal a difference in the rates of deubiquitination between H4<sub>WT</sub> and H4<sub>2RA</sub> cells (Figure S3). Thus, the regulation of Ubp8 by the H4 basic patch may involve either a regulatory mechanism that is not captured in in vitro assays or that depends on the full SAGA complex and not the SAGA-associated DUB module alone.

The H4 basic patch regulates SAGA-dependent H3 acetylation

The SAGA complex consists of four distinct modules and two enzymatic functions—deubiquitination and lysine acetyltransferase (KAT) activities (43). The main target of the KAT module is the histone H3 N-terminus, specifically lysine residues 9, 14, 18, 23, 27, and 36 (44). Although the KAT and DUB modules are distinct structurally, they are in close proximity to one another and the DUB module modestly stimulates KAT activity (45). To determine whether the H4 basic patch also influences SAGA-dependent histone acetylation, we analyzed the histone acetylation levels at H3K9, K14, K18, and K27 in H4<sub>WT</sub> and H4<sub>2RA</sub> cells alongside a gcn5Δ strain as a control (Figure 6). Intriguingly, in the H4<sub>2RA</sub> cells, we observed an increase in H3K18 and H3K27 acetyl levels but little change in H3K9 and H3K14 acetyl levels. However, H3K9 and H3K14 are also targets of other KATs (NuA3 and Rtt109, respectively). Of the acetylation sites tested, H3K18ac and H3K27ac are almost exclusively catalyzed by SAGA, as demonstrated by the complete loss of acetylation at these two sites. Thus, the selective increase in H3K18 and H3K27 acetylation in H4<sub>2RA</sub> cells was likely due to SAGA-specific acetylation changes caused by the loss of H4 basic patch function. To eliminate the possibility that the H4<sub>2RA</sub> mutant alters KAT activity more generally, we analyzed H3K56ac levels in the H4<sub>WT</sub> and H4<sub>2RA</sub> cells; we did not find any difference between the strains (Figure S4). Taken together, these data suggest that, in addition to regulating the DUB module in SAGA, the basic patch also regulates the SAGA KAT module. Thus, these findings document an important inhibitory function of the H4 basic patch in SAGA-associated histone modification functions.

Discussion

We have shown that, by negatively regulating SAGA-associated activities, the H4 basic patch ensures proper levels of H2Bub1 and H3 acetylation. The H4 basic patch regulates many other chromatin-modifying activities, e.g., Dot1-mediated H3K79 methylation and ISWI chromatin remodeling. However, we found that regulation of H2Bub1 and histone acetylation by the H4 basic patch were independent of its regulation of Dot1 or other chromatin remodelers that influence H2Bub1. Thus, these studies document a new function for the H4 tail and reveal another layer of cross-talk regulation of the SAGA complex.

Our study outlines a negative function of the basic patch on SAGA-associated histone-modifying activities. However, it remains unknown precisely how the H4 basic patch restrains the SAGA co-activator complex. Detailed Cryo-EM studies of SAGA and the DUB module in SAGA showed that the histone acetylation and DUB modules are in close proximity to the nucleosomal core (45–49); however, the H4 tail was not resolved and thus these studies do not provide insight into how the H4 basic patch interacts. Although our in vitro DUB assay with the Upb8-DUB module did not reveal a change in the H2Bub1 turnover rate.
when the H4 basic patch was altered (Figure S3), our \textit{in vivo} and time-resolved optogenetic studies did reveal a change in H2Bub1 turnover rate (Figure 5). Thus, the H4 basic patch may be involved in modulating an upstream regulatory pathway during transcription that regulates SAGA activity, perhaps by an indirect mechanism, instead of regulating enzymatic activity by a physical interaction that was perturbed in the mutant. Interestingly, both H4-R17 and SAGA DUB module member Sgf11-R78 interact with acidic patch residue H2A-E64, and DUB activity in the Sgf11-R78A mutant was severely reduced (49, 50). We hypothesize that the H4 basic patch regulates H2Bub1 activity by competing with Sgf11 for association with the H2A acidic patch.

An additional, perhaps related possibility to explain this regulation is the fact that the H4 basic patch-H2A acidic patch interaction affects higher-order chromatin structure that may influence the accessibility of chromatin to chromatin-modifying enzymes. In this scenario, the mutation of the H4 basic patch would relieve its stabilizing effect on chromatin structure and “free” additional nucleosomes for SAGA binding, thereby increasing the overall H2Bub1 and H3 acetylation activities of the SAGA enzyme complex. The precise explanation for how this mechanism operates will be interesting to determine.

In sum, these studies reveal a novel regulatory mechanism for the activity of SAGA that likely is important for proper gene regulation and other functions associated with SAGA. Given the highly conserved nature of yeast, we expect that this function of the H4 tail is conserved in more complex eukaryotes.

\textbf{Experimental procedures}

\textbf{Yeast strains}

Strains and plasmids used in this study are listed in Supporting Information Tables 1 and 2. Strains used in Figures 1 and 3A were derived from the parental histone shuffle strain, YAA524. All other histone shuffle mutants used were derived from the parental histone shuffle strain, yDT51. Gene disruptions and endogenous overexpression were performed as described (51) and verified by PCR and immunoblotting. Plasmids were generated using a standard site-directed mutagenesis protocol and primers were designed with the Agilent QuikChange Primer Design tool (www.agilent.com/store/primerDesignProgram.jsp).

\textbf{Preparation of whole-cell extracts and immunoblots}

Cells were collected by centrifugation and stored at -80 °C. Cell pellets were thawed on ice, resuspended in 200 µL ice-cold TCA buffer (10 mM Tris, 10% TCA, 25 mM NH\textsubscript{4}OAc, 1 mM EDTA), and moved to a microcentrifuge tube and incubated on ice for 10 minutes. Samples were centrifuged at 13K rpm for 5 minutes at room temperature to collect precipitated protein. After aspirating the supernatant, the pellet was resuspended 70-100 µL of 0.1 M Tris pH 11, 3% SDS and heated at 95 °C for 10 minutes followed by centrifugation at 13K rpm for 1 minute to collect cellular debris. Protein concentration was measured using the Bio-Rad DC Protein Assay kit (5000112) and diluted to normalize concentrations across samples. Samples were diluted in 2X loading dye and 10-15 µL of sample was subjected to SDS-PAGE followed by transfer to PVDF membrane for immunoblot analyses.

\textbf{Yeast spotting assays}

Saturated overnight yeast cultures were diluted to an OD\textsubscript{600} of 0.5 and fivefold serial dilutions of the cells were plated onto YPD or SC medium containing the indicated chemotoxic agents. Plates were imaged after 2 to 4 days at 30 °C.

\textbf{Optogenetic and in vivo DUB assays}

LINX-Bre1 optogenetic time courses were performed as described (42). Briefly, colonies of LINX-Bre1 transformed in \textit{bre1\Delta, bre1\Delta ubp8\Delta} or \textit{bre1\Delta ubp10\Delta} strains were grown overnight in SC-LEU-containing medium in the dark (Figure 5) or in the presence of blue light (Figure 4). Cell density was measured and diluted to an OD\textsubscript{600} of 0.5 and grown for 4 hours in the same light condition as the overnight culture. Time courses began when cultures were moved from light to dark or vice versa. At each time point, the same volume of culture was collected and added to the appropriate volume of 100% TCA to yield a final TCA concentration of 20%, followed
immediately by mixing and centrifugation at 5000 rpm for 5 minutes at 4 °C. The supernatant was aspirated and the pellets were stored at -80 °C until processed for Western blot analysis as detailed above.

**Antibodies**

Immunoblots were developed using ECL Prime (Amersham RPN2232). Antibodies:
- Ubiquityl-Histone H2B (Cell Signaling Technologies 5546; 1:5,000), H2B (Active Motif 39237; 1:5,000), H3K79me3 (Abcam 2621; 1:2,500), H3K79me2 (Active Motif 39143; 1:2,500), H3K4me3 (Epicypher 13-0004; 1:5,000), H3K9ac (Active motif 39917, 1:2,500), H3K14ac (Millipore 07-353; 1:2,500), H3K18ac (Millipore 07-354; 1:2,500), H3K27ac (abcam ab4729; 1:2,500), H3K56ac (Active motif 39281; 1:1,000), H3 (EpiCypher 13-0001; 1:5,000), G6PDH (Sigma-Aldrich A9521; 1:100,000),
- FLAG-M2 (Sigma-Aldrich F1804; 1:5,000), Myc (Millipore; 1:2,500), Rabbit (Amersham NA934; Donkey anti-Rabbit) and mouse (Amersham NA931; Sheep anti-mouse) secondary antibodies were used at 1:10,000.

**Galactose induction**

Yeast strains that expressed either the wild-type or mutant histone H4 were grown overnight in media containing 2% raffinose. Cell density was measured and diluted to an OD_600 of 0.25 and grown for 4 hours in media containing 2% raffinose. The induction time course was begun by adding 20% galactose to a final concentration of 2%. At each time point the same volume of each culture was harvested and centrifuged at 4500 rpm for 5 minutes. The supernatant was aspirated and the cell pellets were stored at -80 °C.

**Quantitative real-time PCR (qRT-PCR)**

RNA was isolated by a hot acid phenol method as described (52). Crude RNA was treated with DNase (Promega M6101) followed by RNA cleanup (QIAGEN RNaseasy Mini Kit, 74106). cDNA was synthesized from 500 ng-1 ug of total RNA using random hexamer primers and Superscript Reverse Transcriptase III (Thermo-Fisher Scientific, 108-80044). The cDNA was diluted 1:25 before being subjected to real-time PCR (primers listed in Supporting Information). Quantitative RT-PCR was performed using the iTaq Universal SYBR Green Master mix according to manufacturer’s instructions (Bio-Rad, 1725125), and the relative quantities of transcripts were calculated using the ΔΔCt method (53) and ACT1 as a control. The data shown are the replicates of three independent experiments with three technical replicates in each experiment.

**In vitro DUB assays**

Yeast strains that expressed either wild-type or mutant histone H4, FLAG-tagged histone H2B and lacking both UBP8 and UBP10 were grown overnight in 25 mL YPD and harvested by centrifugation at 4500 rpm for 5 minutes. Cell pellets were frozen and stored at -80 °C. Pellets were resuspended in 500 uL 10 mM Tris.Cl, pH 7.4, 300 mM Sorbitol, 100 mM NaCl, 5 mM MgCl₂, 5 mM EDTA, 10% glycerol, 0.1% Igepal-30 and split into 2 microcentrifuge tubes before being subjected to standard glass bead lysis by vortexing at 4 °C. The tubes were punctured with a push pin and placed into a clean tube and spun to 3000 rpm 3 times to separate the lysate from the beads. Final lysate was clarified by centrifugation at 13K rpm for 10 minutes at 4 °C. Protein concentration was determined using the BioRad Bradford assay. To perform the assay, 50-100 µg of total protein was diluted to a final volume of 20 µL. A master mix containing enough sample to complete the experiment was prepared and preincubated at 30 °C with mixing for 30 minutes before addition of enzyme. The time course was begun by addition of recombinant Ubp8 DUB module (rDUBm) or Ubp10 enzyme. At the indicated timepoints 20 µL of sample was removed and added to a microcentrifuge tube containing 5 µL of 5X loading dye and heated at 95 °C immediately. Twenty µL of the sample was subjected to 12% SDS-PAGE, which was blotted and probed with an M2-FLAG primary antibody to detect both the FLAG-H2Bub1 and FLAG-H2B signal from the same membrane.

**Data Availability** – all data are contained within the manuscript.
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The abbreviations used are: histone H2B monoubiquitylation, H2Bub1; Spt-Ada-Gen5 acetyltransferase coactivator, SAGA; post-translational modifications, PTMs; light-inducible nuclear exporter, LINX; 6-azauracil, 6-AU; lysine acetyltransferase, KAT.
Figure 1. The H4 basic patch is required for proper H2BK123 monoubiquitylation (H2Bub1). A. Sequence of the H4 N-terminus with basic patch residues depicted. Arrows show the arginine residues mutated to alanine to create the basic patch mutant H4_{2RA} used throughout the study. B. Immunoblotting was performed for the indicated proteins or modifications using extracts from cells that expressed wild-type H4 (H4_{WT}) or the basic patch mutant (H4_{2RA}). Strains that had a mutation at H3 lysine 79 (H3K79R) or were deleted for the E3 ubiquitin ligase Bre1 (bre1Δ) were used as antibody controls for H3K79 methylation and H2Bub1, respectively. H3 was used as a loading control. C. Representative immunoblot analysis of H4_{WT} and H4_{2RA} at increasing concentrations for H2B or H2Bub1. D. Relative quantification of H2Bub1/H2B from panel C. Means ± SEM were analyzed from three biological replicates.
Figure 2. Mutation of the H4 basic patch and H2BK123 share phenotypes associated with H2Bub1 loss. Overnight cultures of the indicated strains were spotted at 5-fold dilutions onto solid rich (YPD) medium or media containing the indicated chemotoxic agents and imaged after 2-3 days.
Figure 3. H4 basic patch regulation of H2Bub1 is independent of its interaction with chromatin remodelers. A. Immunoblotting for H2Bub1 and H2B was performed using whole cell extracts from wild-type or strains lacking the indicated chromatin remodelers. B. Blots for H2Bub1 and H2B were derived from cells that expressed H4\textsubscript{WT} or H4\textsubscript{2RA} in combination with a deletion of CHD1 (chd1\textDelta). C. Relative quantification of H2Bub1/H2B from panel B. Means ± SEM were analyzed from three biological replicates.
Figure 4. The H4 basic patch does not interfere with Bre1 ubiquitylation of H2B. A. Immunoblot analysis of H2Bub1 and H2B in H4WT and H42RA in the presence or absence of UBP8 (ubp8Δ). The ability of Bre1 to increase H2Bub1 in the H42RA ubp8Δ strain suggested that the H4 basic patch mutant does not impair Bre1 catalysis. B. H4WT bre1Δ and H42RA bre1Δ cells were transformed with empty vector or ADH1-driven Flag-BRE1 and subjected to immunoblot analysis with the indicated antibodies. Visualizing normal levels of Bre1 protein in the H42RA strain indicated that Bre1 function in catalysis was normal; Bre1 protein stability is normally diminished when Bre1 is unable to catalyze H2Bub1(41). C. Optogenetic-mediated control of the E3 ligase Bre1 (LINX-Bre1) reveals similar kinetics of H2B ubiquitylation in H4WT and H42RA. D. Quantification of data from panel C. Means ± SEM were analyzed from two biological replicates.
**Figure 5.** The H4 basic patch contributes to H2B deubiquitination by influencing Ubp8 but not Ubp10. A. H4WT and H42RA cells lacking UBP10 were grown to log phase in the dark to maintain LINX-Bre1 in the cytoplasm. Timepoint 0 was taken in the dark and subsequent timepoints were collected after exposure to blue light. Immunoblot analysis of H2Bub1 and H2B was performed and quantified. The half-life measured from each curve was determined from single exponential fits of the data. B. Representative immunoblots from panel A. C. H4WT and H42RA cells lacking UBP8 were grown to log phase in the dark to maintain LINX-Bre1 in the cytoplasm. Timepoint 0 was taken in the dark and subsequent timepoints were collected after exposure to blue light. Data were processed as above. D. Representative immunoblots from panel C.
Figure 6. The H4 basic patch regulates SAGA-dependent H3 acetylation in addition to H2B deubiquitination. A. Immunoblot analysis of H3 N-terminal acetyl-lysine residues in H4WT and H42RA cells. gcn5Δ and matched wild-type cells were included as controls.
The histone H4 basic patch regulates SAGA-mediated H2B deubiquitylation and histone acetylation

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