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# Function and Selectivity of Bromodomains in Anchoring Chromatin-Modifying Complexes to Promoter Nucleosomes

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## Summary

**The functions of the SAGA and SWI/SNF complexes are interrelated and can form stable "epigenetic marks" on promoters in vivo. Here we show that stable promoter occupancy by SWI/SNF and SAGA in the absence of transcription activators requires the bromodomains of the Swi2/Snf2 and Gcn5 subunits, respectively, and nucleosome acetylation. This acetylation can be brought about by either the SAGA or NuA4 HAT complexes. The bromodomain in the Spt7 subunit of SAGA is dispensable for this activity but will anchor SAGA if it is swapped into Gcn5, indicating that specificity of bromodomain function is determined in part by the subunit it occupies. Thus, bromodomains within the catalytic subunits of SAGA and SWI/SNF anchor these complexes to acetylated promoter nucleosomes.**

## Introduction

The SWI/SNF ATP-dependent chromatin-remodeling complex and the SAGA histone acetyltransferase complex are each required for expression of a subset of genes in yeast and expression of some genes requires both complexes (Holstege et al., 1998). Genetic interactions between components of these complexes suggest a functional link between them (Pollard and Peterson, 1997; Roberts and Winston, 1997). Both complexes can be recruited to promoters by sequence-specific transcription activators and participate in transcription activation (Natarajan et al., 1999; Neely et al., 1999; Utley et al., 1998; Vignali et al., 2000; Wallberg et al., 2000; Yudkovsky et al., 1999). In vivo crosslinking studies at the yeast *HO* endonuclease promoter have shown that SWI/SNF and SAGA can form a self-sustaining epigenetic mark on the promoter subsequent to the dissociation of the activator, Swi5, which recruited them (Cosma et al., 1999).

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We have recently provided evidence for a functional interaction between HATs and SWI/SNF. Using immobilized template nucleosome assays, we have shown that acetylation of nucleosomal array templates by either the SAGA or NuA4 HAT complexes stabilizes SWI/SNF binding to promoter nucleosomes after the dissociation of the activator that recruited it to the template (Hassan et al., 2001). Thus, stable binding of SWI/SNF has been recapitulated on nucleosome templates in vitro and found to require acetylated histones (Hassan et al., 2001). These results are consistent with other evidence for functional interactions between acetylation and SWI/SNF. Syntichaki et al. have shown the participation of Gcn5, the catalytic subunit of SAGA, in the stabilization of SWI/SNF binding to promoters in vivo (Syntichaki et al., 2000). Histone acetylation by Gcn5 has been shown to be followed by SWI/SNF recruitment during activation of the interferon-β (INF-β) promoter in vitro (Agalioti et al., 2000). Transactivation by RAR/RXR was found to require histone acetylation prior to SWI/SNF action (Dilworth et al., 2000). A transient histone hyperacetylation at the *PHO8* promoter was also shown to be required for nucleosome remodeling by SWI/SNF (Reinke et al., 2001). More recently, the CBP and P/CAF histone acetyltransferases as well as hBrm remodeling complex was shown to be recruited to stimulate transcription of the human $\alpha_1$ antitrypsin promoter (Soutoglou and Talianidis, 2002).

The role of acetylated histones in SWI/SNF promoter occupancy suggest a molecular basis for the functional links between SWI/SNF and SAGA and raises the possibility that the bromodomain of the Swi2/Snf2 subunit might play an important role in anchoring SWI/SNF to promoters. A number of chromatin-modifying complexes, including SWI/SNF and SAGA, contain highly conserved bromodomain(s) (Haynes et al., 1992; Jeanmougin et al., 1997). Bromodomains have been implicated as acetyl-lysine recognition modules (Dhalluin et al., 1999; Hudson et al., 2000; Jacobson et al., 2000; Ornaghi et al., 1999; Owen et al., 2000). The phenotypic affect of the bromodomain varies in different proteins. For example, no phenotype for the bromodomain deletion in the Swi2/Snf2, Spt7, or *Drosophila* Brahma has been observed (Elfring et al., 1998; Gansheroff et al., 1995; Laurent et al., 1991, 1993). In contrast, a bromodomain deletion in one of the human homologs of Swi2/Snf2, hBrm, results in the loss of nuclear localization and a decrease in protein stability (Jacobson et al., 2000). In addition, deletion of the Gcn5 bromodomain affected transcription to some extent (Candau et al., 1997; Marcus et al., 1994; Sterner et al., 1999). Yeast contains a SWI/SNF-related complex, RSC, which appears to play a more global role in the yeast life cycle, is more abundant than SWI/SNF, and contains subunits with multiple bromodomain motifs (Cairns et al., 1996). Some of the bromodomains in the Sth1, Rsc1, or Rsc2 subunits in the yeast RSC complex are essential for cell viability. Deletion of the Sth1 bromodomain results in a conditional phenotype, whereas only one of the bromodomains in the other related RSC subunits (Rsc1 and Rsc2;

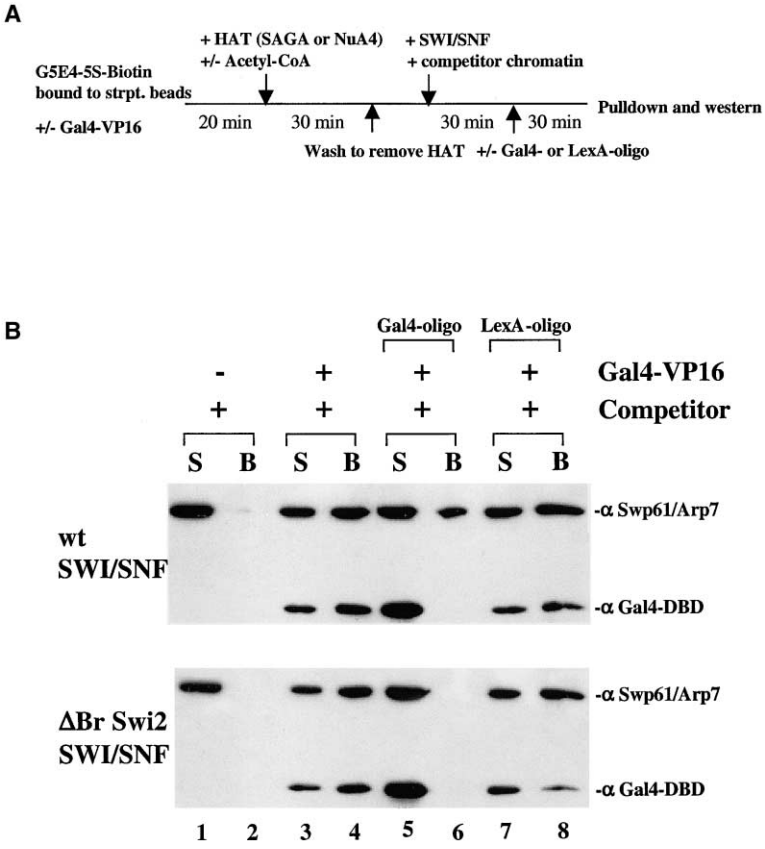


Figure 1. Swi2/Snf2 Bromodomain Deletion Abolishes the Retention of the SWI/SNF Complex on Acetylated Nucleosome Arrays

(A) Diagram of the immobilized template experiment.

(B) Biotinylated G5E4-5S was reconstituted into nucleosome arrays and bound to paramagnetic beads (Dynabeads) coupled to streptavidin as described in Hassan et al. (2001). These templates were then bound by Gal4-VP16 where indicated (lanes 3–8) and acetylated by SAGA, followed by the addition of SWI/SNF (all lanes) and competitor chromatin (all lanes). 1 μg of a Gal4-site oligo (lanes 5 and 6) or LexA-site oligo (as a control, lanes 7 and 8) was subsequently added, and the competition for Gal4-VP16 was performed at 30°C for 30 min prior to separating the supernatants from the beads and Western blot analysis. The blots were then probed with antibodies against the Swp61/Arp7 subunit of the SWI/SNF complex. The antibody against the DNA binding domain of Gal4 (Gal4 (DBD): SC-577; Santa Cruz Biotechnology Inc.) shows the amount of Gal4-VP16 in the supernatant (S) and the bead (B) lanes. The top and the bottom gels show this immobilized template competition experiment using either wild-type SWI/SNF or SWI/SNF lacking the Swi2/Snf2 bromodomain, respectively.

the two forms of the RSC complex contain either of these two different gene products) is essential for function (Cairns et al., 1999).

In this study, we show that the bromodomains of Swi2/Snf2 and Gcn5 were necessary for the stable occupancy of the SWI/SNF and the SAGA complexes, respectively, on acetylated promoter nucleosomes. On the other hand, the Spt7 bromodomain in the SAGA complex was not necessary for the retention of SAGA on acetylated nucleosome arrays. These data illustrate the selectivity and specificity of bromodomain-containing subunits in anchoring chromatin-modifying complexes on promoter nucleosomes. Synthetic phenotypes observed upon combining deletions of the Swi2/Snf2 bromodomain with deletions of the Gcn5 bromodomain or temperature-sensitive mutants in Tra1 suggest related roles of these bromodomains in recruitment and retention of these complexes in chromatin.

## Results

### Requirement of the Swi2/Snf2 Bromodomain for Anchoring the SWI/SNF Complex on Acetylated Nucleosome Arrays

To directly test whether the bromodomain within the Swi2/Snf2 subunit of the SWI/SNF complex contributes to the binding and stabilization of the complex on nucleosome arrays, we purified SWI/SNF from a strain lacking the Swi2/Snf2 bromodomain and tested it in a recruitment/retention assay using a nucleosome array template (shown in Figure 1A; also see Hassan et al., 2001). The loss of the Swi2/Snf2 bromodomain does not affect complex integrity as detected by silver staining (data not shown). The nucleosome templates used in Figure 1 had been previously acetylated by the SAGA complex. While SWI/SNF was able to bind to these arrays by itself (Hassan et al., 2001), this nonspecific binding was not observed in the presence of nontemplate competitor chromatin (Figure 1B, compare lanes 1 and 2). With the addition of Gal4-VP16, which binds the five Gal4 sites in the artificial G5E4 promoter, SWI/SNF binding to the template was recovered, demonstrating the recruitment of the complex to the array by the activator (Figure 1B, compare lanes 3 and 4). When Gal4-VP16 was subsequently removed from the template by a competing Gal4-site oligo (Figure 1B, lanes 5 and 6), wild-type SWI/SNF was retained on these acetylated nucleosome arrays (Figure 1B, lane 6, top gel). By contrast, under the same conditions, SWI/SNF lacking the Swi2/Snf2 bromodomain dissociated from the template with the Gal4-VP16 activator (Figure 1B, lane 6, bottom gel). The same result was observed when templates were acetylated with the NuA4 HAT complex prior to SWI/SNF binding (data not shown). Thus, the bromodomain of SWI/SNF was necessary for anchoring of this complex on H3- or H4-acetylated nucleosome arrays.

### Anchoring of SAGA, but not NuA4, on Acetylated Nucleosome Arrays after Activator Removal

Like SWI/SNF, the SAGA and NuA4 HAT complexes are also recruited to promoters by yeast transcriptional acti-

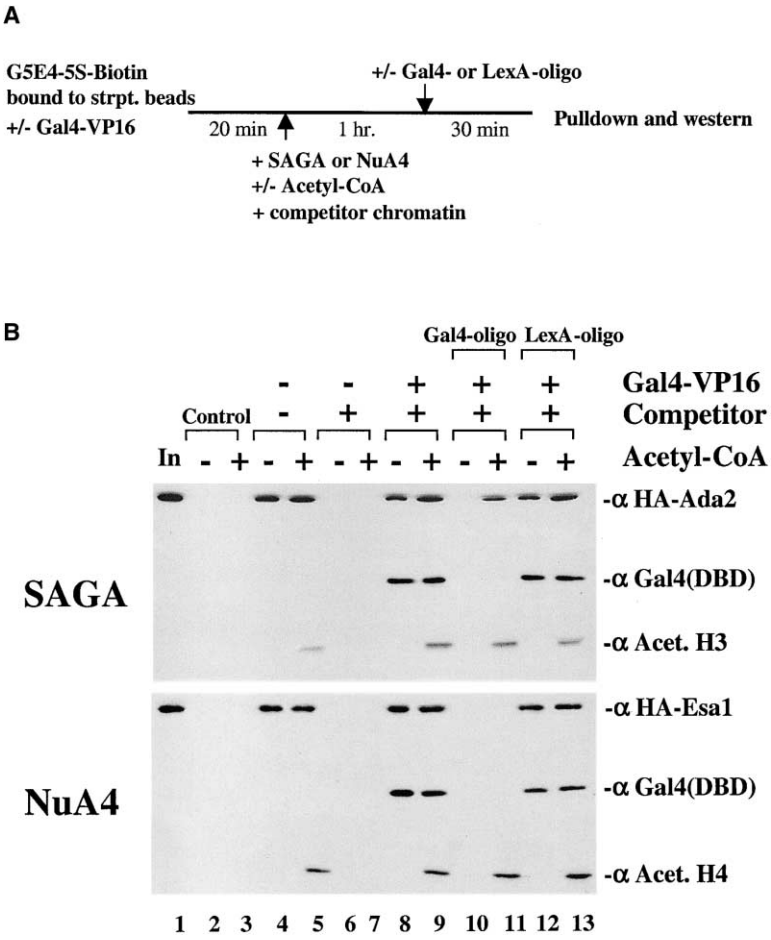


Figure 2. SAGA, but not NuA4, HAT Complex Is Retained on Acetylated Arrays after the Loss of Gal4-VP16

(A) Diagram of the immobilized template experiment.

(B) Immobilized G5E4-5S nucleosome arrays were bound by Gal4-VP16 (lanes 8–13), followed by the addition of either SAGA or NuA4 in the presence or absence of acetyl-CoA and competitor chromatin as indicated followed by oligo competition as in Figure 1. SAGA (top gel) or NuA4 (bottom gel) binding was analyzed using HA-Ada2 or HA-Esa1 antibodies, respectively. Antibodies against acetylated H3 (Lys 9/14) or H4 (Lys 5) (Upstate Biotech.) were used to detect the presence of acetylation in the different lanes. The loss of Gal4-VP16 after the oligo competition was analyzed as in Figure 1 by using a Gal4-DBD antibody.

vators (Brown et al., 2001; Utley et al., 1998; Vignali et al., 2000). Moreover, the SAGA complex contains two bromodomains, one each in the Gcn5 and Spt7 subunits. By contrast, the NuA4 complex does not have any known subunits with bromodomains. We tested whether these HAT complexes might stabilize their own binding to nucleosome arrays by acetylating histones (Figure 2A). Recruitment of SAGA or NuA4 by Gal4-VP16 in the presence of competitor chromatin was independent of the presence of acetyl-CoA or the resulting acetylated histones (Figure 2B, compare lanes 8 and 9). However, when Gal4-VP16 was removed from the template by a Gal4-site oligo, SAGA was retained on these arrays only in the presence of acetyl-CoA and acetylated H3 (Figure 2B, compare lanes 10 and 11, top gel). Thus, modification of the template by SAGA served to anchor the complex. By contrast, while NuA4 was capable of acetylating the template, it did not become anchored and dissociated with the activator (Figure 2B, lanes 10 and 11, bottom gel). Probing the blots with anti-acetylated H3 or H4 confirms the presence of acetylated histones H3 or H4 when acetyl-CoA was present in the reaction (Figure 2B, lanes 5, 9, 11, and 13). These data illustrate that while transcription activators can recruit both SAGA and NuA4 complexes, only the bromodomain-containing SAGA complex became anchored to the acetylated promoter.

### Requirement of the Gcn5 Bromodomain, but not the Spt7 Bromodomain, in Anchoring the SAGA Complex on Acetylated Nucleosome Arrays

To test whether either of the two bromodomains in SAGA was necessary for its retention on promoter nucleosomes following dissociation of the activator, we utilized strains lacking either the Gcn5 or Spt7 bromodomains or lacking both. Purified SAGA complex from these strains was tested in the recruitment/retention assay (Figure 3). The HAT activity of SAGA purified from these different strains was normalized for these experiments. Again, wild-type SAGA was retained on nucleosome arrays following activator removal in a manner dependent on acetyl-CoA and histone acetylation (Figure 3A, lane 7). The SAGA complex lacking the Gcn5 bromodomain was recruited to the template and acetylated H3 (Figure 3B, lanes 4 and 5) but was not retained upon loss of the activator (Figure 3B, lane 7). In contrast, a deletion of the Spt7 bromodomain had no significant effect on binding, targeting, or the retention of the complex in either the presence or absence of acetylation (Figure 3C). Similar to a Gcn5 bromodomain deletion, a double deletion of the Gcn5 and Spt7 bromodomains abolished anchoring of the complex on the nucleosome template (Figure 3D, lane 7). These data distinguish between the functions of the Gcn5 and Spt7 bromodomains. The Gcn5 bromodomain was required to anchor SAGA on the acetylated

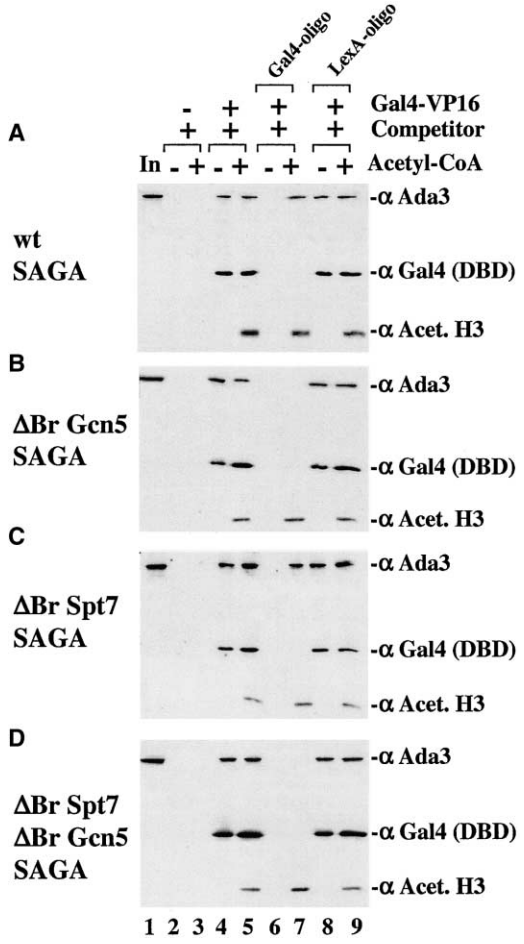


Figure 3. The Gcn5 Bromodomain in the SAGA Complex Is Necessary for Its Retention on Acetylated Nucleosome Arrays

Gcn5 bromodomain deletion, but not an Spt7 bromodomain deletion, abolishes the retention of the SWI/SNF complex on acetylated nucleosome arrays. SAGA was purified from strains with different bromodomain deletions (ΔBr Gcn5 [B], ΔBr Spt7 [C], and ΔBr Gcn5 ΔBr Spt7 double deletion [D]) in the SAGA complex as indicated. The recruitment/retention experiment was performed as in Figure 2 and the antibody used for SAGA binding is against α-Ada3.

promoter nucleosomes, whereas the Spt7 bromodomain was not.

### Replacement of the Gcn5 Bromodomain with That of Spt7 Supports Anchoring of SAGA to Acetylated Nucleosome Arrays

The difference between the ability of the Gcn5 and Spt7 bromodomains to anchor SAGA onto promoter nucleosomes might be due to the specificity of the bromodomains and their ability to recognize acetylated histone H3 or a property of the subunit which each is part of. To test these possibilities, we performed bromodomain swap experiments. We replaced the Gcn5 bromodomain with the Spt7 bromodomain to see if it would now function as part of Gcn5 (Figure 4). As a positive control we also replaced the Gcn5 bromodomain with the Swi2/Snf2 bromodomain. SAGA complexes purified from the

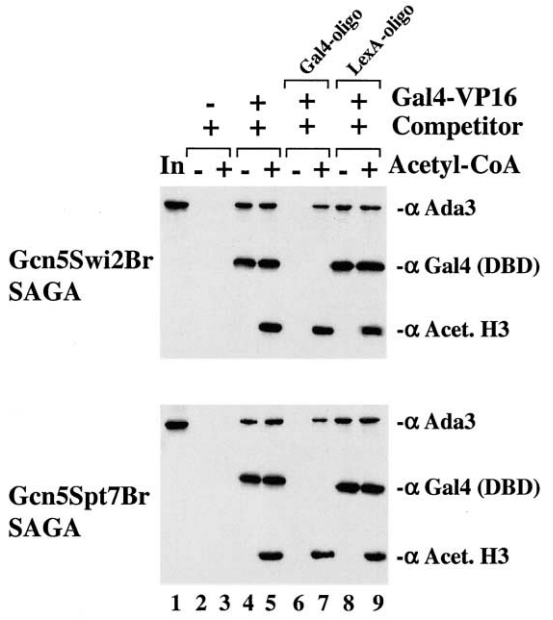


Figure 4. Spt7 Bromodomain in the Context of Gcn5 Can Bind to Acetylated Nucleosome Arrays

The Gcn5 bromodomain was replaced by either the Swi2/Snf2 bromodomain of SWI/SNF (top gel) or the Spt7 bromodomain of SAGA (bottom gel). The SAGA complex was partially purified from these strains and used in the recruitment/retention assays as before.

bromodomain-swapped strains were tested in the recruitment/retention assay. The Swi2/Snf2 bromodomain was able to functionally replace the Gcn5 bromodomain. SAGA complex lacking the Gcn5 bromodomain, but containing the Swi2/Snf2 bromodomain as part of Gcn5, was retained on acetylated templates after activator removal (Figure 4, lane 7, top gel). Surprisingly, when the Gcn5 bromodomain was replaced by the Spt7 bromodomain, the SAGA complex was able to bind to acetylated nucleosomes after the dissociation of the activator (Figure 4, lane 7, bottom gel). These domain-swapping experiments illustrate that the Spt7 bromodomain has the ability to bind to acetylated histone H3; however, Spt7 does not use this activity to effectively anchor SAGA on promoter nucleosomes, which appears to be a particular function of the Gcn5 subunit of SAGA.

### The Gcn5 Bromodomain Also Anchors SAGA onto H4-Acetylated Nucleosome Arrays

While the bromodomain in Spt7 did not contribute to anchoring SAGA onto nucleosomes where it acetylated histone H3, it is possible that the Spt7 bromodomain functions through acetylated H4 tails (i.e., acetylation by NuA4). The H4 tails exit the nucleosome core at quite a different position than the H3 tail (Luger et al., 1997), which could be oriented toward Spt7 when SAGA is bound to the nucleosome. To test whether the Spt7 bromodomain is important in retaining the SAGA complex on acetylated H4 tails, we acetylated nucleosome arrays with NuA4, followed by washing to remove the HAT and acetyl-CoA. This H4-acetylated template was

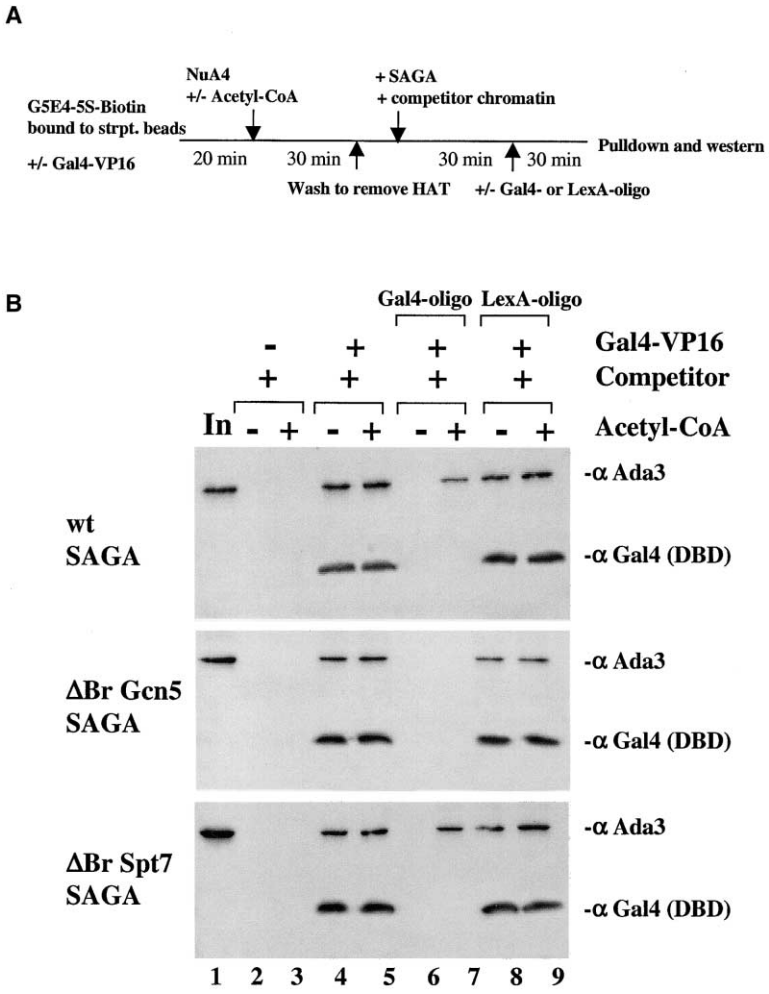


Figure 5. Deletion of the Spt7 Bromodomain Does Not Affect the Binding of SAGA to NuA4-Acetylated Nucleosome Arrays

(A) Diagram of the recruitment/retention experiment.

(B) Immobilized G5E4-5S nucleosome arrays were bound by Gal4-VP16 (lanes 4–9), followed by the addition of NuA4 either in the presence or absence of acetyl-CoA, as indicated. The reactions are then washed to remove any NuA4 or acetyl-CoA from the reaction. Wild-type (top gel) and mutant (ΔBr Gcn5, middle gel; ΔBr Spt7, bottom gel) SAGA complexes and competitor chromatin are added, followed by oligo competition as in Figure 2, and the retention of these complexes on NuA4-acetylated templates is analyzed.

then incubated with SAGA, competitor chromatin, and Gal4-VP16 (Figure 5A). It is important to note that SAGA was added to the reaction after all acetyl-CoA was removed to prevent it from acetylating the template on histone H3. Wild-type SAGA was retained on NuA4-acetylated templates (Figure 5B, lane 7, top gel). Moreover, this could be attributed to the Gcn5 bromodomain since deletion of the Gcn5 bromodomain abolished SAGA retention on NuA4-acetylated nucleosome arrays after activator removal (Figure 5B, lane 7, middle gel). However, loss of the Spt7 bromodomain had no affect on the stabilization of the SAGA complex on these templates (Figure 5B, lane 7, bottom gel). These data illustrate that the bromodomain of Gcn5, but not the Spt7 bromodomain, interacts with acetylated lysines on either the H3 or H4 tails. Thus, anchoring SAGA onto promoter nucleosomes appears to be a dedicated function of the Gcn5 bromodomain, distinguishing it from the bromodomain in Spt7.

### The Bromodomains of Swi2/Snf2 and Gcn5 Participate in the Functions of These Proteins In Vivo

The modest phenotypes observed in Swi2/Snf2 or Gcn5 bromodomain deletion mutants (see Introduction) suggest that neither bromodomain is usually essential for gene activation. However, if the bromodomains contribute to this process, we might expect their importance to be enhanced in the presence of other mutations reducing the efficiency of the pathway. Mutations in SWI/SNF subunits often show strong synthetic phenotypes with mutations in SAGA subunits (Roberts and Winston, 1997; Pollard and Peterson, 1997), and the bromodomain of Gcn5 has been shown to affect chromatin remodeling by SWI/SNF in vivo (Syntichaki et al., 2000). These results suggest complimentary functions of these complexes in vivo. Since each complex has a bromodomain that is capable of binding acetylated histones, we tested for synthetic phenotypes upon combining mutations deleting the bromodomains of Gcn5 and Swi2/Snf2. As can be seen in Figure 6A, deletion of the Swi2/Snf2 or Gcn5 bromodomains usually had at most a moderate affect on cell growth under a variety of conditions. However, when both bromodomains were deleted, a more pronounced affect on cell growth was observed in the presence of galactose or sulfometuron methyl (SMM) or in the absence of histidine. Growth on galactose induces activation of Gal4-driven genes, which requires the SAGA complex in vivo (Larschan and Winston, 2001; Bhaumik and Green, 2001), and the Gal4 activation

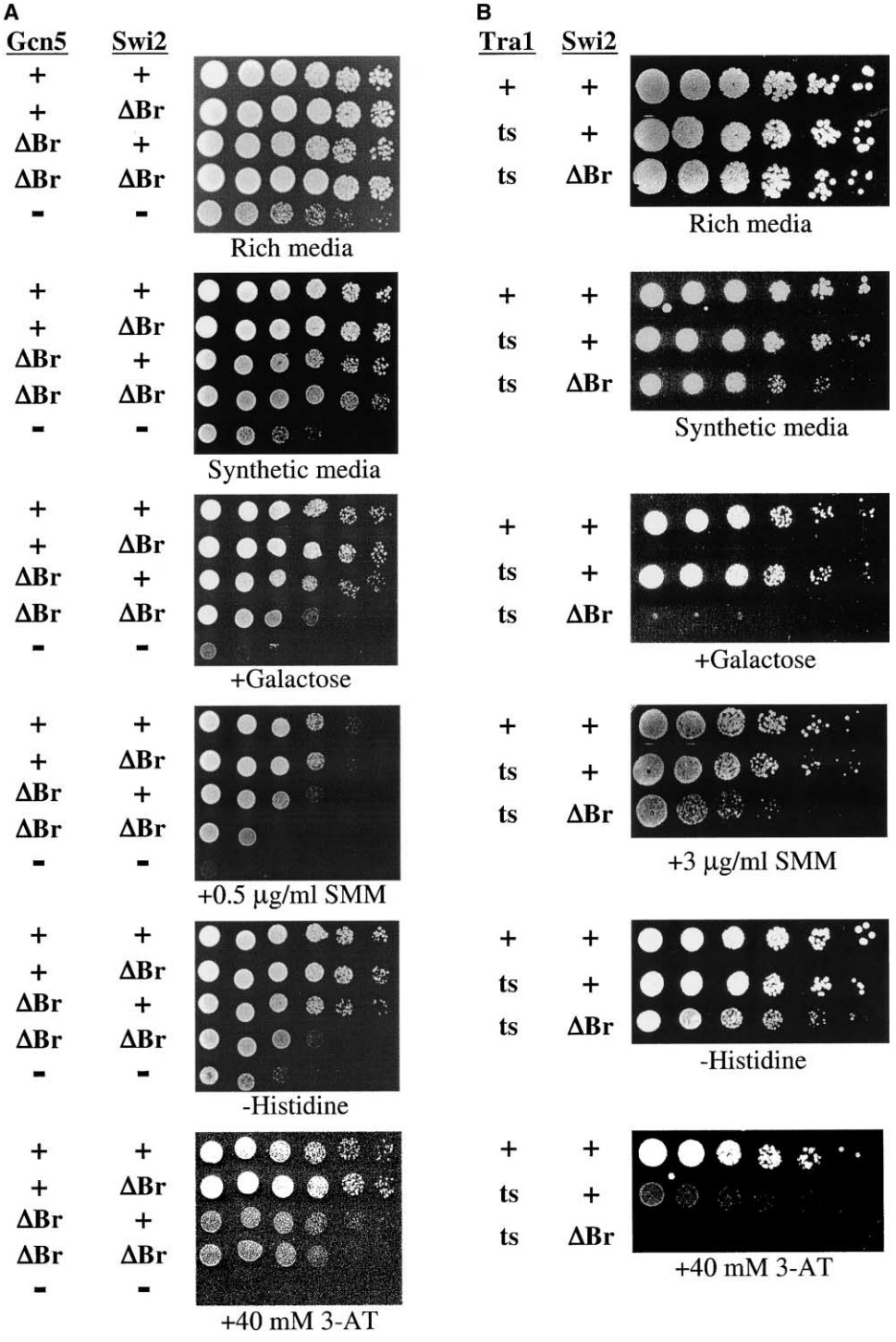


Figure 6. Bromodomain Deletion Mutants Show Reduced Growth under Conditions Requiring Activated Transcription

(A) Yeast strain FY1352 (*MATa ura3-52 lys2-173R2 leu2Δ1 his3Δ200 gcn5Δ::HIS3 snf2Δ::LEU*) was transformed with vector alone (minus) or plasmids encoding either wild-type (plus) or Δbromodomain (ΔBr) Gcn5p or Swi2p. The resulting transformants were plated on either rich media (Rich media); SC media (−Trp−Ura) with dextrose (Synthetic media) or with galactose (Galactose); or SC dextrose media (−Trp−Ura) lacking Histidine (−Histidine) or with 3-aminotriazole (3-AT) or sulfometuron methyl (SMM). Scans of plates are representative of three individual experiments.

(B) Yeast containing a temperature-sensitive (ts) allele of *TRA1* and either wild-type Swi2 (plus) or Δbromodomain Swi2 (ΔBr) were compared for rates of growth to yeast containing wild-type alleles for both *TRA1* and *SWI2*. Growth was measured at the permissive temperature (30°C). Scans of plates are representative of three individual experiments. Plates contained rich media (YPD); SC media (−Trp) with dextrose (Control) or galactose (Galactose); SC dextrose media lacking Histidine (−Histidine) or with 3-aminotriazole (3-AT); or SC dextrose media (−Trp) with sulfometuron methyl (SMM).

domain has been shown to interact with both the SAGA and SWI/SNF complexes in vitro (Carrozza et al., 2002; M.J.C. and J.L.W., unpublished). Growth in the presence of SMM or in the absence of histidine requires activation of Gcn4-dependent genes (Hope and Struhl, 1985; Jia et al., 2000). The Gcn4 activator has been shown to recruit both SAGA and SWI/SNF in vitro and in vivo (Natarajan et al., 1999; Yudkovsky et al., 1999; Neely et al., 1999, 2002; Utley et al., 1998; Kuo et al., 2000). Growth in the presence of aminotriazole (3-AT) also requires the function of the Gcn4 activator (Kanazawa et al., 1988) and was reduced by the deletion of the Gcn5 bromodomain but only slightly further impaired by deletion of the Swi2/Snf2 mutation. We also tested for phenotypes of the Swi2/Snf2 bromodomain deletion in strains bearing a temperature-sensitive mutation in the TRA1 gene (Figure 6B). Tra1 is the largest subunit of the SAGA and NuA4 complexes and plays an important role in their recruitment by activators (Brown et al., 2001). We reasoned that even at the permissive temperature this strain may be weakened in activator function and thus more susceptible to additional mutations affecting gene activation. When combined with the Tra1 temperature-sensitive mutation, strong phenotypes were seen with the Swi2/Snf2 bromodomain deletion with regard to growth on galactose and in the presence of aminotriazole. More modest phenotypes were seen for growth in the presence of SMM or in the absence of histidine. Taken together, these data suggest that the bromodomains of Gcn5 and Swi2/Snf2 play important roles in the activation of Gcn4- and Gal4-driven genes in vivo. The function of these bromodomains seem particularly critical in the presence of mutations in other components affecting recruitment (Tra1) or anchoring (e.g., other bromodomain deletions) of chromatin-modifying/remodeling complexes to promoters.

The Swi2/Snf2 and Gcn5 bromodomain deletions also showed a synthetic defect with regard to growth on raffinose (Figure 7A). Growth on raffinose requires the product of the *SUC2* structural gene for invertase. Transcription of *SUC2* is affected by both SWI/SNF and Gcn5 (Neigeborn and Carlson, 1984; Pollard and Peterson, 1997; Sudarsanam et al., 1999). To address whether bromodomains affected occupancy of these complexes in vivo, we tested whether the Swi2/Snf2 bromodomain deletion had any effect on the occupancy of the SWI/SNF complex at the *SUC2* promoter by in vivo chromatin immunoprecipitation experiments. We integrated 13-Myc epitopes at the C terminus of Snf6 in a *swi2*Δ/*snf2*Δ background and transformed the resulting strain with either wild-type or Δbromodomain Swi2/Snf2-expressing plasmids. Identical strains, lacking the Myc tag, were used as negative controls. Myc-specific antibody was used to immunoprecipitate DNA crosslinked to Myc-tagged Snf6 from whole-cell extracts prepared from formaldehyde-treated cells. Polymerase chain reaction (PCR) was performed to measure the amount of *SUC2* promoter sequence within the immunoprecipitates. The *SUC2* promoter sequence was immunoprecipitated to a greater extent when DNA was purified from the wild-type SWI/SNF strain under derepressed conditions (Figure 7B, compare lanes 13 and 14), as expected. Importantly, the same preferential immunoprecipitation of the *SUC2* promoter that was observed with the wt SWI/SNF was not observed when the bromodomain in Swi2/Snf2 subunit was deleted (Figure 7B, lanes 14–16). This argues for the importance of the Swi2/Snf2 bromodomain in binding of the SWI/SNF complex to the *SUC2* promoter under derepressing conditions. Lanes 9–12 show background binding of the complex in the absence of any tag, and lanes 1–8 are input controls in the absence or presence of the Myc tag for the different strains under different conditions. Figure 7C shows the quantification of the ChIP data from three different experiments. Again, wt SWI/SNF bound to the *SUC2* promoter 3.5- to 4-fold more under derepressed than repressed condition, whereas a deletion in the Swi2/SNf2 bromodomain did not have the same enhanced effect under derepressed conditions. Thus, the Swi2/Snf2 bromodomain contributes to SWI/SNF occupancy of the *SUC2* promoter in vivo.

## Discussion

In this report, we have examined the effect of three different bromodomains in yeast SWI/SNF and SAGA on binding and anchoring these chromatin-modifying complexes on promoter nucleosomes. We show that even though SWI/SNF purified from a Swi2/Snf2 bromodomain deletion strain was recruited to acetylated nucleosome array templates, it was not anchored on these templates and its occupancy was instead dependent on the activator that recruited it. This illustrates the requirement for this bromodomain in anchoring the complex on acetylated templates. In addition, we have shown that SAGA, but not NuA4, is retained on both H3- and H4-acetylated nucleosome arrays after activator removal. The difference in the ability of SAGA versus NuA4 to become stably anchored at the promoter is consistent with the distribution of acetylated nucleosomes resulting from each being recruited by a promoter bound activator. Once recruited, NuA4 leads to a broader range of acetylated nucleosomes spreading from the site of the bound activator. SAGA leads to a narrow patch of acetylation on promoter-proximal nucleosomes, consistent with its catalytic subunit, Gcn5, becoming stably anchored on those nucleosomes (Vignali et al., 2000). Similarly, the SWI/SNF complex is localized and becomes anchored to promoter-proximal nucleosomes (Hassan et al., 2001). Thus, the promoter-proximal patch of acetylated nucleosomes generated by the SAGA complex can serve as a high-affinity interaction site for bromodomain-containing protein complexes. For example, it is easy to envisage these acetylated histones facilitating binding of TFIID to the promoter (as suggested by Cosma et al., 2001) through the bromodomains in this complex (Jacobson et al., 2000; Matangkasombut et al., 2000).

We have also shown that the Spt7 bromodomain can interact with acetylated histones but apparently fails to do so in the intact SAGA complex. This indicates that not all bromodomain-containing proteins will serve this function. The Spt7 bromodomain might instead bind to other acetylated proteins (e.g., transcription factors) that have been acetylated by SAGA or other histone acetyltransferase complexes. It is also possible that the Spt7 bromodomain interacts functionally in some way with

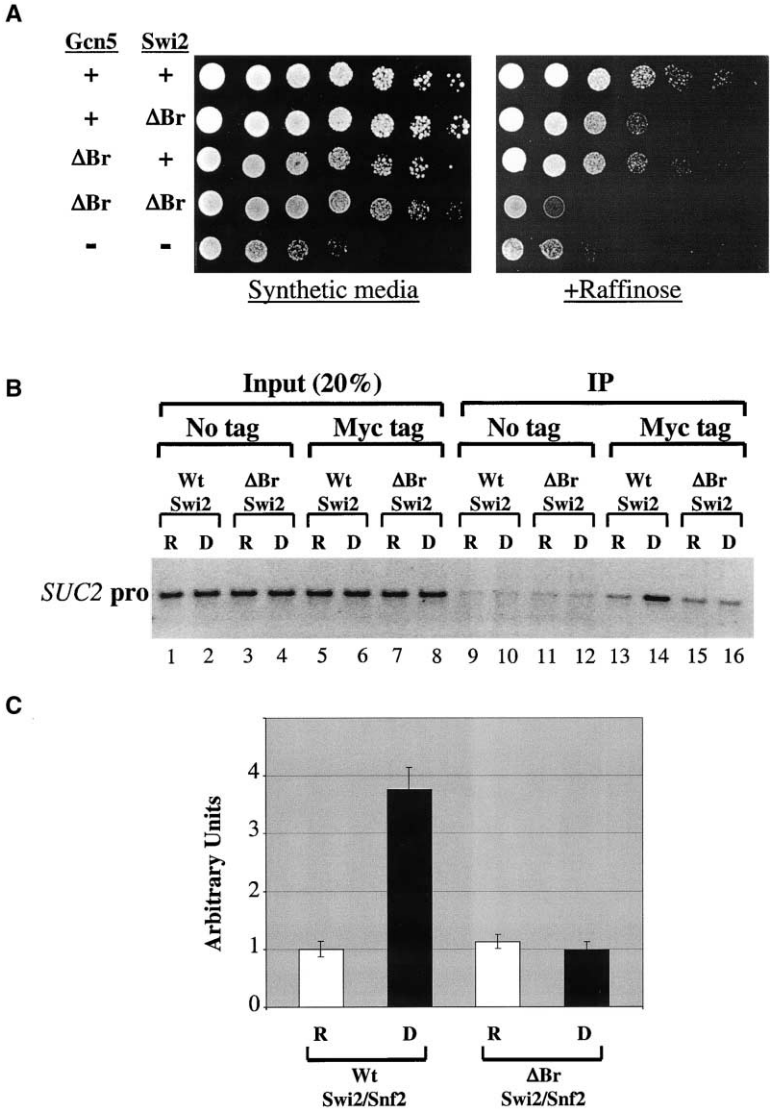


Figure 7. The Swi2/Snf2 Bromodomain Deletion Reduces Occupancy of the SWI/SNF Complex at the *SUC2* Promoter under Derepressing Conditions

(A) Same experiment as in Figure 6A, except that the resulting transformants were plated on SC media (−Trp−Ura) with dextrose (Synthetic media) or with raffinose (Raffinose).

(B) Requirement of the Swi2/Snf2 bromodomain for stable association of the complex in vivo. Appropriate yeast strains were grown under repressed (R), 2% dextrose, or derepressed (D), 0.05% dextrose, states. Chromatin immunoprecipitation was done and PCR performed with a pair of primers for the *SUC2* promoter that gave approximately a 300 bp fragment (−297 to +19) on DNA isolated from the immunoprecipitation for untagged (negative control, lanes 9–12) or 13-Myc-tagged strains (lanes 13–16) or from the whole-cell extracts (20% inputs, lanes 1–8). Lanes 1–4 and lanes 5–8 are inputs for the negative control and the Myc IP experiment, respectively.

(C) Graphical representation of the average values and the standard deviation of data from three different experiments (data shown in [B] and two additional repeats). All the data are normalized to the value of the Myc IP performed with the wt SWI/SNF under repressed conditions.

acetylated lysines in the histone H3 and H4 tails but only when the activator is present to stabilize the binding of SAGA. Loss of the activator might result in a change in the binding of the complex that will not allow its continued interaction of Spt7 with the histone tails. Clearly bromodomain function in Spt7 differs significantly from bromodomain function in Gcn5.

The fact that bromodomains in two different complexes, SWI/SNF and SAGA, recognize the acetylation patterns produced by SAGA or NuA4 suggests possible overlapping functions between these complexes. Indeed, synthetic phenotypes observed for double Gcn5 and Swi2/Snf2 bromodomain deletions support this possibility. Thus, while neither bromodomain deletion had strong phenotypes on its own, combining them to potentially perturb multiple interactions of the complexes with acetylated promoter nucleosomes had stronger phenotypes. It is also possible that there will be redundant functions between these and other homologous chromatin-modifying complexes such as the yeast RSC complex. Indeed, RSC could be dependent on its bromodomains for targeting of the complex to substrates, whereas the SWI/SNF complex is recruited via activators. This may explain why some of the RSC bromodomains are essential. Like in RSC, the bromodomains in human TAFII250 seem to be critical for function. The presence of multiple tandem bromodomains in some proteins (like two bromodomains in TAFII250, Rsc1, and Rsc2) might increase the affinity of these proteins for acetylated histone tails and promote efficient targeting of these proteins by acetylated lysines. This has been observed in the case of TAFII250, where the affinity of this protein for specifically diacetylated histone tails is enhanced compared to singly acetylated tails (Jacobson et al., 2000).

By acetylating histones, the SAGA complex is able to lay down a mark in chromatin that stablizes its own interaction with the template as well as that of SWI/SNF. This releases the SAGA and SWI/SNF complexes from activator dependence for promoter occupancy. Thus,

acetylation by the SAGA can be viewed as putting down an anchor to stabilize its occupancy and that of other protein complexes. An analogous situation occurs with lysine 9 methylation of histone H3 (Grewal and Elgin, 2002). The function of enzymes in chromatin-modifying complexes in laying down marks on which they anchor explains both the phenomenon of these complexes stably binding the products of their enzymes and their ability to form self-sustaining epigenetic marks in chromatin (Cosma et al., 1999).

## Experimental Procedures

### Purification of the SWI/SNF, SAGA, and NuA4 Complexes and Strains

The wild-type and the bromodomain deletion SWI/SNF complexes were purified from yeast whole-cell extract by FLAG immunoprecipitation. The strains are swi2/snf2 Δ and contain a FLAG-tagged on the Snf6 (+ Leucine) subunit of the SWI/SNF complex and either a wt or a bromodomain deletion Swi2/Snf2 (+ Ura) subunit. Briefly, yeast whole-cell extract was added to FLAG M2 resin. The SWI/SNF complex was eluted from the beads by FLAG peptide. Purification was monitored by Western blot, using several antibodies to SWI/SNF subunits as well as silver staining. SAGA and NuA4 complexes were purified over Ni-NTA agarose, followed by mono Q chromatography. Bromodomain deletions of these complexes did not affect complex integrity, as observed by silver staining (data not shown). ΔBr Gcn5 strain (deletion of the gcn5 bromodomain) was created by transforming Δgcn5 with a plasmid containing the first 1044 residues of the gcn5 gene (+ Trp). ΔBr Spt7 strain was a gift from F. Winston (FY 1009). The double bromodomain deletion (ΔBr Gcn5, ΔBr Spt7) strain was created by transforming Δ*gcn5* Δ*spt7* double deletion strain (FY 1438) with two plasmids, one containing the first 1044 residues of the *gcn5* gene (+ Trp) and the other with all but the *spt7* nucleotide residues 1386–1614 (FB 1403), gifts from F. Winston. For the swapping experiments, gcn5 (1–1044 bp) was fused to spt7 (1386–1614 bp) to replace the Gcn5 bromodomain with that of Spt7, and gcn5 (1–1044 bp) was fused to swi2/snf2 (4716–4944 bp) to replace the Gcn5 bromodomain with that of Swi2/Snf2. For the ChIP assays, we transformed Δswi2/snf2 deletion strain that contained either an untagged (control) or an integrated snf6-13 myc-tagged with either wild-type or swi2/snf2 bromodomain deletion plasmids.

### Immobilized Template Recruitment/Retention Assays

The recruitment assay was performed as described previously (Hassan et al., 2001). Briefly, the G5E4-5S fragment was end-labeled with biotin-14-dATP, gel purified, and reconstituted by step dilution. The nucleosomal arrays were then bound to paramagnetic beads coupled to streptavidin (Dynabeads streptavidin, Dynal) as described. 100 nM Gal4-VP16 (50 nM dimers) was added to approximately 200 ng of this template in 20 μl (3.4 nM) binding buffer (10 mM HEPES [pH 7.8], 50 mM KCl, 5 mM DTT, 5 mM PMSF, 5% glycerol, 0.25 mg/ml BSA, and 2 mM MgCl2) and incubated for 20 min at 30°C followed by the simultaneous addition of 4 nM SWI/SNF, SAGA, or NuA4 and 2 mg competitor chromatin. After an additional 1 hr incubation at 30°C, the templates were concentrated on a magnet, the supernatant was removed, and the beads were washed twice before performing Western blot analysis. For the Gal4-VP16 competition experiments, 1 mg of Gal4-oligo or LexA-oligo (control) was added to the reaction after SWI/SNF incubation and the competition performed at 30°C for 30 min prior to concentrating the templates on the beads. In Figures 3 and 4, partially purified SAGA from various deletion or substitution strains were used for the recruitment assay. In Figure 5, NuA4 in the presence or absence of acetyl-CoA was targeted to the templates after the addition of Gal4-VP16. After a 30 min incubation and two washes in the binding buffer to remove the HAT complexes, SAGA and competitor chromatin was added as before. Gal4- or LexA-oligo was then added where indicated for 30 min prior to pull-down.

### Growth Analysis and Strains

The Δ*gcn5* Δ*swi2* double deletion strain used for the study of Gcn5 and Swi2 bromodomain mutants was a gift from F. Winston (FY1352 [*MATa ura3-52 lys2-173R2 leu2Δ1 his3Δ200 gcn5Δ::HIS3 snf2Δ::LEU*]). This strain was transformed with either p*SWI2 wild-type-URA3*-CEN or p*SWI2* Δ *bromodomain-URA3*-CEN (corresponding to the first 1554 amino acids of the Swi2p) and with either p*GCN5 wild-type TRP1*-CEN or p*GCN5* Δ*bromodomain TRP1*-CEN (corresponding to the first 348 amino acids) and were recovered by growth on SCD-Trp-Ura plates. Growth of strains obtained were compared to each other and to the double deletion strain transformed with vector alone. Cells were collected in stationary phase, washed with water, and compared by colony dilution assay (4-fold dilutions from a starting $OD_{600nm}$ of 1.25) after 2 or 3 days at 30°C.

The temperature-sensitive *tra1-28* allele (pCB157-*TRP-CEN*) was created by PCR mutagenesis and transformed into strain YCB650 containing *tra1*::*LEU2* and p*TRA1-URA3-CEN* as previously described (Brown et al., 2001). The *SWI2* gene was replaced in this strain by homologous recombination using a PCR-generated *S. pombe his5*$^+$ cassette from pFA6a-His3MX6, containing 40 bp flanking the ORF for *SWI2* (Longtine et al., 1998). The integration of the *his5*$^+$ gene at the *SWI2* locus was verified by PCR. The p*TRA1-URA3-CEN* plasmid was removed from this strain by growth on synthetic-complete-dextrose (SCD)-Trp plates containing 1 mg/ml 5-FOA. A single colony from this strain (*MAT* **a** *tra1::LEU2, pCB157-TRP-CEN, SWI2::his5*$^+$*, ade2-1, his3-11, 25leu2-3, 112 trp1-1, ura3-1, can1-100*) was transformed with either p*SWI2 wild-type-URA3-CEN* or p*SWI2* Δ *bromodomain -URA3-CEN* and recovered by growth on SCD-Ura plates. Colonies for the wild-type or Δbromodomain *SWI2* strains showed temperature-sensitive growth at 37°C, confirming that only the *tra1-28* allele was present in each. Growth of strains containing *tra1-28* and either wild-type *SWI2* or Δbromodomain *SWI2* were compared to each other and to YCB650 containing p*TRA1-TRP-CEN* (after growth on 5-FOA plates to remove p*TRA1-URA3-CEN*) as a control for native *SWI2* expression. Yeast containing *tra1-28* and lacking *SWI2* were too sick to include in the comparison. Cells for each were collected in stationary phase, washed with water, and compared by colony dilution assay (4-fold dilutions from a starting $OD_{600nm}$ of 0.2) after 3 or 4 days at 30°C.

### In Vivo Chromatin Immunoprecipitation (ChIP) Assay

Appropriate yeast strains that were either untagged (control) or myc-tagged on the Snf6 subunit were grown in 2% dextrose to an $OD_{600}$ of approximately 0.8 and shifted to low (0.05%) dextrose for 2 hr. Following the standard in vivo ChIP protocol (Kuo and Allis, 1999), 1% of the final concentration formaldehyde was added to 100 ml of culture for each condition (repressed and derepressed) and incubated for 15 min at room temperature. After preparing whole-cell extract, anti-myc antibody is used to immmunoprecipitate SWI/SNF and the crosslinked DNA. The beads were washed and the crosslinking reversed. DNA was extracted from the beads, proteinase K digested, phenol-chloroform extracted, and ethanol precipitated, followed by quantitative PCR.

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