The SWI2/Snf2 Bromodomain Is Required for the Displacement of SAGA and the Octamer Transfer of SAGA-acetylated Nucleosomes

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The SWI/SNF and SAGA chromatin-modifying complexes contain bromodomains that help anchor these complexes to acetylated promoter nucleosomes. To study the importance of bromodomains in these complexes, we have compared the chromatin-remodeling and octamer-transfer activity of the SWI/SNF complex to a mutant complex that lacks the Swi2/Snf2 bromodomain. Here we show that the SWI/SNF complex can remodel or transfer SAGA-acetylated nucleosomes more efficiently than the Swi2/Snf2 bromodomain-deleted complex. These results demonstrate that the Swi2/Snf2 bromodomain is important for the remodeling as well as for the octamer-transfer activity of the complex on H3-acetylated nucleosomes. Moreover, we show that, although the wild-type SWI/SNF complex displaces SAGA that is bound to acetylated nucleosomes, the bromodomain mutant SWI/SNF complex is less efficient in SAGA displacement. Thus, the Swi2/Snf2 bromodomain is required for the full functional activity of SWI/SNF on acetylated nucleosomes and is important for the displacement of SAGA from acetylated promoter nucleosomes.

Accessibility of transcriptional factors to promoters in eukaryotes often requires modification of chromatin structure, which is accomplished by the action of multisubunit chromatin-modifying complexes. The yeast SWI/SNF complex is an example of a chromatin-remodeling complex, which uses the energy of ATP hydrolysis to mobilize nucleosomes and affects the expression of a subset of yeast genes (for review, see Refs. 1–5). The SAGA histone acetyltransferase (HAT) complex is another example of a remodeling complex that regulates the acetylation level of histones (for review, see Refs. 6–9). Previous studies have demonstrated genetic interactions between different components of these complexes (10, 11), as well as recruitment of both complexes to promoters by sequence-specific transcription activators (12–22). Moreover, in vivo cross-linking studies have shown that, following activator dissociation, both complexes stay bound to the yeast HO endonuclease promoter (23).

We have previously shown that acetylation of nucleosomal array templates by HAT complexes stabilizes SWI/SNF binding to promoter nucleosomes after the dissociation of the activator (24). Many chromatin-modifying complexes, including SWI/SNF and SAGA, contain highly conserved bromodomains (25–29) that bind to acetylated lysine residues in histone N-terminal tails in vitro (30–35). They can also recognize and bind to acetylated non-histone proteins such as MyoD, the HIV-1 Tat, and the p53 transcription factor (36–39). More recently we have shown that the Swi2/Snf2 and Gcn5 bromodomains play important roles in the anchoring of the SWI/SNF and SAGA complexes to acetylated promoters, respectively (40). When SWI/SNF is recruited to immobilized nucleosomal templates, the retention of SWI/SNF requires both acetylated histones and the Swi2/Snf2 bromodomain. In other words, deletion of the Swi2/Snf2 or Gcn5 bromodomains results in the dissociation of the modifying complex from acetylated nucleosome arrays upon activator removal. Consistent with these in vitro experiments, the Swi2/Snf2 bromodomain was found to be required for SWI/SNF presence at the SUC2 promoter in vivo (40). Furthermore, mutant phenotypes were uncovered for a swi2/snf2 bromodomain deletion when combined with mutations in the SAGA HAT complex, consistent with previous studies that showed redundant roles for the SWI/SNF and SAGA in vivo (40). In addition to the involvement of bromodomains in gene activation, other studies show their role in antisilencing of genes at heterochromatin boundaries (41, 42). Furthermore, using fluorescence resonance energy transfer, it has recently been demonstrated that bromodomain proteins also show selective recognition of acetylated histones in vivo (43).

The stable association of SWI/SNF or SAGA on acetylated nucleosomes provides evidence of the importance of bromodomains and links chromatin-modifying complexes together to regulate gene expression. Recognition of the “histone code” by various domains (such as the bromodomain) in regulatory proteins helps the recruitment and binding of the modifying complexes to the post-translationally modified histones. Thus, the fact, that bromodomains within the catalytic subunit of chromatin-modifying complexes support their binding to acetylated promoter nucleosomes, suggests possible involvement of these bromodomains in targeting, retention, and action of these complexes on acetylated nucleosomal templates. To determine the role of the Swi2/Snf2 bromodomain in the function of the SWI/SNF complex, we have purified a SWI/SNF complex lacking the Swi2/Snf2 bromodomain and tested its functional activity comparing it to that of the wild-type complex. In this study, we show that the Swi2/Snf2 bromodomain is necessary for the functional activity of the complex on acetylated nucleosomal templates. Although the lack of the Swi2/Snf2 bromodomain has no effect on the remodeling as well as octamer-transfer activity of unmodified nucleosomes, the bromodomain-deleted (Δbromodomain) SWI/SNF cannot remodel or transfer SAGA-acetylated nucleosomes as efficiently as the wild type. These results demonstrate the requirement of the Swi2/Snf2 bromodomain for the remodeling and octamer transfer activities of the complex on acetylated nucleosomes. Moreover, we...
show that the SAGA complex bound to the acetylated templates is destabilized and replaced by the SWI/SNF complex to remodel the acetylated nucleosomes. This displacement of SAGA by the SWI/SNF complex on acetylated nucleosomes requires the presence of the Swi2/Snf2 bromodomain. These data illustrate a novel and significant role of the Swi2/Snf2 bromodomain in remodeling of acetylated promoter nucleosomes and in displacing SAGA from promoters.

EXPERIMENTAL PROCEDURES

Purification of the SWI/SNF and SAGA Complexes—The wild-type and the bromodomain deletion SWI/SNF complexes and the SAGA histone acetyltransferase complex were purified from yeast whole cell extract by tandem affinity purification (TAP) over two affinity columns as described previously (44–47). Briefly, whole cell extracts were prepared from six liters of yeast cells grown in YPD media and added to IgG resin (Amersham Biosciences). The complexes were eluted from the beads by TEV Protease (Invitrogen) cleavage in a buffer containing (10 mM Tris (pH 8), 150 mM NaCl, 1 mM MgAc, 1 mM imidazole, 2 mM EDTA, 10% [v/v] glycerol, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, and 1 mM dithiothreitol). Following binding to calmodulin resin (Amersham Biosciences, Uppsala, Sweden), the complexes were eluted using a buffer containing (10 mM Tris (pH 8), 150 mM NaCl, 1 mM MgAc, 1 mM imidazole, 2 mM EDTA, 10% [v/v] glycerol, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, and 0.5 mM dithiothreitol). Purification was monitored by Western blot, using several antibodies as well as silver staining. The Snf6TAP-tag strain was generated by M. Carroza (NIHES, National Institutes of Health) and the Spt7 TAP-tag strain was a gift from F. Winston (Dept. of Genetics, Harvard Medical School) (FY2021 (48). The endogenous Δbromodomain Swi2/Snf2, Snf6-TAP strain was generated using the Cre-Lox recombination system.

Restriction Enzyme Accessibility Assay—The single Gal4-site probe (GUB) was generated by PCR as described (49) and used as naked DNA or a reconstituted mononucleosome in this assay as described above (21). Wild-type or Δbromodomain mutant SWI/SNF was added to ~10 ng of this 32P-labeled GUB template in a binding buffer that contains (10 mM HEPES (pH 7.8), 50 mM KCl, 5 mM dithiothreitol, 5 mM phenylmethylsulfonyl fluoride, 5% glycerol, 0.25 mg/ml bovine serum albumin, and 2 mM MgCl2) in the presence or absence of 2 mM ATP. After incubation for 1 h at 30 °C, the binding reactions were then treated with 10 units of Sall for 30 min at 30 °C. An equal volume of stop buffer (20 mM Tris-HCl (pH 7.5), 50 mM EDTA, 2% SDS, 0.2 mg/ml proteinase K, 1 mg/ml glycogen) was added to the reactions, and incubated at 50 °C for 1 h. Deproteinized samples were precipitated with 200 mM NaCl and 3 volumes of ethanol, and the pellet was resuspended in 5 μl of the formamide dye (95% formamide, 10 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue). After heat denaturation, the samples were resolved on a 6% acrylamide (19:1 acrylamide to bisacrylamide), 8 M urea sequencing gel at 150 volts for 3 h, and then visualized by autoradiography or exposed to a phosphorimaging device and quantified. In Fig. 3, the 32P-labeled GUB mononucleosome templates were first acetylated by SAGA for 30 min at 30 °C, prior to incubation with the SWI/SNF complexes. In Fig. 4, the GUB nucleosome templates were 32P-labeled on one end and biotinylated on the other. After acetylation with the template with SAGA as before, the HAT complex was removed by the addition of 1 µg of competitor chromatin and washed to remove SAGA and the competitor, followed by the addition of the SWI/SNF remodeling complexes and Sall digestion as before.

Octamer Transfer Assay—The same template (GUB) utilized for the restriction enzyme accessibility assay was used in this assay. The reaction conditions for this octamer-transfer assay were the same as the Sall digestion assay described above. Briefly, 10 ng of the 32P-labeled GUB, naked DNA template, which was used as an octamer acceptor in this assay, was incubated with ~10 ng of donor short oligonucleosomes (SONs) in the presence of wild-type or Δbromodomain mutant SWI/SNF and ATP for 2 h at 30 °C in the same binding buffer as before. 0.2 µg of competitor chromatin was added to the reaction to release any bound SWI/SNF complex from the template prior to resolving the binding reactions on a 4% acrylamide (79:1 acrylamide to bisacrylamide ratio) native gel at 150 volts for 2 h. The gel was dried and visualized by autoradiography. In Fig. 4 (C and D), the donor nucleosomes are first acetylated with SAGA prior to the addition of the SWI/SNF complexes.

Immobilized Template Octamer Transfer Assays—The immobilized template octamer-transfer assay is similar to the 32P-labeled GUB octamer-transfer assay described above, except the GUB template was biotinylated here and used as the octamer acceptor. Briefly, the GUB fragment was generated as before using a biotinylated 5′ primer in the PCR reaction. The biotinylated GUB templates were then bound to paramagnetic beads coupled to streptavidin (Dynabeads, Dynal) as described earlier (24, 50). The donor SON nucleosomes were first acetylated with SAGA for 30 min at 30 °C in the presence of [3H]acetyl-CoA (Amersham Biosciences) as described before by Eberharter et al. (51) in the same binding buffer as above. SAGA-acetylated donor nucleosomes were incubated with the biotinylated streptavidin-coupled GUB DNA in the presence or absence of wild-type or mutant SWI/SNF and ATP for 1 h at 30 °C. The biotinylated nucleosome templates were then concentrated on a magnet, the supernatant was removed, and the beads were washed twice in the binding buffer. Acetylated histones on the GUB template were then either 1) eluted by boiling the beads in sample loading buffer, visualized by fluorography after running them on 15% SDS-PAGE gels, and exposed to film (Fig. 4B, top) or 2) bound to filter, washed, and counted in the scintillation counter (Fig. 4B, bottom). In Fig. 5, biotinylated GUB template reconstituted into mononucleosomes and acetylated by SAGA at 30 °C for 1 h was used as the donor of histones, after removing SAGA as above (with the addition of 1 µg of competitor chromatin and several washes). Following the addition of the 32P-labeled GUB DNA template and the SWI/SNF complexes, the experiment was continued as in Fig. 4 (C and D).

Immobilized Template Displacement/Competition Assays—This displacement/competition assay utilizes the same biotinylated streptavidin-bound GUB template used above. Briefly, the immobilized GUB DNA was first reconstituted into mononucleosomes, followed by incubation with SAGA in the presence or absence of cold acetyl-CoA for 30 min at 30 °C. After incubation with ATP and either the wild-type or the mutant SWI/SNF complex for 1 h at 30 °C, the supernatants were separated from the beads, and the beads were washed and resolved on a 12% SDS gel, followed by Western blot analysis as described before (24, 50) looking for both SAGA and SWI/SNF binding.

RESULTS

The Swi2/Snf2 Bromodomain Is Not Required for Activity of the SWI/SNF Complex on Unmodified Nucleosomes—To directly test whether the bromodomain within the Swi2/Snf2 subunit of the SWI/SNF complex contributes to the functional activity of the complex on nucleosomes, we purified wild-type SWI/SNF as well as SWI/SNF from a strain lacking the Swi2/Snf2 bromodomain and tested them in chromatin-remodeling and octamer-transfer assays. For all assays, we used wild-type and mutant SWI/SNF complex that has been highly purified over two affinity columns using the TAP method. The loss of the Swi2/Snf2 bromodomain did not affect complex integrity as detected by silver

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staining (Fig. 1A, compare lanes 2 and 3). The Swi2/Snf2, Swi3, and Swp61 antibodies were used to determine SWI/SNF protein levels by Western blot analysis (Fig. 1B). The same amounts of the wild-type and

the ∆bromodomain SWI/SNF complexes were used in all our assays based on this normalization of the amounts of purified protein.

The activities of these two complexes were first compared on unmodified nucleosomes. The DNA templates used in Fig. 2 is the 183-bp "GUB" fragment generated by PCR using a radiolabeled 5’ primer and the pGALUSFBEND plasmid that we had previously used (14, 21, 49). This DNA fragment was reconstituted into a nucleosome by salt dilution/octamer transfer and was used for the restriction enzyme accessibility assay. We analyzed the ability of the Sall restriction enzyme to digest its cutting site in about the middle of the 183-bp GUB nucleosomal DNA with the wild-type and the ∆bromodomain SWI/SNF complexes in the presence or absence of ATP (Fig. 2A). Both the wild-type and the mutant complex increased the accessibility of the restriction enzyme to its site on the nucleosomal DNA in an ATP-dependent manner (Fig. 2A, lanes 4–11). Moreover, the digestion of the DNA by the restriction enzyme was the same in the presence of either the wild-type or the mutant SWI/SNF complex on this unmodified GUB nucleosome (Fig. 2A, compare lanes 4–6 to 8–10). Quantification of this experiment showed that a similar percentage of the nucleosome template was cleaved in the presence of either of these complexes, indicating that the absence of the bromodomain in the Swi2/Snf2 subunit of the SWI/SNF complex did not affect its remodeling activity on these nucleosomes.

Using the same two complexes in a different functional assay, we compared their ability to transfer octamers from SONs to a radiolabeled GUB, naked DNA fragment. The same GUB DNA fragment used in Fig. 2A was used here and served as an acceptor of histone octamers. As shown in Fig. 2B, both the wild-type and the bromodomain-deleted SWI/SNF complexes were able to transfer histone octamers from the donor SONs to the DNA template in an ATP-dependent manner (Fig. 2B, lanes 3–6). Moreover, the amounts of the octamers transferred by the two complexes (wild-type and the mutant) were similar (Fig. 2B, compare lanes 3–6). These data demonstrate that the ∆bromodomain SWI/SNF complex has the same remodeling and histone octamer-transfer activity as the wild-type SWI/SNF complex. Thus, the deletion
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The Swi2/Snf2 Bromodomain Is Important for the Remodeling and Octamer Transfer Activities of the SWI/SNF Complex on SAGA-acetylated Nucleosomes—Previous observations have shown that acetylation of nucleosomes prior to addition of the SWI/SNF complex helps stabilize the binding of SWI/SNF to a nucleosome array template (24). Moreover, the Swi2/Snf2 bromodomain was found to be important for this enhanced binding (40). These data have led us to investigate the role of the Swi2/Snf2 bromodomain in remodeling of acetylated nucleosomes. To test whether the bromodomain within the Swi2/Snf2 subunit of the SWI/SNF complex contributes to the remodeling activity of the complex on acetylated nucleosomes, we compared the activities of both the wild-type and the mutant SWI/SNF complex on templates that are acetylated with the SAGA HAT complex in the presence or absence of acetyl-CoA, followed by the remodeling reaction and digestion by the restriction enzyme Sall (Fig. 3A), as before. Digestion of the naked DNA template by Sall is not affected by the presence of SAGA or acetylation by SAGA (Fig. 3B, compare lanes 2–4). Lanes 6 and 7 again show the equal remodeling activity of the two SWI/SNF complexes (wild-type and the mutant). No Sall digestion of the nucleosomal template was observed with the SAGA complex in the presence or absence of acetyl-CoA (see lanes 8 and 9), confirming that the SAGA HAT complex has no chromatin-remodeling activity. When SAGA was added to the template in the absence of acetyl-CoA (lanes 10 and 11), the remodeling activity of the wild-type and mutant SWI/SNF complexes were the same, suggesting that the presence of the SAGA complex alone had no effect on the activity of the SWI/SNF in these reactions. However, when the template was acetylated by SAGA in the presence of acetyl-CoA (lanes 12 and 13), reduced digestion by the Sall restriction enzyme was observed with the mutant SWI/SNF complex (lane 13). The activity of the wild-type SWI/SNF on these acetylated nucleosomes did not change compared with unacetylated templates in this experiment (compare lanes 10 and 12). The reason for this seemingly unanticipated decrease in activity is that, because we were not competing away the SAGA complex, some of it had remained bound to the acetylated templates through its Gcn5 bromodomain and thus hindered the complete remodeling by SWI/SNF (see Figs. 5 and 6 below).

These results indicate that the Swi2/Snf2 bromodomain is important for the SWI/SNF-remodeling activity on SAGA-acetylated nucleosomes. Moreover, using a titration of concentrations of the wild-type and the mutant SWI/SNF complexes, we have confirmed these results (Fig. 3C). Three different concentrations of the SWI/SNF wild-type and the mutant complex were compared in their remodeling activity of SAGA-acetylated nucleosomes. Here, we show that, using the same concentrations of the two complexes, the Swi2/Snf2 bromodomain-deleted complex has a significantly reduced remodeling activity on SAGA-acetylated nucleosomes compared with the wild-type complex (Fig. 3C, compare lanes 4–6 to 7–9). Quantification of these results shows that the bromodomain-deleted SWI/SNF complex had a reduction of >60% of remodeling activity on acetylated nucleosomes. These results together demonstrate that the bromodomain of SWI/SNF was necessary for the enzyme digestion and hence remodeling activity of this complex on H3-acetylated nucleosomes.

In addition to restriction digestion activity, we have tested the octamer-transfer ability of the SWI/SNF complex on pre-acetylated nucleosomes and evaluated this ability of the complex when it lacks its Swi2/Snf2 bromodomain. To this end, we have used two different octamer-transfer assays. One is the same as Fig. 2B, where the GUB template was labeled with 32P and in the other, SAGA-acetylated nucleosomes were transferred to a biotinylated GUB DNA template by the wild-type or the bromodomain-deleted SWI/SNF complex as diagrammed in Fig. 4A, followed by fluorography (Fig. 4B, top) or counting on a filter paper (Fig. 4B, bottom) the tritium-labeled nucleosomes. Briefly, SONS were first acetylated by SAGA in the presence of [3H]acetyl-CoA and used as an octamer donor. The H3-acetylated nucleosomes were then added to a reaction containing the biotin-labeled GUB fragment bound to streptavidin magnetic beads and either wild-type or the mutant SWI/SNF complex. After incubation and a pull-down, we followed tritium-labeled histones and the level of acetylated octamer transferred onto the GUB template (Fig. 4A). The results showed ATP-dependent transfer of SAGA-acetylated octamers by the wild-type SWI/SNF complex (Fig. 4B, top, compare lanes 3 and 4). More than half of the input acetylated nucleosomes were transferred to the DNA template by the wild-type complex under these conditions as shown by the amount of acetylated histones in the bead lanes (Fig. 4B, top, compare lanes 1 and 3). The acetylated octamer-transfer activity by the bromodomain-deleted SWI/...
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FIGURE 4. The Swi2/Snf2 bromodomain deletion SWI/SNF complex has reduced octamer-transfer activity on acetylated nucleosomes. A, diagram of the immobilized template octamer transfer experiment. SONs are first acetylated with SAGA in the presence of [3H]acetyl-CoA and used as a donor of histones in this experiment. After the addition of the biotinylated GUB DNA template and SWI/SNF wild-type and mutant complexes, a pull-down assay and a HAT assay were performed as described previously (51) to follow the tritium-acetylated histones. B, octamer-transfer assay shows reduced transfer ability of the Δbromodomain SWI/SNF of SAGA-acetylated nucleosomes compared with the wild-type complex. SAGA-acetylated donor nucleosomes were incubated with biotinylated GUB DNA that was bound to paramagnetic beads (Dynabeads) coupled to streptavidin as described previously (24) and SWI/SNF (wild-type or mutant) in the presence or absence of ATP. The reactions were performed at 30 °C for 1 h prior to separating the supernatants from the beads and checking their acetylation level. The HAT reaction (both supernatants and the beads) was run on a 15% SDS gel and exposed to film (top of B) or bound to filter, washed, and counted in a scintillation counter (bottom of B). The positions of H3 and H4 histones are shown. Lane 1 is the input lane, whereas lanes 2–6 and 7–11 are bead and supernatant lanes, respectively. The bottom panel of A shows the quantification of three independent immobilized octamer transfer/filter binding HAT assay using both the wild-type SWI/SNF and SWI/SNF lacking the Swi2/Snf2 bromodomain. C, diagram of octamer-transfer assay. SONs were first acetylated with SAGA in the presence of acetyl-CoA and used as a donor of histones in this experiment. After the addition of the Δbromodomain SWI/SNF of SAGA-acetylated nucleosomes compared with the wild-type complex. SAGA-acetylated donor nucleosomes were incubated with [3H]labeled GUB DNA and SWI/SNF complexes (wild-type and the mutant) and ATP. The acetylation reaction was performed at 30 °C for 30 min followed by 1-h incubation with the remodeling complex SWI/SNF and acceptor DNA (GUB) prior to running on a 4% acrylamide native gel at 150 volts for 3 h. The gel was dried and visualized by autoradiography. Lanes 5 and 6 compare the octamer-transfer abilities of the two complexes of SAGA-acetylated nucleosomes.

SNF complex was reduced to about one-third of the wild-type activity (Fig. 4B, top; compare lanes 3 and 5). The level of acetylated histones in the supernatant were consistent with the results that the Swi2/Snf2 bromodomain-deleted SWI/SNF has a reduced transfer ability of H3-acetylated nucleosomes to naked DNA fragments compared with the wild-type complex. The depletion of the H3-acetylated histones from the supernatant, when wild-type SWI/SNF was used (lane 6), compared with when the bromodomain-deleted SWI/SNF complex was used (lane 10), showed the higher octamer-transfer ability of the wild-type complex on acetylated nucleosomes. The quantification of this biotinylated pull-down assay and two other repeats is shown at the bottom of Fig. 4B; the dark columns refer to the same lanes as in the top of Fig. 4B.

Fig. 4C shows the outline of the alternative octamer transfer experiment designed to confirm the results in Fig. 4B. Here, the SON was acetylated with SAGA and transferred to [32P]labeled GUB DNA with the help of SWI/SNF complexes (wild-type or the Δbromodomain mutant). Again, both the wild-type and the Δbromodomain SWI/SNF complex transferred acetylated (in the absence of acetyl-CoA) octamers from the SON onto the labeled DNA equally well (Figs. 2B and 4D; compare lanes 3 and 4). On the other hand, the mutant complex had reduced octamer-transfer ability on SAGA-acetylated nucleosomes (compare lanes 5 and 6). These results together demonstrate the requirement of the Swi2/Snf2 bromodomain for the full remodeling and octamer-transfer activities of the SWI/SNF complex on acetylated nucleosomes.

To determine whether the SAGA complex stabilized on acetylated templates affects the activity of the SWI/SNF complex and the role of the Swi2/Snf2 bromodomain in this, we have compared both the restriction enzyme accessibility as well as the octamer-transfer activity of the two SWI/SNF complexes when SAGA is removed following acetylation of the template (Fig. 5). Fig. 5A outlines the restriction enzyme accessibility assay, which is similar to Fig. 3, except the GUB nucleosome templates were [32P]-labeled on one end and biotinylated on the other. After acetylation the template with SAGA as before, the HAT complex was removed by the addition of competitor chromatin, and the beads were washed to remove SAGA and the competitor. Lanes 1 and 2 (Fig. 5B) are repeats of lanes 3 and 4 to lanes 1 and 2, suggesting that SAGA when stabilized on acetylated templates can block binding and activity of the SWI/SNF complex to some extent. Moreover, after SAGA removal, the bromodomain-deleted SWI/SNF complex has reduced activity compared with the wild type. This confirms our previous results that the Swi2/Snf2 bromodomain is required for the full activity of the complex on acetylated nucleosomes.
We have also repeated the octamer-transfer experiment of Fig. 4D with the addition of an extra step to remove SAGA after it has acetylated the template. Fig. 5C is the outline of the experiment where the biotinylated GUB template, reconstituted into mononucleosomes and acetylated by SAGA at 30 °C for 1 h, is used as a donor of histones, after SAGA removal. Lanes 2 and 3 (Fig. 5D) are repeats of lanes 5 and 6 in the Fig. 4D. The results again show a reduction of the octamer-transfer ability of the SWI/SNF complex when it lacks its Swi2/Snf2 bromodomain (Fig. 5D, compare lane 2 to 3). This is also true when SAGA is removed from the template prior to the addition of the SWI/SNF complexes (Fig. 5D, compare lane 4 to 5), even though there is a moderate increase in this activity with the dissociation of SAGA for both the wild-type and the mutant SWI/SNF complex (compare lanes 4 and 5 to lanes 2 and 3). Again this could be due to the fact that the bromodomain-containing SAGA complex competes for binding to acetylated templates with the SWI/SNF complex (Fig. 6).

The Swi2/Snf2 Bromodomain Is Partially Required for the Displacement of SAGA Bound to Acetylated Nucleosomes—Like SWI/SNF, the SAGA complex is recruited to promoters by yeast transcriptional activators. These two complexes are also stabilized in binding to acetylated nucleosomes through their Swi2/Snf2 and Gcn5 bromodomains (24, 40). Moreover, the bromodomains of Swi2/Snf2 and Gcn5 participate in the functions of these proteins in vivo (40, 52). To determine why chromatin remodeling is inhibited on SAGA-acetylated templates, we have asked whether the SAGA and SWI/SNF complexes competed with each other in binding to nucleosomes and investigated the importance of the Swi2/Snf2 bromodomain in this competition.

We purified SAGA complex from an Spt7-TAP strain and used it in the immobilized template competition assay (Fig. 6). Fig. 6A shows the outline of the experiment. Briefly, the SAGA complex was first bound to a biotinylated GUB nucleosomal template in the presence or absence of acetyl-CoA, followed by the addition of either the wild-type or the Δbromodomain SWI/SNF complex. After a pull-down assay, the binding of both complexes to nucleosomes was detected by Western blot analysis using an anti-TAP antibody. Acetylation by the SAGA complex will anchor this complex to the acetylated nucleosomes (40), thus it needs to be displaced before SWI/SNF can remodel or transfer nucleosomes efficiently (observed in Figs. 3 and 4). The dissociation of SAGA might be through removal of the activator that recruited it (shown previously in Ref. 24), addition of competitor chromatin (Fig. 5), or by the SWI/SNF complex itself. In Fig. 6, we have tested the ability of the SWI/SNF complex to displace SAGA bound to acetylated nucleosomes through its Gcn5 bromodomain. Binding of SAGA to the biotinylated GUB nucleosomes was slightly increased in the presence of acetyl-CoA or the resulting acetylated histones as shown by quantification of three independent experiments (Fig. 6B, lanes 1 and 2). Using a more physiological template (such as the GSE4 array) had a more significant affect (40). Moreover, both the wild-type and the bromodomain-deleted SWI/SNF complexes bound equally well to this template (lanes 3 and 4). However, when either the wild-type or the mutant SWI/SNF complex was added to the reaction following SAGA binding, SAGA was not retained completely on these nucleosomes in the absence of acetyl-CoA (Fig. 6B, compare lanes 1 and 2 to 5 and 6). Thus, the SWI/SNF complex seems to displace some of the SAGA complex. This displacement of SAGA from unacetylated nucleosomes did not depend on the Swi2/Snf2 bromodomain of the SWI/SNF complex (Fig. 6B, compare lanes 5 and 6).

In contrast to the result in the absence of acetylation, in the presence of acetyl-CoA, when the nucleosomes were acetylated and the SAGA complex was anchored on the acetylated histones, wild-type SWI/SNF but not the bromodomain-deleted complex could displace SAGA effi-
ciently (Fig. 6B, lanes 7 and 8). Even though the SWI/SNF complex, lacking the Swi2/Snf2 bromodomain, bound to the template to some extent (Fig. 6, lanes 4), it could not displace SAGA as well as the wild-type (Fig. 6, lane 8). Thus, modification of the template by SAGA served to anchor this complex first, followed by ineffective competition from a SWI/SNF complex lacking its Swi2/Snf2 bromodomain. Probing the blots with antibodies against acetylated H3 confirmed the presence of acetylated histones H3 when acetyl-CoA was present on the template (Fig. 6B, lanes 2, 7, and 8). These data illustrate that, although the SAGA complex became anchored to the acetylated promoter, it could only be efficiently displaced by an intact SWI/SNF complex (Figs. 6 and 7). Therefore, the Swi2/Snf2 bromodomain is required for the SWI/SNF complex to displace SAGA from acetylated promoter nucleosomes, leading to remodeling of the nucleosome.

**DISCUSSION**

In this report, we have examined the effect of the Swi2/Snf2 bromodomain in the yeast SWI/SNF complex on the activity of this chromatin-remodeling complex on acetylated promoter nucleosomes. We show that, even though SWI/SNF purified from a Swi2/Snf2 bromodomain deletion strain remodeled unacetylated nucleosome templates as well as the wild-type complex, it could only partially remodel acetylated nucleosome templates. The octamer-transfer ability of bromodomain-deleted SWI/SNF mutant complex was also impaired on acetylated nucleosome templates, even after dissociation of the HAT that acetylated it. These data illustrate the requirement for the Swi2/Snf2 bromodomain in anchoring the complex on acetylated templates prior to any remodeling. In addition, we have examined possible displacement of the previously recruited and retained SAGA complex by the SWI/SNF complex from nucleosomes and the role of the Swi2/Snf2 bromodomain in this competition. Here, we have also shown that SAGA, which is retained on H3-acetylated nucleosomes, can be displaced by the SWI/SNF complex. Moreover, the Swi2/Snf2 bromodomain is required for this displacement. The difference between the ability of the wild-type and the bromodomain-deleted SWI/SNF complex to displace SAGA from acetylated promoter nucleosomes might be due to the fact that
the Swi2/Snf2 bromodomain has the ability to recognize acetylated histone H3 and bind to it (40), effectively competing with SAGA. In other words, bromodomain-deleted SWI/SNF complex cannot be anchored onto H3-acetylated nucleosomes, thus leaving the bromodomain-containing SAGA bound to these nucleosomes.

Mutations in SWI/SNF and SAGA subunits often show strong synthetic phenotypes (10, 11), and the bromodomain of Gcn5 has been shown to affect chromatin remodeling by SWI/SNF in vivo (52). In addition, we observed possible redundant functions between SWI/SNF and SAGA when double Gcn5 and Swi2/Snf2 bromodomain deletions showed synthetic phenotypes in a variety of conditions such as in the presence of galactose, raffinose, sulfometuron methyl, or in the absence of histidine (40). Moreover, both the SWI/SNF and SAGA complexes can recognize and bind to the same nucleosome acetylated pattern produced by SAGA. These data together suggest possible overlapping functions between these complexes and show the importance of their bromodomains in recruitment, binding, retention, and activity on promoter nucleosomes. We have also previously shown that the Swi2/Snf2 bromodomain deletion reduces occupancy of the SWI/SNF complex at the SUC2 promoter under derepressing conditions (40). This is consistent with our data here that the Δbromodomain SWI/SNF complex cannot displace SAGA efficiently or bind and remodel acetylated promoter nucleosomes. Thus, it seems that both acetylation as well as the role of the Swi2/Snf2 bromodomain in competing off SAGA is important for the observed difference in the functional activity of the wild-type and the Δbromodomain SWI/SNF complexes on acetylated nucleosomes.

The SAGA complex, which leads to a localized pattern of acetylation on promoter proximal nucleosomes, is anchored on those nucleosomes through a bromodomain within its catalytic subunit, Gcn5 (16, 40). Similarly, the SWI/SNF complex can be anchored to acetylated promoter nucleosomes through its Swi2/Snf2 bromodomain (40). Thus, nucleosome acetylation by HATs such as the SAGA complex can generate a high affinity docking site for bromodomain-containing protein complexes, including the SAGA complex itself, SWI/SNF, as well as other protein complexes involved in transcription regulation such as TFIIID. For example, it is possible that acetylated nucleosomes serve as substrates for sequential and ordered binding of these bromodomain-containing proteins to the promoter, where these complexes compete with each other for binding. Thus, acetylation by SAGA or NuA4 leading to localized or broad patterns of acetylation, respectively (16), can stabilize SWI/SNF on acetylated nucleosomes to remodel them. This binding of SWI/SNF requires the displacement of some of the SAGA complex stabilized onto the acetylated lysines at promoter proximal nucleosomes through its Swi2/Snf2 bromodomain (40). Thus, through a bromodomain within its catalytic subunit, Gcn5 (16, 40).

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**Swi2/Snf2 Bromodomain Required for SAGA Displacement**