

Histone Acetyltransferase Complexes Stabilize SWI/SNF Binding to Promoter Nucleosomes

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Summary

To investigate the function of SWI/SNF in site-specific chromatin remodeling at promoters, we have used a purified system to analyze its distribution, function, and retention following recruitment by a sequence-specific transcription activator. Activator recruitment of SWI/SNF bound the complex to promoter proximal nucleosomes and led to localized nucleosome disruption. However, retention of SWI/SNF on the promoter required either the continued binding of the transcription activator or acetylated histones. Histone acetylation by either the SAGA or NuA4 HAT complexes increased the retention of SWI/SNF on the promoter. These data illustrate a functional link between HAT complexes and the SWI/SNF chromatin remodeling complex and provide a mechanistic basis for the ordered recruitment of these complexes.

Introduction

Remodeling of chromatin structure at several yeast promoters contributes to the transcriptional regulation of the corresponding genes (Hirschhorn et al., 1992; Peterson and Herskowitz, 1992; Winston and Carlson, 1992; Gavin and Simpson, 1997; Cosma et al., 1999; Krebs et al., 1999). This chromatin remodeling is achieved in part through the action of ATP-dependent chromatin remodeling complexes. One such complex is the yeast SWI/SNF complex, which is a 2 MDa multisubunit complex that uses the energy of ATP hydrolysis to alter nucleosome conformation and/or position (for review, see Armstrong and Emerson, 1998; Workman and Kingston, 1998; Kingston and Narlikar, 1999; Muchardt and Yaniv, 1999; Peterson and Workman, 2000; Vignali et al., 2000a).

The yeast SWI/SNF complex has been found to bind to DNA and nucleosomes with high affinity. It is also able to alter nucleosome structure in a manner resulting in a perturbation of the amount and path of DNA on the surface of the histone octamer (Côté et al., 1994, 1998; Quinn et al., 1996; Bazett-Jones et al., 1999). This disruption changes the pattern of DNase I digestion and enhances the access of DNA binding proteins (transcription factors) and restriction enzymes to nucleosomal DNA (Côté et al., 1994; Logie and Peterson, 1997; Utley et al., 1997; Logie et al., 1999). Studies with human SWI/SNF complexes and a second related complex in yeast,

termed RSC, have revealed similar properties of these complexes. These studies further demonstrated that their action reduced the amount of supercoiling around each nucleosome (Kwon et al., 1994; Imbalzano et al., 1996, 1998; Lorch et al., 1998; Schnitzler et al., 1998). The SWI/SNF complexes have been shown to participate in the transdisplacement of histone octamers from DNA fragments (Owen-Hughes and Workman, 1996; Lorch et al., 1999; Phelan et al., 2000) and to promote the sliding of histones octamers along DNA in cis (Whitehouse et al., 1999). Through these mechanisms the SWI/SNF complex is thought to participate in the formation of nucleosome-free regions or regions of altered nucleosomes at the promoters of specific genes.

Sequence-specific transcription activators can accomplish targeting of the SWI/SNF complex to specific promoters. Both the yeast and human SWI/SNF complexes have been shown to interact directly with transcription activators (Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999; Kadam et al., 2000; Wallberg et al., 2000). The yeast SWI/SNF complex interacts with the activation domains of numerous transcription activators (Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999; Wallberg et al., 2000). Activator recruitment of SWI/SNF allows its participation in the transcription of specific promoters in a milieu of competing chromatin (Neely et al., 1999; Wallberg et al., 2000). In vivo crosslinking/chromatin immunoprecipitation studies of the yeast *HO* promoter have shown that the Swi5p activator is required for the recruitment of the SWI/SNF and the SAGA histone acetyltransferase complex to this promoter (Cosma et al., 1999; Krebs et al., 1999). Interestingly, SWI/SNF crosslinking to the promoter persists after the crosslinking of Swi5p is no longer detected. These data have led to the suggestion that transient interaction of Swi5p with the promoter recruits SWI/SNF, which then becomes stably associated, creating an "epigenetic memory" of Swi5p's interaction (Cosma et al., 1999).

In this study, we have analyzed the localization and retention of the yeast SWI/SNF complex to a promoter in a purified in vitro system. We find that activator recruitment of SWI/SNF to a nucleosome array led to its binding promoter-proximal nucleosomes, which were preferentially disrupted. Retention of SWI/SNF was mediated either by the continued binding of the transcription activator or by acetylated histones. Either the SAGA or NuA4 HAT complexes were able to mediate acetyl-CoA-dependent retention of SWI/SNF on the promoter, demonstrating a functional link between HAT complexes and the SWI/SNF chromatin remodeling complex.

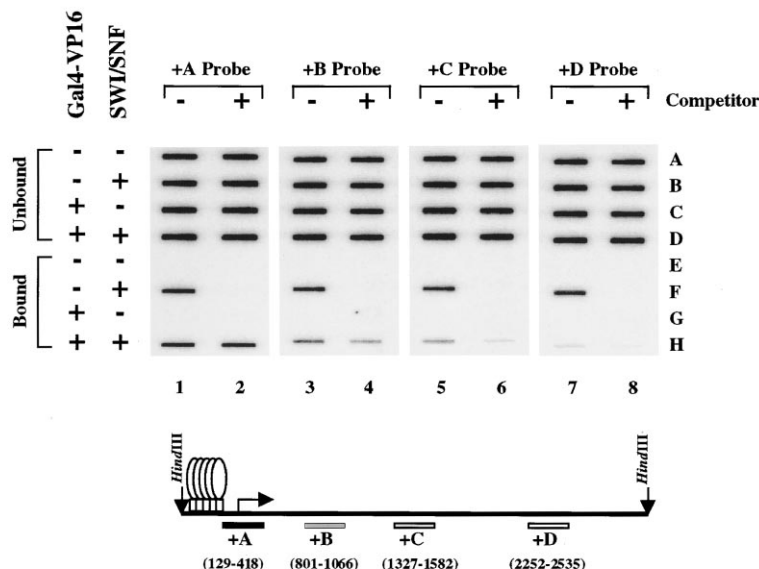
Results

Activator Targeting Localizes SWI/SNF to Promoter-Proximal Nucleosomes

Several studies of SWI/SNF-dependent genes in yeast (i.e., *HO*, *SUC2*, and *PHO8*) have indicated the participation of SWI/SNF in the remodeling of the chromatin

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A.



B.

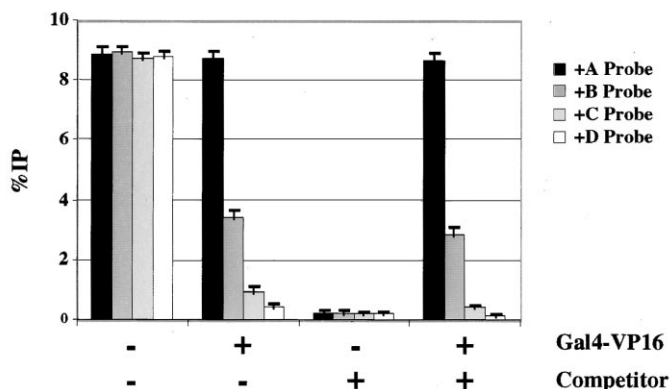


Figure 1. Activator Targeting Localizes SWI/SNF onto Promoter-Proximal Nucleosomes

(A) Biotinylated pG5E4T nucleosomal arrays were bound to paramagnetic beads coupled to streptavidin and incubated in the presence (rows C, D, G, and H) or absence of Gal4-VP16 (rows A, B, E, and F), followed by the addition of SWI/SNF (rows B, D, F, H) and competitor chromatin, as indicated (columns 2, 4, 6, and 8). After crosslinking and washing, the templates were digested with MNase and immunoprecipitated using affinity matrix HA antibody to pulldown SWI/SNF (the Swi2/Snf2 subunit is HA tagged). DNA from the supernatants (rows A–D) and precipitates (rows E–H) were slot blotted and probed with the DNA fragments indicated below the blots. The positions of the different probes when pG5E4T is digested with HindIII is shown at the bottom.

(B) Graphical representation of the average values and the standard deviation of data from 3 different experiments (data shown in [A] and two additional repeats). The background signal (–SWI/SNF) was subtracted from the signal in the presence of SWI/SNF (+SWI/SNF) and the data at four conditions (–Gal4-VP16, –competitor; +Gal4-VP16, –competitor; –Gal4-VP16, +competitor; and +Gal4-VP16, +competitor) represented as % material immunoprecipitated.

structure at their promoters (Hirschhorn et al., 1992; Gavin and Simpson, 1997; Cosma et al., 1999; Gregory et al., 1999; Krebs et al., 1999). In addition, yeast SWI/SNF has been shown to interact directly with activation domains of several promoter binding transcription activators (Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999). The importance of activator recruitment is demonstrated by the fact that under limiting conditions, the SWI/SNF complex will only stimulate transcription from promoters bound by an activator with which it directly interacts (Neely et al., 1999). By determining the nucleosomes bound and disrupted by SWI/SNF when recruited by activators, it is possible to ascertain which nucleosomes need to be remodeled for transcription activation by the complex.

To directly visualize the location of SWI/SNF binding along a nucleosomal array template in a purified system, we utilized an *in vitro* chromatin immunoprecipitation assay, which is a modification of the *in vivo* assay (Kuo and Allis, 1999). We used the pG5E4T plasmid as our nucleosomal template (Lin et al., 1988; Vignali et al., 2000b), which contains 5 Gal4 binding sites upstream

of the adenovirus 2 E4 minimal promoter. The pG5E4T plasmid was linearized by restriction enzyme digestion and assembled into nucleosomes *in vitro* as described (Steger et al., 1998). The pG5E4T plasmid was efficiently assembled into nucleosomes (data not shown, and Vignali et al., 2000b). Upon micrococcal nuclease digestion, at least six to seven nucleosome repeats can be observed, which are distributed along the entire plasmid (Vignali et al., 2000b).

To analyze the binding of SWI/SNF to the template, we performed *in vitro* ChIP assays of micrococcal nuclease-digested nucleosomal templates in the presence or absence of competitor chromatin (see Experimental Procedures). After crosslinking and washing, the pG5E4T nucleosomal array templates were digested with micrococcal nuclease. MNase digested most of the array into mononucleosomes and dinucleosomes with minor amounts of trinucleosomes. This fragmented material was then immunoprecipitated using affinity matrix HA antibody against an HA tag on the Swi2/Snf2 subunit. Following reversal of the crosslinks, the DNA that was associated with the immunoprecipitated SWI/SNF com-

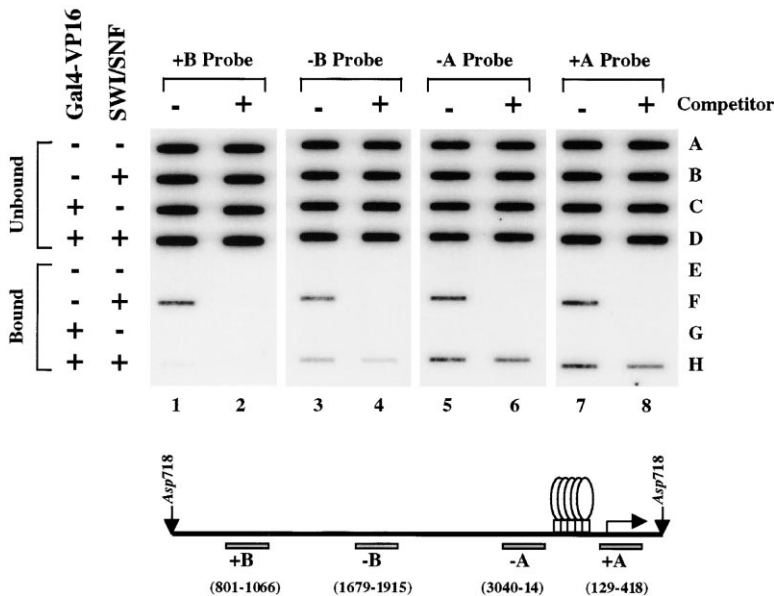


Figure 2. Localization of SWI/SNF on a Nucleosome Array Is Determined by the Location of Activator Binding Sites

The template was linearized so that the 5 Gal4-sites are closer to the opposite end of template from that in Figure 1. Diagram showing the positions of the different probes when pG5E4T is digested with *Asp718* is at the bottom of Figure 2. Addition of SWI/SNF, Gal4-VP16, and competitor chromatin are indicated as in Figure 1.

plex (bound) or left in the supernatant of the reaction (unbound) was purified, slot-blotted, and probed with full-length pG5E4T plasmid and probes to various locations on the plasmid. The positions of the different probes used for these ChIP studies are shown in the bottom of Figures 1A and 2. The intensity of the signal detected after hybridization corresponds to the amount of SWI/SNF complex bound to that particular DNA fragment in the nucleosomal array.

The extent of SWI/SNF binding along the pG5E4T array template was determined in the presence or absence of Gal4-VP16 and competitor chromatin (Figure 1). Figure 1A shows the slot blot exposure of a typical experiment. Figure 1B presents the results of three independent repeats, including the experiment shown in 1A. In the absence of Gal4-VP16 and competitor chromatin, fragments from across the entire length of the template were immunoprecipitated (Figure 1A, row F, lanes 1, 3, 5, and 7), indicating that SWI/SNF had no preference for binding particular nucleosomes along the template. By contrast, upon addition of competitor chromatin in the absence of Gal4-VP16, none of the template plasmid fragments were immunoprecipitated (Figure 1A, row F, lanes 2, 4, 6, and 8), indicating that SWI/SNF was instead bound by the competitor chromatin. However, when Gal4-VP16 was bound to the array, the fragments that are closer to the Gal4 binding sites were preferentially immunoprecipitated in the presence or absence of competitor chromatin (Figure 1A, row H, all lanes). These data illustrate that in the presence of competitor chromatin, Gal4-VP16 both recruited SWI/SNF to the template nucleosome array and localized the complex to the promoter or nearby sequences.

To further illustrate the localization of SWI/SNF by Gal4-VP16, we linearized the template with a different enzyme to locate the Gal4-sites to a different location relative to the ends of the nucleosome array (Figure 2). When the Gal4 sites were localized ~600 base pairs from an end of the array, Gal4-VP16 recruitment of SWI/SNF localized the complex on either side of the Gal4 sites (Figure 2, row H, lanes 6 and 8). This symmetrically

equal binding of SWI/SNF on either side of the activator indicates that there was not a strong sequence preference for SWI/SNF binding once recruited. As in Figure 1A, in the presence of Gal4-VP16, there was little binding of SWI/SNF at sites distal from the activator (Figure 2, row H, lane 2).

Activator Recruitment of SWI/SNF Leads to Localized Nucleosome Disruption

In light of the localized binding of SWI/SNF to nucleosomal arrays observed above, we sought to determine if targeted SWI/SNF binding led to localized nucleosome disruption. To determine the extent of SWI/SNF disruption along an array of nucleosome under targeting conditions, we utilized a restriction endonuclease accessibility assay. In order to assay multiple nucleosomes within an array, we used the G5E4-5S nucleosomal template. This template contains 5 Gal4 binding sites and a minimal adenovirus 2 E4 promoter sequence like in pG5E4T used above. However, in this template the promoter is flanked on both sides by five repeats of 5S rRNA gene nucleosome positioning sequences (Simpson and Stafford, 1983; Ikeda et al., 1999). Within each of these 5S repeats is an *Msp1* digestion site. After end-labeling this fragment, it was assembled into spaced nucleosome arrays (Ikeda et al., 1999). The G5E4-5S nucleosomal arrays were incubated in the presence or absence of Gal4-VP16, followed by the simultaneous addition of SWI/SNF and a large excess of competitor chromatin (where indicated in Figure 3) and the restriction enzyme *Msp1*. The deproteinized samples were then run on an agarose gel (Figure 3A). An increase in the *Msp1* digestion of any site with SWI/SNF addition indicates a disruption of the nucleosome positioned over that region as a result of the action of the complex.

In the absence of SWI/SNF, *Msp1* sites throughout the array were only partially accessible (Figures 3A and 3B, lane 3), however, upon the addition of SWI/SNF, a significant increase in digestion was seen at all the *Msp1* sites (lane 4). When an excess of competitor chromatin

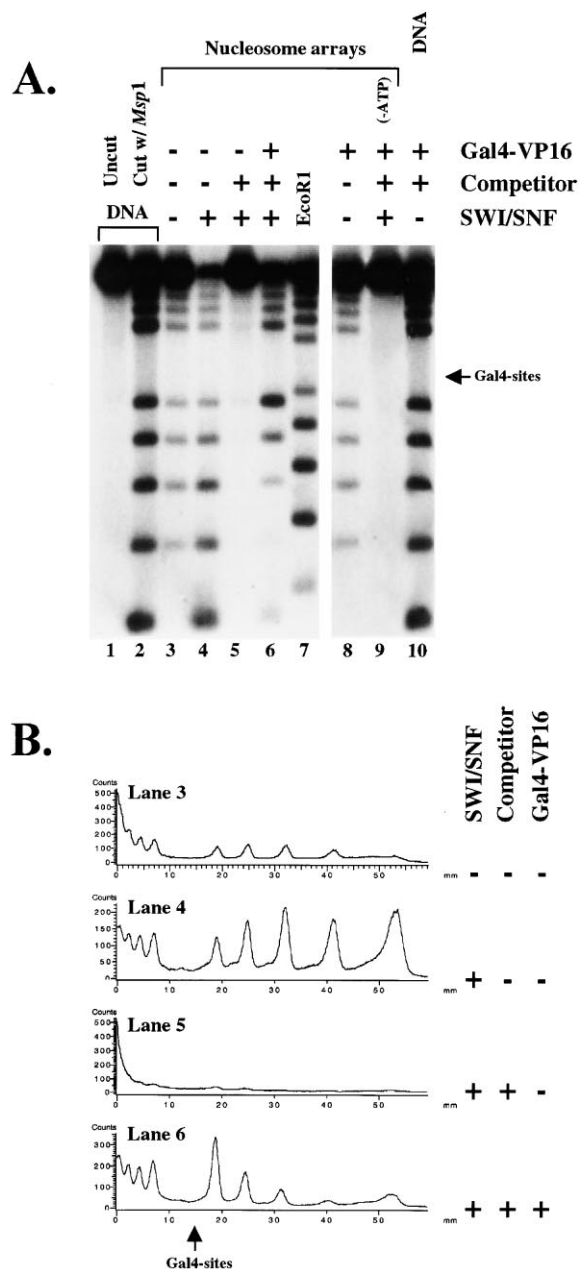


Figure 3. Promoter-Proximal Nucleosomes Are Preferentially Disrupted by SWI/SNF When It Is Targeted by Activators

(A) Restriction enzyme (Msp1) accessibility assays show that Gal4-VP16 targets the disruption activity of SWI/SNF to nucleosomes proximal to the Gal4 binding sites. The G5E4-5S fragment is end labeled with [α - 32 P]dCTP, reconstituted into nucleosomes, and incubated with Gal4-VP16 (lanes 6, 8, 9, and 10), followed by the addition of SWI/SNF and competitor chromatin where indicated. Msp1, which cuts within each of the 5S sequences, was added to the samples at the same time as SWI/SNF. Shown is an exposure of a 0.8% agarose gel that resolved the digestion products. Undigested and Msp1-digested DNA is shown in lanes 1 and 2, respectively. The nucleosomal array template digested with 100-fold more Msp1 than the naked DNA is shown in lane 3. All of the reconstituted nucleosomal array reactions are digested with this higher Msp1 concentration. The addition of SWI/SNF and ATP (lane 4), the excess competitor chromatin (lane 5), and Gal4-VP16 (lanes 6, 8, 9, and 10) result in the observed digestion patterns. Lane 9 shows the digestion pattern in the absence of ATP. Lane 10 is a control showing the

was added, cutting at the Msp1 sites within the G5E4-5S nucleosome array was lost (lane 5). This was largely due to the SWI/SNF complex being competed away from the template, although the Msp1 activity on the template was also reduced (compare lanes 3 and 5). However, a naked DNA control in the presence of the competitor chromatin (lane 10) demonstrates that the Msp1 was active under these conditions. Indeed, when Gal4-VP16 was also included, Msp1 digestion of the G5E4-5S nucleosome array was recovered. Moreover, nucleosomes proximal to the Gal4 sites were preferentially digested, indicating that those nucleosomes were targeted for disruption by SWI/SNF as a result of the binding of Gal4-VP16. The targeted increase in enzyme cutting was due to the recruitment of SWI/SNF and not Msp1. This is demonstrated by the fact that Gal4-VP16 had no effect in the absence of SWI/SNF (Figure 3A, compare lane 3 and lane 8) and by the ATP dependence of the targeted disruption (compare lanes 6 and 9). The ChIP and the restriction enzyme assays together suggest that the SWI/SNF complex can be recruited to promoters by transcription activators, where it remodels only a few nucleosomes proximal to the activator binding sites to stimulate transcription.

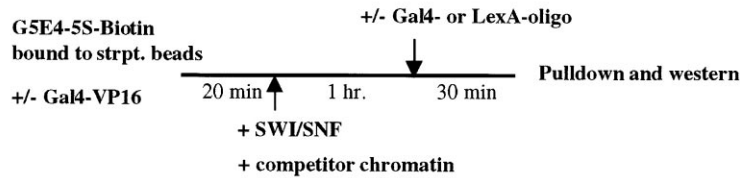
Continued Activator Interactions Retain SWI/SNF on Unmodified Nucleosome Arrays

Recent *in vivo* crosslinking studies have suggested that SWI/SNF binding to the *HO* promoter persists after the dissociation of the Swi5 activator that recruited the complex, providing an epigenetic mark at the promoter (Cosma et al., 1999). To investigate mechanisms by which SWI/SNF might become stably engaged on a promoter once recruited, we tested the retention of SWI/SNF on nucleosome arrays using a pulldown assay similar to that of Yudkovsky and colleagues (Yudkovsky et al., 1999). For this, we used G5E4-5S nucleosome arrays that were end biotinylated and could be immobilized onto streptavidin paramagnetic beads (Dynabeads). After various incubations (Figure 4A), the washed immobilized nucleosome arrays were assayed by Western blots for the presence of Gal4-VP16 with antibodies against the Gal4 DNA binding domain and for SWI/SNF using antibodies against the Swp61/Arp7 subunit. Under the conditions used, ~50% of the SWI/SNF complex was bound to the G5E4-5S nucleosome arrays (Figure 4B, lanes 3 and 4). As expected, the addition of competitor chromatin resulted in a loss of SWI/SNF binding (lanes 5 and 6). Upon addition of Gal4-VP16, SWI/SNF binding to the template array was recovered, illustrating recruitment of SWI/SNF to the array (lanes 7 and 8). To test for the ability of SWI/SNF to be retained on the

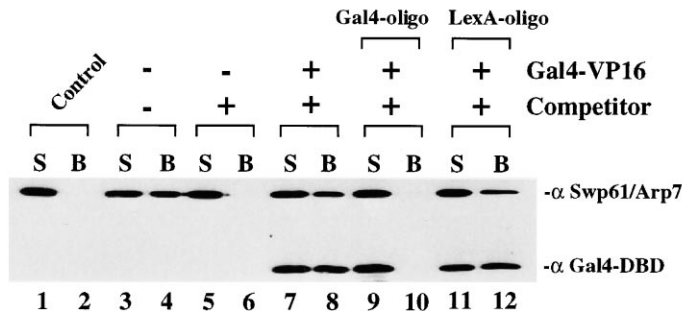
DNA digestion pattern in the presence of Gal4-VP16 and competitor with the same amount of Msp1 as in the nucleosome lanes (100× more than in lane 2). In lane 7, EcoR1 digestion of the nucleosomal array reveals the positions of the 5S rDNA repeats.

(B) Scans of the gel shown in (A), after the gel was exposed to Phosphorimager and quantified. The quantitation of the data at four conditions (lanes 3–6) (–SWI/SNF, –Gal4-VP16, –competitor; +SWI/SNF, –Gal4-VP16, –competitor; +SWI/SNF, +competitor, –Gal4-VP16; and +SWI/SNF, +competitor, +Gal4-VP16) are shown.

A.



B.



template in the absence of activator, we subsequently competed the Gal4-VP16 off the template with a Gal4 site oligo (see diagram in Figure 4A). When Gal4-VP16 was removed from the template by the Gal4 site oligo, the SWI/SNF complex also dissociated (Figure 4B, lanes 9 and 10). By contrast, a nonspecific oligo did not compete Gal4-VP16 or SWI/SNF from the G5E4T-5S nucleosome array (lanes 11 and 12). These data demonstrate that in the presence of competitor chromatin, the stable binding of SWI/SNF to an unmodified nucleosome array required continued interactions with the activator that recruited it.

Histone Acetylation Enhances SWI/SNF Retention on Nucleosome Arrays

The biochemical data presented in Figure 4B suggest that the stable *in vivo* association of SWI/SNF on the *HO* promoter following dissociation of the Swi5 activator (Cosma et al., 1999) likely required interactions of SWI/SNF with the template beyond its DNA binding capacity (Quinn et al., 1996; Côté et al., 1998). As the SAGA histone acetyltransferase complex is also recruited to the *HO* promoter by the Swi5 activator (Cosma et al., 1999; Krebs et al., 1999), we tested whether acetylation of histones might affect the SWI/SNF retention. Control histones (with physiological levels of acetylation) or histones that have been purified from sodium butyrate-treated cells (hyperacetylated histones, see Vettese-Dadey et al., 1996) were used to reconstitute G5E4-5S nucleosome arrays as described previously. The extent of SWI/SNF retention on the hyperacetylated nucleosomal template following dissociation of Gal4-VP16 was analyzed and compared to the control nonacetylated histones (Figure 5A). The competitor chromatin was the same unacetylated chromatin in both cases. The addition of Gal4 oligo to the reaction for 30 min, after the

Figure 4. The Dissociation of SWI/SNF from Nonacetylated Nucleosomal Array Templates
(A) An outline of the immobilized template experiments.

(B) SWI/SNF is targeted to nucleosomal arrays bound by Gal4-VP16 and the loss of Gal4-VP16 from these templates, by Gal4 oligo competition, results in concomitant dissociation of SWI/SNF. Biotinylated G5E4-5S nucleosome arrays were bound to paramagnetic beads (Dynabeads) coupled to streptavidin. Gal4-VP16 was added to the array templates where indicated (lanes 7–12) followed by the addition of SWI/SNF (all lanes) and competitor chromatin (lanes 5–12). The template beads were concentrated with a magnet, and the presence of SWI/SNF in the supernatant and the beads was analyzed by Western blotting, probing with antibodies against the Swp61/Arp7 subunit of the SWI/SNF complex. Using an antibody against the DNA binding domain of Gal4 shows the amount of Gal4-VP16 in the supernatant (S) and the bead (B) lanes. As a control (lanes 1–2), a nonbiotinylated template was used and nonspecific binding of the SWI/SNF to the streptavidin Dynabeads was not observed.

Gal4-VP16 targeting of SWI/SNF, resulted in the complete dissociation of the SWI/SNF complex from nucleosome arrays reconstituted with the control histones (Figure 5A, top panel, lanes 9 and 10). By contrast, the same Gal4 oligo competition failed to efficiently compete SWI/SNF from the nucleosome arrays reconstituted with the hyperacetylated histones (Figure 5A, bottom panel, lanes 9 and 10). These data show that the SWI/SNF complex was retained to a greater extent on hyperacetylated nucleosomal array templates compared to control templates upon the dissociation of Gal4-VP16. This increased retention of SWI/SNF on hyperacetylated nucleosome arrays was not due to a differential dissociation of the Gal4-VP16, as it was efficiently competed from both templates.

Figure 5B is a graphical representation of the average values and the standard deviation of data from 3 different experiments (data shown in [A] and two additional repeats). This graph illustrates that the hyperacetylated histones had no effect on the recruitment of SWI/SNF in the absence of Gal4-VP16 and only slightly increased SWI/SNF recruitment in the presence of Gal4-VP16. The prominent effect of the acetylated histones was to stabilize SWI/SNF binding once Gal4-VP16 was dissociated. These results provide a functional link between histone acetylation and the SWI/SNF complex, namely, that histone acetylation can function in the retention of SWI/SNF on promoters to which it was recruited.

Acetylation by Either SAGA or NuA4 Enhances SWI/SNF Retention Following Activator Dissociation

The SAGA and NuA4 HAT complexes are also recruited to promoters by yeast transcription activators, leading to distinct patterns of acetylated histones on nucleosome arrays (Uitley et al., 1998; Vignali et al., 2000b).

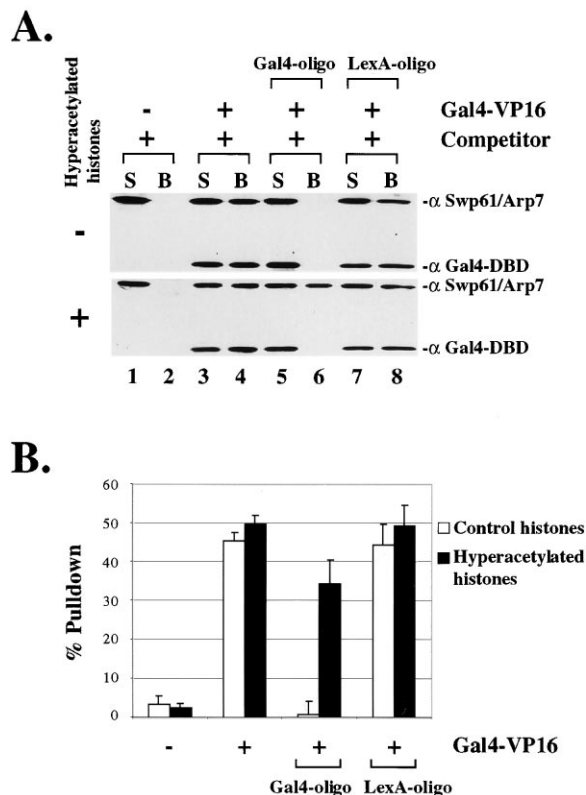


Figure 5. Acetylated Histones Enhance the Retention of the SWI/SNF Complex after the Loss of Gal4-VP16

(A) Biotinylated G5E4-5S was reconstituted into nucleosome arrays with control or hyperacetylated histones and bound to paramagnetic beads. Gal4-VP16 was added to the array templates where indicated (lanes 3–8) followed by the addition of SWI/SNF (all lanes) and competitor chromatin (all lanes) as shown in Figure 4A. One μ g 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 and against Gal4 DNA binding domain. The top and the bottom panels show this immobilized template competition experiment with control and hyperacetylated histones, respectively.

(B) Graphical representation of the average values and the standard deviation of data from 3 different experiments (data shown in [A] and two additional repeats) is shown as % SWI/SNF pulled down.

The SAGA complex primarily acetylates histone H3 on promoter proximal nucleosomes, while the NuA4 complex primarily acetylates histone H4, but over a much broader domain of nucleosomes. To test whether the more biologically relevant acetylation of histones, namely by these specific yeast histone acetyltransferases, can also enhance the SWI/SNF retention on the array, we used the SAGA and NuA4 complexes to acetylate the templates prior to SWI/SNF addition and competition (Figure 6). The SAGA or NuA4 complexes were first incubated with the template in the presence of Gal4-VP16 to generate their specific acetylation patterns (Vignali et al., 2000b). The HAT complexes were removed and these HAT-acetylated templates were used in the SWI/SNF retention assay (Figure 6A). The addition of Gal4

oligo to the reactions resulted in the complete dissociation of the SWI/SNF complex from nucleosome arrays incubated with SAGA or NuA4 in the absence of acetyl-CoA (Figure 6B, first and third panels, lane 6). By contrast, under the same Gal4 oligo competition, SWI/SNF was retained to a much greater degree on the nucleosome arrays incubated with SAGA or NuA4 in the presence of acetyl-CoA (Figure 6B, second and fourth panels, lane 6). Thus, acetylation of the nucleosome array by either the SAGA or NuA4 complexes increased the retention of SWI/SNF once the activator dissociated. These data further indicate that either H3 or H4 acetylation stabilizes binding of the SWI/SNF complex to the target nucleosome array and that either localized acetylation by SAGA or broadly distributed acetylation by NuA4 is sufficient.

Acetylation Enhances SWI/SNF Retention on Promoter-Proximal Nucleosomes

In Figures 1 and 2 we demonstrated that when recruited by a DNA bound activator, the SWI/SNF complex becomes localized to promoter proximal nucleosomes near the activator binding sites. However, when the activator has dissociated and the continued binding of SWI/SNF to the nucleosome array is enhanced by acetylated histones, it is possible that the complex would be free to migrate on the nucleosome array. In particular, when the arrays are reconstituted with hyperacetylated histones or acetylated by NuA4, the acetylated nucleosomes would extend beyond those to which SWI/SNF was originally recruited. By contrast, promoter localized acetylation by SAGA might function to retain SWI/SNF at the promoter.

To investigate these possibilities, we repeated the SWI/SNF in vitro chromatin immunoprecipitation assay with hyperacetylated templates or templates acetylated by SAGA following dissociation of Gal4-VP16 (Figures 7B and 7C, respectively). We used hyperacetylated histones to reconstitute the template (Figure 7B) or acetylated the template with SAGA, similar to the procedure in Figure 6, prior to SWI/SNF addition (Figure 7C). The ChIP was done as in Figure 1, except that a 30 min incubation to allow competition of the activator was included prior to crosslinking. As in Figure 1, in the absence of Gal4-VP16 and competitor chromatin, SWI/SNF bound anywhere on the template (Figures 7B and 7C, row E, lanes 1, 3, 5, 7, 9, 11, 13, and 15). When Gal4-VP16 was prebound to the array, regardless of the presence of competitor chromatin, SWI/SNF was preferentially bound near the Gal4 binding sites (Figures 7B and 7C, row F, all lanes). The addition of Gal4 oligo resulted in the complete dissociation of SWI/SNF from nonacetylated templates (Figures 7B and 7C, row G, lanes 1, 2, 5, 6, 9, 10, 13, and 14) as was observed in the immobilized template assay (Figures 4, 5, and 6). The fact that competition with the Gal4-site oligo dissociated SWI/SNF even in the absence of competitor chromatin suggests that SWI/SNF had a higher affinity for the activator than for the nucleosome array template. However, with acetylation, SWI/SNF was retained on the template following the addition of Gal4 oligo (Figures 7B and 7C, row G, compare lanes 1–2 and 3–4). Importantly, SWI/SNF was retained on the promoter proximal nucleo-

G5E4-5S-Biotin bound to strpt. beads
 +/- Gal4-VP16
 HAT (SAGA or NuA4) +/- Acetyl-CoA
 + SWI/SNF + competitor chromatin
 20 min 30 min 1 hr 30 min
 Wash to remove HAT +/- Gal4- or LexA-oligo
 Pulldown and western

Acetyl-CoA	HAT	-		+		Gal4-oligo		LexA-oligo		Gal4-VP16 Competitor	
		S	B	S	B	S	B	S	B		
-	SAGA										
+	SAGA										
-	NuA4										
+	NuA4										

Discussion

plexes (e.g., SAGA and NuA4) and an activator-recruited ATP-dependent chromatin remodeling complex (SWI/SNF) and provide a mechanistic basis for their ordered recruitment.

An important question regarding the role of ATP-driven chromatin remodeling complexes is their function in gene-specific transcription regulation. Several reports have demonstrated that gene-specific activators interact with the SWI/SNF complex and that this interaction leads to transcriptional stimulation from nucleosomal templates (Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999; Kadam et al., 2000; Wallberg et al., 2000). We sought to determine the location of nucleosomes bound and disrupted by SWI/SNF when recruited by a transcription activator to facilitate transcription. We have found that binding of SWI/SNF and the disruption of nucleosomes following activator targeting of the complex are restricted to promoter-proximal nucleosomes. Indeed, it is striking that a 2 MDa complex, which is ten times the size of a nucleosome (for example, see Bazett-Jones et al., 1999) should preferentially bind those nucleosomes that are so near the site where it is targeted. A previous electron microscopy study indicated that SWI/SNF can simultaneously bind DNA or nucleosomes at two different locations, which can create loops of variable length with an average of 133 base pairs (Bazett-Jones et al., 1999). The localized binding seen in this study suggests that activator recruit-

(B) Immobilized G5E4-5S nucleosome arrays were bound by Gal4-VP16 (lanes 3–8), followed by the addition of either SAGA or NuA4 in the presence or absence of acetyl-CoA as indicated. After a 30 min incubation and several washes to remove the HAT complexes (Figure 6A), SWI/SNF and competitor chromatin were added followed by oligo competition as in Figures 4 and 5.

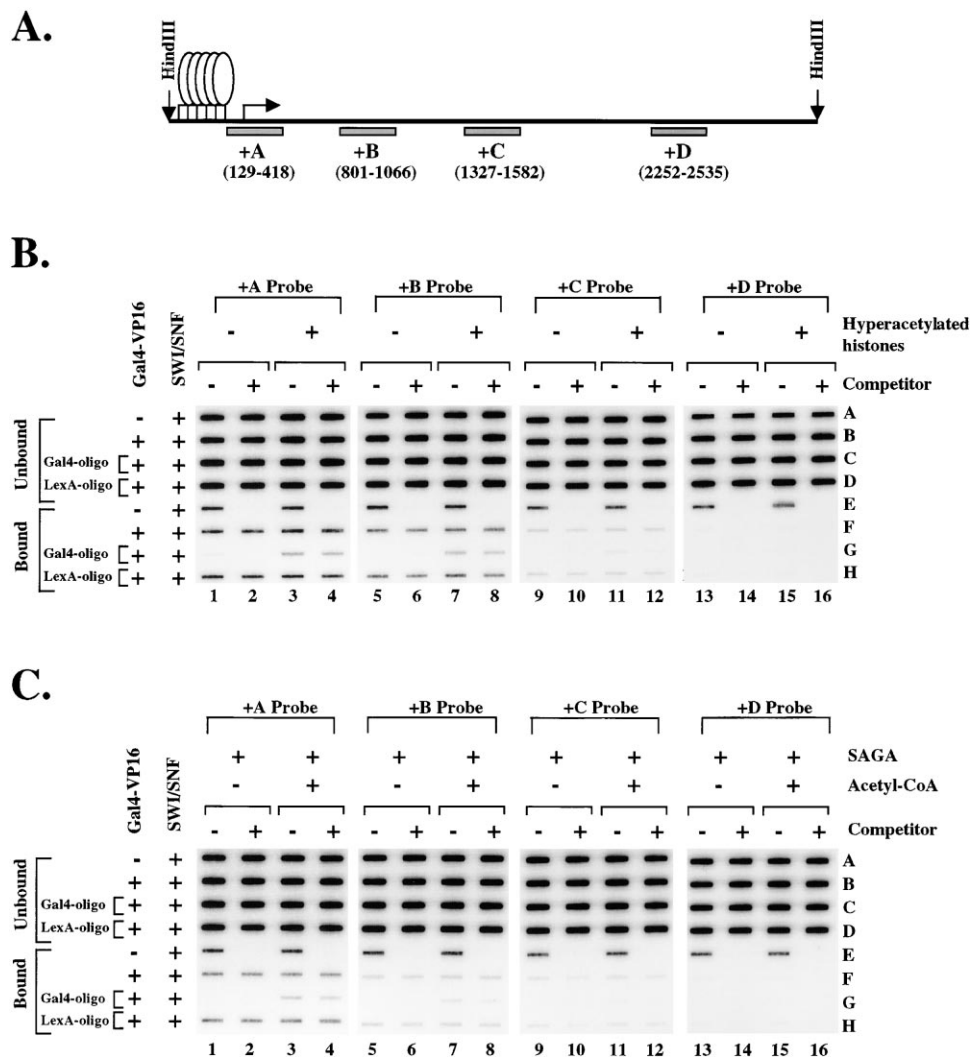


Figure 7. Acetylated Histones Enhance the Retention of SWI/SNF on Promoter-Proximal Nucleosomes

(A) Diagram showing the positions of the different probes used relative to the Gal4 binding sites when pG5E4T is digested with HindIII.

(B) Retention of SWI/SNF on promoter-proximal nucleosomes by hyperacetylated histones. pG5E4T was linearized, biotinylated, and reconstituted with control (columns 1, 2, 5, 6, 9, 10, 13, and 14) or hyperacetylated (columns 3, 4, 7, 8, 11, 12, 15, and 16) histones, bound to paramagnetic beads coupled to streptavidin and incubated in the presence (rows B, C, D, F, G, and H) or absence of Gal4-VP16 (rows A and E), followed by the addition of SWI/SNF (all rows) and competitor chromatin as indicated (columns 2, 4, 6, 8, 10, 12, 14, and 16). Gal4 oligo (rows C and G) or LexA oligo (rows D and H) was added and incubated for 30 min prior to immunoprecipitation and blotting as in Figure 1. The membrane was then hybridized successively with probes of 250–300 bp shown in Figure 7A. Note that the hyperacetylated histones retain SWI/SNF on promoter proximal nucleosomes (lanes 1–4, row G).

(C) Localized retention of SWI/SNF on promoter-proximal nucleosomes that were previously acetylated by SAGA. pG5E4T was linearized, biotinylated, and reconstituted with histones, bound to paramagnetic beads coupled to streptavidin, and incubated in the presence (rows B, C, D, F, G, and H) or absence of Gal4-VP16 (rows A and E), followed by the addition of SAGA in the presence (columns 3, 4, 7, 8, 11, 12, 15, and 16) or absence (columns 1, 2, 5, 6, 9, 10, 13, and 14) of acetyl-CoA. After a 30 min incubation, SWI/SNF (all rows) and competitor chromatin were added as indicated (columns 2, 4, 6, 8, 10, 12, 14, and 16). Gal4 oligo (rows C and G) or LexA oligo (rows D and H) followed and incubated for 30 min prior to immunoprecipitation and blotting as in Figure 1. The membrane was then hybridized successively with probes shown in Figure 7A. Incubation with SAGA led to an acetyl-CoA dependent enhancement of subsequent SWI/SNF binding to promoter-proximal nucleosomes (compare lanes 1–4, row G).

ment may favor short loops where both sites of binding are near the activator, or target one site of interaction at the promoter with the other randomly distributed on the array. In addition, the localized nucleosome disruption shown in this study suggests that the site of activator interaction on the complex may reside relatively near the ATPase site required for nucleosome disruption. It also suggests that disruption of nucleosome most near the promoter is crucial for transcription activation, con-

sistent with a long history of reports indicating that nucleosomes are inhibitory to preinitiation transcription complex formation (reviewed in Felsenfeld, 1992).

Role of HAT Complexes in Stabilizing Promoter Occupancy by SWI/SNF

Cosma et al. have shown that the activator Swi5 is not continually required for the maintenance of an active chromatin state at the *HO* promoter and that it disap-

appears shortly after it has recruited the SWI/SNF complex (Cosma et al., 1999). To address whether the SWI/SNF complex would still be associated with nucleosomes after the loss of the activator, we tested the status of the binding of the SWI/SNF complex to nucleosomes after the removal of the activator Gal4-VP16. In this purified *in vitro* system, we found that with the loss of Gal4-VP16, SWI/SNF also dissociated from the nucleosomal templates, suggesting a requirement for continued interaction between the complex and the activator. Thus, our data suggest that the retention of SWI/SNF at the *HO* promoter *in vivo* is unlikely to be a result of stable nucleosome binding of the complex alone. Instead, these results suggest that once Swi5p is dissociated, other interactions are likely to participate in retention of SWI/SNF at the promoter.

One candidate for participating in SWI/SNF retention is the acetylation of histones resulting from the action of the subsequently recruited Gcn5-dependent SAGA histone acetyltransferase complex. Indeed, we have found that acetylation of the template nucleosomes by either the SAGA or NuA4 HAT complexes enhances the retention of SWI/SNF on promoter nucleosomes after the loss of the activator. This retention could result from an interaction of the Swi2/Snf2p bromodomain with acetylated histones, which could stabilize SWI/SNF binding. Bromodomains have been shown to bind acetylated histones (Dhalluin et al., 1999; Jacobson et al., 2000). Our biochemical result is also consistent with a report that Gcn5p can participate in stabilizing SWI/SNF binding to a promoter *in vivo* (Syntichaki et al., 2000) and a report that histone acetylation decreases the ability of SWI/SNF to remodel numerous nucleosome arrays *in vitro* (Logie et al., 1999).

Interrelated Functions of HAT Complexes and SWI/SNF

In addition to the SWI/SNF complex, the SAGA and NuA4 histone acetyltransferase complexes are also recruited by transcription activators in yeast. This raises the question of which comes first on genes that employ both complexes. The answer is likely to depend on the particular requirements for chromatin remodeling to activate a given gene. For example, there is strong evidence that the SWI/SNF complex is recruited first to the *HO* promoter and is required for the subsequent recruitment of SAGA and for acetylation of histones at the promoter (Cosma et al., 1999; Krebs et al., 1999). In this instance, recruitment of SWI/SNF may provide an initial remodeling of chromatin structure at the promoter, making it more accessible to acetylation by the SAGA complex. This may be a unique requirement for activation in mitotic chromatin, where the *HO* gene is transcribed (Krebs et al., 2000). However, our data also suggest that the subsequent stable binding of SWI/SNF to form an "epigenetic mark" on the *HO* promoter (Cosma et al., 1999) may require acetylation of promoter nucleosomes by SAGA. Our finding that histone acetylation can stabilize the binding of the SWI/SNF complex to promoter nucleosomes suggest that in many instances, the function of HAT complexes may precede that of SWI/SNF during transcription activation. Indeed, while this manuscript was under revision two papers appeared that indicate such a pathway. Agalioti and colleagues have found

that Gcn5 recruitment and histone acetylation precedes recruitment of the SWI/SNF complex during activation of the interferon β promoter *in vitro* (Agalioti et al., 2000). Dilworth and colleagues have shown that transactivation by RAR/RXR involves histone acetylation in a step prior to the action of the human SWI/SNF complex (Dilworth et al., 2000).

While this study has clearly shown that promoter binding by SWI/SNF is enhanced by histone acetylation, it is important to note that the effect of acetylated histones in stabilizing protein interactions may extend to other proteins (for example, other bromodomain containing proteins). Moreover, the function of histone acetylation in facilitating interactions with other proteins may be part of a greater "histone code" that includes other histone modifications, providing specific interaction sites for regulatory proteins and epigenetic marks in chromatin (Strahl and Allis, 2000; Turner, 2000).

Experimental Procedures

Purification of the SWI/SNF Complex

The SWI/SNF complex was purified from yeast (strain CY396) as described (Côté et al., 1994; Neely et al., 1999). This strain is HA tagged on the Swi2/Snf2 subunit of the SWI/SNF complex. Briefly, yeast whole-cell extract was added to Ni^{2+} -NTA resin. The SWI/SNF complex eluted from Ni^{2+} with 300 mM imidazole. The eluted fraction was run on a Mono Q column, followed by a heparin sepharose column, and a DNA cellulose column. The SWI/SNF complex eluted at 200 mM NaCl on the DNA cellulose column. Purification was monitored by Western blot, using several antibodies to SWI/SNF subunits.

Purification of Gal4-Derivative Proteins

Gal4 fusion proteins were expressed in bacteria and purified as described (Lin et al., 1988; Chasman et al., 1989; Baichwal and Tjian, 1990; Tanese et al., 1991). Protein purity was checked by SDS-PAGE.

Histone Preparation and Nucleosome Reconstitution

Core histones and long oligonucleosomes (LON) were purified from HeLa cells as described (Côté et al., 1995; Owen-Hughes et al., 1999). These long oligonucleosomes were used as competitor chromatin in all of the assays. Control and hyperacetylated histones were purified from HeLa cells that were incubated in the absence or presence of 10 mM sodium butyrate as described (Vettese-Dadey et al., 1996). Nucleosomal arrays were reconstituted with purified core histones by step dilution (Steger et al., 1998; Owen-Hughes et al., 1999).

Chromatin ImmunoPrecipitation (ChIP) Assays

pG5E4T was prepared as described (Vignali et al., 2000b) with the exception that the plasmid was linearized with either HindIII or Asp718 so that the Gal4-sites are closer to one end or the other of the template fragment (shown in Figures 1A and 2). The fragments were then biotinylated, gel purified, and reconstituted with core histones by step dilution as described previously (Steger et al., 1998). The biotinylated nucleosomal arrays were incubated at 30°C for 1 hr with paramagnetic beads coupled to streptavidin (Dynabeads streptavidin, Dynal) in a binding buffer containing 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 MgCl_2 , supplemented with 300 mM KCl. Templates were washed twice with 50 μl of the binding buffer to remove excess KCl and then resuspended in the binding buffer to a concentration of 25 ng/ μl . Following the standard ChIP protocol (Kuo and Allis, 1999) modified for *in vitro* conditions (Vignali et al., 2000b), 100 nM Gal4-VP16 (50 nM dimers) was added to ~250 ng of biotinylated nucleosomal array in 20 μl binding buffer (4.2 nM) and incubated for 30 min at 30°C. Eight nM SWI/SNF and about 10-fold excess competitor chromatin was then added. After a 30 min incubation at 30°C, formaldehyde was added to 1% of the final

reactions and incubated for 15 min at room temperature. The excess crosslinking reagent was washed away using magnets to pulldown the immobilized templates (two washes in 50 μ l binding buffer). The templates were released from biotin and the Dynabeads by digestion with PstI or EcoRI at 37°C for 4 hr. The samples were then digested with 10 mU MNase in 3 mM CaCl₂ for 5 min at room temperature. The digestions were stopped with the addition of a 2-fold excess EGTA, and the samples were immunoprecipitated using affinity matrix HA (Covance) antibody to pulldown SWI/SNF and the cross-linked DNA. The beads were washed and the crosslinking reversed by adding 4 μ l of 5 M NaCl in 100 μ l binding buffer and incubated at 65°C overnight. DNA was extracted from the beads with 50 μ l of 1% SDS, 0.1 M NaHCO₃ solution twice followed by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation of the unbound and bound materials. Denatured samples were applied to Zeta-Probe membranes by slot blot. The membranes were then hybridized successively with probes of 250–300 bp generated by PCR and labeled by random hexanucleotide primer extension (Boehringer Mannheim) as described (Vignali et al., 2000b) and shown in Figures 1, 2, and 7. The membranes were exposed to Phosphorimager and quantified. In Figure 7B, control or hyperacetylated histones were used to reconstitute the templates and 1 μ g of Gal4 or LexA oligo was added where indicated and incubated for 30 min to compete Gal4-VP16 prior to crosslinking. In Figure 7C, SAGA in the presence or absence of 2 μ M acetyl-CoA was targeted to the templates after the addition of 100 mM Gal4-VP16 as before. After a 30 min incubation and two washes with the binding buffer to remove the SAGA complex, SWI/SNF and competitor chromatin was added and the experiment was continued as before.

Restriction Endonuclease (Msp1) Accessibility Assays

pG5E4-5S containing a dinucleosome length G5E4 fragment flanked on both sides by 5S sequences was prepared as described (Ikeda et al., 1999). The G5E4-5S fragments were produced by digesting pG5E4-5S with Asp718 and treating the digestion products with Klenow in the presence of [α -³²P]dCTP. The 2.5 kb end-labeled G5E4-5S fragments were then gel purified away from the backbone by digesting with ClaI and EaeI. This array was then reconstituted by step dilution as described previously. This assay was performed similarly to the one described earlier. One hundred nM Gal4-VP16 was added to about 100 ng of template in 20 μ l binding buffer (1.7 nM) and incubated for 30 min at 30°C, followed by the addition of 4 nM SWI/SNF and 1 μ g of competitor chromatin where indicated. Msp1 (0.4 mU for naked DNA templates and 40 mU for nucleosomal templates) was added to the samples simultaneously with SWI/SNF. An equal volume of stop buffer (20 mM Tris-HCl [pH 7.5], 50 mM EDTA, 2% SDS, 0.2 mg/ml proteinase K, 0.25 mg/ml yeast tRNA) was added to the Msp1-digested reactions and incubated at 50°C for 1 hr. Deproteinized samples were precipitated and the pellets resuspended in 10 μ l of agarose loading dye. The samples were run on a 0.8% agarose gel, fixed, dried, and visualized by autoradiography. Graphical representation of several lanes of this experiment is shown in Figure 3B.

Immobilized Template Pulldown Assays

The G5E4-5S fragment was end labeled with biotin-14-dATP as described earlier for the end-labeled template, gel purified, and reconstituted by step dilution. The nucleosomal arrays were then bound to paramagnetic beads coupled to streptavidin (Dynabeads streptavidin, Dynal) as described before. 100 nM Gal4-VP16 (50nM dimers) was added to ~200 ng of this template in 20 μ l binding buffer (3.4 nM) and incubated for 20 min at 30°C, followed by the simultaneous addition of 4 nM SWI/SNF and 2 μ g 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. The blots were probed with antibodies against the Swp61/Arp7 subunit of the SWI/SNF complex as well as an antibody against the DNA binding domain of Gal4 (Gal4[DBD]: SC-577; Santa Cruz Biotechnology, Inc.). For the Gal4-VP16 competition experiments, 1 μ g 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

Figure 5A, templates were either reconstituted with control or hyperacetylated histones. In Figure 6, two different HATs (SAGA and NuA4) in the presence or absence of acetyl-CoA were individually 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, SWI/SNF and competitor chromatin was added as before. Gal4 or LexA oligo was then added where indicated for 30 min prior to pulldown.

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References

- Agalioti, T., Lomvardas, S., Parekh, B., Yie, J., Maniatis, T., and Thanos, D. (2000). Ordered recruitment of chromatin modifying and general transcription factors to the IFN-promoter. *Cell* 103, 667–678.
- Armstrong, J.A., and Emerson, B.M. (1998). Transcription of chromatin: these are complex times. *Curr. Opin. Genet. Dev.* 8, 165–172.
- Baichwal, V.R., and Tjian, R. (1990). Control of c-Jun activity by interaction of a cell-specific inhibitor with regulatory domain delta: differences between v- and c-Jun. *Cell* 63, 815–825.
- Bazett-Jones, D.P., Cote, J., Landel, C.C., Peterson, C.L., and Workman, J.L. (1999). The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol. Cell. Biol.* 19, 1470–1478.
- Chasman, D.I., Leatherwood, J., Carey, M., Ptashne, M., and Kornberg, R.D. (1989). Activation of yeast polymerase II transcription by herpesvirus VP16 and GAL4 derivatives in vitro. *Mol. Cell. Biol.* 9, 4746–4749.
- Cosma, M.P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* 97, 299–311.
- Côté, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* 265, 53–60.
- Côté, J., Utley, R.T., and Workman, J.L. (1995). Basic analysis of transcription factor binding to nucleosomes. *Meth. Mol. Genet.* 6, 108–129.
- Côté, J., Peterson, C.L., and Workman, J.L. (1998). Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding. *Proc. Natl. Acad. Sci. USA* 95, 4947–4952.
- Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 399, 491–496.
- Dilworth, F.J., Fromental-Ramain, C., Yamamoto, K., and Chambon, P. (2000). ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR in vitro. *Mol. Cell* 6, 1049–1058.
- Felsenfeld, G. (1992). Chromatin as an essential part of the transcriptional mechanism. *Nature* 355, 219–224.
- Gavin, I.M., and Simpson, R.T. (1997). Interplay of yeast global transcriptional regulators Ssn6p-Tup1p and Swi-Snf and their effect on chromatin structure. *EMBO J.* 16, 6263–6271.

- Gregory, P.D., Schmid, A., Zavari, M., Munsterkotter, M., and Horz, W. (1999). Chromatin remodelling at the PHO8 promoter requires SWI-SNF and SAGA at a step subsequent to activator binding. *EMBO J.* 18, 6407-6414.
- Hirschhorn, J.N., Brown, S.A., Clark, C.D., and Winston, F. (1992). Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.* 6, 2288-2298.
- Ikeda, K., Steger, D.J., Eberharter, A., and Workman, J.L. (1999). Activation domain-specific and general transcription stimulation by native histone acetyltransferase complexes. *Mol. Cell. Biol.* 19, 855-863.
- Imbalzano, A.N. (1998). Energy-dependent chromatin remodelers: complex complexes and their components. *Crit. Rev. Eukaryot. Gene. Expr.* 8, 225-255.
- Imbalzano, A.N., Schnitzler, G.R., and Kingston, R.E. (1996). Nucleosome disruption by human SWI/SNF is maintained in the absence of continued ATP hydrolysis. *J. Biol. Chem.* 271, 20726-20733.
- Jacobson, R.H., Ladurner, A.G., King, D.S., and Tjian, R. (2000). Structure and function of a human TAFII250 double bromodomain module. *Science* 288, 1422-1425.
- Kadam, S., McAlpine, G.S., Phelan, M.L., Kingston, R.E., Jones, K.A., and Emerson, B.M. (2000). Functional selectivity of recombinant mammalian SWI/SNF subunits. *Genes Dev.* 14, 2441-2451.
- Kingston, R.E., and Narlikar, G.J. (1999). ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. *Genes Dev.* 13, 2339-2352.
- Krebs, J.E., Kuo, M.H., Allis, C.D., and Peterson, C.L. (1999). Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. *Genes Dev.* 13, 1412-1421.
- Krebs, J.E., Fry, C.J., Samuels, M.L., and Peterson, C.L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. *Cell* 102, 587-598.
- Kuo, M.H., and Allis, C.D. (1999). In vivo cross-linking and immunoprecipitation for studying dynamic Protein:DNA associations in a chromatin environment. *Methods* 19, 425-433.
- Kwon, H., Imbalzano, A.N., Khavari, P.A., Kingston, R.E., and Green, M.R. (1994). Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature* 370, 477-481.
- Lin, Y.S., Carey, M.F., Ptashne, M., and Green, M.R. (1988). GAL4 derivatives function alone and synergistically with mammalian activators in vitro. *Cell* 54, 659-664.
- Logie, C., and Peterson, C.L. (1997). Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. *EMBO J.* 16, 6772-6782.
- Logie, C., Tse, C., Hansen, J.C., and Peterson, C.L. (1999). The core histone N-terminal domains are required for multiple rounds of catalytic chromatin remodeling by the SWI/SNF and RSC complexes. *Biochemistry* 38, 2514-2522.
- Lorch, Y., Cairns, B.R., Zhang, M., and Kornberg, R.D. (1998). Activated RSC-nucleosome complex and persistently altered form of the nucleosome. *Cell* 94, 29-34.
- Lorch, Y., Zhang, M., and Kornberg, R.D. (1999). Histone octamer transfer by a chromatin-remodeling complex. *Cell* 96, 389-392.
- Muchardt, C., and Yaniv, M. (1999). ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. *J. Mol. Biol.* 293, 187-198.
- Natarajan, K., Jackson, B.M., Zhou, H., Winston, F., and Hinnebusch, A.G. (1999). Transcriptional activation by Gcn4p involves independent interactions with the SWI/SNF complex and the SRB/mediator. *Mol. Cell* 4, 657-664.
- Neely, K.E., Hassan, A.H., Wallberg, A.E., Steger, D.J., Cairns, B.R., Wright, A.P., and Workman, J.L. (1999). Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. *Mol. Cell* 4, 649-655.
- Owen-Hughes, T., and Workman, J.L. (1996). Remodeling the chromatin structure of a nucleosome array by transcription factor-targeted trans-displacement of histones. *EMBO J.* 15, 4702-4712.
- Owen-Hughes, T., Utley, R.T., Steger, D.J., West, J.M., John, S., Cote, J., Havas, K.M., and Workman, J.L. (1999). Analysis of nucleosome disruption by ATP-driven chromatin remodeling complexes. *Methods Mol. Biol.* 119, 319-331.
- Peterson, C.L., and Herskowitz, I. (1992). Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. *Cell* 68, 573-583.
- Peterson, C.L., and Workman, J.L. (2000). Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr. Opin. Genet. Dev.* 10, 187-192.
- Phelan, M.L., Schnitzler, G.R., and Kingston, R.E. (2000). Octamer transfer and creation of stably remodeled nucleosomes by human SWI-SNF and its isolated ATPases. *Mol. Cell. Biol.* 20, 6380-6389.
- Quinn, J., Fyrberg, A.M., Ganster, R.W., Schmidt, M.C., and Peterson, C.L. (1996). DNA-binding properties of the yeast SWI/SNF complex. *Nature* 379, 844-847.
- Schnitzler, G., Sif, S., and Kingston, R.E. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell* 94, 17-27.
- Simpson, R.T., and Stafford, D.W. (1983). Structural features of a phased nucleosome core particle. *Proc. Natl. Acad. Sci. USA* 80, 51-55.
- Steger, D.J., Eberharter, A., John, S., Grant, P.A., and Workman, J.L. (1998). Purified histone acetyltransferase complexes stimulate HIV-1 transcription from preassembled nucleosomal arrays. *Proc. Natl. Acad. Sci. USA* 95, 12924-12929.
- Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. *Nature* 403, 41-45.
- Syntichaki, P., Topalidou, I., and Thireos, G. (2000). The Gcn5 bromodomain co-ordinates nucleosome remodelling. *Nature* 404, 414-417.
- Tanese, N., Pugh, B.F., and Tjian, R. (1991). Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. *Genes Dev.* 5, 2212-2224.
- Turner, B.M. (2000). Histone acetylation and an epigenetic code. *Bioessays* 22, 836-845.
- Utley, R.T., Cote, J., Owen-Hughes, T., and Workman, J.L. (1997). SWI/SNF stimulates the formation of disparate activator-nucleosome complexes but is partially redundant with cooperative binding. *J. Biol. Chem.* 272, 12642-12649.
- Utley, R.T., Ikeda, K., Grant, P.A., Côté, J., Steger, D.J., Eberharter, A., John, S., and Workman, J.L. (1998). Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature* 394, 498-502.
- Vettese-Dadey, M., Grant, P.A., Hebbes, T.R., Crane-Robinson, C., Allis, C.D., and Workman, J.L. (1996). Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. *EMBO J.* 15, 2508-2518.
- Vignali, M., Hassan, A.H., Neely, K.E., and Workman, J.L. (2000a). ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol.* 20, 1899-1910.
- Vignali, M., Steger, D.J., Neely, K.E., and Workman, J.L. (2000b). Distribution of acetylated histones resulting from Gal4-VP16 recruitment of SAGA and NuA4 complexes. *EMBO J.* 19, 2629-2640.
- Wallberg, A.E., Neely, K.E., Hassan, A.H., Gustafsson, J.A., Workman, J.L., and Wright, A.P. (2000). Recruitment of the SWI-SNF chromatin remodeling complex as a mechanism of gene activation by the glucocorticoid receptor tau1 activation domain. *Mol. Cell. Biol.* 20, 2004-2013.
- Whitehouse, I., Flaus, A., Cairns, B.R., White, M.F., Workman, J.L., and Owen-Hughes, T. (1999). Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* 400, 784-787.
- Winston, F., and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* 8, 387-391.
- Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* 67, 545-579.
- Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C.L. (1999). Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev.* 13, 2369-2374.