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Activation Domain–Mediated Targeting of the SWI/SNF Complex to Promoters Stimulates Transcription from Nucleosome Arrays

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Summary

The yeast SWI/SNF complex is required for the transcription of several yeast genes and has been shown to alter nucleosome structure in an ATP-dependent reaction. In this study, we show that the complex stimulated in vitro transcription from nucleosome templates in an activation domain–dependent manner. Transcription stimulation by SWI/SNF required an activation domain with which it directly interacts. The acidic activation domains of VP16, Gcn4, Swi5, and Hap4 interacted directly with the purified SWI/SNF complex and with the SWI/SNF complex in whole-cell extracts. The similarity of activation domain interactions and transcriptional stimulation between SWI/SNF and the SAGA histone acetyltransferase complex may account for their apparent overlapping functions in vivo.

Introduction

The SWI/SNF complex is a multisubunit complex that alters the conformation of nucleosomal DNA via an ATP-dependent reaction (for review, see Imbalzano, 1998; Workman and Kingston, 1998). The genes encoding subunits of the SWI/SNF complex were discovered in yeast genetic screens as genes that are required for *HO* and for *SUC2* gene expression (Neigeborn and Carlson, 1984; Stern et al., 1984; Breeden and Nasmyth, 1987; Laurent et al., 1990; Peterson and Herskowitz, 1992). The SWI/SNF complex contains 11 known subunits, one of which is a highly conserved DNA-dependent ATPase, the Swi2/Snf2 subunit (Laurent et al., 1993).

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Nucleosome disruption by the SWI/SNF complex involves a perturbation of histone DNA interactions that alters the path and length of DNA bent around the histone octamer (Côté et al., 1998; Bazett-Jones et al., 1999). The altered conformation of nucleosome cores generated by the action of SWI/SNF or the related RSC complex (Cairns et al., 1996c) persists after depletion of ATP and detachment of the complex (Imbalzano et al., 1996; Côté et al., 1998; Lorch et al., 1998; Schnitzler et al., 1998). The action of the SWI/SNF complex can facilitate the sliding of histone octamers on DNA in *cis* (Whitehouse et al., 1999) as well as the displacement of histone octamers from DNA in *trans* (Owen-Hughes et al., 1996; Lorch et al., 1999). Consequences of SWI/SNF action include increased accessibility of nucleosomal DNA to transcription factors and restriction enzymes (Côté et al., 1994; Kwon et al., 1994; Logie and Peterson, 1997; Logie et al., 1999) and the release of nucleosome-induced paused RNA polymerase II (Brown et al., 1996).

An important question regarding SWI/SNF function is how the complex might be targeted to specific promoter regions in chromatin. The possibility that SWI/SNF is targeted by transcription activators is consistent with several results. Interactions between the SWI/SNF complex and the glucocorticoid receptor (Yoshinaga et al., 1992; Fryer and Archer, 1998) and the lymphoid-specific Ikaros DNA-binding proteins (Kim et al., 1999) have been reported in cell extracts. A SWI/SNF-related complex was purified as an essential cofactor for the erythroid transcription activator EKLF (Armstrong et al., 1998). Moreover, recent chromatin immunoprecipitation studies in yeast suggest that interaction of the SWI/SNF complex with the *HO* endonuclease gene promoter follows the binding of the Swi5 transcription activator (Cosma et al., 1999; Krebs et al., 1999). Transcription activation via a Swi5-binding site in yeast is highly dependent on components of the SWI/SNF complex (Cairns et al., 1996b).

In this study, we show activation of Gal4-VP16-driven transcription from chromatin templates by the yeast SWI/SNF complex. Transcription activation by SWI/SNF is attributed to recruitment of the SWI/SNF complex by acidic activation domains. These results and those in the accompanying manuscript (Natarajan et al., 1999 [this issue of *Molecular Cell*]) reveal the role of sequence-specific transcription activators in targeting the SWI/SNF chomatin remodeling complex to promoters.

Results

Stimulation of Gal4-VP16-Driven Transcription by the SWI/SNF Complex

To test the transcriptional activities of SWI/SNF, we utilized a purified in vitro chromatin reconstitution system, which generates spaced nucleosome arrays, to form transcription templates. This approach has demonstrated acetyl-CoA-dependent transcription activation by histone acetyltransferase complexes (Steger et al.,

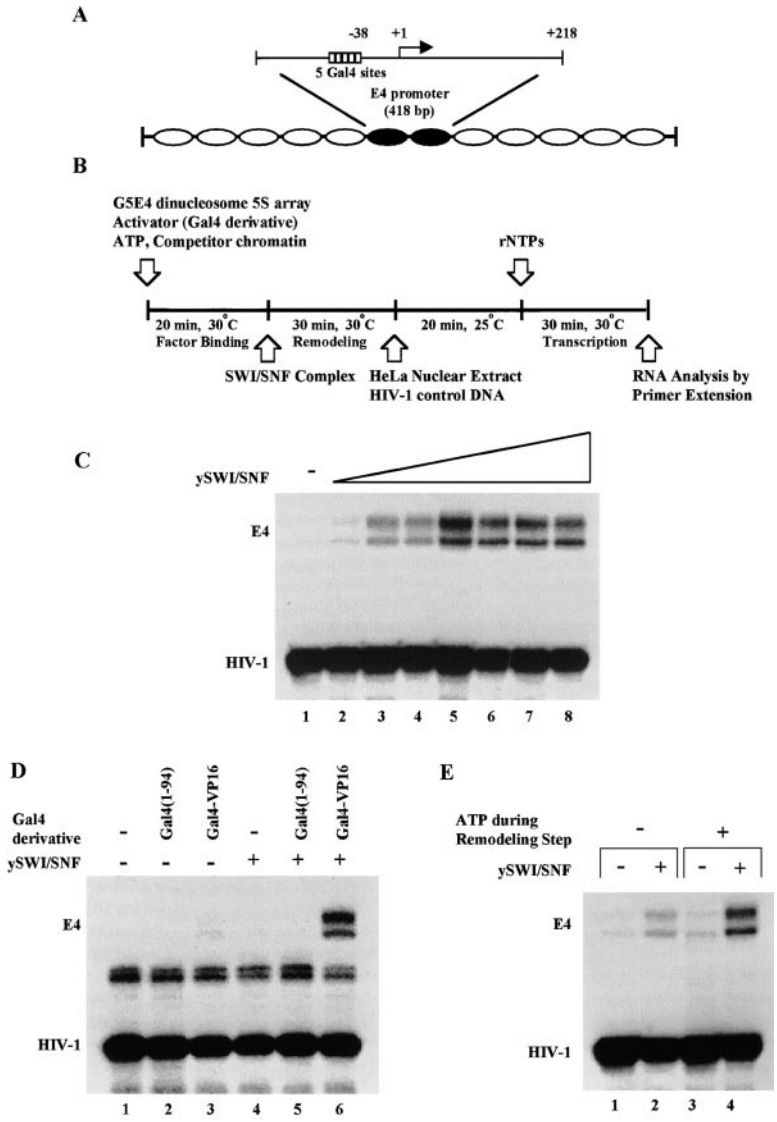

Figure 1. SWI/SNF-Dependent Stimulation of Transcription from Chromatin Templates

(A) The 5S-G5E4 nucleosomal template used for the transcription assays contains five Gal4-binding sites upstream of a minimal adenovirus E4 promoter. The promoter region is contained in two nucleosomes (closed ovals) and is flanked by five 5S rDNA nucleosomes on either side (open ovals).

(B) A schematic of the steps in the transcription assay, including the order of addition of reagents and the lengths of incubations.

(C) Transcription of the nucleosome template in the presence of increasing amounts of SWI/SNF. All lanes contain Gal4–VP16. E4 indicates the nucleosomal template and a plasmid containing HIV-1 sequence served as an internal recovery control.

(D) Activation domain dependence of SWI/SNF stimulation. Transcription was assayed on G5E4 nucleosomal DNA following the binding of Gal4(1–94), Gal4–VP16, or no activator in the presence (lanes 4–6) or absence (lanes 1–3) of SWI/SNF.

(E) Remodeling of the nucleosome array plays a role in SWI/SNF stimulation. Transcription was assayed in the presence (lanes 3 and 4) or absence (lanes 1 and 2) of ATP during the "remodeling" step of the assay. All lanes contain Gal4–VP16. ATP was added to lanes 1 and 2 with the rNTPs to assure equal levels of ATP at the start of transcription.

1998; Ikeda et al., 1999; Steger and Workman, 1999). The nucleosomal array template contained five Gal4-binding sites upstream of a minimal adenovirus E4 promoter (Figure 1A). Figure 1B shows a schematic of the transcription assays including the order of addition of reagents and the lengths of incubations.

SWI/SNF stimulated Gal4-VP16-driven transcription from the nucleosome array templates. The reactions contained a 50-fold excess of competitor chromatin, which reduced the molar ratio of SWI/SNF to nucleosomes to approximately 1 SWI/SNF per 50 nucleosomes. While Gal4-VP16-driven transcription from the nucleosome array was low in the absence of SWI/SNF, addition of increasing amounts of the SWI/SNF complex led to substantial transcription stimulation (Figure 1C, compare lane 1 to lanes 2–8). SWI/SNF transcriptional stimulation was dependent on the presence of the VP16 activation domain. Reactions that contained only the DNA-binding domain of Gal4, Gal4(1–94), did not support SWI/SNF stimulation (Figure 1D, compare lanes 1 and 4 and lanes 2 and 5 to lanes 3 and 6). SWI/SNF stimulation of Gal4-VP16-driven transcription also depended on the presence of Gal4-binding sites (data not shown).

To address the involvement of the ATPase activity of SWI/SNF, and therefore the potential role of nucleosome remodeling, in transcriptional stimulation, we omitted ATP from the "remodeling" step (see Figure 1B). When ATP was omitted from the reaction until the start of transcription, stimulation by SWI/SNF was reduced (Figure 1E, compare lanes 1 and 2 with lanes 3 and 4). The reduced but still apparent stimulatory activity of SWI/SNF when ATP was omitted from the remodeling step may be due to nucleosome remodeling by SWI/SNF during the "transcription" step. Alternatively, this remaining stimulation may reflect a separate "transcription adaptor" function of the complex (see Discussion).

Direct Interaction of the SWI/SNF Complex with Acidic Activation Domains

The activation domain dependence of transcriptional stimulation by SWI/SNF raised the possibility that SWI/

SNF stimulation was dependent on an interaction with the VP16 activation domain. Thus, we tested for interactions of GST activation domain fusion proteins with purified SWI/SNF complex by pulldown assays. The presence of SWI/SNF in the supernatants or attached to the GST fusion protein–bound beads was detected by immunoblotting with antibodies against an HA epitope of an HA-tagged Swi2/Snf2 subunit or the Swp61/Arp7 subunit (Cairns et al., 1998; Peterson et al., 1998). The SWI/SNF complex directly interacted with the acidic activation domains from VP16 (amino acids 413–490), yeast Gcn4 (amino acids 9–172), and yeast Hap4 (amino acids 330–554) (Figure 2A, lanes 4–9), as indicated by the presence of SWI/SNF in the bound fraction. Recently, the yeast activator Swi5 has been implicated in recruitment of SWI/SNF to the *HO* promoter in vivo (Cosma et al., 1999; Krebs et al., 1999). Figure 2B demonstrates a direct interaction between the full-length Swi5 protein (as a fusion with GST) and the purified SWI/SNF complex (compare lanes 2 and 3 with lanes 6 and 7).

To investigate the specificity of the interaction of SWI/SNF with VP16, we tested GST–VP16 fusion proteins in which the activation domain was mutated. The "wild-type" VP16 activation domain contains amino acids 413 through 490 of the VP16 protein. GST–VP16Δ456 has a C-terminal truncation in the VP16 activation domain, which reduces transcriptional activity to 30%–50% of the wild-type (Triezenberg et al., 1988; Berger et al., 1990). GST–VP16Δ456 FP442 contains the truncated activation domain with a point mutation causing a phenylalanine to proline change at amino acid 442. This mutated activation domain does not support activated transcription (Berger et al., 1990; Cress and Treizenberg, 1991; Regier et al., 1993). Using GST pulldown assays, we found that GST–VP16Δ456 still interacted with SWI/SNF, albeit with reduced affinity (Figure 2A, compare lanes 10 and 11 to lanes 8 and 9). By contrast, while equally acidic, GST–VP16Δ456 FP442 did not interact with SWI/SNF (Figure 2A, lanes 12 and 13), indicating that the structure of the activation domain is important. Similarly, mutations in hydrophobic clusters in the GCN4 activation domain reduce the interaction of GCN4 with the SWI/SNF complex (Natarajan et al., 1999).

To test activation domain interactions with SWI/SNF in a more natural context, we incubated the GST activation domain fusion proteins with yeast whole-cell extract. Proteins that associated with the beads were run on an SDS-PAGE gel and immunoblotted with antibodies against SWI/SNF subunits, Swp61/Arp7, Swp82, and $TAF_{II}30$/Tfg3 (Cairns et al., 1996a). For comparison, 20% of the input was loaded (Figure 2B, lane 1). GST–Gcn4, GST–VP16, and GST–Hap4 pulled the SWI/SNF complex out of the extract, indicating a stable interaction (lanes 3, 4, and 7, respectively). GST alone and GST–VP16Δ456 FP442 did not pull down a significant amount of the SWI/SNF complex (lanes 2 and 6). While GST–VP16Δ456 demonstrated a weakened but reproducible interaction with the purified SWI/SNF complex (Figure 2A), it did not pull down detectable amounts of SWI/SNF from the whole-cell extract (Figure 2B, lane 5), illustrating the effect of competition by other yeast proteins.

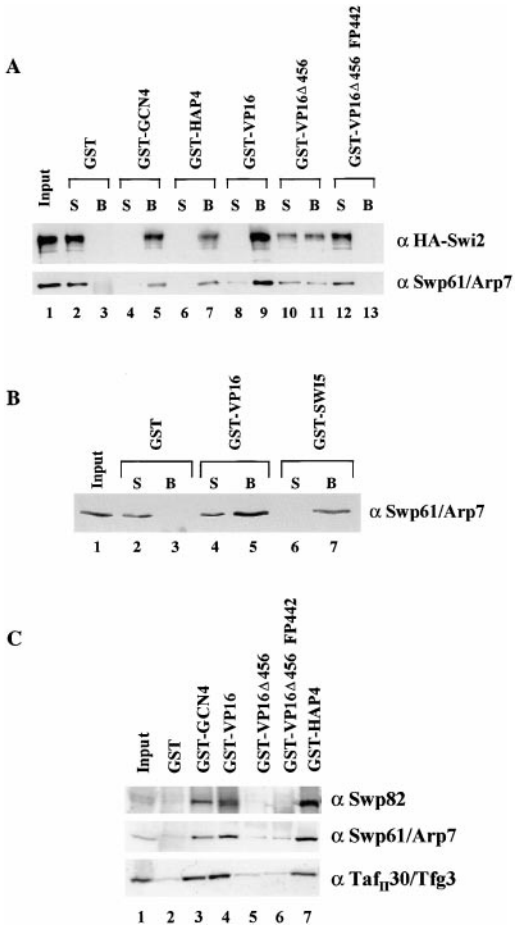

Figure 2. Direct Interaction of SWI/SNF with Acidic Activation Domains

(A) GST pulldown assays were performed using the GST fusion proteins indicated bound to glutathione sepharose beads and purified SWI/SNF. Equal amounts of supernatants, S, and beads, B, were run on a 10% SDS-PAGE gel and immunoblotted with antibodies against the HA-tagged Swi2/Snf2 and Swp61/Arp7 SWI/SNF subunits.

(B) GST pulldown assays were performed using GST–$SWI5_{1–709}$ and purified SWI/SNF as described in (A). SWI/SNF was detected with antibodies against the Swp61/Arp7. Note that the SWI/SNF preparations were found to be devoid of detectable levels of RSC complex, which also contains the Arp7 subunit. GST–VP16 and GST pulldowns are shown for comparison.

(C) Acidic activators interact with SWI/SNF in a yeast whole-cell extract. GST pulldown assays were done using a yeast whole-cell extract. Proteins that came down with the beads were run on a 10% SDS-PAGE gel and immunoblotted with antibodies against SWI/SNF subunits, Swp82, Swp61/Arp7, and $TAF_{II}30$/Tfg3.

Preferential Binding of SWI/SNF to Activator-Bound DNA

The SWI/SNF complex can bind directly to DNA and nucleosomes (Quinn et al., 1996; Côté et al., 1998). If the interactions of SWI/SNF with acidic activation domains play a role in targeting the SWI/SNF complex to promoters, they should direct SWI/SNF preferentially to DNA bound by transcription activators. To test this, we used a 183 bp probe containing one Gal4-binding site in a

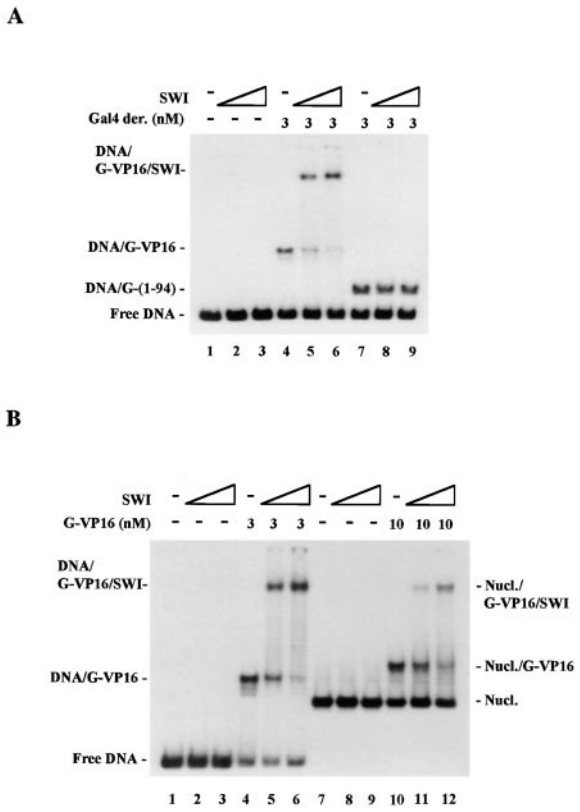

Figure 3. Targeting of SWI/SNF to Gal4-VP16-Bound DNA and Nucleosomes

(A) SWI/SNF is targeted to Gal4-VP16-bound DNA. A 183 bp DNA fragment containing one Gal4 site was bound by Gal4–VP16 (lanes 4–6), Gal4(1–94) (lanes 7–9), or no Gal4 derivative (lanes 1–3). SWI/SNF was subsequently added to the reactions as indicated and resolved on a native acrylamide gel (3.5% 79:1 acrylamide:bisacrylamide).

(B) SWI/SNF is targeted to Gal4-VP16-bound nucleosomes. The same 183 bp probe was reconstituted into a nucleosome core and bound by Gal4–VP16 (lanes 10–12) or no activator (lanes 7–9) followed by incubation in the presence or absence of SWI/SNF as indicated. Similar reactions using the labeled DNA fragment are shown in lanes 1–6 for comparison.

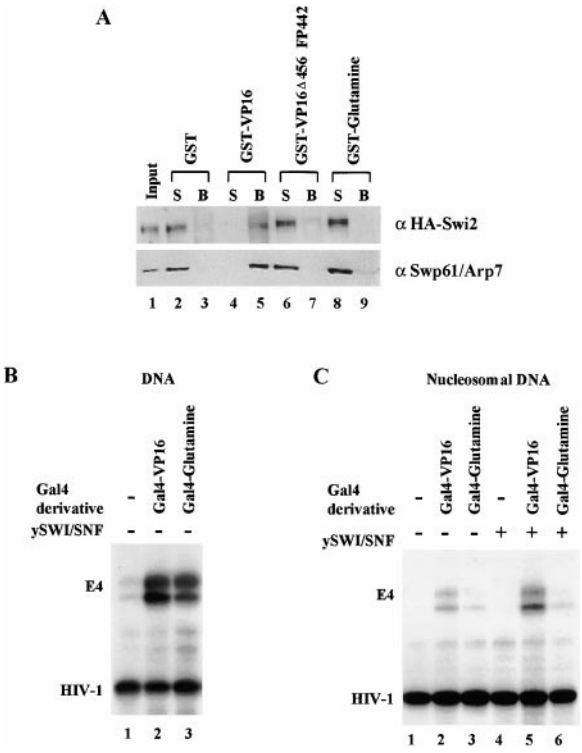

Figure 4. Activation Domain Specificity of SWI/SNF Transcription Stimulation

(A) SWI/SNF failed to interact with a glutamine-rich activation domain. GST pulldown assays were performed using the GST fusion proteins indicated and purified SWI/SNF. SWI/SNF interactions were detected as described in Figure 2.

(B) Gal4–VP16 and Gal4–glutamine stimulate transcription from naked DNA templates. Transcription was performed with the array template as naked DNA in the presence of Gal4–VP16 or Gal4–glutamine or in the absence of activator, as indicated.

(C) Preferential SWI/SNF stimulation of transcription driven by an acidic activator. Transcription was assayed from the G5E4 nucleosomal template in the presence of 50-fold excess nonspecific nucleosomes. The template was bound by Gal4–VP16, Gal4–glutamine, or no activator in the presence of absence of SWI/SNF as indicated.

gel shift assay. Gal4 derivatives were added at concentrations near the K_d so the reactions would contain both bound and free DNA (Figure 3A, lanes 4 and 7). When SWI/SNF was subsequently added to the reactions, the Gal4-VP16-bound DNA was supershifted in a large complex with SWI/SNF. By contrast, the free DNA (lanes 5 and 6) and Gal4(1–94)-bound DNA (lanes 8 and 9) were not bound by SWI/SNF at these concentrations. These data demonstrate the interaction of SWI/SNF with a DNA-bound transcription activator.

To demonstrate that the SWI/SNF complex can be targeted to activator-bound nucleosomes, we used the same 183 bp probe reconstituted into a nucleosome core. As above, Gal4–VP16 was added to a concentration near its K_d (Figure 3B, lane 10). When SWI/SNF was added, Gal4-VP16-bound nucleosomes were supershifted into the larger complex with SWI/SNF (lanes 11 and 12). In the absence of Gal4–VP16, the nucleosomes were not bound by the SWI/SNF at these concentrations (lanes 8 and 9). Reactions are shown with the DNA probe for comparison (lanes 1–6).

Activation Domain Specificity of SWI/SNF in Transcription Activation

We sought to test if transcription activation by SWI/SNF required the presence of an activation domain with which it interacted or whether it would permissively stimulate transcription by any activator. To do this, we tested if SWI/SNF interacted with other activation domains that are functional in vitro. In contrast to VP16, the SWI/SNF complex did not directly interact with the glutamine-rich activation domain from the human transcription factor Sp1 in GST pulldown assays (Figure 4A, compare lanes 4 and 5 with lanes 8 and 9). Moreover, GST–glutamine did not "pull down" the SWI/SNF complex from yeast whole-cell extracts (data not shown).

To test for activity of the glutamine-rich activation domain in SWI/SNF-mediated transcription stimulation,

we used a Gal4 DNA-binding domain/glutamine-rich activation domain fusion protein. Both Gal4–VP16 and Gal4–glutamine were able to stimulate transcription from the Gal4 site/E4 promoter on naked DNA templates (Figure 4B). By contrast, when the template was reconstituted into an array of nucleosomes, SWI/SNF stimulated Gal4-VP16-driven transcription but did not affect transcription in the presence of Gal4–glutamine (Figure 4C, compare lanes 2 and 5 to lanes 3 and 6). These data demonstrate that transcriptional enhancement by SWI/SNF required the presence of an activator with which it directly interacts. Thus, activation domain–mediated targeting of the complex to the template nucleosome array is an important step in SWI/SNF transcription activation.

Discussion

In this study, we have shown that activation domain–mediated targeting of the SWI/SNF complex to promoters within nucleosome arrays activates transcription from nucleosome templates in vitro. Importantly, the accompanying paper from Hinnebusch and colleagues (Natarajan et al., 1999) demonstrates an in vivo function for these interactions.

These results support a model for activator-mediated targeting of the SWI/SNF complex to promoters in vivo. Targeting of SWI/SNF could occur by two non–mutually exclusive pathways. SWI/SNF may be recruited by activators that are previously bound to promoter sequences in chromatin. Alternatively, acidic activators may interact with SWI/SNF in solution and then direct the complex to the promoter region via the sequence specificity of their DNA-binding domains. Our data are consistent with both possibilities, illustrating stable interactions of activation domains with SWI/SNF both in solution (Figure 2) and when bound to DNA or nucleosomes (Figure 3).

The transcriptional stimulation we observe most likely occurs before, during, or shortly after the initiation step of transcription, since the labeled primer used in primer extension for RNA analysis anneals only 110 bp downstream of the start site. A function of SWI/SNF in preinitiation complex assembly could be mediated by its nucleosome disruption activity and/or by it potentially functioning as a "transcriptional adaptor" complex since SWI/SNF interacts both with acidic activators and RNA polymerase II holoenzyme (Wilson et al., 1996). SWI/SNF might also stimulate further elongation on our templates beyond the position of the primer used. Indeed, using a different system, Brown et al. (1996) have shown that SWI/SNF can release a nucleosome-induced pause of RNA polymerase II elongation.

The interaction of SWI/SNF with acidic activators resembles that of the SAGA and NuA4 histone acetyltransferase (HAT) complexes (Utley et al., 1998). The biochemical activities of these two types of chromatin remodeling complexes are quite distinct. However, targeting of either SWI/SNF or the HAT complexes to promoters is able to stimulate transcription from nucleosome arrays. This similarity in the outcome of SWI/SNF or SAGA activity in vitro may be related to the apparent functional overlap of these two complexes in vivo (Pollard and Peterson, 1997; Roberts and Winston, 1997; Biggar and Crabtree, 1999).

Experimental Procedures

SWI/SNF Purification

SWI/SNF complex was purified from yeast as described (Côté et al., 1994), with some minor variations. Elution from Ni^{2+} agarose was at 300 mM imidazole. The Mono Q column was followed by a heparin sepharose and a DNA cellulose column with the complex eluting at 340 mM NaCl and 200 mM NaCl, respectively. Purification was monitored using antibodies to SWI/SNF subunits. The purified SWI/SNF used in these experiments was tested for the presence of SRB/mediator with antibodies against Med4 (data not shown) and against Med2 and Srb2 (Natarajan et al., 1999) and found to be devoid of these SRB/mediator subunits. The fractions were also tested for the presence of SAGA ($\alpha TAF_{II}90$, $\alpha TAF_{II}17$ and αTra1) and RSC components (αRsc6) and found to be devoid of these complexes.

Purification of Gal4 Derivative Proteins

Gal4 fusion proteins were expressed in bacteria and purified as described previously: Gal4(1–94) and Gal4–VP16 (Lin et al., 1988; Chasman et al., 1989). Gal4–VP16 was step-eluted at 0.4 M NaCl from the DEAE column. Gal4–glutamine was purified as described (Tanese et al., 1991). Protein purity was checked by SDS-PAGE and Coomassie staining. The DNA-binding ability of the proteins was checked by gel shift assays using a Gal4 site probe.

Purification of GST Fusion Proteins

GST fusion proteins were expressed in bacteria and purified as in the manufacturer's (Pharmacia) protocol, except that protease inhibitors (PMSF, leupeptin, pepstatin A, aprotinin) and DTT were added to the resuspension buffer. The fusion proteins were cross-linked to glutathione sepharose 4B with dimethylpimelimidate (DMP). Protein purity and cross-linking efficiency were checked by SDS-PAGE and Coomassie staining. Protein amounts for pulldown assays were normalized by Coomassie staining.

Histone Preparation and Nucleosome Reconstitution

Core histones and oligonucleosomes were purified from HeLa cells as described (Côté et al., 1995). Long oligonucleosomes (LON) were used in the transcription assays and gel shifts as competitor nucleosomes. Nucleosomal arrays were reconstituted with core histones by step dilution as described (Steger et al., 1998; Steger and Workman, 1999).

Preparation of Yeast Whole-Cell Extract

A yeast whole-cell extract was prepared as described previously (Woontner et al., 1991), except with some minor variations (Reese et al., 1994).

GST Pulldown Assays

GST fusion proteins cross-linked to glutathione sepharose 4B were incubated with purified yeast SWI/SNF complex in a pulldown buffer (50 mM HEPES [pH 7.5], 1 mM EDTA, 150 mM NaCl, 10% glycerol, 0.1% Tween 20, 0.5 mM DTT, 1 mM PMSF, 2 μg/mL leupeptin, and 2 μg/mL pepstatin A) for at least 2 hr at 4°C while mixing slowing on a rotating wheel. The supernatants were collected, and the beads were then washed with pulldown buffer three times and left as a 50% slurry after the final wash. Equal fractions of both supernatants and beads were loaded on a 10% SDS-PAGE gel, and the presence of SWI/SNF was detected by immunoblotting. The yeast whole-cell extract pulldowns were done similarly, except that 1250–1500 μg of total yeast whole-cell extract protein was incubated with the GST fusion proteins at 150 mM salt.

In Vitro Transcription

Transcription reactions were carried out as described previously (Steger et al., 1998; Ikeda et al., 1999; Steger and Workman, 1999), except that ATP (1 mM final) and $MgCl_2$ (3 mM final) were added to the binding buffer (for remodeling by SWI/SNF). Acetyl CoA and sodium butyrate were not added to any reactions. Approximately 15–20 ng of reconstituted G5E4 nucleosomal array (pIC-2085S/G5E4R) or G5E4 DNA was assayed, and 1–5 ng of HIV-1 DNA [pHIV(D,N)] was added to each reaction as an internal recovery

control. We added 1 μg of competitor nucleosomes (LON) to each reaction. Purified yeast SWI/SNF (heparin sepharose fraction 37) was added where indicated. A 10–20 nM final concentration of the Gal4 derivative was added as indicated. For primer extension analysis of the RNA, 25,000–50,000 cpm of ^{32}P-labeled E4 (+86 to +110) and HIV-1 (+50 to +81) primers were used per reaction.

Gel Shift Assays

The single Gal4 site probe (GUB) used for the gel shift assays was generated by PCR as described (Juan et al., 1997) and used as DNA or as a reconstituted mononucleosome. Gal4 derivatives were added to approximately 20 ng of the probe 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 4 mM $MgCl_2$ and incubated for 30 min at 30°C followed by the addition of SWI/SNF. After an additional 30 min incubation at 30°C, the samples were run on a 3.5% (79:1 acrylamide to bisacrylamide) gel, dried, and visualized by autoradiography.

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