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The Yeast SWI-SNF Complex Facilitates Binding of a Transcriptional Activator to Nucleosomal Sites In Vivo

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The *Saccharomyces cerevisiae* SWI-SNF complex is a 2-MDa protein assembly that is required for the function of many transcriptional activators. Here we describe experiments on the role of the SWI-SNF complex in activation of transcription by the yeast activator GAL4. We find that while SWI-SNF activity is not required for the GAL4 activator to bind to and activate transcription from nucleosome-free binding sites, the complex is required for GAL4 to bind to and function at low-affinity, nucleosomal binding sites in vivo. This SWI-SNF dependence can be overcome by (i) replacing the low-affinity sites with higher-affinity, consensus GAL4 binding sequences or (ii) placing the low-affinity sites into a nucleosome-free region. These results define the criteria for the SWI-SNF dependence of gene expression and provide the first in vivo evidence that the SWI-SNF complex can regulate gene expression by modulating the DNA binding of an upstream activator protein.

The SWI-SNF complex is required for the expression of a number of diversely regulated genes in the yeast *Saccharomyces cerevisiae*. The results of genetic experiments indicate that the SWI-SNF requirement reflects the ability of the complex to antagonize chromatin-mediated transcriptional repression (26). In these studies, mutations in genes that encode chromatin components, including *HHT1* (encoding histone H3), *HHF1* (encoding histone H4), and *SIN1* (encoding an HMG-1-like protein which is thought to be a chromatin component), were found to alleviate the transcriptional defects of *swi* mutants (14, 15). In addition, alteration of relative histone levels by deletion of one of the two endogenous *HTA1-HTB1* gene clusters (encoding histones H2A and H2B) is also able to partially restore transcriptional activity to *swi-snf* cells (12). These results suggest that the SWI-SNF complex acts to promote the expression of genes within the context of a compact eukaryotic genome.

The primary level of chromatin architecture in vivo is the nucleosome. The results of in vivo and in vitro studies indicate that nucleosomes inhibit transcription by competing with transcription factors for occupancy of DNA binding sites (for a review, see reference 27). In vitro, nucleosomes can inhibit transcription initiation either by blocking access of the general transcription machinery to promoter sequences or by hindering the binding of upstream activator proteins. It is not known at present which of these steps might be overcome by the SWI-SNF complex in vivo. The results of biochemical studies have shown that yeast or human SWI-SNF complex can stimulate the binding of an activator protein to a nucleosomal binding site (5, 16). Furthermore, the purified human SWI-SNF complex can facilitate the binding of the general transcription factor TATA box binding protein (TBP) to a positioned nucleosome (13). Therefore, the SWI-SNF complex may function at one or more discrete steps to facilitate gene expression in vivo.

In this study, we address the ability of the SWI-SNF complex to facilitate the binding of a transcriptional activator to upstream activation sites (UASs). To do this, we monitored the transcriptional activity and DNA binding of the yeast activator GAL4 to derivatives of the UAS of the divergently transcribed *GAL1* and *GAL10* genes. These UAS derivatives differ in the affinity of the GAL4 binding sites as well as the nucleosomal context of these sites. We find that the SWI-SNF complex is required for GAL4 to activate transcription from a promoter containing two low-affinity, nucleosomal binding sites in vivo. In addition, we show that the SWI-SNF dependence of this promoter reflects, in part, the ability of the complex to facilitate GAL4 binding to these nucleosomal sites.

MATERIALS AND METHODS

β-Galactosidase assays. Strains were grown in minimal medium containing 2% galactose, 0.5% sucrose, and all the amino acids except histidine to an optical density at 600 nm (OD_{600}) of 0.5 to 0.9. Assays were performed (23) on three transformants, and Miller units (19) were averaged. Standard deviations were <25%. β-Galactosidase activity from all strains in the absence of GAL4 expression was <1 Miller unit.

In vivo footprinting. Intact cells were treated with dimethyl sulfate (DMS), and the methylated DNA was isolated essentially as previously described (10), except final pellets were dissolved in water and quantitated with a spectrophotometer. DNA samples were then digested with *Hae*III and analyzed via a cyclic primer extension reaction (1) using a labeled oligonucleotide. Primer extension products were extracted with chloroform-isoamyl alcohol (24:1), precipitated, and electrophoresed on a 5% National Diagnostics sequencing gel. The gel was dried and exposed to film for 12 to 48 h. For a control for spurious primer extension products, DNA from a strain that was not treated with DMS was also prepared. Footprinting at the wild-type *GAL1,10* UAS (see Fig. 2) was analyzed by using an oligonucleotide with the following sequence: 5′-GAG CCC CAT TAT CTT AGC-3′, which anneals to a sequence located 60 bp upstream of GAL4 binding site 1. Footprinting at the integrated reporter loci (see Fig. 3) was analyzed by using an oligonucleotide with the following sequence: 5′-CCG GCT CGT ATG TTG TGT GG-3′. This oligonucleotide anneals to the unique pUC sequences located upstream of the GAL4 binding sites in the reporters (see below).

Indirect end labeling. Nuclei were prepared as described previously (22). To prepare free DNA, 80 μl of 10% sodium dodecyl sulfate (SDS) and 30 μl of 20-mg/ml proteinase K were added to a 1-ml aliquot of nuclei (approximately 5×10^9 cell equivalents), and the sample was incubated at 37°C for 2 h. Lysed nuclei were treated with 180 μl of 5 M potassium acetate on ice for 1 h and subjected to centrifugation, and the supernatant was precipitated with isopropanol. This DNA sample was resuspended in water and reprecipitated with ethanol. The resultant free DNA was resuspended in 1 ml of buffer D2 (10 mM HEPES [pH 7.3], 5 mM $MgCl_2$, 2 mM $CaCl_2$). Free DNA and nuclei samples (approximately 1.7×10^9 cell equivalents in 300-μl aliquots) were digested with 0 to 50 U of micrococcal nuclease (MNase) for 5 min at 37°C. Reactions were halted by adding 26 μl of 10% SDS and 10 μl of 20-mg/ml proteinase K and incubating the

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mixtures at 37°C for 2 h. Samples were treated with 60 μl of 5 M potassium acetate on ice for 1 h and subjected to centrifugation, and the supernatant was precipitated with isopropanol. DNA was then digested with either *Cla*I (see Fig. 4A and B) or *Eco*RI (see Fig. 4C) in the presence of RNase. Digested fragments were extracted with buffered phenol, and the aqueous layer was precipitated with ethanol. Final pellets were electrophoresed on a 1.5% agarose gel overnight. DNA was transferred to a nylon membrane, and blots were probed with an internally labeled 870-bp *lacZ* fragment (see Fig. 4A and B) or an internally labeled 550-bp fragment from the *GAL1* gene (see Fig. 4C).

***Hga*I restriction enzyme accessibility assay.** Permeabilized spheroplasts were prepared from exponentially growing cells (OD_{600} of 0.6, in glucose medium) by treatment with yeast lyticase (24). Briefly, cells were harvested, resuspended in 1/10 culture volume of prespheroplasting buffer (100 mM Tris [pH 8.0], 60 mM β-mercaptoethanol), and shaken for 10 min at room temperature. Cells were harvested and resuspended in 1/20 culture volume of spheroplasting buffer (0.7 M sorbitol, 0.75% yeast extract, 1.5% peptone, 10 mM Tris [pH 7.5], 10 mM β-mercaptoethanol), lyticase was added, and cells were incubated at 37°C until the OD_{600} of cells diluted with water was decreased by 90% (30 to 40 min). Spheroplasts were harvested by centrifugation, washed once with nystatin buffer (50 mM NaCl, 1.5 mM $CaCl_2$, 20 mM Tris [pH 8], 1 M sorbitol, 10 mM $MgCl_2$), and resuspended in 1/150 culture volume of nystatin buffer. Nystatin was added to a final concentration of 15 μg/ml, and spheroplasts were incubated at 37°C for 5 min. *Hga*I (New England BioLabs) was added to the permeabilized spheroplasts at a concentration of 120 U/ml, and samples were incubated at 37°C for 0 to 30 min. Reactions were stopped by the addition of 1/5 volume of 5% SDS–50 mM EDTA, and DNA was purified as described above (a negative control lacked *Hga*I). Purified DNA was digested with *Hin*dIII (New England BioLabs), electrophoresed on a 1.5% agarose gel, Southern blotted to a nylon membrane, and probed with a 500-bp *Eco*RI fragment from plasmid pLB8 that was internally labeled by random priming (see Fig. 5). Results were quantitated by phosphorimager analysis.

Plasmid and strain construction. The following plasmids have been described previously: pRY171 (*GAL1-lacZ* fusion [30]), pEG202 (pLEXA [11]), and pMA210 (pGAL4) and pSD15 (17). Plasmids pLB7 and pLB8 were constructed by inserting the 194-bp *Sma*I-*Pvu*II fragment of pUC18 (29) between the *URA3* gene and the GAL4 binding sites of the plasmids pEG44 and pEG28, respectively (9). Plasmid pEG28 contains GAL4 binding sites 3 and 4 positioned 190 bp upstream of the *GAL1* TATA element. These sites are separated by 62 bp (center-to-center distance). Plasmid pEG44 contains two synthetic, consensus GAL4 binding sites positioned 150 bp upstream of the *GAL1* TATA element (9). These two sites are separated by 32 bp (center-to-center distance). These plasmids were targeted for integration at the *ura3-52* locus of yeast strains CY524, CY525, and CY526 (Table 1) by digestion with *Apa*I. A DNA fragment containing the 5S nucleosome positioning element (NPE) of sea urchin was amplified from plasmid pICN5S182 (gift from Jerry Workman) by PCR using the following primers: 5′-CCACGAATAACTTCCAGGG-3′ and 5′-CCCCGAGGAATTAA GTAC-3′ (18). The 182-bp PCR product was inserted into plasmid pLB8, 20 bp upstream of GAL4 binding site 3 (see Fig. 1). This new plasmid, called pLB16, was targeted for integration at the *ura3-52* locus of CY524, CY525, and CY526 by digestion with *Apa*I. Yeast transformations were performed via the lithium acetate method described previously (8).

TABLE 1. Yeast strains used in this study

Strain	Genotype
CY257	*MAT***a** *swi1*Δ::*LEU2 lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-*Δ*99*
CY296	*MAT***a** *gal4*Δ::*LEU2 lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-*Δ*99*
CY297	*MAT***a** *gal4*Δ::*LEU2 swi1*Δ::*LEU2 lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-*Δ*99*
CY341	*MAT***a** *lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-*Δ*99*
CY353	Same as CY524 but contains *URA3*::pRY171 (*GAL1,10*)
CY366	*MAT***a** *swi1*Δ::*LEU2 ura3-52 ade2-101 his3-*Δ*200 leu2-*Δ*1 lys2-801 URA3*::pRY171 (*GAL1,10*)
CY401	Same as CY524 but contains *URA3*::pEG28 (2 low)[a]
CY422	Same as CY526 but contains URA3::pEG28 (2 low)
CY524	*MAT***a** *lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-52*
CY525	*MAT***a** *gal4*Δ::*LEU2 lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-52*
CY526	*MAT***a** *swi1*Δ::*LEU2 lys2-801 ade2-101 leu2-*Δ*1 his3-*Δ*200 ura3-52*
CY528	Same as CY524 but contains *URA3*::pLB7 (2 high)[b]
CY529	Same as CY525 but contains *URA3*::pLB7 (2 high)
CY530	Same as CY526 but contains *URA3*::pLB7 (2 high)
CY532	Same as CY524 but contains *URA3*::pLB8 (2 low; pUC spacer)
CY533	Same as CY525 but contains *URA3*::pLB8 (2 low; pUC spacer)
CY534	Same as CY526 but contains *URA3*::pLB8 (2 low; pUC spacer)
CY586	Same as CY524 but contains *URA3*::pLB16 (2 low; NPE)
CY587	Same as CY525 but contains *URA3*::pLB16 (2 low; NPE)
CY588	Same as CY526 but contains *URA3*::pLB16 (2 low; NPE)

[a] 2 low, two low-affinity GAL4 binding sites.
[b] 2 high, two high-affinity GAL4 binding sites.

RESULTS

SWI-SNF activity is required for transcriptional activation by GAL4 at some promoters. Isogenic *SWI*$^+$ and *swi*$^-$ strains that contain similar single-copy integrated reporters were constructed and are shown schematically in Fig. 1A. Strains CY353 (*SWI*$^+$) and CY366 (*swi1*$^-$) contain a *GAL1,10* UAS reporter consisting of four GAL4 binding sites upstream of a *GAL1-lacZ* fusion gene (reporter a). Strains CY401 (*SWI*$^+$) and CY422 (*swi1*$^-$) contain a derivative of this reporter that contains only two of these low-affinity GAL4 binding sites (sites 3 and 4; reporter b); strains CY532 (*SWI*$^+$) and CY534 (*swi1*$^-$) contain a version of reporter b that contains 192 bp of additional plasmid sequences 20 bp upstream of GAL4 site 3 (reporter c); strains CY528 (*SWI*$^+$) and CY530 (*swi1*$^-$) contain a reporter which contains two high-affinity, consensus GAL4 binding sites in place of the two low-affinity sites (reporter d) (9). β-Galactosidase assays were performed on these strains to monitor GAL4 transcriptional activity either in the presence of endogenous concentrations of GAL4 (pLEXA columns) or under conditions in which GAL4 is overexpressed (pGAL4 columns) (Fig. 1A).

In the absence of functional SWI-SNF complex, transcriptional activation by GAL4 from the wild-type *GAL1,10* UAS region is reduced only 1.5-fold from the activity in the respective wild-type strain (Fig. 1A, reporter a). This result is consistent with a previous study in which it was found that *GAL1* expression was only weakly affected by *swi-snf* mutations (21). In contrast, GAL4 activity on the two reporters with two low-affinity GAL4 binding sites is SWI-SNF dependent; transcriptional activity is reduced 22- to 32-fold in the *swi1*$^-$ strains from that of the wild-type strain (Fig. 1A, reporters b and c). When these two low-affinity sites are replaced by two high-affinity, consensus GAL4 binding sites, transcriptional activation is reduced only 1.3-fold in the *swi1*$^-$ strain from that of the wild-type strain (Fig. 1A, reporter d).

Strains harboring the two low-affinity site reporters were also distinct from those carrying the two high-affinity site reporters in that *lacZ* expression was increased 2.5- to 3.5-fold when GAL4 protein was overexpressed (Fig. 1A, reporters b and c; also data not shown). The simplest interpretation of this result is that physiological levels of GAL4 are not sufficient to fully occupy the two low-affinity sites (9; also see below). Importantly, overexpression of GAL4 does not overcome the SWI-SNF dependence of this reporter (Fig. 1A, reporters b and c). It should be noted that the *swi1* mutation does not affect expression of GAL4 from this overexpression plasmid in this strain background (Fig. 1B) (20).

SWI-SNF is required for GAL4 occupancy of low-affinity binding sites in vivo. To directly assess the ability of GAL4 to bind its sites in the different reporter strains in the presence or absence of SWI-SNF, we used an in vivo DMS footprinting assay. GAL4 protects guanine residues at each end of its 17-bp

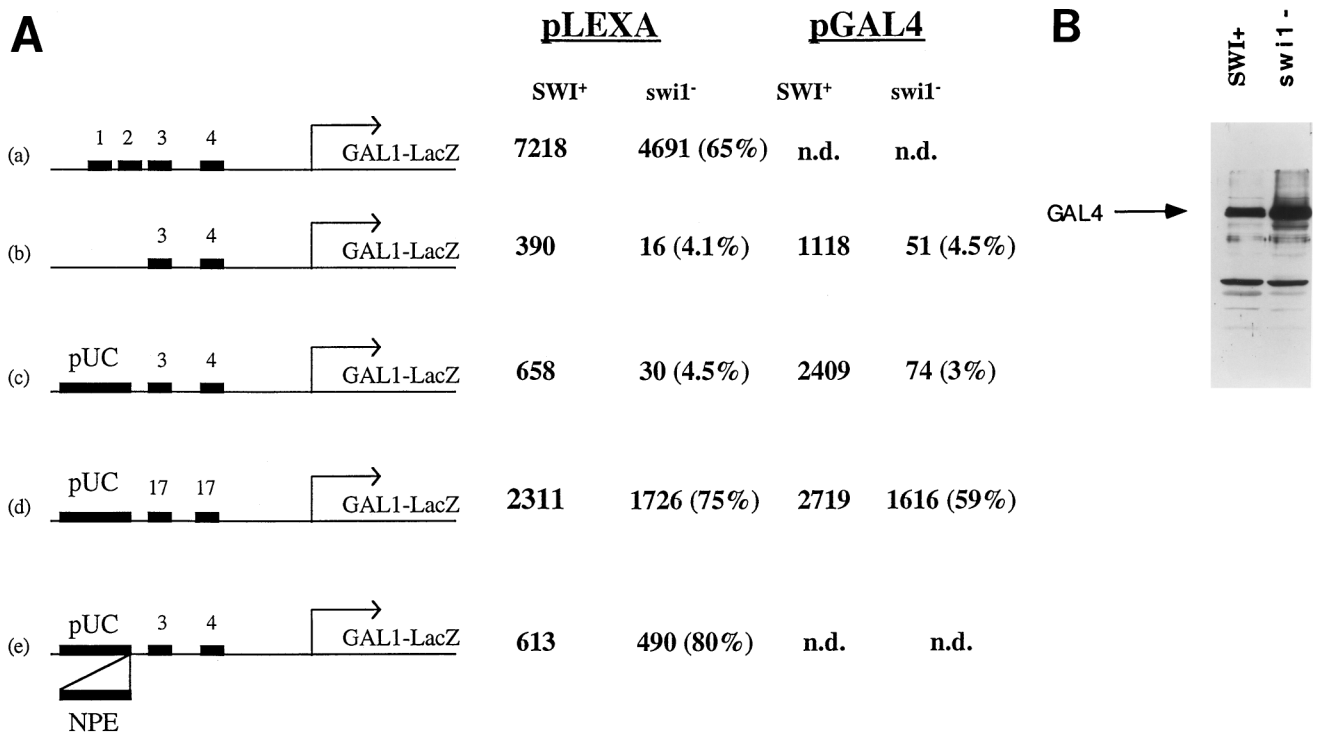

FIG. 1. GAL4 requires functional SWI-SNF complex to activate transcription from two low-affinity binding sites. (A) β-Galactosidase assays were performed on isogenic *SWI*$^+$ or *swi1*$^-$ strains harboring the integrated *GAL1-lacZ* UAS reporter plasmids pRY171 (a), pEG28 (b), pLB8 (c), pLB7 (d), or pLB16 (e). GAL4 binding sites are denoted by the small black boxes. GAL4 sites numbered 1 to 4 indicate sites from the *GAL1,10* UAS region; GAL4 sites numbered 17 indicate synthetic, consensus GAL4 binding sites. Strains contained plasmids that overexpressed either the bacterial LexA DNA binding domain (pEG202) or the full-length GAL4 protein (pMA210). Numbers in parentheses show activities as percentages of the wild-type levels. β-Galactosidase activities of all reporters were <1 Miller unit in the absence of GAL4. Drawings are not to scale. n.d., not determined. (B) Western blot analysis of GAL4 levels. Whole-cell extracts (21) were prepared from equal numbers of *SWI*$^+$ (CY296) and *swi1*$^-$ (CY297) cells containing the GAL4 overexpression plasmid pMA210. Expression of GAL4 protein (indicated by the arrow) was identified by Western blotting using an antibody directed against the C terminus of GAL4 (21).

recognition site both in vivo (10) and in vitro (3). As shown in Fig. 2, these guanine residues within the four GAL4 binding sites of the *GAL1,10* UAS are protected from DMS methylation in the presence of GAL4 (lane 3) but are accessible to methylation in an isogenic *gal4*$^-$ strain (lane 2). Introduction of GAL4 expression plasmids into the *gal4*$^-$ strain restores protection of these guanines (lanes 6 and 8), while introduction of a plasmid expressing the bacterial LexA protein does not (lane 7). In the absence of SWI-SNF activity, protection of all four sites is retained (lane 4). Furthermore, as shown in Fig. 3A, GAL4 also protects the guanine residues at each of the two high-affinity binding sites in the presence (lane 4) and absence (lane 5) of SWI-SNF. Similar results were obtained in at least six different experiments with five independent DNA preparations. These results are consistent with the results of our functional experiments (Fig. 1) in which SWI-SNF is not required for the activity of GAL4 from either the wild-type *GAL1,10* UAS or the two-high-affinity-site reporter.

Figure 3B shows the results of an in vivo footprinting analysis of the two-low-affinity-site reporter in strains CY532 (*SWI*$^+$) and CY534 (*swi1*$^-$). We detect little protection of the two low-affinity sites in our footprinting assay, even in a *SWI*$^+$ strain (lane 3). As discussed above, this probably reflects the low occupancy of these sites at physiological GAL4 levels, because when GAL4 is overexpressed in this *SWI*$^+$ strain, protection is restored (lane 5). Overexpression of GAL4 in the absence of a functional SWI-SNF complex, however, does not restore complete protection of either low-affinity binding site (lane 6). This is most apparent at GAL4 site 3, where strong protection in the *SWI*$^+$ strain (lane 5) and are only weak protection in the *swi1*$^-$ strain (lane 6) are observed. Quantitation of these results by phosphorimager analysis indicates that protection of the relevant guanine residue in site 3 in the *swi1*$^-$ strain is decreased (40%) from that seen in a wild-type strain. For an internal control, we also analyzed these samples for GAL4 binding at the endogenous *GAL1,10* UAS locus. In all cases, we observe complete occupancy of the four GAL4 sites in the presence and absence of SWI-SNF (data not shown). The lack of complete occupancy at the two low-affinity sites in the *swi1*$^-$ strain indicates that the SWI-SNF complex modulates GAL4 binding in vivo.

Introduction of an NPE into a SWI-SNF-dependent promoter region can suppress the requirement for SWI-SNF. The results of genetic and biochemical experiments have indicated that SWI-SNF complex facilitates activator function by disrupting chromatin structure. To assess whether the chromatin structure of the GAL4 binding sites was related to their SWI-SNF dependence, a strong NPE was inserted directly upstream of the two low-affinity binding sites in strains CY532 (*SWI*$^+$) and CY534 (*swi1*$^-$). This 182-bp NPE sequence has been shown previously to translationally and rotationally position a nucleosome in vivo in yeast (20) (Fig. 4B). Insertion of the NPE had a dramatic effect on the SWI-SNF dependence of GAL4 function. While GAL4 transcriptional activity at the parental two-low-affinity-site reporter was decreased over 20-fold in the absence of SWI-SNF (Fig. 1A, reporters b and c), there was less than a 1.5-fold decrease when the NPE was inserted (Fig. 1A, reporter e). This effect of the NPE is not due

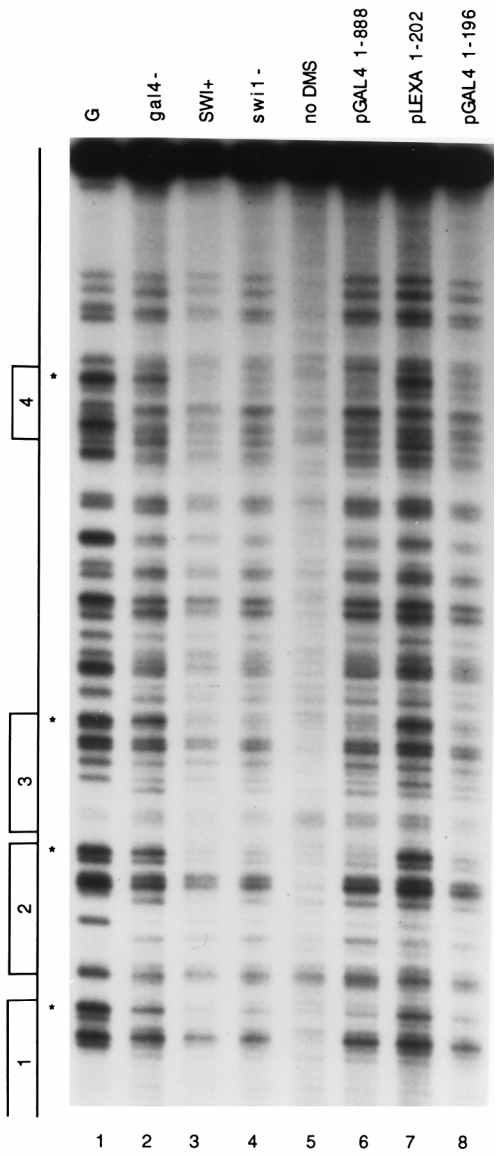

FIG. 2. GAL4 can bind its sites in the *GAL1,10* UAS region in the presence or absence of SWI-SNF. In vivo DMS footprinting analysis of the *GAL1,10* UAS region was performed on strains CY341 (*SWI*$^+$ GAL4$^+$) (lane 3), CY257 (*swi1*$^-$ GAL4$^+$) (lane 4), CY296 (*SWI*$^+$ gal4$^-$) (lane 2), CY296 containing a GAL4 expression plasmid (pMA210 or pSD15) (lanes 6 and 8), and CY296 containing a LexA expression plasmid (pEG202) (lane 7). Lane 1 contains a genomic guanine sequencing ladder. Boxes to the left of the gel denote the GAL4 binding sites of the *GAL1,10* UAS, and asterisks denote guanine residues shown previously to be protected by GAL4 binding.

simply to insertion of foreign DNA sequences directly upstream of the two low-affinity GAL4 binding sites, as insertion of 192 bp of pUC plasmid sequences at an identical position does not alter the SWI-SNF dependence of GAL4 activity (Fig. 1A, reporters b and c).

Nucleosomal context of GAL4 binding sites dictates SWI-SNF dependence. The results of functional experiments described above suggest that the SWI-SNF dependence of GAL4 can be modulated by nucleosome positioning. In the case of the *GAL1,10* locus, the four GAL4 binding sites are maintained in a constitutive nucleosome-free region which is flanked on both sides by an array of positioned nucleosomes (6, 7) (Fig. 4). This precise nucleosome positioning at the *GAL1,10* locus is believed to require the GRF2 protein which binds to a sequence that overlaps GAL4 binding sites 1 and 2 (4, 7). The *GAL1,10* UAS derivatives that we have analyzed in this study (Fig. 1A, reporters b to e) lack this GRF2 binding site, and therefore, they may not maintain nucleosome positioning surrounding the GAL4 binding sites. To confirm this possibility, we analyzed the chromatin structure of the two-low-affinity-site reporters (with and without an NPE) and compared these structures to that of the *GAL1* locus in the same strains.

Nuclei and free DNA were prepared from the *SWI*$^+$ strain CY532, which contains the parental two-low-affinity-site reporter (without an NPE), and samples were analyzed by MNase digestion and indirect end labeling. First, we analyzed the chromatin structure at the *GAL1* locus in this reporter strain (Fig. 4C). Comparison of the MNase cleavage patterns for free and chromatin DNA samples reveals a repeating pattern of MNase protections (each about 140 bp in size) flanked on each side by MNase-hypersensitive sites. These results are essentially identical to a previous study (6) and are consistent with an array of positioned nucleosomes downstream of the nucleosome-free GAL4 binding sites.

A similar analysis of the two-low-affinity-site reporter locus (without an NPE) yielded very different results (Fig. 4A). In the region upstream of the two GAL4 binding sites, the MNase digestion pattern of the chromatin sample is similar to the pattern of digestion of free DNA, although we do reproducibly observe several preferred cleavage sites in free DNA that appear to be enhanced in the chromatin sample. Importantly, the MNase cleavage sites directly upstream and adjacent to the GAL4 binding sites are cleaved with equal efficiency in the free and chromatin DNA samples. A region of about 80 bp that contains the GAL4 binding sites is not cleaved efficiently in either the free or chromatin DNA samples; this is due to the inherent sequence specificity of MNase I and is not due to a positioned nucleosome (6). In addition, this protected region is not flanked on both sides by hypersensitive sites. Directly downstream of the GAL4 binding sites, however, a 140-bp region of MNase resistance is detected, which is flanked on each side by MNase-hypersensitive sites, suggesting the presence of a positioned nucleosome. The location of this nucleosome corresponds to a positioned nucleosome mapped between the GAL4 sites and the *GAL1* TATA box of the endogenous *GAL1,10* locus (2, 6) (Fig. 4C and data not shown). Thus, although one nucleosome still appears to be positioned at this *GAL1,10* UAS derivative, the majority of nucleosome positioning has been lost. Consequently, nucleosomes appear to be located randomly upstream of the two remaining GAL4 binding sites. In the absence of a positioned array of nucleosomes, the two low-affinity GAL4 binding sites are unlikely to reside in a constitutive nucleosome-free region.

MNase digestions and indirect end labeling were also used to confirm that the NPE positioned a nucleosome upstream of the two low-affinity GAL4 binding sites (Fig. 4B). Comparison of the free and chromatin DNA samples reveals an MNase-protected region of about 250 bp which contains the NPE sequences as well as the MNase-resistant GAL4 binding site sequences. This result is consistent with the positioning of a nucleosome over the NPE sequence. In addition, the putative positioned nucleosome located directly downstream of the two low-affinity GAL4 binding sites is also detected at the NPE-containing reporter locus in CY586 (Fig. 4B).

These results are consistent with the model shown in Fig. 6. In the absence of the NPE, the two low-affinity GAL4 binding sites are encompassed by randomly positioned nucleosomes;

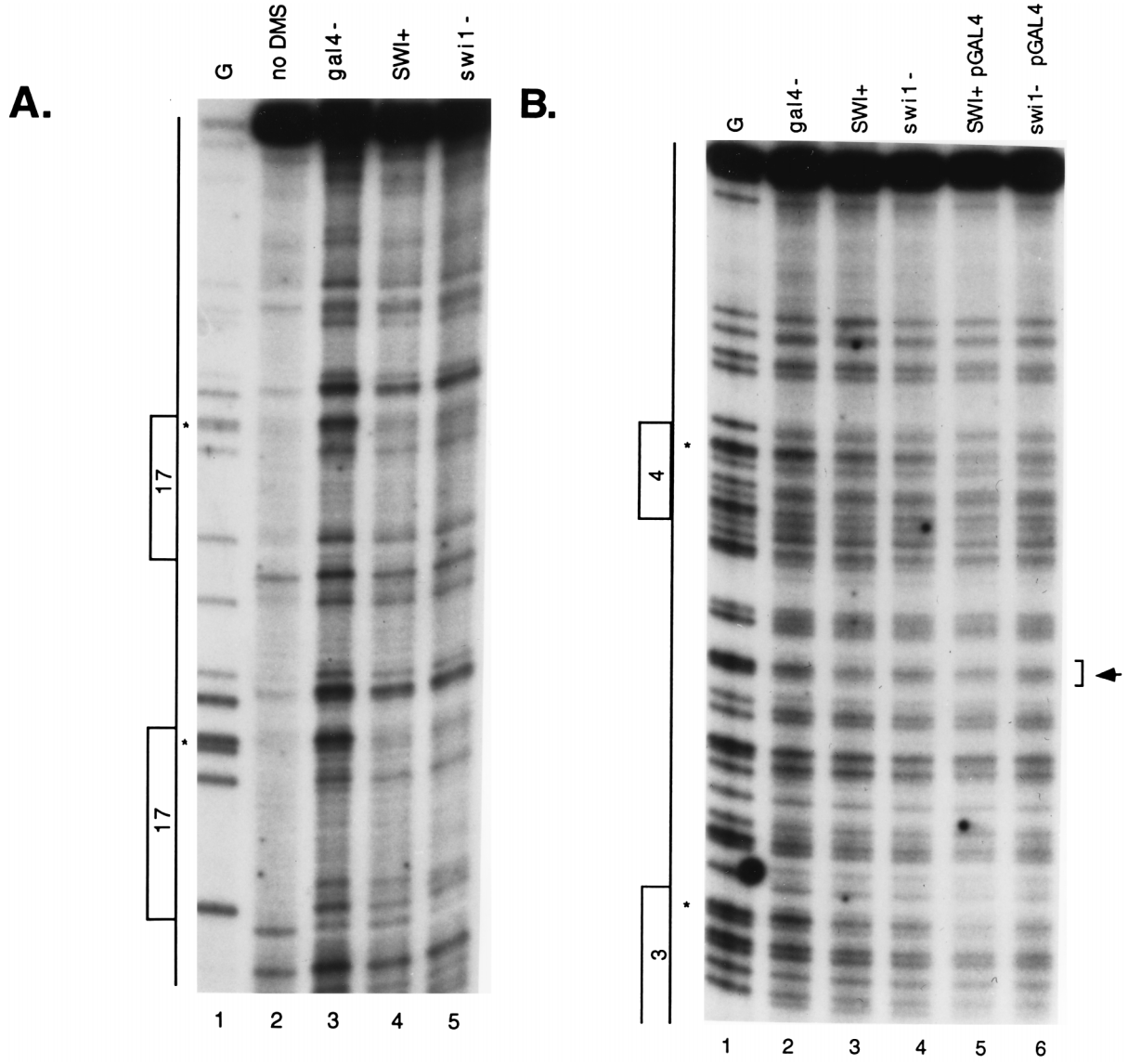

FIG. 3. SWI-SNF is required for complete occupancy of two low-affinity GAL4 binding sites in vivo. In vivo DMS footprinting was performed on strains that harbor the two-high-affinity-site reporter (A) or the two-low-affinity-site reporter (B). The strains used were as follows: for panel A, CY529 (*gal4*$^-$ *SWI*$^+$) (lane 3), CY528 (*GAL4*$^+$ *SWI*$^+$) (lane 4), and CY530 (*GAL4*$^+$ *swi1*$^-$) (lane 5); for panel B, CY533 (*gal4*$^-$ *SWI*$^+$) (lane 2), CY532 (*GAL4*$^+$ *SWI*$^+$) (lane 3), CY534 (*GAL4*$^+$ *swi1*$^-$) (lane 4), CY532 harboring a GAL4 expression plasmid (lane 5), and CY534 harboring a GAL4 expression plasmid (lane 6). For quantitation of lanes 5 and 6 in panel B, phosphorimager data were normalized to the doublet band indicated by the arrowhead (G449 and G450 [numbering from reference 30]). Symbols and lane 1 are as described in the legend to Fig. 2.

insertion of the NPE repositions the GAL4 binding sites into a nucleosome-free region between two positioned nucleosomes (see Fig. 6). To further test the possibility that insertion of the NPE changes the accessibility of the GAL4 binding sites, we investigated the ability of a restriction enzyme to cleave a site within GAL4 site 3 in permeabilized spheroplasts (Fig. 5). Spheroplasts were prepared from the *SWI*$^+$ strains CY532 (which contains the parental two-low-affinity-site reporter) and CY586 (which harbors the NPE-containing two-low-affinity-site reporter). Spheroplasts were made permeable by treatment with nystatin (24) and incubated with *Hga*I restriction endonuclease for 15 to 30 min. Genomic DNA was then purified and digested to completion with *Hin*dIII, and the *Hga*I-*Hin*dIII cleavage products were analyzed by Southern blotting using a DNA probe from the *GAL1* upstream region. This DNA probe will detect the *Hga*I-*Hin*dIII cleavage products derived from the *GAL1,10* locus (1.6 kb) or the reporter locus (0.5 kb) in the same DNA sample (see the schematic in Fig. 5A). In the case of the *GAL1,10* locus, we expected that the *Hga*I site within GAL4 site 3 would be highly accessible in permeabilized spheroplasts, since this GAL4 site is known to be nucleosome-free (6). In contrast, the model presented in Fig. 6 predicts that the *Hga*I site at the parental two-low-affinity-site reporter will be less accessible than the same site present at *GAL1,10*. Furthermore, the model predicts that insertion of the NPE will enhance the accessibility of the *Hga*I site at the reporter locus.

Figure 5B shows the phosphorimager quantitation of a typical *Hga*I accessibility assay. At 15 and 30 min of digestion, *Hga*I cleavage at the *GAL1,10* locus was nearly complete (value set at 100%) (data not shown). However, in the case of the

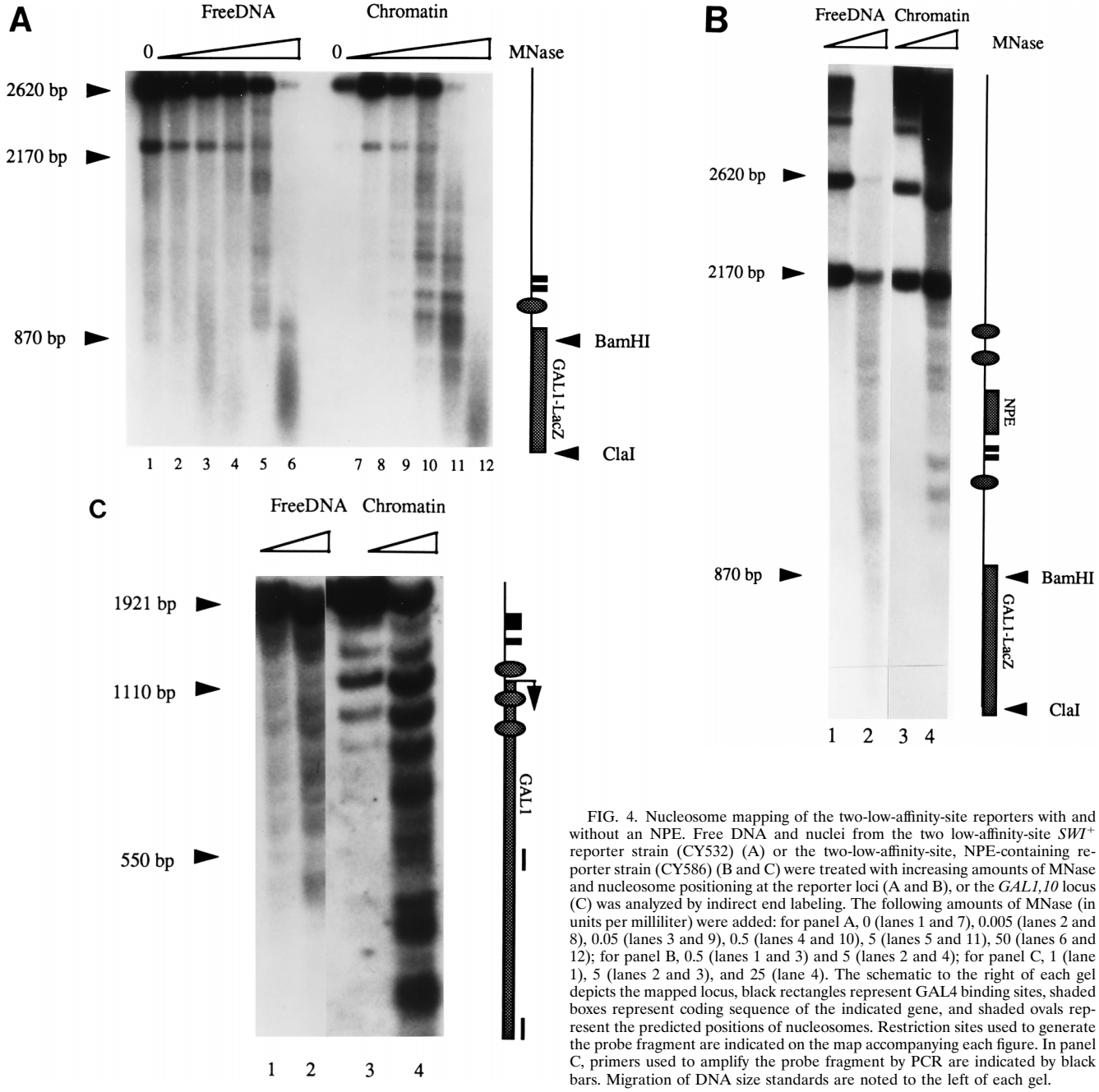

FIG. 4. Nucleosome mapping of the two-low-affinity-site reporters with and without an NPE. Free DNA and nuclei from the two low-affinity-site *SWI*$^+$ reporter strain (CY532) (A) or the two-low-affinity-site, NPE-containing reporter strain (CY586) (B and C) were treated with increasing amounts of MNase and nucleosome positioning at the reporter loci (A and B), or the *GAL1,10* locus (C) was analyzed by indirect end labeling. The following amounts of MNase (in units per milliliter) were added: for panel A, 0 (lanes 1 and 7), 0.005 (lanes 2 and 8), 0.05 (lanes 3 and 9), 0.5 (lanes 4 and 10), 5 (lanes 5 and 11), 50 (lanes 6 and 12); for panel B, 0.5 (lanes 1 and 3) and 5 (lanes 2 and 4); for panel C, 1 (lane 1), 5 (lanes 2 and 3), and 25 (lane 4). The schematic to the right of each gel depicts the mapped locus, black rectangles represent GAL4 binding sites, shaded boxes represent coding sequence of the indicated gene, and shaded ovals represent the predicted positions of nucleosomes. Restriction sites used to generate the probe fragment are indicated on the map accompanying each figure. In panel C, primers used to amplify the probe fragment by PCR are indicated by black bars. Migration of DNA size standards are noted to the left of each gel.

parental two-low-affinity-site reporter, cleavage of GAL4 site 3 by *Hga*I was less efficient, indicating that these sequences are less accessible than they are at *GAL1,10*. Furthermore, insertion of the NPE upstream of the GAL4 binding sites had a dramatic effect on *Hga*I cleavage. Cleavage at the NPE-containing reporter locus was at least threefold greater than the parental reporter locus, and the cleavage appeared to be even more efficient than cleavage at the *GAL1,10* locus. These differences in *Hga*I accessibility are not due to the binding of GAL4, since similar results were also obtained when permeabilized spheroplasts were prepared from *gal4* deletion strains (data not shown). Thus, the accessibility of GAL4 site 3 to *Hga*I cleavage correlates well with the nucleosome mapping data and the SWI-SNF dependence of GAL4 activity (Fig. 6).

DISCUSSION

In vitro experiments have demonstrated that the SWI-SNF complex can stimulate the binding of an activator protein to a nucleosomal binding site (5, 16). Here we have tested whether the complex exhibits this activity in vivo. We have shown that the SWI-SNF complex facilitates the binding of GAL4 to two low-affinity binding sites in vivo. Furthermore, we find that the inability of GAL4 to occupy these low-affinity sites in the absence of SWI-SNF activity can be overcome by (i) replacing the low-affinity binding sites with high-affinity GAL4 binding sites or (ii) placing the low-affinity binding sites into a nucleosome-free region. These results indicate that SWI-SNF is able to facilitate GAL4 binding in vivo, perhaps by helping GAL4 to

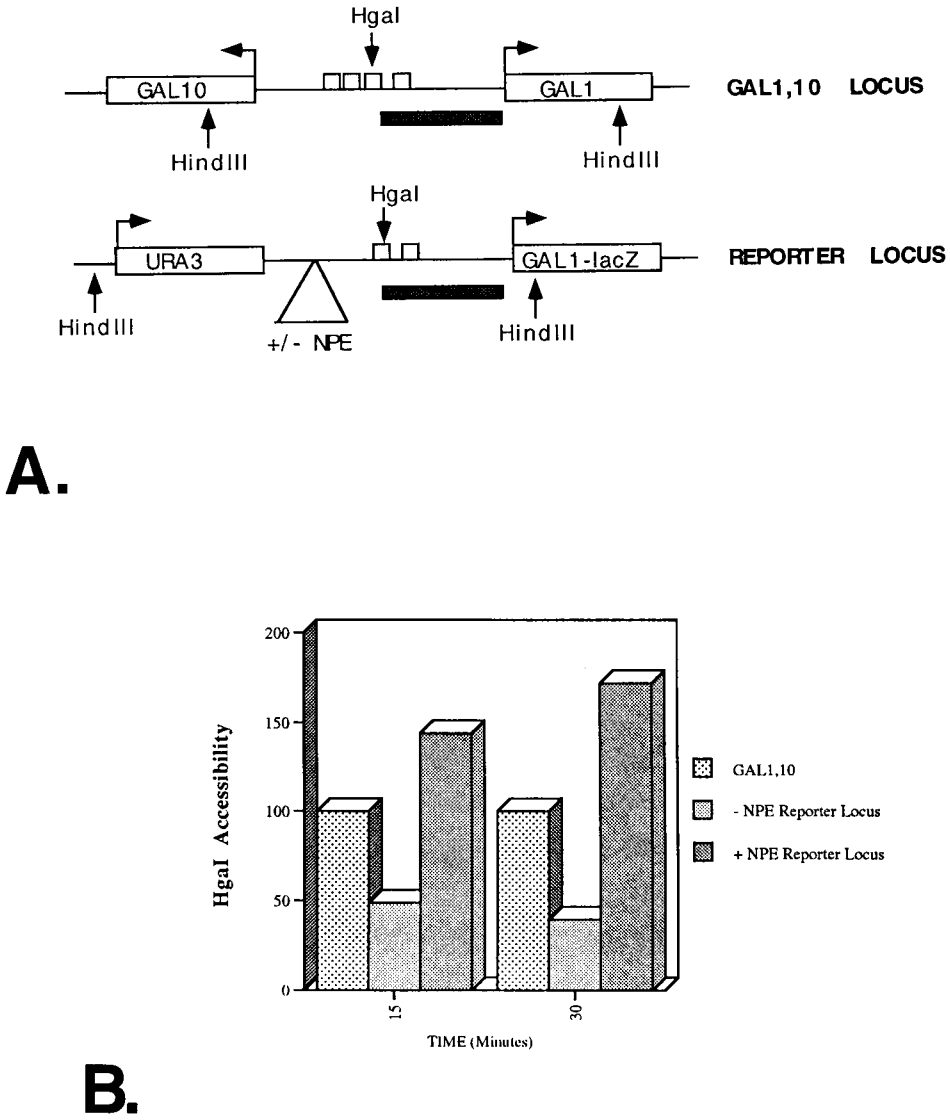

FIG. 5. An NPE leads to enhanced accessibility of a GAL4 binding site. (A) Schematic of the *GAL1,10* and reporter loci. The black bar denotes the DNA fragment used as a probe in Southern blotting. The *Hga*I site is located at the 3′ end of GAL4 site 3. +/− NPE, with or without an NPE. (B) *Hga*I accessibility assay. *Hga*I was added to nystatin-permeabilized spheroplasts prepared from *SWI*$^+$ strains harboring either the parental two-low-affinity-site reporter (− NPE; CY532) or the NPE-containing two-low-affinity-site reporter (+ NPE; CY586). DNA digested in situ was purified, digested to completion with *Hin*dIII, and analyzed by Southern blotting using the DNA probe denoted in panel A. Phosphorimager quantitation of the *Hga*I-*Hin*dIII fragments is shown for the 15- and 30-min *Hga*I digestion time points. The yields of the 0.5-kb *Hga*I-*Hin*dIII fragments from the two different reporter loci are presented as a percentage of the 1.6-kb *Hga*I-*Hin*dIII fragment derived from the *GAL1,10* locus which was set at 100% accessibility.

compete more effectively with histones for occupancy of its binding sites.

A definition of SWI-SNF dependence. Our results suggest that the SWI-SNF dependence of a transcriptional activator protein is not an innate feature of the activator protein per se but rather reflects the chromosomal context of the activator binding sites. We propose that when the activator binding sites are encompassed in a nucleosome, as in the two-low-affinity-site reporter used in this study, SWI-SNF function is necessary for the activator protein to bind to and activate transcription from those sites (Fig. 6). On the other hand, when binding sites are nucleosome-free, as is the case at the *GAL1,10* UAS region or at the NPE-containing two-low-affinity-site reporter, then SWI-SNF function is dispensable for GAL4 activity (Fig. 6). In this case, GAL4 can bind to its sites and activate transcription in the absence of SWI-SNF activity. Furthermore, SWI-SNF dependence does not appear to correlate with the level of transcriptional activation (i.e., promoter strength [Fig. 1A]). Thus, based on the results presented here, we predict that the subset of genes whose expression requires SWI-SNF will have activator binding sites that are encompassed in nucleosomes; genes that do not require SWI-SNF will have such sites positioned in the linker regions between nucleosomes or in other nucleosome-free regions. This positioning may be determined by DNA sequence, as in the NPE-containing reporter, or by abundant DNA binding proteins like GRF2, as in the case of the intact *GAL1,10* reporter.

Our results also require that binding site affinity be incorporated into the definition of SWI-SNF dependence. While GAL4 requires SWI-SNF in order to access two low-affinity

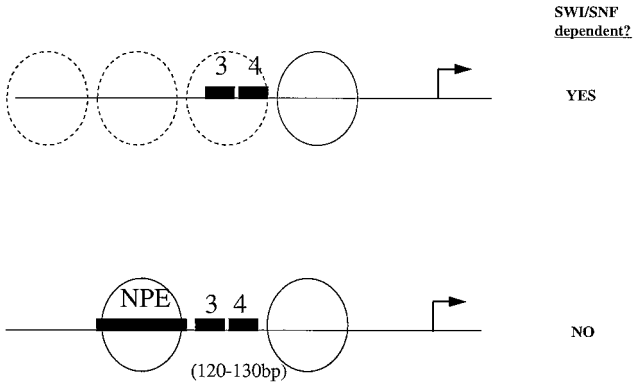

FIG. 6. Predicted nucleosome structure of SWI-SNF-dependent and -independent promoters. Schematics depicting the predicted nucleosome structure of the two-low-affinity-site reporter construct and the same construct containing an NPE directly upstream of the GAL4 binding sites. Solid-line ovals represent positioned nucleosomes; broken-line ovals represent randomly located nucleosomes. Nucleosomes located randomly over the GAL4 binding sites of the former cause the expression of this reporter gene to require SWI-SNF activity. In the NPE-containing construct, on the other hand, the GAL4 binding sites are nucleosome-free and therefore reporter gene expression is SWI-SNF independent.

binding sites, high-affinity sites are occupied even in the absence of SWI-SNF. The ability of higher-affinity binding sites to alleviate SWI-SNF dependence provides strong evidence that the SWI-SNF complex modulates transactivation at the level of DNA binding. Furthermore, if these high-affinity binding sites are encompassed by nucleosomes, then our results suggest that GAL4 binding to high-affinity, nucleosomal sites can occur in vivo via a SWI-SNF-independent mechanism. These results are consistent with those of Workman and Kingston (28), who found that GAL4 can bind to a nucleosomal site to form a tripartite transcription factor-histone-DNA complex.

Does the SWI-SNF complex play an additional role in transcriptional activation? As noted above, we detect a consistent level of GAL4 binding to the low-affinity sites in the *swi1*$^-$ strain (Fig. 3B, compare lanes 5 and 6); phosphorimager analysis indicates site 3 may be occupied at 60% of the level seen in a *SWI*$^+$ strain (Fig. 3C). The fact that this level of occupancy results in negligible levels of transcription (3% of wild-type level [Fig. 1A, reporter c]) may indicate a role for the SWI-SNF complex in transcriptional activation beyond the modulation of activator binding. In light of recent reports suggesting that the SWI-SNF complex may associate with the RNA polymerase II holoenzyme (25), it is interesting to consider the possibility that an activator–SWI-SNF interaction is involved in preinitiation complex assembly. On the other hand, our in vivo footprinting procedure does not allow us to determine the extent to which site 3 and site 4 are simultaneously occupied and we cannot distinguish whether the 60% occupancy in a *swi-snf* strain reflects the stable binding of GAL4 in 60% of the cells or reflects weak, unstable binding in 100% of the cells. Therefore, our results may support an additional role for the SWI-SNF complex in facilitating the cooperative binding of GAL4 or in enhancing the stability of GAL4 binding to low-affinity, nucleosomal sites in vivo.

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