SWI/SNF Stimulates the Formation of Disparate Activator-Nucleosome Complexes but Is Partially Redundant with Cooperative Binding

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Rhea T. Utley, Jacques Côté, Tom Owen-Hughes, and Jerry L. Workman†

From the Department of Biochemistry and Molecular Biology and The Center for Gene Regulation, The Pennsylvania State University, University Park, Pennsylvania 16802-4500

To investigate the potential mechanisms by which the SWI/SNF complex differentially regulates different genes we have tested whether transcription factors with diverse DNA binding domains were able to exploit nucleosome disruption by SWI/SNF. In addition to GAL4-VP16, the SWI/SNF complex stimulated nucleosome binding by the Zn\(^{2+}\) fingers of Sp1, the basic helix-loop-helix domain of USF, and the rel domain of NF-κB. In each case SWI/SNF action resulted in the formation of a stable factor-nucleosome complex that persisted after detachment of SWI/SNF from the nucleosome. Thus, stimulation of factor binding by SWI/SNF appears to be universal. The degree of SWI/SNF stimulation of nucleosome binding by a factor appears to be inversely related to the extent that binding is inhibited by the histone octamer. Cooperative binding of 5 GAL4-VP16 dimers to a 5-site nucleosome enhanced GAL4 binding relative to a single-site nucleosome, but this also reduced the degree of stimulation by SWI/SNF. The SWI/SNF complex increased the affinity of 5 GAL4-VP16 dimers for nucleosomes equal to that of DNA but no further. Similarly, multimerized NF-κB sites enhanced nucleosome binding by NF-κB and reduced the stimulatory effect of SWI/SNF. Thus, cooperative binding of factors to nucleosomes is partially redundant with the function of the SWI/SNF complex.

In eukaryotic cells the DNA is packaged into nucleosomes, the primary order of chromatin structure that provides an inherent barrier for activator proteins and the basal transcription machinery. There are several mechanisms that help transcription factors contend with nucleosomal DNA (reviewed in Refs. 1 and 2). One such mechanism involves energy-dependent remodeling of chromatin, which allows transcription factors to access otherwise inaccessible promoters. Such activities have been purified from yeast (3–5), Drosophila (6), and humans (7–9). The first of these activities to be identified was the yeast SWI/SNF complex.

SWI/SNF was originally identified in yeast as a set of positive regulators of the HO gene (mating type switch, SWI) and the SUC2 gene (sucrose non-fermenting, SNF) (reviewed in Ref. 10). The connection to chromatin was revealed when mutant suppressors were identified in histones as well as in other chromatin proteins (11, 12). These SWI/SNF proteins were biochemically isolated as an 11-subunit complex of approximately 2 megadaltons. Members of this complex include SWI1, SWI2-SNF2, SWI3, SNF5, SNF6 (3, 4), SWP73 (13), SNF11 (14), and TFG3 (TAF30) (15), although the latter two do not demonstrate a 'swi\(^{+}\)' phenotype. The SWI2-SNF2 subunit contains a conserved ATPase domain and has been shown to function as a DNA-dependent ATPase. This domain is required for SWI/SNF function in vivo (16). Interestingly, SWI/SNF homologs have also been identified in mammals and have a conserved function. In contrast to yeast, however, multiple forms of the complexes can be found within the same cell (17). Two SWI2-SNF2 homologs (brm and BRG1) have been identified in mouse and human systems. Both are found in large (2-megadalton) complexes, but each is present in separate complexes (8). In vitro binding experiments showed that ySWI/SNF can enhance the binding of GAL4 derivatives to nucleosomes in an ATP-dependent reaction (18). This enhancement required only the GAL4 DNA binding domain and was accompanied by an ATP-dependent disruption of histone-DNA contacts. The human SWI/SNF complex was also shown to enhance the binding of GAL4 derivatives (7, 8) as well as TBP to nucleosomes (19).

The SWI/SNF complex is able to disrupt nucleosome structure in a sequence-independent manner (20, 21) suggesting that it may be able to function at diverse promoters. Consistent with this possibility is the fact that transcription of a set of diversely regulated genes was found to require SWI/SNF. These genes include HO, SUC2, ADH1–2, INO1, Ty elements, and to some degree the GAL1–10 genes (10, 22). In addition, certain yeast activators as well as the function of several heterologous activators were shown to require the SWI/SNF complex in yeast (22–24). However, transcription of several other yeast genes does not depend on the SWI/SNF complex in vivo. For example, transcription of the PHO5 gene is SWI/SNF-independent (13). Moreover, transcription from several yeast promoters or from artificial promoters driven by heterologous activators in yeast is differentially affected by mutations in the SWI/SNF subunit Swp73 (13).

The differential requirement for SWI/SNF function in different promoter contexts might be explained in part by at least two possibilities. First, SWI/SNF function may be available to only a subset of transcriptional activators. In other words, the disruption of DNA-histone interactions by the SWI/SNF complex may be effectively exploited by activators with particular DNA binding domains but insufficient to enhance the affinity of others. Second, SWI/SNF function may be redundant with alternative mechanisms mediating access of transcription fac-

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology and The Center for Gene Regulation, The Pennsylvania State University, University Park, PA 16802-4500. Tel.: 814-863-8256; Fax: 814-863-0099; E-mail: JLW10@psu.edu.

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tors to binding sequences in chromatin. To test these possibilities we have analyzed the ability of the SWI/SNF complex to stimulate nucleosome binding by transcription factors with diverse DNA binding domains and whether stimulation by SWI/SNF was affected by cooperative binding of activators to nucleosomes.

**EXPERIMENTAL PROCEDURES**

**Plasmids and DNA Probes**—To generate plasmid pUGB-NFX1, a 20-bp oligonucleotide (5'-CTAGGGGAGTTCCGCGTAT-3') harboring a high affinity NF-\(\beta\) site was inserted into a unique BamHI site between the USF and GAL4 sites of pUSF/GALBend (28). Plasmid pUGB-NFX3 was similarly generated by inserting three copies of the same oligonucleotide into the BamHI site in pGALUSFBend (25). Plasmid pG5_SUE4T was created by the insertion of a 37-bp oligonucleotide (5'-ACGGGGGCGGGTTACCTTCAGACACGTGGCCTG-3') containing an Sp1 site, a USF site, and XhoI cohesive ends into p\(\alpha\)ET47 (26) at the unique XhoI site.

All of the purified DNA fragments were labeled by Klenow reaction after the first enzyme digest and then cleaved with the second enzyme. The 149-bp USF site probe with a USF site centered 31 bp from the end of the fragment was generated by cutting pGALUSFBend (25) with SpeI followed by XhoI. pUGB-NFX1 was digested with XhoI and BamHI to create a 156-bp fragment with an NF-\(\beta\) site 30 bp from the end and a competitor GAL4 site 58 bp from the end. A 167-bp probe harboring an Sp1 site 35 bp from the end was cleaved from pG5_SUE4T with enzymes HindIII and BamHI. The 5 GAL4 site probe was obtained from pG540HSF70CAT (27) by cutting with NheI followed by PstI giving rise to a 156-bp fragment with the first GAL4 site centered at 17 bp from the end. Plasmid pUGB-NFX3 was digested with XhoI followed by BstEII to generate a 166-bp molecule containing 3 NF-\(\beta\) sites centered at 15, 35, and 53 bp from the end. All probe DNA fragments were isolated from 8% polyacrylamide (1 x Tris borate/EDTA) gels.

**Protein Purification**—Recombinant proteins USF and NF-\(\beta\) were overexpressed in *Escherichia coli* using the pET system (Novagen) and purified as described by Pognonec et al. (28). The form of NF-\(\beta\) used for this study was a 42-kDa truncated form of the p50 derivative (29). The form of NF-\(\beta\) used for this study was a 42-kDa truncated form of the p50 derivative (29). The form of NF-\(\beta\) used for this study was a 42-kDa truncated form of the p50 derivative (29). The form of NF-\(\beta\) used for this study was a 42-kDa truncated form of the p50 derivative (29).

**Nucleosome Reconstitution and Binding Reactions**—Nucleosomes were reconstituted from heLa octamer monomers prepared as described previously (20, 31). Gel shift binding reactions included 12.5 ng of total nucleosomes (12.5 nM) in 10 \(\mu\)l with final conditions of 20 mM HEPES, pH 7.5, 3 mM MgCl\(_2\), 1 \(\mu\)M ZnCl\(_2\), 0.1 mg/ml bovine serum albumin, 5% glycerol, 2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride. The concentration of nucleosomes and/or DNA fragments as described previously (20, 31). Gel shift binding reactions included 12.5 ng of total nucleosomes (12.5 nM) in 10 \(\mu\)l with final conditions of 20 mM HEPES, pH 7.5, 3 mM MgCl\(_2\), 1 \(\mu\)M ZnCl\(_2\), 0.1 mg/ml bovine serum albumin, 5% glycerol, 2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride. The concentration of nucleosomes and/or DNA fragments actually bearing the transcription factor binding sites was less than 0.1 nM in all instances. Each transcription factor was serially diluted in its stock buffer as follows: USF dilution buffer (10 mM HEPES, pH 8.0, 1 mM EDTA, pH 8.0, 3 mM MgCl\(_2\), 0.1% (v/v) Nonidet P-40, 1 mg/ml bovine serum albumin, 20% glycerol, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride), NF-\(\beta\) dilution buffer (20 mM Tris-Cl, pH 7.8, 1 mM EDTA, pH 8.0, 100 mM KCl, 0.1% (v/v) Nonidet P-40, 20% glycerol, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride), GAL4 dilution buffer (20 mM HEPES, pH 7.5, 100 mM KCl, 10 \(\mu\)M ZnCl\(_2\), 1 mM bovine serum albumin, 20% glycerol, 10 mM \(\beta\)-mercaptoethanol), and Sp1 dilution buffer (12 mM HEPES-KOH, pH 7.7, 50 mM KCl, 6 mM MgCl\(_2\), 5 \(\mu\)M ZnSO\(_4\), 0.05% Nonidet P-40, 50% glycerol, 1 mM dithiothreitol). The yeast SWI/SNF complex was added at a concentration of 0.1 nM (0.8:1 molar ratio of SWI/SNF to nucleosomes) for all gel shift experiments except the experiment illustrated in Fig. 6 where 20 nM yeast SWI/SNF was added. MgATP (1 mM) was included where indicated in the figure legends. Binding proceeded for 30 min at 30 °C. Interaction of ySWI/SNF with competitor resulting in a clear footprint at 1 nM Sp1 (lane 8). When the nucleosomes were treated with the SWI/SNF complex, the DNase I cutting pattern was perturbed (compare lanes 5 and 9), illustrating SWI/SNF interaction with these nucleosomes. While Sp1 readily bound the mock-reconstituted naked DNA control resulting in a clear footprint at 1 nM Sp1 (lane 2), binding was not observed to the reconstituted nucleosomes at even 100 nM Sp1 (lane 8). However, Sp1 binding was observed when the nucleosomes were also treated with the SWISNF complex (compare lanes 9 and 12).

The effect of SWI/SNF treatment on nucleosome binding by...
USF and NF-κB is shown in Fig. 1, C and D, respectively. Each of these factors also bound the mock-reconstituted naked DNA probes with nanomolar affinities (lanes 1–4 of each panel). In the case of USF, at high concentrations of the factor alone some protection of the USF site from DNase I cleavage was observed on the nucleosome template (Fig. 1C, lanes 5–8). However, in the presence of the SWI/SNF complex nucleosome binding by USF was stimulated greater than 10-fold (Fig. 1C, lanes 9–12). There was no detectable binding of NF-κB to the nucleosome template at 110 nM factor (Fig. 1C, lanes 5–9). By contrast, when the NF-κB site nucleosome was treated with the SWI/SNF complex a clear footprint was observed at 11 nM (lane 10) indicating greater than 10-fold stimulation of NF-κB binding. Similar to the Sp1 site nucleosome (Fig. 1B) the action of SWI/SNF also altered the DNase I digestion pattern of the USF site and NF-κB site nucleosomes (compare lanes 5 and 9 of Fig. 1, C and D, respectively). Thus, as with GAL4 derivatives the disruption of nucleosomal DNA by the SWI/SNF complex also resulted in enhanced affinity of Sp1, USF, and NF-κB for nucleosomal DNA.

Since the nucleosomes in Fig. 1 did not display a strong rotational phasing pattern by DNase I (i.e. a 10-bp ladder), it is

**Fig. 1.** Yeast SWI/SNF enhances binding of Sp1, USF, and NF-κB to their sites within a nucleosome. A, nucleosome reconstitutions of various DNA fragments. *Lane 1*, a 167-bp fragment bearing an Sp1 site 35 bp from the end. *Lane 2*, a 149-bp fragment containing a USF site 33 bp from the end is shown in lane 2. *Lane 3* contains a 156-bp fragment harboring an NF-κB site 34 bp from the end. B, DNase I analysis of the Sp1 probe used in A (lane 1), either mock-reconstituted (DNA lanes 1–4) or reconstituted into nucleosomes (lanes 5–12). Binding reactions for lanes 8–12 also contained 20 nM SWI/SNF. All lanes contained 1 mM ATP. Sp1 was included at the amounts indicated. The position of the Sp1 site is shown at the right. C, the USF fragment from A (lane 2) was subjected to DNase I footprinting. USF titrations on naked DNA (lanes 1–4) and reconstituted nucleosomes in the absence (lanes 5–8) and presence (lanes 9–12) of 20 nM SWI are shown. 1 mM ATP was included in all lanes. Concentrations of USF are given. The USF binding site is indicated by a bar at the right. D, analysis of NF-κB binding to DNA (lanes 1–4) and nucleosomes (lanes 5–12) by DNase I footprinting (same probe from A, lane 3). 20 nM ySWI/SNF was included in lanes 9–12. NF-κB was present at the indicated concentrations. All binding reactions contained 1 mM ATP. The recognition site for NF-κB is shown at the right.
likely that a mixed population of rotational orientations of the DNA on nucleosomes were present. Thus, in these cases the precise orientation of the binding sites with respect to the histone octamer was heterogeneous. However, stimulation of factor binding by SWI/SNF was observed in all cases indicating that stimulation by SWI/SNF was not dependent on a particular rotational phasing of the DNA relative to the histone octamer.

The data in Fig. 1 illustrate that the SWI/SNF complex is able to stimulate nucleosome binding by transcription factors with diverse DNA binding domains. However, the SWI/SNF complex also binds to DNA (37) and nucleosomes\textsuperscript{2} with nanomolar affinities. Thus, in the DNase I footprinting experiments shown in Fig. 1 as well as in footprinting experiments from previous reports (7, 8, 18, 19) the enhanced affinity of the factors represents a comparison of its affinity to a SWI/SNF-nucleosome complex\textit{versus} that of a nucleosome alone. Therefore, it is useful to determine whether the observed enhanced binding is dependent on the continued binding of the SWI/SNF-complex or whether it persists once SWI/SNF has detached from the nucleosome. Indeed, only GAL4 derivatives have been shown (i.e. by gel shift analysis) to form a stable ternary complex with nucleosomes as a result of SWI/SNF action that persists following detachment of the SWI/SNF complex (18, 21).

While the SWI/SNF complex binds to nucleosomes with nanomolar affinity, it can be competed off of nucleosomes to which it is bound by the addition of cold nucleosomes and/or naked DNA (21). Thus, to determine whether the SWI/SNF-induced nucleosome binding by Sp1, USF, or NF-κB resulted in a stable ternary factor-nucleosome complex we competed SWI/SNF off the target nucleosomes following the binding reactions (Fig. 2). Following this competition we tested for the presence of stable factor-nucleosome complexes by gel shift analysis. This analysis is shown in Fig. 3. Fig. 3A shows mobility shift of Sp1 site nucleosomes that were incubated with SWI/SNF in the presence or the absence of ATP and Sp1 followed by competition of the SWI/SNF complex. In the absence of ATP very little Sp1-nucleosome complex was observed (lanes 3 and 5). By contrast, when ATP was present to allow SWI/SNF function there was a clear stimulation of Sp1-nucleosome complex formation (compare lane 3 to 4 and lane 5 to 6). The loss of a fraction of the Sp1-nucleosome complexes into Sp1 aggregates (not resolved on the gel) due to the self-association properties of Sp1 accounts for the reduction of total radioactivity in lanes 4 and 6. While this effect and nonspecific competition of Sp1 by the added competitor chromatin and nonspecific DNA reduced the apparent amount of Sp1 binding relative to the footprinting reactions above, it is clear that Sp1-nucleosome ternary complexes persisted following the detachment of SWI/SNF. Thus, the ATP-dependent Sp1-nucleosome complexes formed by the action of SWI/SNF did not require the continued presence of SWI/SNF.

Both USF and NF-κB also formed stable factor nucleosome complexes as a consequence of SWI/SNF action (Fig. 3, B and C). Fig. 3B clearly indicates that stable USF binding to nucleosomes was also stimulated by the SWI/SNF complex in the presence of ATP. The formation of a stable USF-nucleosome complex that persisted after competition of the SWI/SNF complex was stimulated by the presence of ATP (compare lane 3 to 4 and 5 to 6). Fig. 3C shows a similar analysis using NF-κB. In the presence of SWI/SNF and ATP the formation of a stable NF-κB/nucleosome complex was enhanced (Fig. 3C, compare lanes 3 and 4, and 5 and 6). Thus, the data in Fig. 1 illustrate that the SWI/SNF complex stimulates the binding of disparate transcription factors to nucleosomes. The data in Fig. 3 illustrate that SWI/SNF action also results in the formation of transcription factor-nucleosome complexes, which persisted independent of continued SWI/SNF binding.

The experiments described above argue that SWI/SNF stimulation of transcription factor binding to nucleosomes is universal and therefore unlikely to provide distinction between SWI-dependent and-independent promoters. We therefore considered the alternative possibility that SWI/SNF function in stimulating factor binding is in part redundant with alternative mechanisms of nucleosome binding. For example, many promoter/enhancer regions contain multiple transcription factor binding sites, which may lead to cooperative binding in chromatin. Cooperative binding of 5 GAL4 dimers to nucleosomes has been shown to occur \textit{in vitro} (27, 38). The observed cooperativity is in response to inhibition from the histone octamer and stimulates the binding of multiple GAL4 dimers relative to binding of individual GAL4 dimers to a single GAL4 site (especially near the center of the nucleosome). We thus tested whether cooperative binding of GAL4-VP16 to nucleosome cores would enhance or reduce the stimulation observed by the SWI/SNF complex.

Analysis of GAL4-VP16 binding to DNA or nucleosome cores bearing 5 GAL4 sites is illustrated Fig. 4. When increasing amounts of GAL4-VP16 were added to the mock-reconstituted naked DNA (lanes 1–6), complete protection of the 5 sites was observed at 14 nM protein (lane 4). When reconstituted into a nucleosome, this DNA fragment generates a distinct 10–11-bp

\textsuperscript{2} J. Côté and J. L. Workman, unpublished data.
repeating pattern of hypersensitive cutting by DNase I indicating that this fragment has a preferred path of DNA bending around the histone octamer (lane 7). When GAL4-VP16 was titrated into reactions containing the reconstituted 5-site nucleosome, complete protection of the GAL4 sites was apparent at 140 nM protein (lane 12). Thus, a 10-fold repression of GAL4-VP16 binding to the 5 GAL4 site probe resulted from the presence of the histone octamer. When SWI/SNF was also included, the DNase I digestion pattern of the 5-site nucleosome was disrupted (lane 14). Concurrent with nucleosome disruption by SWI/SNF, the affinity of GAL4-VP16 was enhanced allowing full protection of the GAL4 sites at 14 nM GAL4-VP16 (lanes 14–20).

The data in Fig. 4 illustrate that under conditions of cooperative nucleosome binding the SWI/SNF complex stimulated the binding of GAL4-VP16 to nucleosomes 10-fold. By contrast, stimulation of GAL4-VP16 binding to a nucleosome bearing only a single GAL4 site was greater. Fig. 5 illustrates the binding of GAL4-VP16 to a mock-reconstituted naked DNA fragment (lanes 1–6) or the same fragment after reconstitution into nucleosome cores (lanes 7–13). Greater than 50% protection of the GAL4 site on the naked DNA was observed at 4.5 nM GAL4-VP16 with complete protection at 14 nM (lanes 3 and 4). When binding to the nucleosome core, however, half-protection of the GAL4 site required 1400 nM GAL4-VP16 (lane 12) indicating that GAL4-VP16 binding to this single site in the nucleosome was inhibited approximately 300-fold relative to naked DNA. However, when the SWI/SNF complex was included, half-protection of the GAL4 site occurred between 14 and 45 nM GAL4-VP16 (lane 16). Thus, the SWI/SNF complex stimulated the binding of a single GAL4 dimer to a nucleosome core by approximately 100-fold.

The data in Figs. 4 and 5 illustrate several important conclusions regarding GAL4-VP16 binding to nucleosome cores and the function of the SWI/SNF complex. 1) Five GAL4-VP16 dimers bound nucleosomes with an affinity that was more than 10-fold greater than the binding of a single GAL4-VP16 dimer. Indeed, the cooperative binding of 5 dimers was only inhibited by the presence of the histone octamer 10-fold relative to GAL4-VP16 binding to naked DNA. 2) The SWI/SNF complex stimulated the binding of GAL4-VP16 to the single-site nucleosome 100-fold but only stimulated the binding of GAL4-VP16 to the...
5-site nucleosome 10-fold. Thus, SWI/SNF exerted the most dramatic effect where inhibition of GAL4-VP16 binding to nucleosomes was greatest. 3) The SWI/SNF complex did not enhance the affinity of GAL4-VP16 for nucleosomes beyond its affinity for naked DNA. The binding of 5 GAL4-VP16 dimers to nucleosomes in the presence of SWI/SNF occurred with an affinity equal to that of naked DNA.

To determine whether cooperative binding might similarly reduce the extent of SWI/SNF stimulation of different factors we have also tested SWI/SNF stimulation of NF-κB binding to nucleosomes containing either 1 or 3 NF-κB sites. As we were unable to obtain a DNase I footprint of NF-κB binding to a
single-site nucleosome in the absence of SWI/SNF we utilized the gel shift assay where partial occupancy could be measured. Fig. 6 shows a mobility shift analysis of binding to a 3 NF-\(\kappa\)B site (NFX3) and a single-site (NFX1) nucleosome. Mock-reconstituted DNA lanes (1, 2, 6, and 7) were incubated with 3.3 nm NF-\(\kappa\)B and used to illustrate the migration of NF-\(\kappa\)B/DNA complexes (lanes 2 and 7). Binding of NF-\(\kappa\)B to the first site of the 3-site nucleosome occurred easily due to its proximity to the edge of the nucleosome (13 bp from the end). However, binding to the next two sites (33 and 53 bp into the nucleosome, respectively) occurred in a “cooperative” manner as very few two NF-\(\kappa\)B nucleosome complexes were observed relative to three NF-\(\kappa\)B complexes (lane 4). In the presence of 330 nm NF-\(\kappa\)B, 16.9% of the total nucleosomes were bound by 3 NF-\(\kappa\)B molecules (lane 4). When the SWI/SNF complex was added to the reaction mixture, this binding was stimulated 2.9-fold resulting in the occupancy of 49.7% of the nucleosomes with 3 NF-\(\kappa\)B proteins (lane 5). In the case of the nucleosome with a single NF-\(\kappa\)B site 30 bp into the nucleosome core, NF-\(\kappa\)B binding was more greatly inhibited by the histone octamer than binding to the 3-site nucleosome indicating that multiple sites increased NF-\(\kappa\)B binding (compare levels of the 3 NF-\(\kappa\)B-nucleosome complex in lane 4 to the levels of 1 NF-\(\kappa\)B-nucleosome complex in lane 9). Addition of 330 nm NF-\(\kappa\)B resulted in NF-\(\kappa\)B binding to only 4.6% of the single-site nucleosomes (lane 9). However, upon incubation with SWI/SNF binding of NF-\(\kappa\)B to the single-site nucleosome was increased to 36.5%, a 7.9-fold increase. Thus, the extent of SWI/SNF stimulation of NF-\(\kappa\)B binding to a nucleosome bearing 3 sites relative to a single site was reduced approximately 3-fold.

The data in Figs. 4–6 illustrate that multimerization of binding sites for either GAL4-VP16 or NF-\(\kappa\)B enhanced the extent of binding to sites well within the nucleosome and in turn reduced the extent of SWI/SNF stimulation observed. These observations indicate that degree of SWI/SNF stimulation of factor binding to nucleosomes is inversely related to the degree of inhibition by the histone octamer. Thus, parameters that reduce nucleosome-mediated inhibition of factor binding, such as cooperative binding, are partially redundant with the function of the SWI/SNF complex in facilitating binding of transcription factors to nucleosomal DNA.

DISCUSSION

The SWI/SNF complex participates in the activation of several yeast genes in vivo (3); however, many inducible genes do not require SWI/SNF, even some that have been shown to undergo chromatin remodeling upon induction, such as PHO5 (13, 39, 40). We have tested the possibility that the differential effect of SWI/SNF might reflect a selective ability of different transcription activators to exploit SWI/SNF-mediated disruption of nucleosomes to gain access to their binding sites in nucleosomes. By biochemical analysis we have found that the purified SWI/SNF complex stimulated nucleosome binding by three different transcription factors, Sp1, USF, and NF-\(\kappa\)B concomitant with the ATP-dependent SWI/SNF disruption of histone-DNA contacts. Moreover, the function of SWI/SNF resulted in the formation of stable factor nucleosome complexes containing any of these factors that persisted after detachment of the SWI/SNF complex. The fact that these factors all contain very different types of DNA binding domains indicates that the stimulation of stable transcription factor binding to nucleosomes by SWI/SNF is a general mechanism. Most DNA binding proteins are inhibited by the presence of nucleosomes. Our data illustrate that SWI/SNF perturbation of histone-DNA interactions involves contacts which are generally important for repression of transcription factor binding. Thus, the effect of
SWI/SNF action on activator binding is universal and likely does not play a major role in determining which genes are SWI-dependent.

A common quality of many promoter/enhancer regions is the occurrence of multiple binding sites for upstream activators which may lead to cooperative binding to nucleosomes (25). The presence of multiple adjacent GAL4 sites within a nucleosome leads to cooperative binding by GAL4 derivatives in vitro. (27, 38). This cooperativity led to only a 10-fold repression of GAL4-VP16 binding to nucleosomes relative to naked DNA, which was completely reversed by the SWI/SNF complex (Fig. 4). By contrast, SWI/SNF was able to stimulate the binding of a single GAL4-VP16 dimer by over 100-fold since the repression of GAL4-VP16 binding for a single dimer was greater than 300-fold. Thus, the requirement for SWI/SNF function in the vitro binding of GAL4-VP16 to nucleosome cores is partially redundant with cooperative nucleosome binding. Furthermore, SWI/SNF had a greater stimulatory effect on the binding of a single NF-κB to its nucleosomal recognition site as compared with its effect on NF-κB binding to 3 sites in a nucleosome. The fact that similar effects are seen with a different type of transcription factor supports the general idea that reducing the repressive effect of nucleosomes in turn reduces the stimulation effect of the SWI/SNF complex. This observation raises the possibility that SWI/SNF function may be redundant with cooperative nucleosome binding. Furthermore, SWI/SNF had a greater stimulatory effect on the binding of a single NF-κB to its nucleosomal recognition site as compared with its effect on NF-κB binding to 3 sites in a nucleosome.

Two other nucleosome remodeling activities have been biochemically identified that are distinct from SWI/SNF but have some similar biochemical activities. The NURF (ISWI) complex was purified from Drosophila (6, 41) and the RSC (STH1) complex from yeast (5). ISWI is the catalytic subunit of NURF for which homologs have been identified in yeast as well as humans (42). These complexes may also function by antagonizing the repressive effects of chromatin and thus might in principle be functionally redundant with SWI/SNF. It will be interesting to see if these distinct nucleosome disrupting activities provide overlapping functions in the yeast nucleus.

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Rhea T. Utley, Jacques Côté, Tom Owen-Hughes and Jerry L. Workman

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