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3. Vertebrae T7 through T10 were exposed in adult 250-g female Sprague-Dawley rats while the rats were anesthetized with halothane (1.5%; respiratory rate, ≈60 per minute; rectal temperature, <3°C below normal). After T8 and T9 posterior laminectomies and bipolar cauterization to control hemorrhage, a 5-mm T8 spinal cord segment was removed with microscissors (Fig. 1A). Eighteen intercostal nerves collected in Hanks' buffered saline solution (HBSS) were used to reconnect and redirect pathways between the spinal cord stumps (Fig. 1E). The floor of the cavity between the spinal cord stumps was covered by a thin gelfoam layer. Minced pieces of peripheral nerve were used to fill the angle between the gelfoam bottom and the ventralmost (white matter) surface of the cut end of the distal spinal cord stump, producing a slanted floor that allowed some physical support of the nerve bridges that went from deep (white matter) proximal sites to somewhat more dorsal (gray matter) distal sites. The cut ends of the intercostal nerve bridges were attached to the semidry spinal cord stump surfaces. The two fibrin glue sealant elements (Beriplast P, generously provided by Behring, Germany) were prepared (*8, 13*). The final glue solution, of which 10 μl was applied to the engrafted area, consisted of aFGF (2.1 μg/ml), fibrinogen (100 mg/ml), aprotinin (200 kIU/ml), thrombin (40 IU/ml), and 8 mM $CaCl_2$. The T7 and T10 spinal processes were fixed in dorsiflexion with an S-shaped monofilament surgical steel (DS-20, Ethicon) loop and fastened to the spinal column with nonabsorbable circumspinal threads. One experimental group was subjected to unilateral redirection, and aFGF was included in the glue (URDaFGF); the other two groups were subjected to bilateral redirection and aFGF glue through use of autografts (50%) mixed with allografts (BRDaFGF I) or through use of only autografts (BRDaFGF II). Control animals were subjected to transection at T8 (C1), to removal of a 5-mm cord segment (C2), or to grafting with the same methods as in the experimental groups except that HBSS replaced aFGF (BRD) or that grafting was done with only white matter–to–white matter connections (RBaFGF). Animals were caged on thick soft bedding, with heating from below during the first postoperative days. The rats' bladders were emptied manually twice daily as long as needed. Antibiotics (Borgal, Hoechst, 15 mg per kilogram of body weight, subcutaneously) were injected once daily for 7 days. Decubitus sores on hind limbs were treated with iodine-soaked dressings. Animals were killed if severe sepsis (urinary tract infection), infected decubitus sores, or other wounds occurred. For the major experiments described here, mortality was <10%. Experiments were approved by the animal research ethical committee of Stockholm. Animals were killed at different time points for histological analyses but no earlier than 1 month after surgery to ensure complete degeneration of the cut descending fibers in the distal stump (*9*).
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Persistent Site-Specific Remodeling of a Nucleosome Array by Transient Action of the SWI/SNF Complex

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The SWI/SNF complex participates in the restructuring of chromatin for transcription. The function of the yeast SWI/SNF complex in the remodeling of a nucleosome array has now been analyzed in vitro. Binding of the purified SWI/SNF complex to a nucleosome array disrupted multiple nucleosomes in an adenosine triphosphate–dependent reaction. However, removal of SWI/SNF left a deoxyribonuclease I–hypersensitive site specifically at a nucleosome that was bound by derivatives of the transcription factor Gal4p. Analysis of individual nucleosomes revealed that the SWI/SNF complex catalyzed eviction of histones from the Gal4-bound nucleosomes. Thus, the transient action of the SWI/SNF complex facilitated irreversible disruption of transcription factor–bound nucleosomes.

The remodeling of chromatin structures to generate deoxyribonuclease (DNase)-hypersensitive sites at regulatory elements precedes or occurs concurrently with the induction of transcription at many genes (*1*). Genetic and biochemical studies implicate an adenosine triphosphate (ATP)–dependent multisubunit protein complex, the SWI/SNF complex, in this process (*2*). The 2000-kD SWI/SNF complex binds to DNA and nucleosomes, perturbs histone-DNA interactions, and enhances the affinity of transcription factors for nucleosomal DNA (*3–7*). To investigate the mechanism by which SWI/SNF might remodel chromatin at transcriptional regulatory elements, we analyzed the action of the *Saccharomyces*

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cerevisiae SWI/SNF complex on an array of nucleosomes.

For these studies, it was necessary to avoid contamination from chromatin assembly factors or other nucleosome-remodeling activities [for example, nucleosome remodeling factor (NURF)] that can remain associated with templates reconstituted into nucleosomes in crude systems (*8–10*). Thus, we reconstituted nucleosome arrays in a purified system composed of only histones and DNA. Nucleosome cores were reconstituted on a 2.3-kb DNA fragment that contains a central nucleosome-length sequence with five Gal4 binding sites flanked on either side by five repeats of a nucleosome-positioning sequence from 5S ribosomal RNA genes (*11*). The 5S sequences sequester a nucleosome core on each repeat and also allow one to form over the Gal4 sites, which results in an array of 11 nucleosome cores (*12, 13*). Nucleosome core reconstitution onto the construct results in a pattern of nuclease protection and sensitivity indicative of a repeating array of positioned nucleosomes (Fig. 1B) (*12, 14*).

The action of a Gal4p derivative, Gal4-AH, and the SWI/SNF complex on the nucleosome array was analyzed by DNase I digestion (Fig. 1B) (*15, 16*). Five Gal4-AH dimers bound to the central nucleosome within the array, as was observed previously with mononucleosomes (*17, 18*). The addition of Gal4-AH resulted in a low-resolution footprint and DNase hypersensitivity flanking the five Gal4 sites in the center of the nucleosome array. When the purified yeast SWI/SNF complex was also included, the boundaries between adjacent nucleosomes became less defined, indicating that SWI/SNF affected the structure of all nucleosomes within the array in an ATP-dependent manner. The hypersensitivity flanking the Gal4 sites persisted in the presence of SWI/SNF, suggesting that this nucleosome remained bound by Gal4-AH.

Because there is insufficient SWI/SNF in yeast cells for it to remain stably associated with each potentially active promoter (*3, 4*; but see *19, 20*), SWI/SNF is likely to act transiently at promoters in vivo. To examine whether SWI/SNF induced persistent structural alterations in the nucleosome array, we diluted the complex to substoichiometric concentrations with a 20-fold excess of unlabeled oligonucleosomes after the binding reactions (Fig. 1A). This dilution removed the SWI/SNF complex from the disrupted nucleosome array (*21*). To examine the nucleosome occupying the Gal4 sites, the bound Gal4-AH was also removed by competition with a double-stranded Gal4-site oligonucleotide. These competition reactions had no effect on the nucleosome array that was not exposed to SWI/SNF or Gal4-AH (Fig. 1C). Moreover, competition with the array bound by Gal4-AH in the absence of SWI/SNF resulted in a loss of the Gal4-AH footprint and adjacent DNase I sensitivity, with the return to a configuration that resembled that of the array that had not been bound by Gal4-AH. This result suggests that, as when Gal4-AH binds to single nucleosomes (*9, 22*), the central nucleosome remained intact during Gal4-AH binding. When the SWI/SNF complex was removed from the array, the nuclease digestion pattern of the repeating positioned nucleosomes on the 5S sequences reappeared (Fig. 1C). In contrast, the nucleosome bound by Gal4-AH was affected differently. When the SWI/SNF complex and Gal4-AH were both removed, a site of DNase hypersensitivity remained at this location. Formation of this hypersensitive site within the array was dependent on Gal4-AH binding, the presence of SWI/SNF, and ATP. Thus, the combined action of Gal4-AH binding and the SWI/SNF complex resulted in an ATP-dependent permanent alteration to the nucleosome array, detected as a sequence-specific DNase-hypersensitive site.

The SWI/SNF-induced permanent DNase hypersensitivity at the Gal4 binding sites must represent either a structurally altered nucleosome core that is nuclease-sensitive or a region of naked DNA that results from loss of histones. Because nuclease digestion analysis of chromatin structure cannot distinguish between these possibilities, we performed more definitive experiments with mononucleosome substrates to address this issue.

The ability of the SWI/SNF complex to interact with both the Gal4 site and 5S nucleosomes was confirmed by the data shown in Fig. 2. Addition of the SWI/SNF complex perturbed the DNase digestion pattern of both the 5S mononucleosome (Fig. 2A) and the Gal4-site mononucleo-

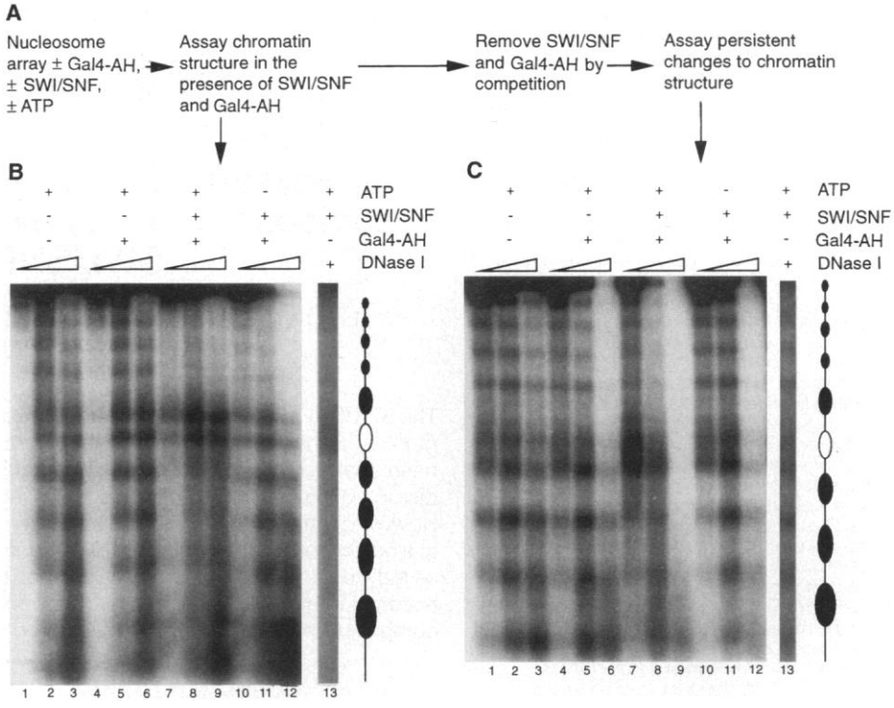

Fig. 1. Transient and persistent changes in chromatin structure mediated by the SWI/SNF complex. (**A**) The order of Gal4-AH and SWI/SNF binding to the nucleosome array, subsequent competition reactions, and the points of DNase I digestion analysis (vertical arrows). The end-labeled Mlu I–Pvu II fragment from plasmid pG5208-10 (*11*) was reconstituted into a nucleosome array (*13*) before incubation in the presence or absence of 50 nM Gal4-AH, 5 nM SWI/SNF, and 1 mM ATP (Mg^{2+} salt) as indicated (*15*). (**B**) Binding reactions were performed for 30 min at 30°C and then directly analyzed by DNase I digestion (*16*). (**C**) Binding was performed under conditions identical to those in (B) and was followed by competition of Gal4-AH and SWI/SNF with Gal4 oligonucleotide and nucleosomes (*15*) before DNase I digestion. Each set of conditions is represented by three lanes of increasing amounts of DNase I digestion (ramps) with the exception of lane 13 (B and C), which presents a single digestion condition. The positions of the 5S nucleosomes (black ovals) and the Gal4-site nucleosome (white oval) within the array are indicated. A DNase I–hypersensitive site that formed at the location of the Gal4-site nucleosome when the array was treated with SWI/SNF and ATP in the presence of Gal4-AH (B, lane 8) persisted after competition (C, lane 7), whereas the 5S nucleosome positioning was recovered after competition both above (C, lane 7) and below (C, lane 8) the hypersensitive site.

some (Fig. 2B). This disruption enhanced the affinity of Gal4-VP16 for the Gal4-site nucleosome. Both footprinting (Fig. 2B) and gel mobility-shift analysis (Fig. 2C) revealed that the binding of five Gal4-VP16 dimers to nucleosomal DNA in the presence of SWI/SNF was achieved at similar concentrations to those required for Gal4-VP16 binding to the corresponding naked DNA fragment. Thus, in the presence of the SWI/SNF complex, histone-mediated inhibition of Gal4-VP16 binding is not apparent. This result, together with the DNase-sensitive site formed in the region occupied by the central nucleosome, raises the possibility that the histones were evicted from the Gal4-site sequences as a consequence of Gal4-derivative binding and SWI/SNF action. We investigated this possibility with an oligonucleotide-competition gel mobility-shift assay that is capable of determining the fate of the core histones after transcription factor binding (*23*).

To examine the effects of SWI/SNF action and Gal4-AH binding on the underlying nucleosome core, we removed Gal4-AH by oligonucleotide competition after its binding in the presence of SWI/SNF (Fig. 3). In the absence of SWI/SNF, removal of bound Gal4-AH resulted in the reappearance of the original nucleosome core. Thus, Gal4-AH binding alone did not efficiently displace the underlying histone octamer. In contrast, when the SWI/SNF complex was present during Gal4-AH binding, removal of bound Gal4-AH and SWI/SNF released the fragment as naked DNA. Thus, disruption of this nucleosome by the SWI/SNF complex resulted in eviction of the histones from the Gal4-AH-bound nucleosome. Eviction of the histones by SWI/SNF was dependent on the binding of Gal4-AH and a hydrolyzable form of ATP (Fig. 3).

The data in Fig. 3 provide a molecular explanation for the appearance of the persistent hypersensitive site in the nucleosome array in response to the combined action of SWI/SNF and Gal4-AH binding (Fig. 1). Whereas the SWI/SNF complex interacts with multiple nucleosomes in the array, only the nucleosome bound by Gal4-AH underwent histone eviction. Thus, the nucleosome destabilization attributable to the binding of Gal4-AH (*9*, *22–24*) predisposed this nucleosome to histone loss in response to further disruption by the SWI/SNF complex (*25*). However, at different regulatory elements, the action of SWI/SNF and bound factors might result in persistent nucleosome disruption without the actual loss of histones (*26*).

These results suggest that ATP-dependent chromatin-remodeling machines like the SWI/SNF complex function in conjunction with nucleosome-bound transcription factors to generate regions of nucleosome disruption in chromosomal DNA.

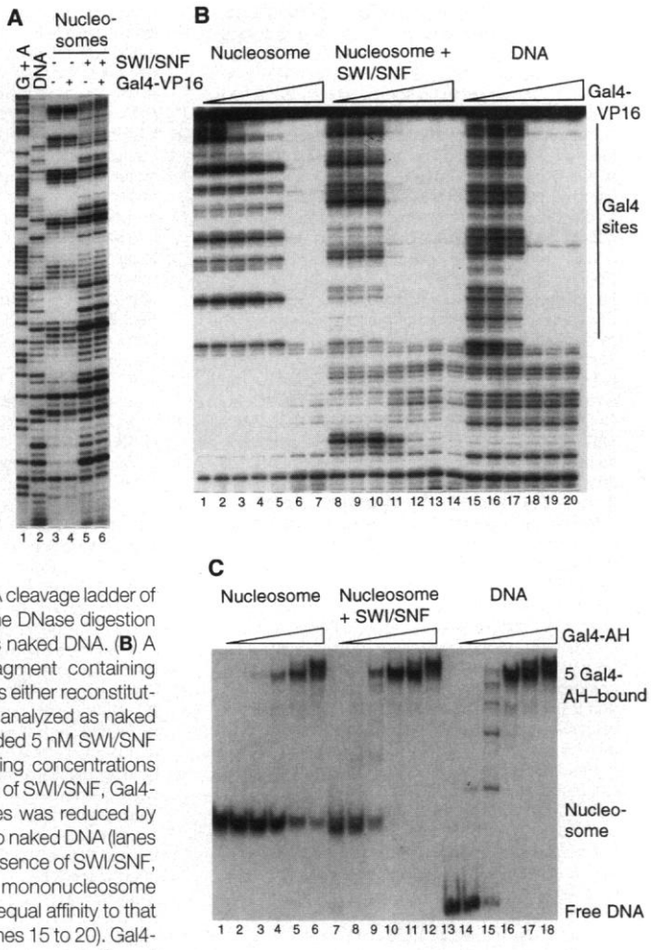

Fig. 2. Perturbation of both 5*S* and Gal4-site mononucleosomes and stabilization by SWI/SNF of Gal4 derivatives binding to their recognition sites. (**A**) A single copy of the 5*S* nucleosome-positioning sequence (*31*) was reconstituted into mononucleosome cores, incubated in the presence or absence of 105 nM Gal4-VP16 and 5 nM SWI/SNF as indicated, and analyzed by DNase I digestion. SWI/SNF perturbed the DNase digestion pattern of the 5*S* nucleosome independent of the presence of Gal4-VP16 (lanes 5 and 6). DNase I digestions of the mononucleosomes were performed as previously described (*4*). Lane 1, a G + A cleavage ladder of the 5*S* DNA probe; lane 2, the DNase digestion pattern of the same probe as naked DNA. (**B**) A nucleosome-length DNA fragment containing five Gal4 binding sites (*32*) was either reconstituted into nucleosome cores or analyzed as naked DNA. Binding reactions included 5 nM SWI/SNF where indicated and increasing concentrations of Gal4-VP16. In the absence of SWI/SNF, Gal4-VP16 affinity for the Gal4 sites was reduced by 10-fold (lanes 1 to 7) relative to naked DNA (lanes 15 to 20). However, in the presence of SWI/SNF, Gal4-VP16 binding to the mononucleosome (lanes 8 to 14) occurred with equal affinity to that observed with naked DNA (lanes 15 to 20). Gal4-VP16 concentrations were 0 in lanes 1, 8, and 15; 0.7 nM in lanes 2, 9, and 16; 2.1 nM in lanes 3, 10, and 17; 7 nM in lanes 4, 11, and 18; 21 nM in lanes 5, 12, and 19; 70 nM in lanes 6, 13, and 20; and 210 nM in lanes 7 and 14. DNase I digestions were performed as in (A). (**C**) Gel mobility-shift analysis of the effect of SWI/SNF on Gal4-AH binding to the five Gal4-site mononucleosome cores. Increasing amounts of Gal4-AH were incubated with the probe containing five Gal4 binding sites as naked DNA or as nucleosome cores in the presence or absence of 5 nM SWI/SNF (*33*). In the presence of SWI/SNF, the binding of five Gal4-AH dimers to the mononucleosomes occurred with an affinity similar to that for naked DNA (lanes 7 to 12 and 13 to 18). Gal4-AH concentrations were 0 in lanes 1, 7, and 13; 1.5 nM in lanes 2, 8, and 14; 5 nM in lanes 3, 9, and 15; 15 nM in lanes 4, 10, and 16; 45 nM in lanes 5, 11, and 17; and 135 nM in lanes 6, 12, and 18.

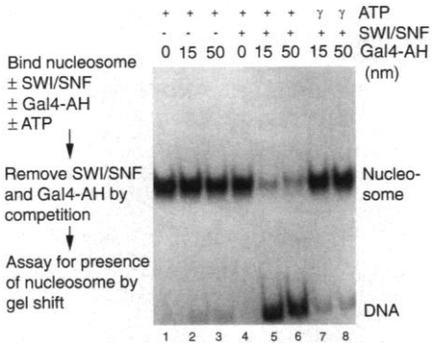

Fig. 3. SWI/SNF facilitation of histone eviction from transcription factor–bound nucleosomes. Binding reactions were performed with five Gal4-site nucleosomes (*34*) in the presence or absence of Gal4-AH at the concentrations indicated, 5 nM SWI/SNF, and either 1 mM ATP (+) or the nonhydrolyzable ATP analog adenosine 5′-*O*-(3-thiotriphosphate) (γ) as Mg^{2+} salts, as indicated. After the binding reactions, Gal4-AH and SWI/SNF were removed by competition with a Gal4 oligonucleotide and HeLa nucleosomes, enabling the fate of the Gal4-AH–bound nucleosome to be determined by gel mobility-shift assay. In this assay, if the histones were retained on DNA during the binding reaction, the mononucleosome core (nucleosome) reappears after Gal4-AH and SWI/SNF competition. If the histones were dissociated from nucleosomal DNA during the binding reaction, the DNA fragment is released as naked DNA after Gal4-AH and SWI/SNF competition. Histones were evicted from the Gal4-site DNA only in the presence of Gal4-AH, SWI/SNF, and a hydrolyzable form of ATP (lanes 5 and 6).

The persistence of the hypersensitive site after removal of the SWI/SNF complex may explain how this complex of relatively low abundance functions at a diverse range of loci in vivo. The creation of transcriptionally active chromatin conformations may require only the transient presence of SWI/SNF. The ability of transcription factors to predispose specific nucleosomes to persistent disruption by the SWI/SNF complex, coupled with the possible targeting of the SWI/SNF complex to particular regions of chromatin by interactions with transcription activators or RNA polymerase II holoenzyme (*6*, *19*, *27*), indicates how the SWI/SNF complex might generate specific changes in chromatin structure that are important in inducible or developmental pathways of gene regulation.

Additional ATP-dependent activities capable of chromatin remodeling have been identified in *Drosophila* embryo extracts (*10*, *28–30*). Although these activities include NURF, a distinct protein complex, the existing data are consistent with the possibility that these activities function by a mechanism similar to that described here for SWI/SNF. For example, the NURF complex also interacts with nucleosomes independent of transcription factors, but still contributes to the formation of factor-dependent DNase I–hypersensitive sites (*10*, *28*).

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15. Purification of the SWI/SNF complex and Gal4p derivatives, and their use in binding reactions (20 μl), was essentially as described (*4*). Binding reactions contained 2 μl of 10× binding buffer [200 mM Hepes (pH 7.5), 500 mM KCl, 30 mM $MgCl_2$, 50% (v/v) glycerol, 20 mM dithiothreitol, 10 μM $ZnCl_2$, 2 mM phenylmethylsulfonyl fluoride, bovine serum albumin (1 mg/ml)] and 2 μl each of SWI/SNF, Gal4p derivative, ATP (Mg^{2+} salt), or the appropriate control buffer as described (*4*). Gal4-AH was used unless indicated otherwise. Gal4(1–94) (NH_2-terminal 94 amino acids containing DNA binding and dimerization domains), Gal4-AH, and Gal4-VP16 all functioned equally in the assays described here (T. Owen-Hughes and J. L. Workman, unpublished results). For analysis of the array construct, 1 fmol of probe DNA and 62.5 fmol of donor nucleosomes were included in each reaction. When Gal4-AH and SWI/SNF were to be removed by competition, 1 μg of duplex Gal4 site–containing DNA (created by the annealing of the primers 5′-CTAGACCGGACGACAGTACTCCGACT-3′ and 5′-CTAGAGTCGGAGTACTGTCGTCCGGT-3′), 0.5 μg of HeLa nucleosomes, and 0.5 μg of calf thymus DNA (Sigma) were added to each binding reaction in 2 μl of CB [0.5 mM tris (pH 7.5), 0.5 mM EDTA, 1 M NaCl]. Incubation was extended for 1 hour at 37°C. Competition was judged to be complete by the inability of Gal4-AH or SWI/SNF to induce a shift in the mobility of nucleosomes subsequent to this treatment (Fig. 3).
16. DNase I digestions were performed at room temperature for 4 min. Gal4 oligonucleotide and nucleosomes were added immediately before DNase I digestion to binding reactions that were not subject to competition. Digestions were performed with 0.3, 1, and 3 U of DNase I (Boehringer Mannheim). Reactions were stopped with 1 volume of 20 mM tris-HCl (pH 7.5), 50 mM EDTA, 2% SDS, yeast tRNA (0.25 mg/ml), and proteinase K (0.2 mg/ml). Samples were incubated at 50°C for 1 hour before precipitation with 0.25 M NaCl and 3 volumes of ethanol. Digestion products were resuspended in 1× TAE loading buffer (5% glycerol, 40 mM tris-acetate, 1 mM EDTA 0.25% bromophenol blue) and resolved on 1% agarose gels.
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20. There is a discrepancy between estimates of the in vivo amounts of SWI/SNF in yeast. Purification of yeast SWI/SNF complexes by two different procedures led to estimates of 100 to 200 complexes per yeast cell (*3*, *4*). A 10-fold higher number was suggested by others on the basis of copurification of SWI/SNF complexes with RNA polymerase II holoenzyme (*19*). Resolution of the exact number of SWI/SNF complexes in a yeast cell and the extent of SWI/SNF interactions with RNA polymerase II holoenzyme awaits further analysis.
21. SWI/SNF binds to DNA (*7*) and to nucleosomes to form a high molecular mass complex. Addition of excess unlabeled nucleosomes or DNA can disrupt these complexes, indicating that SWI/SNF is effectively exchanged by this competition (J. Côté, T. Owen-Hughes, J. L. Workman, unpublished observations). In (*4*), the ratio of SWI/SNF to nucleosomes was such that this competition was not required in the gel mobility-shift analysis.
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26. Histone loss (in trans or in cis) is likely to occur from many enhancers or promoters in vivo in response to factor binding and disruption by SWI/SNF or other nucleosome-remodeling activities. However, at other regulatory elements, the action of SWI/SNF may stabilize factor binding without actually evicting histones, leading to a persistently disrupted factor-bound nucleosome [E. H. Bresnick, M. Bustin, V. Marsaud, H. Richard-Foy, G. L. Hager *Nucleic Acids Res.* **20**, 273 (1992); M. Truss, J. Bartsch, A. Schelbert, R. J. Hache, M. Beato, *EMBO J.* **14**, 1737 (1995)].
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31. A single copy of the 5*S* nucleosome-positioning sequence was generated by digestion of p207-12 with Ava I and Sca I, and labeled at the Ava I ends with the Klenow fragment.
32. The nucleosome-length DNA fragment containing five Gal4 binding sites was excised from plasmid pG5H (*18*) with the enzymes Nhe I and Pst I, and end-labeled at the Nhe I site.
33. Binding reactions for the gel mobility-shift analysis were performed for 30 min at 30°C. SWI/SNF was then removed from the DNA by competition with 0.5 μg of HeLa oligonucleosomes and 0.5 μg of calf thymus DNA at 30°C for 30 min, enabling Gal4-AH bound complexes to be resolved by native polyacrylamide gel electrophoresis in 0.5× tris-borate-EDTA. Binding reactions shown in Fig. 2 all contained 25 ng of total nucleosomes and 0.2 ng of probe DNA.
34. The DNA sequence containing the five Gal4 sites was excised from plasmid pG5208-10 (*11*) by digestion with Sal I, end-labeled with the Klenow fragment, and reconstituted into nucleosome cores.

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