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RESEARCH ARTICLE

Stimulation of GAL4 Derivative Binding to Nucleosomal DNA by the Yeast SWI/SNF Complex

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The SWI/SNF protein complex is required for the enhancement of transcription by many transcriptional activators in yeast. Here it is shown that the purified SWI/SNF complex is composed of 10 subunits and includes the SWI1, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products. The complex exhibited DNA-stimulated adenosine triphosphatase (ATPase) activity, but lacked helicase activity. The SWI/SNF complex caused a 10- to 30-fold stimulation in the binding of GAL4 derivatives to nucleosomal DNA in a reaction that required adenosine triphosphate (ATP) hydrolysis but was activation domain-independent. Stimulation of GAL4 binding by the complex was abolished by a mutant SWI2 subunit, and was increased by the presence of a histone-binding protein, nucleoplasmin. A direct ATP-dependent interaction between the SWI/SNF complex and nucleosomal DNA was detected. These observations suggest that a primary role of the SWI/SNF complex is to promote activator binding to nucleosomal DNA.

The yeast SWI1, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products are required for the induced expression of a large set of genes (*1*). Furthermore, SWI/SNF products are required for the enhancement of transcription by several gene-specific activator proteins in yeast, such as GAL4 (*2*), *Drosophila ftz* (*2*), mammalian glucocorticoid and estrogen receptors (*3*), and LexA-GAL4 and LexA-Bicoid fusion proteins (*4*). The SWI/SNF gene products function as components of a large multi-subunit protein complex of approximately 2×10^6 daltons (*5*, *6*). One activity of this complex is to associate with the mammalian glucocorticoid receptor (*3*). These observations suggested that homologs of the *SWI/SNF* genes would be present in *Drosophila* and mammals (*3*). Candidate homologs of the *SWI2* gene have been identified in *Drosophila* (*7*), mouse (*8*), and human (*9*, *10*). Protein chimeras between either BRG1 (one of two putative human homologs) or brahma (brm, the putative *Drosophila* homolog) and SWI2 are functional in yeast, which suggests that these relatives are functional homologs (*9*, *11*). Gel filtration data suggest that the BRG1 protein may also be a subunit of a large protein complex (*9*).

A current hypothesis of how the SWI/SNF complex facilitates activator function suggests that the complex antagonizes chromatin-mediated transcriptional repression. The relation between the SWI/SNF complex and chromatin structure was suggested because mutations in genes that encode chromosomal proteins alleviate the phenotypes of *swi* and *snf* mutants. Mutations that inactivate the *SIN1* gene, which encodes a putative nonhistone chromatin component, or in the *SIN2* gene, which encodes histone H3, alleviate the defects in growth and in transcription caused by mutations in

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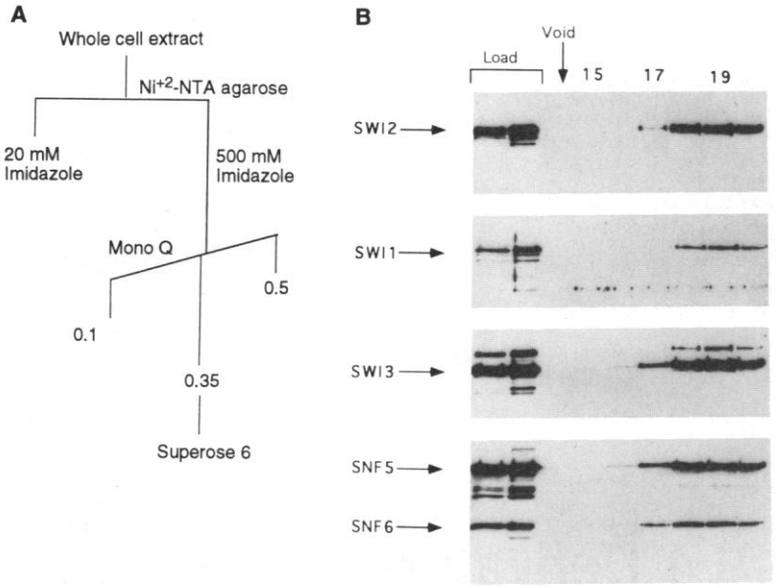
Table 1. Purification of the SWI/SNF complex was followed by protein immunoblots probing for the SWI2-HA-6HIS fusion protein. Similar levels of purification were obtained in at least four preparations.

	Volume (ml)	Concentration (mg/ml)	Total protein (mg)	Specific activity (units*)
WCE	130	21.5	2800	1
Ni^{2+} eluate	43	1	43	65
Mono Q	2	0.35	0.7	3,965
Superose	1.5	0.02	0.03	91,195

*One unit is equivalent to the amount of SWI2-HA-6HIS fusion protein in 100 μg of whole cell extract as measured by immunoblots. The overall yield is estimated to be approximately 25 percent.

SWI1, *SWI2*, or *SWI3* (*12*, *13*). A deletion of one of the two gene clusters that encodes histones H2A and H2B can suppress the defect in *SUC2* gene expression, which results from mutations in *SWI2*, *SNF5*, or *SNF6* (*14*).



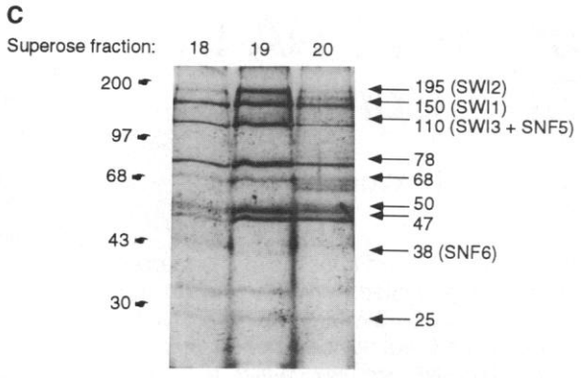


Fig. 1. Purification of the yeast SWI/SNF complex. (**A**) Purification scheme (*45*). (**B**) Protein immunoblot analysis of Superose 6 gel filtration fractions. 10-μl aliquots of Superose 6 fractions (500 μl) were electrophoresed on a 10 percent SDS gel, transferred to nitrocellulose, and processed for protein immunoblot analysis as described (*5*). Arrows to the left of the figure show immunoreactive polypeptides specific for the indicated SWI or SNF gene product. One blot was probed sequentially by inactivating the peroxidase-coupled secondary antibodies by treatment with 15 percent hydrogen peroxide in TBS for 30 min at room temperature. (**C**) SDS-PAGE analysis of Superose 6 fractions. 200-μl aliquots of the indicated Superose 6 fractions were trichloroacetic acid (TCA)–precipitated, resuspended in SDS loading buffer, and subjected to electrophoresis on a 10 percent SDS gel. SWI and SNF polypeptides were identified on the basis of their relative migration in the protein immunoblot in (B), which was run in parallel. Size markers are indicated in kilodaltons.

Table 2. GAL4 derivatives require SWI/SNF function for transcription enhancement. Strains were grown in S medium (*38*) containing 2 percent galactose and 0.5 percent sucrose. β-Galactosidase assays were performed (*39*) on at least three independent transformants, and the Miller units (*40*) were averaged. Standard deviations were <20 percent. The activity for GAL4 in the *SWI*+ strain was 786 Miller units. The activity of GAL4-AH in the *SWI*+ strain was 386 units. The activity of GAL4 in the *swi1*− strain is taken from (*2*). ND, not determined. For GAL4-AH studies, two plasmids were introduced into strains CY296 (*SWI*+ *gal4Δ::LEU2*) and CY297 (*swi1Δ::LEU2 gal4Δ::LEU2*): (i) plasmid pEG50 (*41*), a 2 μM vector that expresses GAL4-AH from the yeast ADH1 promoter, and (ii) plasmid p632-17b-2 (*42*), a 2 μM reporter that contains two GAL4 binding sites upstream of a *GAL1-lacZ* reporter gene. For studies of intact GAL4, a GAL4 reporter plasmid, p121-Δ10 (*43*), which contains two GAL4 binding sites, was introduced into CY407 (*swi2Δ::HIS3 GAL4*+ with one of the following three plasmids: (i) CP337 contains wild-type *SWI2* gene in vector RS315 (*44*), (ii) CP359 contains *swi2K798A-HA-6HIS* in RS315, or (iii) RS315.

Strain	GAL4 (%)	GAL4-AH (%)
SWI+	100	100
swi1−	7	13
swi2−	1	ND
swi2K798A	5	ND

The SWI2 subunit contains seven sequence motifs that are characteristic of nucleic acid–stimulated ATPases (*15*). A small subset of these ATPases are also known to be DNA or RNA helicases. The SWI2 ATPase motifs are well conserved among the putative SWI2 homologs, and mutational studies in yeast and in mammalian cells indicate that these sequences are crucial for SWI2 function (*9*, *16*). On the basis of these sequence homologies, it was suggested that the SWI/SNF complex might function as a DNA helicase that disrupts chromatin structure (*17*).

In vitro and in vivo studies of nucleosome function in transcription indicate that two primary steps prior to the initiation of transcription are inhibited by nucleosomes (reviewed in *18*). The first is the ability of upstream activators to bind to their recognition sites, and the second is the ability of the general factors and RNA polymerase II to form a preinitiation complex at the TATA box and transcription initiation site. In this second step, nucleosomes suppress basal transcription, but this suppression can be overcome by the activation domains of upstream regulatory factors (for example, GAL4-VP16) (*19*, *20*). Several factors that influence the initial binding of upstream regulatory factors to nucleosomes include: differential affinity of different factors for nucleosomal DNA, position of the recognition sites on the nucleosome, histone acetylation, and the cooperative binding of multiple factors (*21*, *22*). Furthermore, the binding of GAL4 derivatives, USF, and Sp1 to nucleosomes is enhanced by the pentameric histone-binding protein, nucleoplasmin (*23*). This raises questions concerning the role of accessory protein complexes in the assistance of transcription factor binding to nucleosomal DNA in vivo.

Purification and characterization of the SWI/SNF protein complex. We have described a yeast strain that contained a *SWI2* fusion gene with a hemaglutinin (HA) epitope tag and six tandem histidines at the extreme COOH-terminus of the SWI2 protein (*5*). Whole cell extracts (WCE) were made from a small scale culture of this strain and the SWI/SNF complex was partially purified by a combination of affinity chromatography on Ni^{2+}-NTA (Ni^{2+}-nitrilotriacetic acid) agarose and gel filtration chromatography. This purification procedure was scaled up for a 36-liter culture, and an ion exchange step was added (Fig. 1A and Table 1). The three-step purification resulted in greater than a 90,000-fold enrichment of the SWI2 subunit, with an overall yield of about 25 percent (Table 1). The SWI1, SWI2, SWI3, SNF5, and SNF6 polypeptides all co-eluted from gel filtration in a

large complex of approximately 2 megadaltons (Fig. 1B). To determine whether the SWI/SNF complex might contain additional polypeptides, we analyzed the peak fractions from Superose 6 chromatography by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1C). Five additional polypeptides (78 kD, 68 kD, 50 kD, 47 kD, and 25 kD) co-eluted with the other SWI/SNF subunits from Superose 6 (peak in fraction 19). Each of these were present in at least four independent preparations of the SWI/SNF complex; furthermore, all 10 polypeptides were immunoprecipitated from the Mono Q pool by a monoclonal antibody to the HA epitope (*24*). A similar spectrum of SWI/SNF subunits was also immunoprecipitated by antibodies to the SWI3 and SNF6 subunits (*6*).

We assayed fractions from the Superose 6 chromatography step for DNA-stimulated ATPase activity (*25*) and detected an activity that peaked with the SWI/SNF complex, in agreement with other results (*6*). The ATPase activity was proportional to the amount of protein added to the reaction and was greatly diminished when extracts were purified from a *swi2* mutant. The SWI/SNF ATPase activity was stimulated 31-fold by double-stranded DNA, 43-fold by nucleosomal DNA, and 51-fold by single-stranded DNA (*25*). Poly (U), which is a substrate for an RNA-stimulated ATPase (*26*), did not stimulate the ATPase activity of the SWI/SNF complex. The specific activity of the SWI/SNF ATPase in the presence of nucleosomal DNA was approximately 60 pmol of inorganic phosphate (Pi) released per minute per microgram of SWI/SNF complex, a value within the range of activities measured for other nucleic acid–stimulated ATPases (*26*). An analysis of ATPase kinetics with constant protein and nucleosomal DNA was carried out over a 200-fold range of ATP concentrations (5 to 1000 μM). The K_m value, which represents the ATP concentration at half maximal velocity, was calculated from double reciprocal plots to be 4.5×10^{-5} M ATP.

It has been proposed that the SWI/SNF complex might function as a DNA helicase that disrupts chromatin structure (*17*). We tested the purified SWI/SNF complex for helicase activity in an oligonucleotide release assay (*27*) using three different types of substrates: an annealed oligonucleotide with flushed ends, an annealed oligonucleotide with a 3′ single-strand extension, and an annealed oligonucleotide with a 5′ single-strand extension. Either SV40 T-antigen or high concentrations of yeast replication protein A (yRPA) were used as positive controls. In all cases, we were unable to detect helicase activity associated with the SWI/SNF complex (*28*).

SWI/SNF complex stimulates binding of GAL4 derivatives to nucleosomal DNA. In vivo transcriptional enhancement by the yeast GAL4 activator was reduced at least tenfold in the absence of SWI products (*2*). One possibility is that the SWI/SNF complex stimulated the binding of GAL4 to the in vivo chromatin template. Because the transcriptional activity of GAL4-AH, a small derivative of GAL4, was also dependent upon SWI function in vivo (Table 2), we tested the effect of the SWI/SNF complex on the binding of GAL4-AH to nucleosomal DNA in a purified in vitro system. A 154-bp DNA probe that contained a single GAL4 binding site 32 bp from one end (*29*), was assembled into a single nucleosome core by histone octamer transfer (*30*). A gel retardation assay performed with this nucleosome-reconstituted probe is shown (Fig. 2A). In this nucleosome-reconstitution, approximately 85 percent of the probe DNA was assembled into nucleosome cores (nucleosome), and 15 percent remained naked DNA (DNA). When increasing amounts of GAL4-AH protein were added to the reconstituted nucleosome, binding was initially observed to the free DNA, which produced a GAL4-DNA complex. At higher concentrations, GAL4-AH bound to the nucleosome, which yielded the ternary, GAL4-nucleosome complex (Fig. 2A). These results are identical to those of stud-

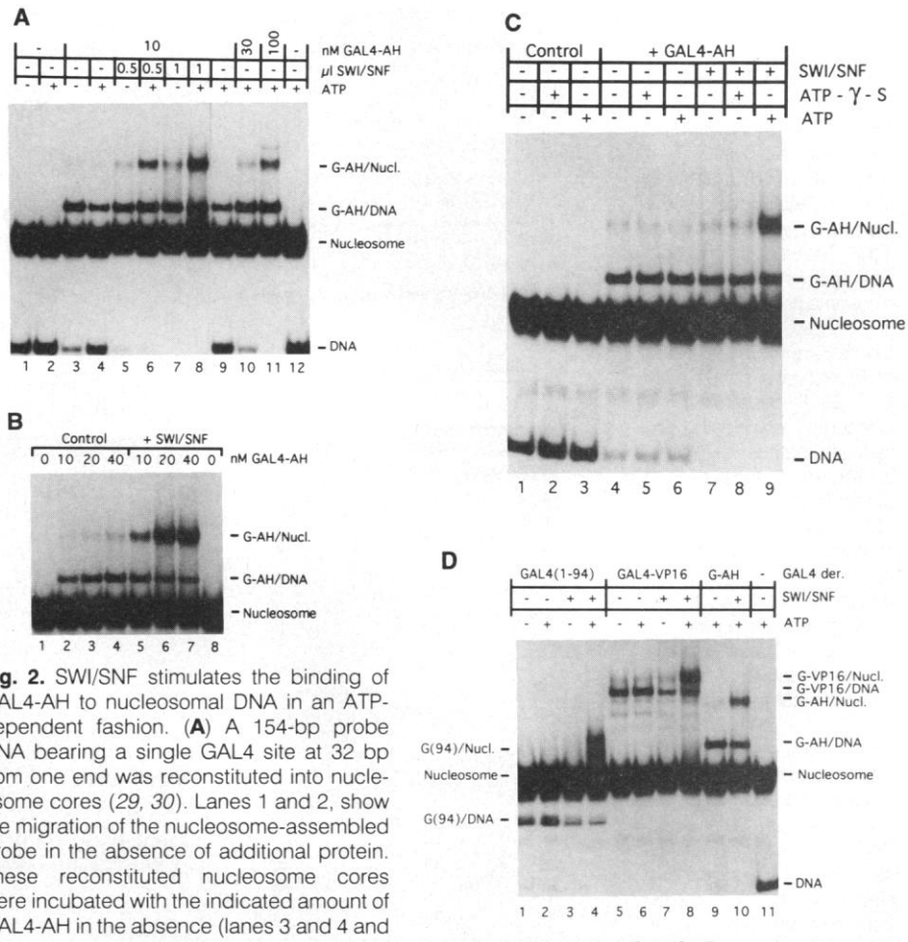


Fig. 2. SWI/SNF stimulates the binding of GAL4-AH to nucleosomal DNA in an ATP-dependent fashion. (**A**) A 154-bp probe DNA bearing a single GAL4 site at 32 bp from one end was reconstituted into nucleosome cores (*29, 30*). Lanes 1 and 2, show the migration of the nucleosome-assembled probe in the absence of additional protein. These reconstituted nucleosome cores were incubated with the indicated amount of GAL4-AH in the absence (lanes 3 and 4 and 9 to 11) or presence (lanes 5 to 8) of the indicated amount of purified SWI/SNF complex (50 or 100 ng, 2.5, or 5 nM). In addition to the probe, each reaction also contained 25 ng (25 nM) of cellular donor nucleosome core DNA. 1 mM ATP was also added to the binding reaction where indicated. The position of complexes resulting from GAL4-AH binding to the small fraction of naked DNA (G-AH/DNA) and GAL4-AH binding to the nucleosome cores (G-AH/Nucl.) are indicated. (**B**) Reconstituted nucleosome cores (as in A) were incubated with increasing concentrations of GAL4-AH in the absence (lanes 1 to 4) or presence (lanes 5 to 8) of 1 μl of purified SWI/SNF complex (100 ng, 5 nM). 1 mM ATP was present in each binding reaction. (**C**) Nucleosome cores (as in A) were incubated with 10 nM of GAL4-AH alone (lanes 4 to 6) or in the presence of 1 μl of purified SWI/SNF complex (lanes 7 to 9). 1 mM ATP or 1 mM ATP-γ-S (nonhydrolyzable analog) was also present when indicated. (**D**) Reconstituted nucleosome cores (as in A) were incubated with either 10 nM GAL4(1-94) (lanes 1 to 4), GAL4-VP16 (lanes 5 to 8), or 10 nM GAL4-AH (lanes 9 and 10) in the absence (lanes 1, 2, 5, 6, and 9), or the presence (lanes 3, 4, 7, 8, and 10) of 1 μl of purified SWI/SNF complex (100 ng, 5 nM). Adenosine triphosphate (1 mM) was also present in the binding reaction when indicated (lanes 2, 4, 7, and 9 to 11).

ies in which the identity of these respective complexes was established (*20*, *22*, *23*).

The binding of GAL4-AH to the reconstituted nucleosome probe was stimulated by the SWI/SNF complex in an ATP-dependent manner. At a GAL4-AH concentration of 10 nM, very little probe was present in the GAL4-nucleosome complex (Fig. 2A). Moreover, GAL4-AH binding to the nucleosomes was not stimulated by the addition of 0.5 μl (50 ng) of SWI/SNF complex added in the absence of ATP. By contrast, upon addition of ATP, the amount of GAL4-nucleosome complex was increased four- to fivefold in the presence of 50 ng of the SWI/SNF complex. Addition of twice the amount of SWI/SNF complex increased the GAL4-AH binding eight- to tenfold and the stimulation remained ATP-dependent. Stimulation of GAL4-AH binding to nucleosome cores by the SWI/SNF complex was also observed at several GAL4-AH concentrations (Fig. 2B). The ATP requirement for stimulation could not be substituted by a nonhydrolyzable analog, ATP-γ-S (*31*), which indicates that ATP hydrolysis was required for the stimulation (Fig. 2C). Because a fourfold increase in the amount of the GAL4-AH-nucleosome complex required at least tenfold higher concentrations of GAL4-AH (Figs. 2A and 4C), the SWI/SNF complex appears to increase the affinity of GAL4-AH for nucleosomal DNA by over an order of magnitude.

We tested whether the stimulation in GAL4-AH binding by the SWI/SNF complex was also dependent upon a transcription activation domain. A gel retardation experiment was performed with a nucleosomal probe and two additional GAL4 derivatives, GAL4 (1-94), which contains only the GAL4 DNA binding domain of GAL4, and GAL4-VP16, which contains the potent VP16 activation domain (Fig. 2D). In a manner similar to GAL4-AH, the SWI/SNF complex stimulated the binding of GAL4 (1-94) and GAL4-VP16 to nucleosomal DNA to similar extents in an ATP-dependent fashion. These results indicate that this function of the SWI/SNF complex does not require a functional transcription activation domain, and are consistent with a model in which SWI/SNF complex functions by increasing the affinity of the DNA binding domain of GAL4. This is also consistent with previous studies that illustrate activation domain-independent disruption of nucleosomes by GAL4 derivatives both in vitro and in vivo (*32*).

During our analyses of nucleosomal binding, we consistently observed that the addition of the SWI/SNF complex also led to a small increase in the amount of the GAL4-AH-DNA complex and a decrease in free DNA (Fig. 2C). This modest stimulation of the binding of GAL4-AH to naked DNA (less than twofold) did not appear to require ATP (Fig. 2C). We have further investigated this activity and found that the ability of SWI/SNF complex to stimulate GAL4-AH binding to naked DNA was more apparent with a probe bearing two low-affinity GAL4 sites with which we observed a two- to fourfold stimulation of GAL4-AH binding (*28*). In contrast to the dramatic 10- to 30-fold stimulation of nucleosomal binding, the small effect on naked DNA binding did not require ATP.

We performed deoxyribonuclease I (DNase I) footprinting on nucleosomal templates to further quantitate the increase in binding affinity. A titration of GAL4-AH binding to naked DNA or nucleosome-reconstituted DNA assayed by DNase I footprinting is shown (Fig. 3A). At a GAL4-AH concentration of 3 nM, the GAL4 site on the naked DNA probe was more than 50 percent occupied by GAL4-AH. In contrast, at least 100-fold more GAL4-AH (>300 nM) was required for the

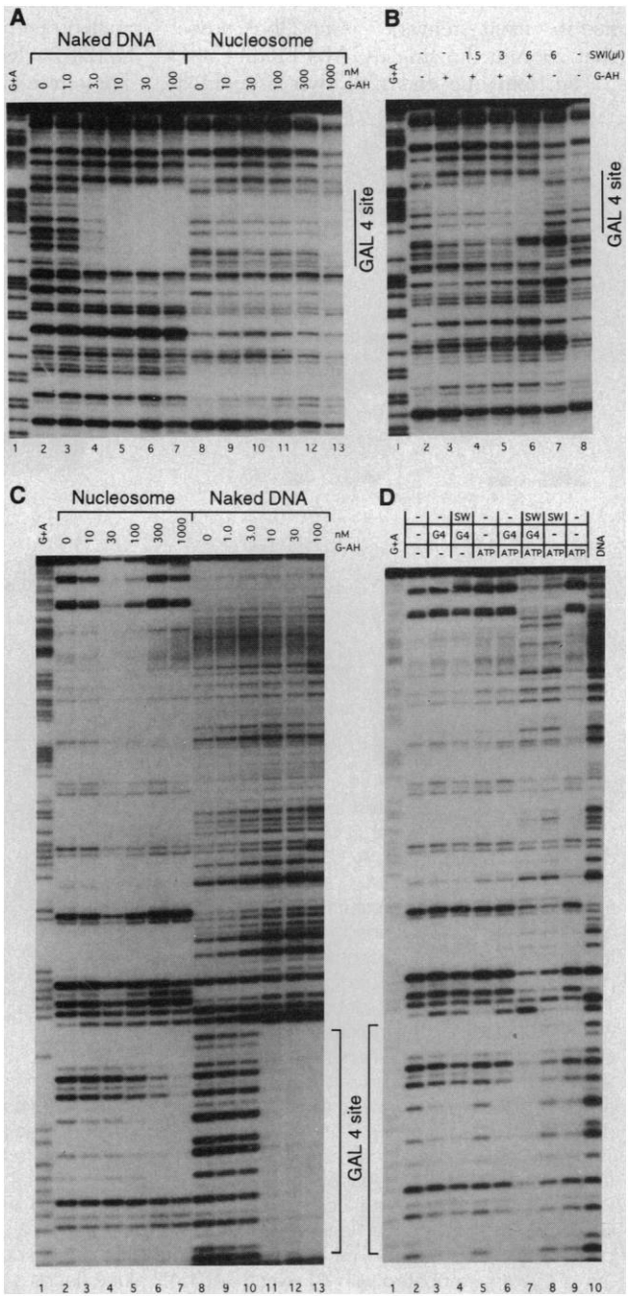


Fig. 3. Enhancement of GAL4-AH binding to nucleosomal DNA by SWI/SNF complex is specific for its binding site. (**A**) A 153-bp DNA probe containing a single GAL4 site at 43 bp from one end (*29*) was incubated with the indicated concentrations of GAL4-AH (lanes 2 to 7). Binding was analyzed by DNase I footprinting (*46*) as shown and the GAL4 binding site is indicated by the bar on the right. The same fragment was reconstituted into nucleosome cores and similarly analyzed for GAL4-AH binding at the indicated concentrations (lanes 8 to 13). (**B**) Reconstituted nucleosome cores as in (A) were incubated with 100 nM of GAL4-AH in the presence of increasing amount of purified SWI/SNF complex (150 to 600 ng, 3.8 to 15 nM) (lanes 4 to 6). Binding was analyzed by DNase I footprinting as in (A); 1 mM ATP was present in each binding reaction. Note that lane 6 has to be compared to lane 7 (600 ng of SWI/SNF complex, no GAL4-AH) because SWI/SNF complex by itself modified the DNase I digestion pattern of nucleosomal DNA. See also (D). (**C**) A 154-bp probe harboring a single GAL4 site at 32 bp from one end (same as in Fig. 2) was analyzed by footprinting assay using increasing concentration of GAL4-AH like in (A) as naked DNA (lanes 8 to 13) or nucleosome cores (lanes 2 to 7). The nucleosome cores reconstituted on that DNA probe are rotationally phased as shown by the periodicity of DNase I hypersensitivity every ten base pairs (lane 2 compared to lane 8). (**D**) Footprinting assay of the reconstituted nucleosome cores as in (C) with 0 or 100 nM (lanes 3, 4, 6, and 7) of GAL4-AH in the absence or the presence (lanes 4, 7, and 8) of purified SWI/SNF complex (6 μl, 600 ng). Adenosine triphosphate was also present in lanes 5 to 9. The DNase I digestion pattern was disrupted in the presence of SWI/SNF complex only when ATP was also present (lanes 7 and 8 versus lane 4).

same degree of occupancy when the GAL4-AH site was contained in the reconstituted nucleosome core. At a GAL4-AH concentration of 100 nM, little protection of the GAL4 site was observed, whereas significant protection was observed with 1000 nM GAL4-AH. However, upon addition of increasing concentrations of SWI/SNF complex in the presence of ATP, complete occupancy of the nucleosomal site was observed with 100 nM GAL4-AH (Fig. 3B).

Stimulation of GAL4-AH binding was also apparent on nucleosome cores in which the DNA helix was rotationally phased on the surface of the histone octamer. This is indicated by the approximately 10-bp repeating pattern of DNase I cleavage of the nucleosomal DNA (Fig. 3C). The GAL4 site on the naked DNA probe was completely protected at 10 nM GAL4-AH, whereas the nucleosome-reconstituted probe was only partly protected at 1000 nM GAL4-AH. The affinity of GAL4-AH for the rotationally phased nucleosome core was increased by over an order of magnitude by the presence of the SWI/SNF complex and ATP (Fig. 3D). At a concentration of 100 nM, GAL4-AH was insufficient to protect the GAL4 site on the nucleosome core by itself or in the presence of SWI/SNF complex in the absence of ATP. However, in the presence of SWI/SNF complex and ATP, 100 nM GAL4-AH resulted in more efficient protection of the GAL4 site than that provided by 1000 nM GAL4-AH in the absence of SWI/SNF. These footprinting studies confirm that the SWI/SNF complex increases the affinity of GAL4-AH for nucleosomal DNA by at least an order of magnitude in an ATP-dependent reaction.

Deoxyribonuclease I digestion experiments also indicated a direct interaction between the SWI/SNF complex and nucleosomal DNA in the absence of GAL4-AH (Fig. 3D). Addition of SWI/SNF complex to this rotationally positioned nucleosome core resulted in a perturbation of the 10-bp DNase I digestion ladder in the presence or absence of GAL4-AH. In the presence of SWI/SNF complex, new DNase I cleavage sites were observed throughout the nucleosome digest that were also present in the digestions of naked DNA but not in the digests of the nucleosome cores in the absence of SWI/SNF. In a manner similar to other SWI/SNF functions on nucleosomal DNA, this activity required ATP. In addition, no change in the DNase I digestion pattern of naked DNA was observed in the presence of the SWI/SNF complex and ATP (*33*). Thus, the SWI/SNF complex specifically perturbs the rotational orientation of DNA on the surface of the nucleosome core in an ATP-dependent manner, which leads to a stimulation in the binding of GAL4 derivatives.

A SWI/SNF complex containing a mutant SWI2 subunit defective in the stimulation of nucleosomal DNA binding by GAL4-AH. The requirement for ATP hydrolysis suggested that the SWI2 subunit of the SWI/SNF complex participates in the stimulation of nucleosome binding by GAL4-AH. We purified SWI/SNF complex from a *swi2* mutant, *swi2K798A*, in which the conserved lysine within the putative ATP binding loop had been changed to an alanine, which is a mutation that does not affect the assembly of the complex (*5*). This *swi2* mutant does not complement a *swi2Δ* allele and exerts a dominant negative phenotype in the presence of wild-type *SWI2* (*9*). Enhancement of transcription by GAL4 is also crippled by mutation of this putative ATP binding loop (Table 2). Changing this conserved lysine to an arginine decreases the ATP hydrolysis activity of a bacterially expressed SWI2 fusion protein (*16*).

SWI/SNF complex that contains the SWI2K798A subunit was purified through the same three fractionation steps described in Fig. 1 (Fig. 4A). At equal concentrations of wild-type and mutant complexes however, the ATPase activity of the mutant complex was reduced about eightfold (Fig. 4B). Likewise, the mutant SWI/SNF complex was defective in the stimulation of GAL4 binding to nucleosomal DNA (Fig. 4C). Although the SWI2K798A mutation might exert an indirect effect on other subunits of the SWI/SNF complex, these results are consistent with the view that SWI2 encodes the ATPase subunit of the SWI/SNF complex that is required to stimulate nucleosome binding by GAL4-AH. Furthermore, these data confirm that the stimulation of nucleosomal binding is the result of the SWI/SNF complex.

SWI/SNF complex function complemented by the presence of a histone binding protein. Transcription studies of 5S RNA genes bound to H3-H4 tetramers have shown that transcription is enhanced relative to 5S RNA genes bound to complete histone octamers (octamers that also contain 2 H2A-H2B dimers) (*34*). In addition, the binding of GAL4-AH, USF, and Sp1 to nucleosomal DNA was stimulated by the histone-binding protein, nucleoplasmin (*23*), which is a specific chaperone of histones H2A and H2B (*35*). Nucleoplasmin appeared to increase the avidity of GAL4-AH binding to nucleosomal DNA by providing a specific chaperone onto which H2A-H2B dimers could transfer upon GAL4-AH binding (*23*). We reasoned that if stimulation of GAL4-AH binding to nucleosomes by the SWI/SNF complex involved relief of repression by the histone H2A-H2B dimers, then addition of small amounts of nucleoplasmin might facilitate the reaction.

Figure 5 shows the results of adding nucleoplasmin to a GAL4-AH-nucleosome binding reaction in the presence or absence of the SWI/SNF complex. In the absence of

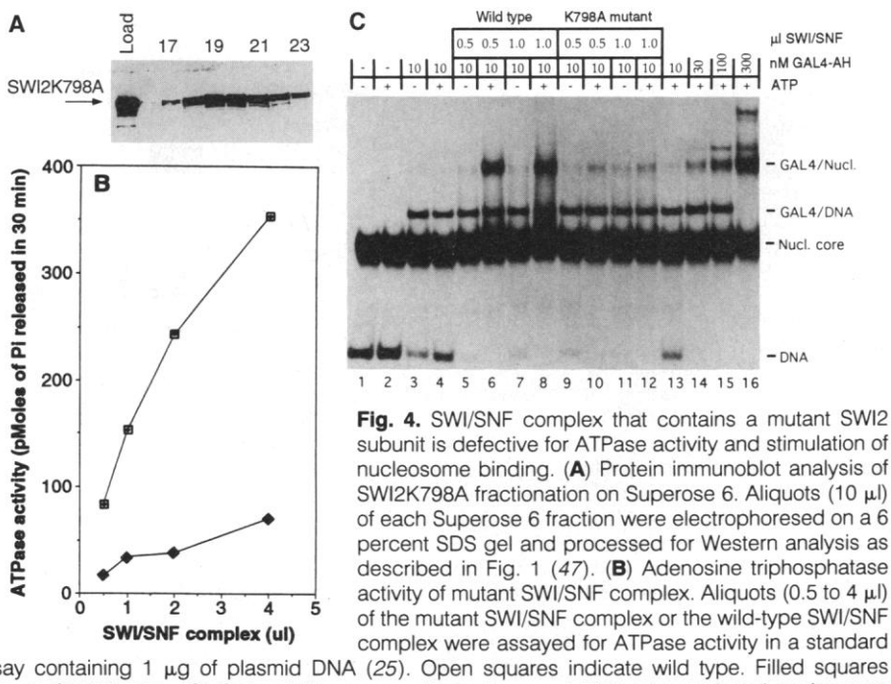


Fig. 4. SWI/SNF complex that contains a mutant SWI2 subunit is defective for ATPase activity and stimulation of nucleosome binding. (**A**) Protein immunoblot analysis of SWI2K798A fractionation on Superose 6. Aliquots (10 µl) of each Superose 6 fraction were electrophoresed on a 6 percent SDS gel and processed for Western analysis as described in Fig. 1 (*47*). (**B**) Adenosine triphosphatase activity of mutant SWI/SNF complex. Aliquots (0.5 to 4 µl) of the mutant SWI/SNF complex or the wild-type SWI/SNF complex were assayed for ATPase activity in a standard assay containing 1 µg of plasmid DNA (*25*). Open squares indicate wild type. Filled squares indicate SWI2K798A. (**C**) Gel mobility assay with nucleosomal DNA. Reconstituted nucleosome cores as in Fig. 2 were incubated with the indicated amount of GAL4-AH in the absence (lanes 4 and 5 and 13 to 16) or the presence (50 or 100 ng) of either the wild-type SWI/SNF complex (lanes 5 to 8), or the mutant SWI/SNF complex (lanes 9 to 12). Adenosine triphosphate (1 mM) was present in the binding reaction when indicated.

nucleoplasmin, SWI/SNF complex induced a four- to fivefold increase in the GAL4-nucleosome complex (Fig. 5A). Addition of 5 ng of nucleoplasmin increased the SWI/SNF effect and doubled the amount of the GAL4-nucleosome complex. In the absence of SWI/SNF complex, this low concentration of nucleoplasmin (5 ng) had little effect on GAL4-AH binding. At tenfold higher concentrations (50 ng), nucleoplasmin stimulated the binding of GAL4-AH two- to threefold. But, in the presence of SWI/SNF complex, there was no additional stimulation observed above that seen with 5 ng of nucleoplasmin. Thus, at limiting concentrations of nucleoplasmin, the stimulatory function was additive with SWI/SNF, and the SWI/SNF stimulation precluded further stimulation by higher concentrations of nucleoplasmin. This result is consistent with the view that the mechanisms by which SWI/SNF complex and nucleoplasmin stimulate GAL4-AH binding are related.

At high concentrations of the SWI/SNF complex, the migration of the GAL4-AH-nucleosome complex and the nucleosome core alone became smeared or stuck in the wells of the gel (Fig. 5, B and C). This is consistent with a direct interaction between the nucleosome and the SWI/SNF complex (Fig. 3D). The addition of nucleoplasmin to this reaction resolved this putative SWI/SNF-nucleosome interaction and generated distinct GAL4-nucleosome and nucleosome complexes (Fig. 5B). Although the basis for this effect is unknown, it may indicate that the histone-binding protein allowed the release of SWI/SNF-nucleosome interactions.

The interaction of SWI/SNF complex with nucleosome cores not bound by GAL4-AH indicates that at high concentrations the complex also interacted with the cellular donor nucleosome cores present in the binding reactions (*30*). (This assumes that the complex does not distinguish between nucleosome cores bearing labeled probe DNA and those bearing unlabeled cellular DNA.) These results permitted an analysis of the core histones in the presence of a high concentration of SWI/SNF complex to determine if any proteolysis occurred that might have contributed to the increase in GAL4-AH binding (*22*). Binding reactions in the presence or absence of an excess of SWI/SNF were scaled up and analyzed by both mobility shift and SDS-PAGE of the core histones. Under these conditions, all of the nucleosome cores, regardless of GAL4-AH binding, were shifted to the well in the presence of the high concentrations of complex indicating that all of the nucleosomes interacted with SWI/SNF complex (Fig. 5C). However, as illustrated in the protein gel of the same reactions (Fig. 5D), the core histones were intact in the presence of SWI/SNF complex. The only additional bands in the histone region of the gel were contaminants of the SWI/SNF preparation. This indicates that the function of the complex in the stimulation of GAL4-AH binding to nucleosome cores was neither the result of contaminating proteases (which is also indicated by the loss of activity in the mutant complex; Fig. 4) nor the result of SWI/SNF functioning as a nonspecific ATP-dependent protease.

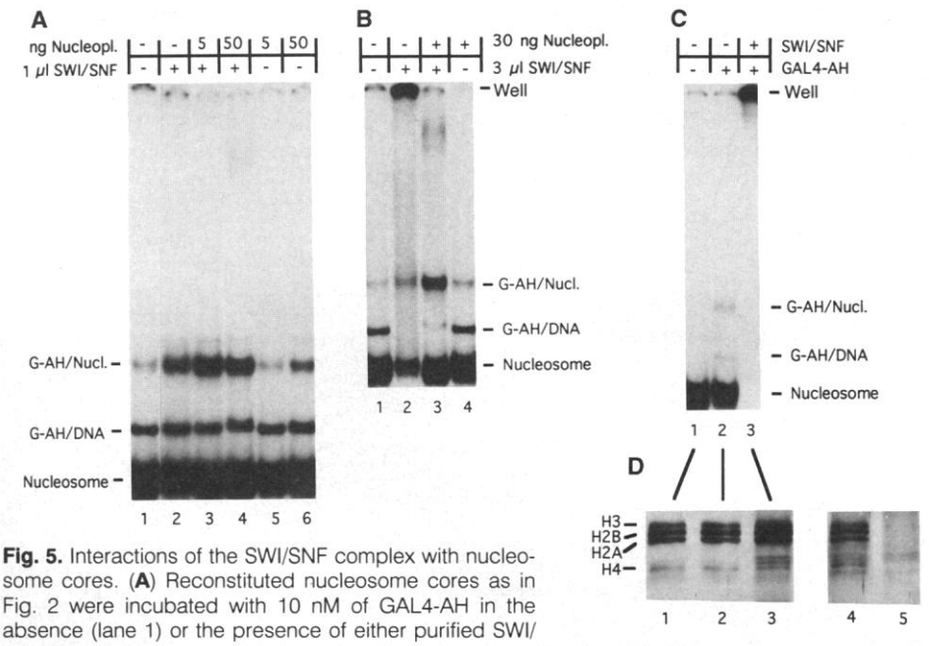


Fig. 5. Interactions of the SWI/SNF complex with nucleosome cores. (**A**) Reconstituted nucleosome cores as in Fig. 2 were incubated with 10 nM of GAL4-AH in the absence (lane 1) or the presence of either purified SWI/SNF complex (100 ng, 5 nM) (lane 2), indicated amount of nucleoplasmin (5 or 50 ng, 3.3 or 33 nM of nucleoplasmin pentamer) (lanes 5 and 6) or a combination of both (lanes 3 and 4) (*48*). 1 mM ATP was present in all binding reactions. (**B**) Reconstituted nucleosome cores as in (A) were incubated with 10 nM of GAL4-AH in the absence (lane 1) or the presence of either high amount of purified SWI/SNF complex (300 ng, 15 nM) (lane 2), 30 ng of nucleoplasmin (20 nM of pentamer) (lane 4) or a combination of both (lane 3). 1 mM ATP was present in all binding reactions. (**C**) Reconstituted nucleosome cores as in (A) were incubated with 25 nM of GAL4-AH in a four times upscaled binding reaction in the absence (lane 2) or the presence of high amount of purified SWI/SNF complex (1600 ng, 20 nM) (lane 3) and one fourth of the binding reactions were studied by mobility shift assay. (**D**) Three quarters of the binding reactions from (C) were TCA-precipitated and electrophoresed on an 18 percent SDS-PAGE and the gel was then silver-stained (*49*). Lane 2 contains nucleosomes plus GAL4-AH and lane 3 contains nucleosomes, GAL4-AH and the SWI/SNF complex. Note that the bands above H4 come from the SWI/SNF fraction as shown from the samples of a similar experiment represented in lanes 4 and 5. Lane 4 contains a binding reaction prepared exactly like the one in lane 3; sample in lane 5 was also prepared similarly but omitting the nucleosomes cores and thus represents proteins in the SWI/SNF fraction alone.

Implications for the mechanism of SWI/SNF function. In vivo, the SWI/SNF complex is required for the expression of only a subset of genes. Furthermore, during induction of *SUC2* gene transcription, the SWI/SNF complex is required for a disruption of only a few nucleosomes (*14*). On the basis of the yields of several purifications, we estimate that there are 50 to 150 copies of the SWI/SNF complex in a yeast cell, which suggests that it is not a general component of chromatin. Thus, the SWI/SNF complex must be targeted to specific chromosomal positions. We envision that the SWI/SNF complex is targeted by interaction either with activators in solution or by recognition of an activator bound weakly to a nucleosomal binding site or by both. The former possibility is supported by previous studies in which it was shown that the SWI/SNF complex associates with the glucocorticoid receptor in the absence of DNA (*3*). We also note that the SWI/SNF complex appears to have a weak intrinsic affinity for nucleosomal DNA, which results in a distortion of the migration of the nucleosome cores on mobility shift gels at high SWI/SNF concentrations (Fig. 5, B and C) and disruption of DNase I digestion patterns of nucleosomal DNA at even lower SWI/SNF concentrations (Fig. 3D) (*33*).

It was suggested that the SWI/SNF complex might counteract the repressive action of H2A-H2B dimers on transcription because a deletion of one of the two gene clusters in yeast that encodes histones H2A and H2B alleviated the defects in growth and in transcription associated with mutations in *SWI2/SNF2*, *SNF5*, or *SNF6* (*14*).

This raises the possibility that the SWI/SNF complex could function in a manner similar to that of nucleoplasmin by interacting directly with H2A-H2B dimers (*23*). For example, the highly acidic NH_2-terminus of the SWI3 subunit might interact with basic histones and facilitate their displacement (*2*). However, such an activity alone does not account for the ATP dependence of SWI/SNF stimulation of GAL4-AH binding to nucleosomes that is not observed with nucleoplasmin (*23*). Thus, the mechanism of the SWI/SNF complex stimulation of GAL4-AH binding to nucleosome cores appears to differ from that of nucleoplasmin or involve additional activities. We favor a model in which the SWI/SNF complex interacts primarily with the activator and the DNA component of the nucleosome, which leads to a disruption of histone-DNA contacts (Fig. 3) that destabilizes the histone octamer. A SWI/SNF interaction with DNA is also consistent with previous in vitro studies that demonstrate SWI/SNF function in transcription activation in crude extracts; in this case nucleosomes were absent but other nonspecific DNA binding inhibitory proteins were present (*3*).

The ATP dependence of SWI/SNF stimulation of GAL4-AH binding to nucleosome cores suggests that the complex has a catalytic function. One possibility is that the complex might use the energy of ATP hydrolysis to unwind part of the DNA duplex within the nucleosome core which could destabilize the histone octamer. This is consistent with the observed dissociation of the histone H2A-H2B dimers from nucleosome core particles by intercalation of EtBr (*36*). Dissociation of H2A-H2B dimers onto other chromosomal DNA or onto histone chaperones, such as nucleoplasmin, could result in a permanent increase in the affinity of a transcription factor allowing reuse of the SWI/SNF complex. Such a pathway is also consistent with the illustration of ATP-dependent nucleosome disruption by the GAGA factor in a *Drosophila* embryo nucleosome assembly extract (*37*). However, the molecular weight of the SWI/SNF complex and its multisubunit structure indicate that it might perform several functions in stimulating the activation of transcription.

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24. For immunoprecipitations, monoclonal antibody 12CA5 was coupled to goat α-mouse immunoglobulin G agarose (Sigma) with dimethylpimelimidate as described [E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988)]. 15 μl of the 12CA5 resin or 15 μl of goat α-mouse IgG agarose that had been cross-linked in the absence of the monoclonal antibody was added to a 1-ml sample of the Mono Q pool (~350 mM NaCl) and rocked on a nutator (Adams) for 3 hours at 4°C. Beads were isolated by centrifugation, washed four times with 1 ml of extraction buffer, and 2 times with extraction buffer without detergent. Beads were resuspended in 15 μl of SDS sample buffer, boiled, and subjected to electrophoresis on a 10 percent SDS-PAGE. Gels were stained with silver with a Bio-Rad Silver Stain Plus kit.
25. For ATPase assays, standard reaction mixtures (20 μl) contained 2 μl of the Superose 6 fractions or the Superose 6 pool (concentrated) and nucleic acid in 20 mM tris (pH 8), 5 percent glycerol, 0.1 percent Tween-20, 30 mM NaCl, 0.2 mM dithiothreitol (DTT), 5 mM $MgCl_2$, 100 μM ATP, bovine serum albumin (BSA; 0.5 mg/ml), and 1 μCi of γ-^{32}P-ATP (3000 Ci/mmol, Amersham). Reactions were incubated for 30 s at 30°C and terminated by the addition of 150 μl of 5 percent activated charcoal (acid washed, Sigma) in 20 mM phosphoric acid. Samples were incubated 5 min on ice and then centrifuged to remove the charcoal and unreacted ATP. This procedure was repeated once. A sample of the supernatant was counted by scintillation to determine the amount of released inorganic phosphate. A background of 1.5 × 10^3 cpm obtained in the absence of protein was subtracted from all values. For K_m determinations, cold ATP concentrations were varied from 5 μM to 1000 μM and reaction times were reduced to 10 min to ensure linearity. Data was plotted as 1/[ATP] vs 1/reaction velocity (picamoles of Pi released in 10 min) and the x-intercept (K_m value) was calculated by linear regression. An average from three experiments gave a value of 4.5 ± 1 × 10^{-5} M ATP.
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27. Helicase substrates were prepared by annealing oligonucleotides to M13MP18 DNA (Pharmacia). For the flush-end and 5′ overhang substrates, either the −40 universal primer (Pharmacia) or oligonucleotide BSO35 (C(19)AGGAACGGTACGCCAGAATC) was annealed and then 3′ end-labeled with Klenow fragment of DNA polymerase I and α-^{32}P-2′-deoxyadenosine 5′-triphosphate (dATP), thymidine 5′-triphosphate (dTTP), and 2′-deoxyguanosine 5′-triphosphate (dGTP). Exclusion of 2′-deoxycytidine 5′-triphosphate (dCTP) resulted in a flushed duplex of 26 base pairs (flushed substrate) or a 20-base duplex region and 19 bases of 5′ single-strand extension (5′ overhang substrate). For the 3′ overhang substrate, oligonucleotide 3′ tail [GGCCGATTAAAGGGATTTTAGACAGGAACGGTACGCCAGAT (*20*)] was first kinased with T4 polynucleotide kinase and γ-^{32}P-ATP prior to annealing to an excess (1 μg) of M13MP18 DNA. This yielded an annealed oligo with a 40-base duplex region and a 20-base 3′ extension. In all cases, substrates were purified on 1 ml Sepharose CL-4B columns to remove unannealed oligonucleotide. Helicase reactions (20 μl) contained 10 to 25 ng of substrate, 1 mM ATP, 2 to 6 μl of SWI/SNF complex (200 to 600 ng), 10 mM $MgCl_2$, 10 mM Hepes (pH 7.3), 50 mM NaCl, 5 μM $ZnCl_2$, BSA (0.25 mg/ml), and 0.5 mM DTT. 10 to 100 ng of T-antigen or 400-ng yeast replication protein A (gifts of T. Melendy, Cold Spring Harbor Laboratory) were used as positive controls. Reactions were incubated for 30 min at 30°C and terminated by the addition of 5 μl of helicase stop (1.7 percent SDS, 0.17 M EDTA, 25 percent glycerol, bromophenol blue). Reactions were electrophoresed on 8 percent polyacrylamide gels; dryed, and exposed to films overnight at −80°C.
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29. Plasmid pBEND401G1 containing 1 GAL4 site has been described (*22*). Plasmid pBEND30G1 contains an extra 37 bp of bending sequence at the Sal I site. Digestion of plasmid pBEND401G1 with Sal I and Mlu I produces a 154-bp fragments with the middle of the GAL4 site (17-mer) 32 bp from the end. Digestion of pBEND30G1 with Xho I and Ssp I produces a 153-bp fragment with the middle of GAL4 site 43 bp from the end. These probes were labeled on one end by Klenow or polynucleotide kinase and purified on 8 to 10 percent acrylamide, (acrylamide:bis = 29:1) 1X TBE native gels.
30. Nucleosome core reconstitution on labeled probes were performed by the octamer transfer method [D. Rhodes and R. A. Laskey, *Methods Enzymol.* **170**, 575 (1989)]. HeLa H1-depleted oligonucleosome cores (5 μg) were prepared as described (*22*) and were mixed with 400,000 cpm (20 ng) of probe (10^6 cpm, 50 ng for footprint substrates) and then the NaCl concentration was brought to 1 M in a final volume of 20 μl. After a first incubation at 37°C for 20 min, the transfer reaction was serially diluted to 0.85, 0.65, 0.5, and 0.3 M NaCl with 50 mM Hepes (pH 7.5), 1 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) with incubations at 30°C for 30 min at each dilution step. The reaction was finally brought to 0.1 M salt with final dilution buffer [10 mM tris-HCl (pH 7.5), 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.1 percent NP-40, 20 percent glycerol, 0.5 mM PMSF, BSA (100 μg/ml)] and incubated at 30°C for 30 min. GAL4(1-147)-AH, GAL4(1-147)-VP16, and GAL4(1-94) were purified from bacterial strains with the use of DEAE-cellulose (DE52, Waters) and heparin-Sepharose

CL-6B (Pharmacia) chromatography [Y.-S. Lin, M. F. Carey, M. Ptashne, M. R. Green, *Cell* **54**, 659 (1988)]. Serial dilutions of GAL4 derivatives protein stocks were made in GAL4 dilution buffer [10 mM Hepes (pH 7.5), 100 mM NaCl, 10 μM $ZnCl_2$, 10 mM 2-mercaptoethanol, 0.2 mM PMSF, and BSA (1 mg/ml)]. Binding reactions were done in 10 μl volume containing 20 mM Hepes (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, 1 μM $ZnCl_2$, 2 mM DTT, 5 percent glycerol, 0.2 mM NaCl, 3 mM $MgCl_2$, 1 μM $ZnCl_2$, 2 mM DTT, 5 percent glycerol, 0.2 mM PMSF, BSA (200 μg/ml) and 2000 cpm of reconstituted nucleosome cores (0.1 ng of probe and 25 ng of cold donor nucleosome cores). Purified SWI/SNF complex was added last just after GAL4 derivatives. Binding reactions were incubated at 30°C for 30 min, and then directly loaded onto a 4 percent acrylamide (acrylamide:bis = 29:1), 0.5X TBE native gel and run at 150 V for 3 hours. Gels were dried and exposed to Kodak films overnight with an intensifying screen at −80°C. Results were quantified by counting each gel on a Betascope blot analyzer (Betagen Corp.).

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45. Whole cell extracts were prepared from 36 liters of strain CY396 (*swi2Δ::HIS3 HO-lacZ SWI2-HA-6HIS::URA3*) with cells grown in YEPD medium (1 percent yeast extract, 2 percent bactopeptone, and 2 percent glucose) and harvested at an optical density $(OD)_{600}$ of 2 to 2.5. Cells were washed once in extraction buffer (*5*) and lysed in 150 ml of extraction buffer by glass beads with 4 × 30 s pulses on a BioSpec Products beadbeater, and clarified by centrifugation for 1 hour at 43 K in a Beckman Ti45 rotor. Extract was bound batchwise with 20 ml of Ni^{2+}-NTA agarose (Qiagen) for 2.5 hours at 4°C. The resin was poured into a column and washed sequentially with 100 ml of extraction buffer, 50 ml of buffer A (10 percent glycerol, 100 mM NaCl, 0.1 percent Tween-20) with 20 mM imidazole (pH 7), and 50 ml of buffer A with 500 mM imidazole (pH 7). The 500 mM imidazole eluate was loaded onto an FPLC Mono Q HR 5/5 column, equilibrated in buffer B [50 mM tris (pH 8), 10 percent glycerol, 0.1 percent Tween-20] with 100 mM NaCl. Bound protein was eluted with a 25-ml salt gradient of 100 mM to 500 mM NaCl in Buffer B. Peak fractions (~350 mM NaCl) were pooled (2 ml), concentrated to 0.25 ml on a Centricon-30 concentrator (Amicon), and loaded at 0.3 ml/min onto an FPLC Superose 6 column equilibrated in extraction buffer. Fractions containing SWI2 were pooled (fractions 18 to 20), bovine insulin (Sigma) was added to 50 μg/ml, and the pool was concentrated to 0.3 ml as above. In some cases, the salt concentration of the Superose 6 pool was reduced to 100 mM NaCl by sequential dilution and re-concentration on a Centricon-30 concentrator.
46. Deoxyribonuclease I footprinting assays were performed on twofold upscaled binding reactions. Naked DNA controls used probes that have instead been added at the final dilution step of the octamer transfer protocol. Deoxyribonuclease I (2 U for nucleosome cores and 0.2 U for naked DNA) was added at the end of the binding reactions and incubated at room temperature for 1 min. Reactions were stopped by the addition of 1 volume of 20 mM tris-HCl (pH 7.5), 50 mM EDTA, 2 percent SDS, yeast tRNA (0.25 mg/ml), and proteinase K (0.2 mg/ml). Samples were then incubated for 1 hour at 50°C and precipitated at room temperature with 2.5 M NH_4Ac and 3 volumes of absolute ethanol. The pellets were washed with 80 percent ethanol and resuspended in 2 μl of H_2O, 3 μl of 95 percent formamide, 0.1 percent xylene cyanol, 0.1 percent bromophenol blue and 10 mM EDTA were added and samples were then heated at 90°C for 5 min and cooled on ice before loading on an 8 percent acrylamide (acrylamide:bis = 19:1), 8 M urea, 1X TBE sequencing gel. Gels were run at 60 W constant power for 1 hour 45 min and exposed wet to Kodak films with an intensifying screen for two days at −80°C. G+A ladders were produced as described [A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980)].
47. SWI/SNF complex that contains the SWI2K798A mutant subunit was purified from a 20-liter culture of strain CY397 (*swi2Δ::HIS3 HO-lacZ swi2K798A-HA-6HIS::URA3*), which is isogenic to strain CY296. All manipulations were performed as described above for the wild-type complex.
48. Nucleoplasmin was purified from *Xenopus* eggs through the DEAE-cellulose chromatography step [L. Sealy, R. R. Burgess, M. Cotten, R. Chalkley, *Methods Enzymol.* **170**, 612 (1989)] and was added last to the binding reaction just after the SWI/SNF complex. In all reactions in which SWI/SNF, GAL4 derivatives, or nucleoplasmin was omitted, an equal volume of the corresponding buffer containing BSA to equivalent protein concentration was added.
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