RESEARCH ARTICLE

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

Jacques Côté, Janet Quinn, Jerry L. Workman, Craig L. Peterson*

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), Dro sophila ftz (2), mammalian glucocorticoids 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 \times 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 phenotype of transgenic embryos. 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

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.

<table>
<thead>
<tr>
<th></th>
<th>Volume (ml)</th>
<th>Concentration (mg/ml)</th>
<th>Total Protein (mg)</th>
<th>Specific Activity (units)</th>
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<tbody>
<tr>
<td>WCE</td>
<td>130</td>
<td>21.5</td>
<td>2800</td>
<td>1</td>
</tr>
<tr>
<td>Np2-</td>
<td>eluate</td>
<td>43</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>Mono C</td>
<td>2</td>
<td>0.35</td>
<td>0.7</td>
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<tr>
<td>Superose</td>
<td>1.5</td>
<td>0.02</td>
<td>0.03</td>
<td>91,195</td>
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*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).

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 hemagglutinin (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 Ni2+-NTA (Ni2+-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

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**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 pHS3-1b-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:HS3 GAL4+ with one of the following three plasmids: (i) CP337 contains wild-type SWI2 gene in vector RS315 (44), (ii) CP359 contains swi2K798A-HA-HIS in RS315, or (iii) RS315.

<table>
<thead>
<tr>
<th>Strain</th>
<th>GAL4 (%)</th>
<th>GAL4-AH (%)</th>
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</thead>
<tbody>
<tr>
<td>SWI+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>swi1−</td>
<td>7.7</td>
<td>13</td>
</tr>
<tr>
<td>swi2−</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>swi2K798A</td>
<td>5</td>
<td>ND</td>
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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 SWI6 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 K₅₀ 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 (yRPAA) 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-
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, ATPγ-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-AH 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
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.

Deoxynucleobonucleoside 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 digests 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
nucleoplasm, SWI/SNF complex induced a four- to fivefold increase in the GAL4-nucleosome complex (Fig. 5A). Addition of 5 ng of nucleoplasm 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 nucleoplasm (5 ng) had little effect on GAL4-AH binding. At tenfold higher concentrations (50 ng), nucleoplasm 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 nucleoplasm. Thus, at limiting concentrations of nucleoplasm, the stimulatory function was additive with SWI/SNF, and the SWI/SNF stimulation precluded further stimulation by higher concentrations of nucleoplasm. This result is consistent with the view that the mechanisms by which SWI/SNF complex and nucleoplasm 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 nucleoplasm 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.

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 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 nucleoplasmid by interacting directly with H2A-H2B dimers (23). For example, the highly acidic NH1-termini
of the SWI/SNF subunit might interact with basic histones and facilitate their dis-
placement (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 nucleoplasmid (23). Thus, the mechanism of the SWI/SNF complex stimulation of GAL4-AH binding to nucleosome cores appears to differ from that of nucleoplasmid or involve additional activi-
ties. 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 destabi-
lates the histone octamer. A SWI/SNF in-
teraction with DNA is also consistent with previous in vitro studies that demonstrate SWI/SNF function in transcription activation in crude extracts; in this case nucleosome
cores were absent but other nonspecific DNA binding inhibitory proteins were pres-
tant (3).

The ATP dependence of SWI/SNF stimula-
tion of the factor allowing release of the complex has a catalytic function. One possibility is that the complex might use the energy of ATP hy-
drolysis to unwind part of the DNA duplex within the nucleosome core which could destabilize the histone octamer. This is con-
sistent with the observed dissociation of the histone H2A-H2B dimers from nucleosome core particles by intercalation of EB(r39). Dissociation of H2A-H2B dimers onto other chromosomal DNA or onto histone chaper-
ones, such as nucleoplasmid, could result in a permanent increase in the affinity of a transcription factor for DNA, and might be a feature 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 nucleo-
osome assembly extract (37). However, the molecular weight of the SWI/SNF complex and its multisubunit structure indicate that it might perform several functions in stimulat-
ing the activation of transcription.

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26. Plasmid pBEND401G1 containing 1 GAL4 site has been described (22). Plasmid pBD3001G1 con-
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34. H. Chen, C. L. Peterson, unpublished data.
CL-6B (Pharmacia) chromatography [Y.-S. Lin, M. F. Carey, M. Plaschne, 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), 50 mM NaCl, 3 mM MgCl₂, 1 mM ZnCl₂, 2 mM DTT, 5 percent glycerol, 0.2 mM Na3HPO₄, 1 mM MgCl₂, 1 mM ZnCl₂, 2 mM DTT, 5 percent glycerol, 0.2 mM PMSF, and BSA (1 mg/ml)]. Binding reactions were done in 10 μl volume containing 20 μM Hepes (pH 7.5), 50 mM NaCl, 3 mM MgCl₂, 1 mM ZnCl₂, 2 mM DTT, 5 percent glycerol, 0.2 mM Na3HPO₄, 1 mM MgCl₂, 1 mM ZnCl₂, 2 mM DTT, 5 percent glycerol, 0.2 mM Na3HPO₄, and 20 μM Na3HPO₄ and 2000 cpm of recombinant nucleiosome cores (0.1 ng of probe and 25 ng of cold donor nucleiosome 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.).

31. ATP-γ-S is a competitive inhibitor of the ATPase activity of the SWI/SNF complex (29).
33. U. Clerc and J. L. Workman, unpublished observations.
37. S. P. reports contains 6.7 grams per liter yeast nitrogen base without amino acids (Difco Laboratories) and is supplemented with amino acids as described (39).
44. Whole cell extracts were prepared from 36 liters of strain CY396 (swi2Δ-HIS3 HO-lacZ SWI2-HA-6×His:URA4) with cells grown in YPD medium (1 percent yeast extract, 2 percent Bacto-peptone, 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 x 30 s pulses on a BioSpec Products beadbeater, and clarified by centrifuga-
tion for 1 hour at 43 K in a Beckman Ti45 rotor. Extract was bound batchwise with 20 ml of Ni²⁺-NTA agarose (Qiagen) for 2 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 imid-
azole (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 gradient and M. Plaschne of 100 ml to 500 ml of 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, 300 ml in total) and the protein 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 dialysis and re-concentration on a Centricron-30 concentrator.
45. Deoxyribonuclease I footprinting assays were performed on hexoluid upshifted binding reactions. Naked DNA controls used probes that have in-
stead been added at the final dilution step of the octamer transfer protocol. Deoxyribonuclease I (2 U for nucleiosome 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 volumes of absolute ethanol. The pellets were washed with 80 percent ethanol and resuspended in 2 μl of H₂O, 3 μl of 95 percent formamide, 0.1 percent xylene cyanol, 0.1 percent bromopho-

46. SWI/SNF complex that contains the SWI2/K798A mutant subunit was purified from a 20-liter cul-
ture of strain CY397 (swi2Δ-HIS3 HO-lacZ swi2Δ/K798A-HA-6×His:URA4), which is isogenic to strain CY296. All manipulations were performed as described above for the wild-type complex.
47. Nucleoplasmin was purified from Xenopus eggs through the DEAE-cellulose chromatography step (64) (Swi4, 20-liter cul-
ture of strain CY397). Nucleoplasmin was then separated from SWI2/SNF complex by ammonium sulfate precipitation and dialysis (65).
48. We thank L.-J. Juan, P. Walter and H. Chen for contributing the purified GAL4-4H donor nucleo-

49. M. Betjes and C. Adams for plasmids pBEND401G1 and pBEND390GI, S. Bell and T. Melendy for providing monoclonal anti-
lides, T. Melendy for purified T-antigen and yeast RPA, E. T. Young for α-SWI antibodies, J. Flanagan for excellent technical assistance, G. Vigliani, C. Adams and T. Owen-Hughes for critically reading the manuscript, and R. E. Kingston for useful discussions. This work was supported by NIH grant GM43852-01 and March of Dimes Birth Defects Foundation research grant 5-FY93-0622 to C.L.P., and NIH grant GM47867-02 to J.L.W. J.C. is the recipient of a Canadian Medical Research Council Postdoctoral Fellowship. J.L.W. and C.L.P. are Scholars of the Leukemia Society of America.