dilution and a further period of preincubation, the Ins(1,4,5)P_3-induced inactivation recovered over a period of about 60 s (Fig. 4*a*). A time-dependent recovery from inactivation could also be induced by chelation of medium Ca^{2+} with EGTA in the sustained presence of saturating Ins(1,4,5)P_3 (Fig. 4*b*). This again shows the interdependent nature of inhibition by Ins(1,4,5)P_3 and Ca^{2+} and demonstrates that recovery from the inactivated state can occur as a result of decreased levels of either Ins(1,4,5)P_3 or Ca^{2+}.

The present study represents the first demonstration that the Ins(1,4,5)P_3 receptor Ca^{2+} channel is inactivated as a result of Ins(1,4,5)P_3 binding. This observation has been helped by the Mn^{2+} quench technique, which permits dissociation of the permeability measurement from Ins(1,4,5)P_3 binding and Ca^{2+} flux events, and allows ion permeability to be studied *in situ* over an extended period. Some earlier reports have concluded that Ins(1,4,5)P_3 does not desensitize its receptor[6,10,12]. However, in these studies the measurements of channel activity relied on Ins(1,4,5)P_3-induced Ca^{2+} fluxes that are short lived and known to have feedback effects on the receptor. Moreover, the data presented here demonstrate that Ins(1,4,5)P_3-induced inactivation occurs in a novel incremental manner, whereby submaximal Ins(1,4,5)P_3 doses inactivate only the subpopulation of channels responding to that Ins(1,4,5)P_3 dose, rather than causing global inactivation of all receptors. In the intact cell, Ins(1,4,5)P_3-induced inactivation may provide a mechanism for adaptation to partially elevated basal levels of Ins(1,4,5)P_3. However, inactivation is likely to be manifest most strongly once channel opening is initiated and the process is potentiated by the interaction between Ins(1,4,5)P_3 and released Ca^{2+}. This could serve to limit the duration of channel opening and may play an important role in the termination phase of $[Ca^{2+}]_i$ spikes. □

Received 4 February; accepted 20 May 1994.

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ACKNOWLEDGEMENTS. We thank B. Ehrlich, S. Joseph and J. Hoek for helpful suggestions. This work was supported by grants from the NIH.

Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex

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CHROMATIN structure can affect the transcriptional activity of eukaryotic structural genes by blocking access of sequence-specific activator proteins (activators) to their promoter-binding sites[1]. For example, the DNA-binding domain of the yeast GAL4 protein interacts very poorly with nucleosome cores compared with naked DNA[2] (and see below), and binding of other activators is even more strongly inhibited[2,3]. The way in which activators bind to nucleosomal DNA is therefore a critical aspect of transcriptional activation. Genetic studies have suggested that the multi-component SWI/SNF complex of *Saccharomyces cerevisiae* facilitates transcription by altering the structure of the chromatin[4,5]. Here we identify and partially purify a human homologue of the yeast SWI/SNF complex (hSWI/SNF complex). We show that a partially purified hSWI/SNF complex mediates the ATP-dependent disruption of a nucleosome, thereby enabling the activators, GAL4–VP16 and GAL4–AH, to bind within a nucleosome core. We conclude that the hSWI/SNF complex acts directly to reorganize chromatin structure so as to facilitate binding of transcription factors.

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TABLE 1 Amount of GAL4 derivative required for half-maximal binding to nucleosomal templates in the presence of the hSWI/SNF complex

	Footprinting (U)		
	Expt. 1	Expt. 2	Expt. 3
GAL4–VP16	20	70	40
GAL4–AH	200	300	100
GAL4(1–94)	1,000	2,000	1,000

DNase I footprinting was as described for Fig. 2 using increasing amounts of each GAL4 derivative, 0.3 ng (1.12×10^{-10} M) specific DNA and 3 ng carrier DNA (*Hae*III-digested pUC18). Percentage protection was determined at each concentration of a GAL4 derivative by phosphorimager analysis as previously described[19]. The amount of each GAL4 derivative required for 50% protection of naked DNA was defined as 1 unit (U). Increasing amounts of each GAL4 derivative were then used to determine the amount required for 50% protection of nucleosomal DNA in the presence of 1.3 μg hSWI/SNF complex and 4 mM ATP. 50% protection of nucleosomal DNA was not achieved for any GAL4 derivative in the absence of ATP. *E. coli*-expressed GAL4 derivatives were purified as described[20,21].

Previous studies have shown that human cells contain homologues of the yeast protein, SWI2 (refs 6, 7). We therefore sought to identify and purify a SWI/SNF complex from a human (HeLa) cell nuclear extract. Our experimental strategy was to fractionate a nuclear extract and monitor the chromatographic fractions for the human SWI2 homologue. Figure 1*a* shows the chromatographic scheme and Fig. 1*b* the polypeptide composition of the fractions. The hSWI2 in a HeLa cell nuclear extract was present in two distinct phosphocellulose fractions (0.5 M and 0.7 M KCl) and is a component of two chromatographically separable hSWI2-containing complexes, designated hSWI/SNF complexes A and B.

Figure 1*b* shows the polypeptide composition of the two hSWI/SNF complexes after the final Superose 6 gel filtration (complex A, lane 1) or glycerol gradient (complex B, lane 2) step. In addition to hSWI2, two polypeptides of high relative molecular mass (M_r), p170 and p150, are present in both complexes. The hSWI/SNF complex A preparation contains four smaller polypeptides (p60, p50, p45 and p43) which were also detected in the purified hSWI/SNF complex B but seem to be present in lower relative amounts. These seven polypeptides

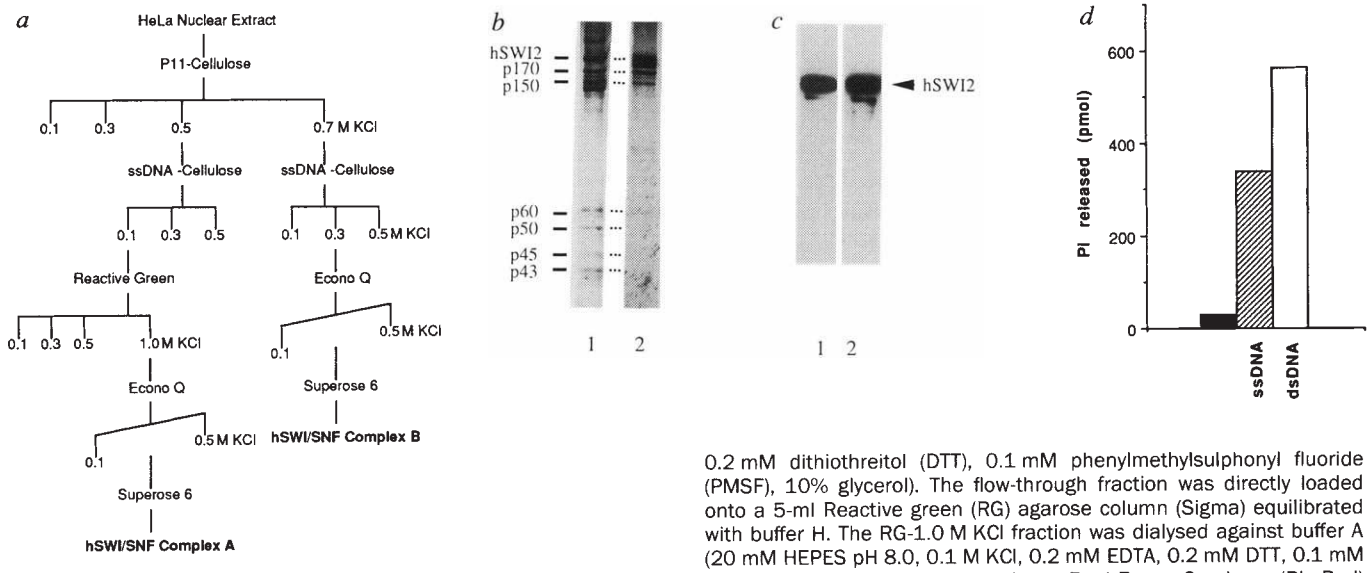
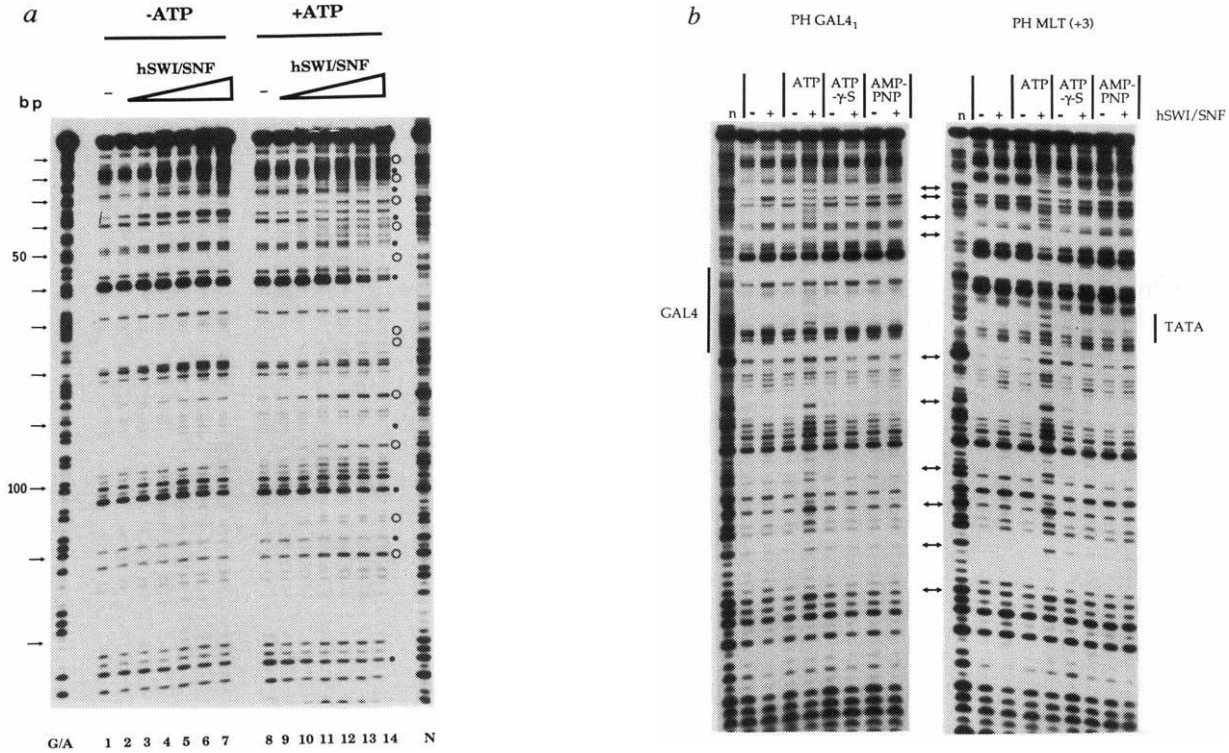
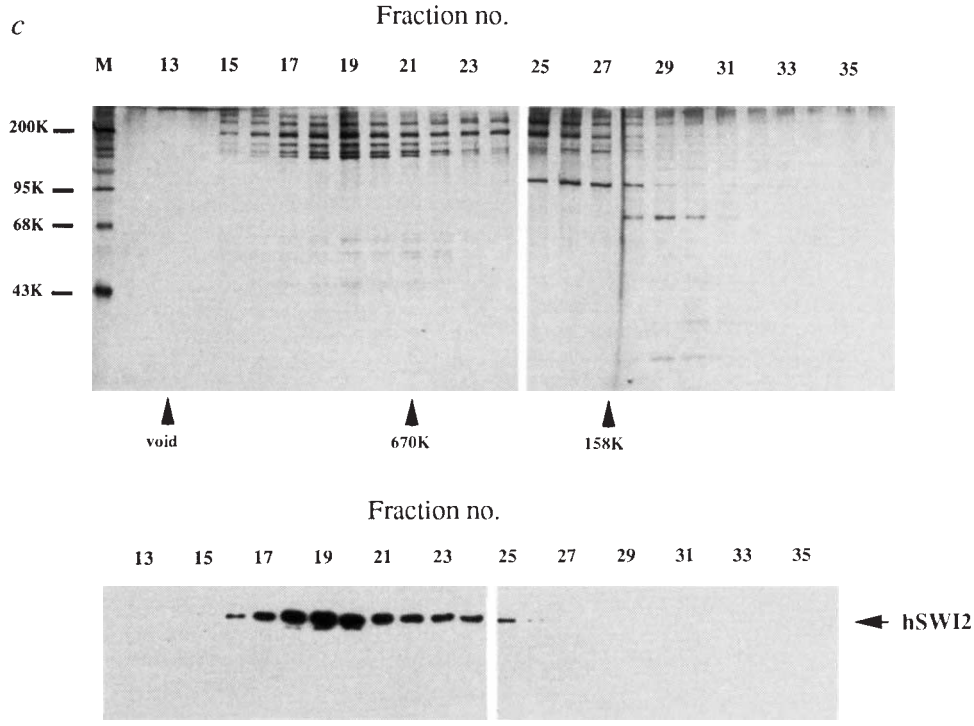
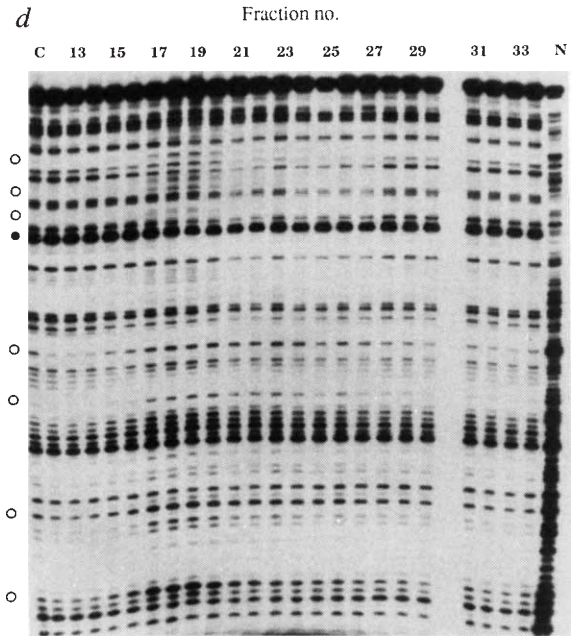
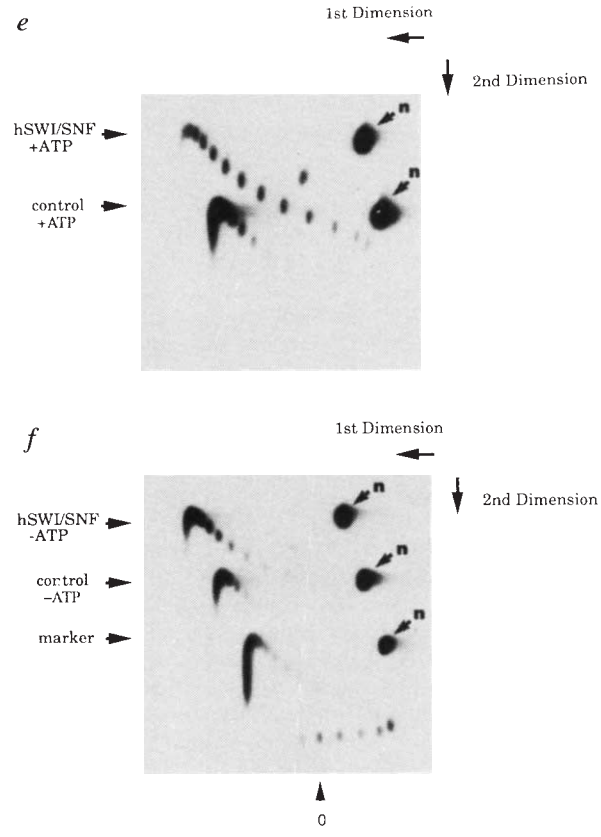


FIG. 1 Purification of hSWI/SNF complexes. *a*, Purification scheme; *b*, *c*, polypeptide composition of the chromatographic fractions. Silver staining (*b*) and an immunoblotting assay using the α-BRG1 antibody[7] (*c*) of an SDS–PAGE gel showing the hSWI/SNF complex A (Superose 6 fraction) from the P11–0.5 M (lane 1) and the hSWI/SNF complex B (glycerol gradient fraction) from the P11–0.7 M KCl fractions (lane 2). *d*, The hSWI/SNF complex contains a DNA-dependent ATPase activity. ATP hydrolysis was measured by the release of $^{32}P_i$ from [γ-$^{32}P_i$]ATP. ATP hydrolysis was measured in the absence (solid bar) or presence of 1 μg single-stranded DNA (hatched bar) or double-stranded DNA (open bar). Stimulation by ssDNA and dsDNA was 11-fold and 19-fold, respectively. METHODS. The P11–0.5 M KCl fraction of HeLa cell nuclear extract (1 g) was applied to a ssDNA cellulose column (10 ml) equilibrated with buffer H (20 mM HEPES pH 8.0, 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 10% glycerol). The flow-through fraction was directly loaded onto a 5-ml Reactive green (RG) agarose column (Sigma) equilibrated with buffer H. The RG-1.0 M KCl fraction was dialysed against buffer A (20 mM HEPES pH 8.0, 0.1 M KCl, 0.2 mM EDTA, 0.2 mM DTT, 0.1 mM PMSF, 10% glycerol) and applied to a 5-ml Econo Q column (Bio-Rad) equilibrated with buffer A. Protein was eluted with a 90-ml linear gradient from 0.1 M to 1.0 M KCl (flow rate, 2 ml min^{-1}). The Econo Q fraction of the hSWI/SNF complex A (200 μl) was fractionated on a Superose 6 HR 10/30 gel filtration column (Pharmacia) equilibrated with buffer A at 0.2 ml min^{-1} and 0.6-ml fractions were collected. The Econo Q fraction of the hSWI/SNF complex B (440 μl) was loaded onto a 15–40% linear glycerol gradient (5 ml) in buffer H. Centrifugation was at 35,000 r.p.m. for 12 h in an SW55 rotor (Beckman) and the gradient divided into 24 fractions. *d*, ATPase activity of the hSWI/SNF complex (300 ng, Superose 6 fraction) was measured in the presence or absence of 1 μg of single-stranded pBluescript DNA or double-stranded pUC18 DNA as described[22]. The background hydrolysis (absence of hSWI/SNF complex) was 6 pmol and this value was subtracted. The results shown are average values of duplicate reactions.









◄ FIG. 2 The hSWI/SNF complex disrupts a nucleosome core structure in an ATP-dependent manner. *a*, The ATP-dependent disruption of nucleosome (PH GAL4$_1$) by the hSWI/SNF complex A. The amount of hSWI/SNF complex A added was: lanes 1, 8, 0 ng; lanes 2, 9, 50 ng; lanes 3, 10, 100 ng; lanes 4, 11, 200 ng; lanes 5, 12, 400 ng; lanes 6, 13, 800 ng; lanes 7, 14, 1,500 ng. N, naked DNA; open circles, increased DNase I cleavage; closed circles, decreased DNase I cleavage. Ten-base-pair repeats calculated from the 5′ end of the DNA fragment are indicated on the left. *b*, ATP hydrolysis requirement for nucleosome disruption. PH GAL4$_1$ nucleosome was incubated with 1.3 μg of the hSWI/SNF complex A (left panel) and PH MLT (+3) nucleosome was incubated with 240 ng of the hSWI/SNF complex B (right panel). Arrows indicate modifications of DNase I digestion pattern similarly observed in both nucleosomes (PH GAL4$_1$ and PH MLT (+3)). *c*, *d*, The hSWI/SNF complex and nucleosome-disrupting activity co-fractionate. hSWI/SNF complex A (Econo Q fraction) was fractionated by Superose 6 gel filtration chromatography, and each fraction was assayed for nucleosomal disruption activity. *c*, Upper part, Silver-stained SDS–PAGE gel; lower part, immunoblotting assay with the α-BRG1 antibody, *d*, nucleosome disruption assay. Open circles, increased DNase I cleavage; closed circles, decreased DNase I cleavage. *e*, *f*, The hSWI/SNF complex decreases DNA superhelical density in reconstituted chromatin. DNA topoisomers were resolved by two-dimensional gel electrophoresis[23]. The direction of electrophoresis in the first dimension (absence of chloroquine) and second dimension (presence of 4.1 μM chloroquine) are indicated. To avoid overlap, the samples were loaded in separate wells within a 1.5-h time interval. The nicked DNA (n) serves as a reference point for the migration of DNA. ►

METHODS. A 149-bp DNA containing two copies of a 20-bp artificial nucleosome positioning sequence[12] at the one end and a GAL4 binding site (PH $GAL4_1$) or adenovirus major late promoter TATA box (PH MLT (+3)) at the dyad position was reconstituted with purified HeLa histone octamers by a salt dilution method[15]. Glycerol gradient sedimentation revealed that >95% of the DNA was nucleosomal (data not shown). The integrity of the reconstituted nucleosome was confirmed by micrococcal nuclease digestion (data not shown; and see accompanying manuscript[8]). The reconstituted nucleosome (3.3 ng total DNA), isolated by a 5–30% glycerol gradient centrifugation (5 ml, 35,000 r.p.m., 15 h, Beckman SW55 rotor), was incubated in 25-μl reaction buffer (12 mM HEPES pH 7.9, 60 mM KCl, 6 mM $MgCl_2$, 60 μM EDTA, 2 mM DTT, 13% glycerol) with the hSWI/SNF complex for 30 min at 30°C in the presence or the absence of 4 mM Mg–ATP, ATP-γS or AMP-PNP. The nucleosome was digested with DNase I (0.32 U) for 2 min at room temperature, and the DNA products fractionated on an 8% polyacrylamide/8 M urea sequencing gel. *e*, *f*, pG_5HC_2AT (3.35 kb), internally labelled with ^{32}P, was reconstituted with HeLa histone octamers in a *Xenopus* oocyte heat-treated extract[24]. Reconstituted chromatin was isolated on a 10–40% glycerol gradient (5 ml, 35,000 r.p.m., 4 h, SW55 Beckman rotor). The reconstituted chromatin (2.2 ng total DNA) in 25 μl reaction buffer (12 mM HEPES pH 7.9, 60 mM KCl, 6 mM $MgCl_2$, 60 μM EDTA, 4 mM Mg-ATP, 2 mM DTT, 13% glycerol, 3 units topoisomerase I (Promega)) was incubated at 30 °C for 40 min in the presence or absence (controls) of 900 ng of the hSWI/SNF complex. Two-dimensional gel electrophoresis was as previously described[23].

(hSWI2, p170, p150, p60, p50, p45 and p43) co-fractionated on a Superose 6 gel filtration column (hSWI/SNF complex A; Fig. 2*c*) and on a glycerol gradient (hSWI/SNF complex B; data not shown), consistent with the notion that they are components of a single complex with a native molecular mass greater than 700,000 (700 K). However, additional experiments will be required to define the subunit composition of the two hSWI/SNF complexes, to determine which polypeptides in these fractions are required for activity, and to ascertain whether there are functional differences between the two hSWI/SNF complexes. We note that in all the assays presented here, and in the accompanying manuscript[8], the activities of the two hSWI/SNF complexes were similar.

Yeast SWI2 (ySWI2) and hSWI2 have a DNA helicase motif[6,7,9,10] and indeed an *Escherichia coli*-derived ySWI2 has a DNA-dependent ATPase activity[11]. Figure 1*d* shows that hSWI/SNF complex A contained an ATPase activity which was significantly stimulated by addition of DNA. Double-stranded DNA stimulated ATPase activity more effectively than single-stranded DNA, analogous to the results obtained with a recombinant ySWI2 protein[11].

We next examined the ability of the hSWI/SNF complex to alter nucleosome structure. A 149-base-pair (bp) DNA fragment containing two copies of a 20-bp artificial nucleosome positioning sequence[12] at one end and a single GAL4-binding site at its centre was reconstituted into a nucleosome using purified HeLa core histones. The DNase I digesion pattern of the reconstituted nucleosome showed the expected 10-bp periodicity (Fig. 2*a*, lane 1). In the absence of ATP, increasing amounts of the hSWI/SNF complex produced no qualitative change in the DNase I digestion pattern (Fig. 2*a*, lanes 2–7), but with ATP present it was dramatically changed; in particular, the characteristic 10-bp periodicity was progressively lost with increasing amounts of the hSWI/SNF complex (Fig. 2*a*, lanes 9–14). The complex had no effect on the DNase I digestion pattern of naked DNA (data not shown; and see accompanying manuscript[8]).

Figure 2*b* shows that the non-hydrolysable ATP analogues, AMP-PNP and ATP-γS, did not support the nucleosome disrupting activity of the hSWI/SNF complex (left panel). This activity was not DNA-template-specific as the hSWI/SNF complex had a comparable effect on a nucleosome core containing the adenovirus major late promoter TATA box (Fig. 2*b*, right panel).

To obtain more evidence that the hSWI/SNF complex, and not a contaminant, caused the nucleosome disruption, partially purified hSWI/SNF complex A (Econo Q fraction) was fractionated on a Superose 6 column and the fractions were tested for their ability to disrupt nucleosome structure. Figure 2*c* shows that hSWI/SNF complex A, which included hSWI2, eluted from the Superose 6 column as a broad peak with a native molecular mass greater than 700K. Significantly, Fig. 2*d* shows that hSWI/SNF complex A and the nucleosome-disrupting activity co-fractionated, with a peak at fraction 19. Similarly, hSWI/SNF complex B co-fractionated with the nucleosome disrupting activity on a glycerol gradient (data not shown). Taken together, these results indicate that hSWI/SNF complexes A and B disrupt nucleosome structure in an ATP-dependent manner.

If nucleosome disruption is accompanied by either unwinding of nucleosomal DNA or removal of histones, the linking number per nucleosome will be altered. To test this possibility, ^{32}P-labelled closed circular DNA was reconstituted with purified histone octamers in a *Xenopus* oocyte heat-treated extract and the reconstituted chromatin was isolated by glycerol gradient centrifugation. On average, 15.5 nucleosomes were deposited on 3.35-kilobase (kb) DNA (one nucleosome per 216 bp of DNA). Figure 2*e*, *f* shows that following incubation with the hSWI/SNF complex, ATP and topoisomerase I, the average linking number of the isolated DNA was dramatically altered from 15.5 to 10.5, but in the absence of ATP, the complex had no effect

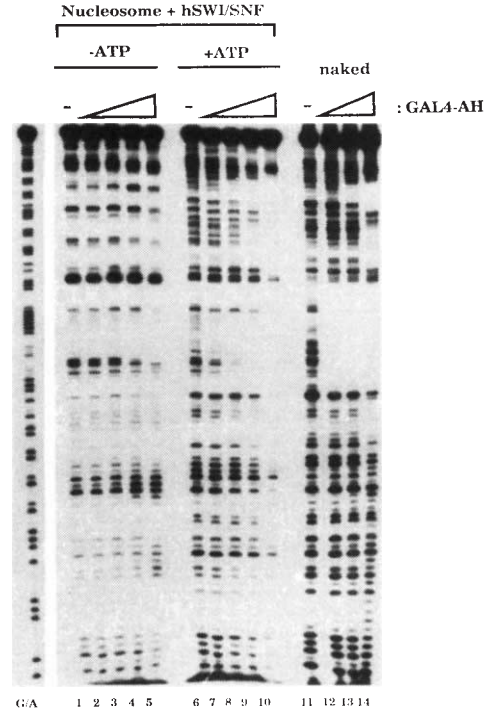


FIG. 3 The hSWI/SNF complex facilitates binding of GAL4–AH to nucleosomal DNA.
METHODS. The nucleosome core was reconstituted as described for Fig. 2. The reconstituted nucleosome (3.3 ng total DNA) was incubated in 25 μl reaction buffer (12 mM HEPES pH 7.9, 60 mM KCl, 6 mM $MgCl_2$, 60 μM EDTA, 2 mM DTT, 13% glycerol) with 1.3 μg of the hSWI/SNF complex for 30 min at 30 °C in the presence or absence of 4 mM Mg–ATP. GAL4–AH was added followed by further incubation for 30 min at 30 °C. The nucleosome was digested with DNase I (0.4 U) for 2 min at room temperature, and analysed on an 8% polyacrylamide/8 M urea sequencing gel. The amount of GAL4–AH added was: lanes 1, 6, 11, 0 M; lanes 2, 7, 12, 2.4×10^{-8} M; lanes 3, 8, 13, 2.4×10^{-7} M; lanes 4, 9, 14; 2.4×10^{-6} M; lanes 5, 10, 2.4×10^{-5} M.

on the linking number of DNA isolated from reconstituted chromatin. These results further support the notion that the hSWI/SNF complex mediates the ATP-dependent disruption of a nucleosome core and that this disruption is accompanied by a dramatic change in DNA topology. In addition, our data indicate that the hSWI/SNF can alter the chromatin structure of a closed circular DNA template as well as a mono-nucleosome.

The observation that a hSWI/SNF complex disrupted a nucleosome raised the possibility that the hSWI/SNF complex could help transcription factors bind to their sites within a nucleosomal DNA template. To test this possibility, we first examined the effect of the hSWI/SNF complex on binding of the activator, GAL4–AH, to its DNA site located within a nucleosome core (Fig. 3). In the absence of the hSWI/SNF complex, GAL4–AH bound to its site within a nucleosome $\sim 10^4$-fold less well than to naked DNA. In contrast, in the presence of both the hSWI/SNF complex and ATP, GAL4–AH binding was enhanced more than 100-fold. The hSWI/SNF complex had no effect without ATP.

We next investigated whether a transcriptional activation domain affected the ability of the hSWI/SNF complex to facilitate binding of a GAL4 derivative to a nucleosomal DNA template. Three GAL4 derivatives were analysed: GAL4(1–94), which lacks an activation domain; GAL4–AH, which has a relatively weak acidic activation domain[13]; and GAL4–VP16, which has a well-characterized, extremely potent acidic activation domain[14]. Previous studies have shown that on naked DNA or purified nucleosomal DNA templates, these activation domains do not affect GAL4 DNA binding (ref. 15; and data not shown). Table 1 shows that in three independent experiments GAL4–VP16 and GAL4–AH bound significantly better than GAL4(1–94) to nucleosomal DNA. Furthermore, binding of GAL4–VP16 was 5–10-fold higher than that of GAL4–AH.

In this and the accompanying manuscript[8] we have shown that in the absence of promoter-specific and general transcription factors, a partially purified hSWI/SNF complex can disrupt the structure of a nucleosome core. On disruption of the nucleosome, binding of an activator and TATA-box binding protein (TBP) to nucleosomal DNA templates is augmented. The ySWI/SNF complex was originally identified as being required for the *in vivo* function of diverse activators[4,16,17]. Our results strongly suggest that the SWI/SNF complex is required for activator function because it directly disrupts nucleosomal structure to increase access of transcription factors to promoters. The requirement for the SWI/SNF complex *in vivo* implies that disruption of chromatin structure is an essential aspect of transcriptional activation.

We have shown that the hSWI/SNF-complex-facilitated binding of three GAL4 derivatives parallels their ability to activate transcription. In particular, the VP16 activation domain was extremely active in this assay. Although the physical basis for this effect is unknown, the VP16 activation domain could interact with components of the hSWI/SNF complex or histones.

In vivo, GAL4–VP16 is a significantly more potent activator than GAL4–AH[14]. However, *in vitro* on naked DNA templates, GAL4–AH is a comparable, and in some instances an even better, activator than GAL4–VPI6 (ref. 18). Thus, part of the high activity of the VP16 activation domain is lost on naked DNA templates. We speculate that the ability of the VP16 activation domain to enhance binding to nucleosomal DNA is part of the basis for its unusual potency. □

Received 4 February; accepted 7 July 1994.

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ACKNOWLEDGEMENTS. We thank L. L. Chiang for technical assistance; S. A. Brown for reagents; and C. Peterson for helpful discussion and communicating results before publication. This work was supported by grants from the NIH to M.R.G. and R.E.K.

Facilitated binding of TATA-binding protein to nucleosomal DNA

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BINDING of the TATA-binding protein (TBP) to the TATA box is required for transcription from many eukaryotic promoters in gene expression. Regulation of this binding is therefore likely to be an important determinant of promoter activity. Incorporation of the TATA sequence into nucleosomes dramatically reduces transcription initiation[1–3], presumably because of stereochemical constraints on binding of general transcription factors. Biochemical and genetic studies imply that cellular factors such as yeast SWI/SNF are required for activator function and might alter chromatin structure[4–11]. One step that could be regulated during the activation process is TBP binding in chromatin[12,13]. We show here that binding of TBP to the TATA sequence is severely inhibited by incorporation of this sequence into a nucleosome. Inhibition can be overcome by ATP-dependent alterations in nucleosomal DNA structure mediated by hSWI/SNF, a putative human homologue of the yeast SWI/SNF complex. Additionally, the orientation of the TATA sequence relative to the surface of the histone core affects the access of TBP. We propose that the dynamic remodelling of chromatin structure to allow TBP binding is a key step in the regulation of eukaryotic gene expression.

We constructed a 149-base pair (bp) DNA fragment containing two rotational phasing sequences[14] at the 5′ end and the adenovirus major late promoter TATA box in the middle (PH MLT). The phasing sequence favours specific positioning of the histone core. Two variant templates positioned the TATA box either 3 or 6 bp closer to the phasing sequences (PH MLT(+3) and PH MLT(+6)), which should rotate the position of the TATA relative to the face of the histone core (see Fig. 1*c* and below). This should ensure that at least one of the templates contains the TATA sequences in an accessible position on the surface of the histones. *In vitro* assembled mononucleosomes[15]

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