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Glucocorticoid Receptor-Glucocorticoid Response Element Binding Stimulates Nucleosome Disruption by the SWI/SNF Complex

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The organization of DNA in chromatin is involved in repressing basal transcription of a number of inducible genes. Biochemically defined multiprotein complexes such as SWI/SNF (J. Côté, J. Quinn, J. L. Workman, and C. L. Peterson, Science 265:53–60, 1994) and nucleosome remodeling factor (T. Tsukiyama and C. Wu, Cell 83:1011–1020, 1995) disrupt nucleosomes in vitro and are thus candidates for complexes which cause chromatin decondensation during gene induction. In this study we show that the glucocorticoid receptor (GR), a hormone-inducible transcription factor, stimulates the nucleosome-disrupting activity of the SWI/SNF complex partially purified either from HeLa cells or from rat liver tissue. This GR-mediated stimulation of SWI/SNF nucleosome disruption depended on the presence of a glucocorticoid response element. The in vitro-reconstituted nucleosome probes used in these experiments harbored 95 bp of synthetic DNA-bending sequence in order to rotationally position the DNA. The GR-dependent stimulation of SWI/SNF-mediated nucleosome disruption, as evaluated by DNase I footprinting, was 2.7- to 3.8-fold for the human SWI/SNF complex and 2.5- to 3.2-fold for the rat SWI/SNF complex. When nuclear factor 1 (NF1) was used instead of GR, there was no stimulation of SWI/SNF activity in the presence of a mononucleosome containing an NF1 binding site. On the other hand, the SWI/SNF nucleosome disruption activity increased the access of NF1 for its nucleosomal binding site. No such effect was seen on binding of GR to its response element. Our results suggest that GR, but not NF1, is able to target the nucleosome-disrupting activity of the SWI/SNF complex.

DNA in eukaryotic cells associates with proteins called histones to form nucleosomes, which together with nonhistone proteins form a higher-order structure, chromatin (22). It is now well established that chromatin not only provides a DNA storage function but is also involved in gene regulation (41, 63). When the synthesis of histone H4 is inhibited in yeast cells, several genes which are tightly regulated are expressed in a constitutive manner (14). The mouse mammary tumor virus (MMTV) promoter, which is repressed in the absence of glucocorticoid hormone, becomes constitutively expressed when the nucleosome density is decreased by coinjection of competitor DNA, as shown in *Xenopus* oocytes (43). Injection of single-stranded DNA into *Xenopus* oocytes leads to nucleosome assembly coupled to DNA synthesis. This results in a tighter chromatin structure which confers a more stringent repression of transcription than chromatin formed on DNA injected in the double-stranded form (2). Several studies suggest that chromatin acts both by excluding certain upstream-gene-specific transcription factors from their recognition sites (1, 4) and by inhibiting access of the basic transcription machinery to the transcriptional initiation site (17, 30, 33, 64).

Regulatory regions of many inducible genes in mammals and yeast have positioned nucleosomes. Examples include the MMTV promoter (53), the glucocorticoid-responsive enhancer region of the rat tyrosine aminotransferase gene (9, 52), the mouse *Cyp1A1* promoter (37), and the yeast *PHO5* promoter (16). The array of nucleosomes occupying defined sites on DNA is thought to prevent more efficiently the binding of transcription factors to their cognate DNA sites, thereby preventing constitutive basal transcription (55, 62). Upon gene activation, the structure of a subset of these nucleosomes is rapidly altered in vivo into a more open form, experimentally revealed as the appearance of a DNase I-hypersensitive site. This is seen after glucocorticoid hormone activation of the MMTV promoter (66) and the tyrosine aminotransferase gene (25). These hormone-induced DNase I-hypersensitive sites appear only in a restricted segment of the regulatory region and coincide with the site of the hormone response element. The structural and mechanistic bases for the transition from a positioned nucleosome(s) to a DNase I-hypersensitive site are not known.

Several in vitro studies have indicated that binding of a hormone-receptor complex, such as glucocorticoid receptor (GR) (42), estrogen receptor (47), or progesterone receptor (48), to its response element in a nucleosome is insufficient for nucleosome disruption. It has been suggested that a nucleosome remodeling activity is required for gene activation, in addition to gene-specific activators. Biochemical and genetic studies have identified a set of genes in the yeast *Saccharomyces cerevisiae* that may have such a function. These genes are called the *SWI/SNF* genes. They are essential for the activation of a number of inducible genes (29, 46). Genetic and biochemical evidence suggests that the *SWI/SNF* gene products have a function which is associated with chromatin and may be involved in remodeling of nucleosomes. First, deletions of some of the *SWI/SNF* genes led to an altered chromatin structure (23), and second, mutations in chromatin-associated proteins, such as the histones, override the phenotype caused by deletion of some of the *SWI/SNF* genes (5, 23, 27).

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The *SWI/SNF* gene products in yeast form a large protein complex, the SWI/SNF complex (7, 45), containing at least 10 subunits. The purified yeast SWI/SNF complex can disrupt the structure of nucleosomes in the presence of ATP in vitro and thereby facilitate the binding of transcription factors, such as GAL4 (12). The SWI2/SNF2 protein is highly conserved evolutionarily (8, 26). One apparently functional homolog, brahma, has been identified in *Drosophila melanogaster* (56). An additional and more distantly related protein, ISWI (15), is a constituent of another protein complex, the nucleosome remodeling factor (NURF) (57), which can also disrupt chromatin in vitro. In mammals, two close homologs of *SWI2/SNF2* have been identified, human and mouse *brahma* (10, 38) and *brahma*-related gene (*BRG1*) (10, 26, 51). The *Drosophila* brahma and the human BRG1 proteins are components of large multiprotein complexes, as is yeast SWI2/SNF2, and these complexes have been implicated in counteracting chromatin-mediated repression of transcription (13, 24, 28, 56). The human SWI/SNF protein complex has an ATP-dependent nucleosome-disrupting activity similar to that of the yeast SWI/SNF complex in vitro (24, 28), and the human BRG1 ATPase domain complements the yeast homolog in vivo (26). It remains to be shown whether either of these in vitro nucleosome remodeling complexes (or others which have yet to be discovered) have a role in the nucleosome disruption which occurs during transcriptional induction (see above).

Gene activation in vivo is tightly regulated, as is the changing of nucleosome structure. The alterations of the chromatin structure, observed as DNase I-hypersensitive sites, are usually limited to regulatory regions (21). Studies performed with the yeast and human SWI/SNF complexes, NURF, and related complexes in vitro have demonstrated that these protein complexes remodel the nucleosome structure so that certain transcription factors, such as GAL4 derivatives (12, 28), TATA-box-binding protein (TBP) (24), the GAGA factor (58), and the heat shock factor (59), gain access to their binding sites in a nucleosome. The yeast SWI/SNF complex binds DNA, but the binding is not sequence specific; rather, it depends on the length and the structure of DNA, with a strong preference for four-way junction DNA (50). If the SWI/SNF complex, or any other chromatin remodeling protein complex, participates in the site-specific chromatin opening process, then the chromatin remodeling complex would have to be targeted to or its activity stimulated at that site. We postulate that GR belongs to a class of transcription factors which can mediate this function. This is based on five experimental observations. (i) The DNase I-hypersensitive site in the nucleosome which contains the glucocorticoid response element (GRE) in the MMTV promoter appears within minutes of glucocorticoid hormone treatment (66). (ii) GR-mediated transcription in yeast requires *SWI/SNF* gene products (26, 65). (iii) Human SWI/SNF homologs enhance the function of mammalian nuclear receptors, such as estrogen receptor, retinoic acid receptor (10), and glucocorticoid receptor (38), in tissue culture cells. (iv) A component(s) of the SWI/SNF complex coprecipitates with a GR derivative in a yeast extract (65). (v) GR has an unparalleled capacity to recognize its cognate GRE within a nucleosome (32) and hence should be able to find its site in chromatin before nucleosome disruption.

Here we have evaluated the capacity of GR to aid nucleosome disruption in cooperation with partially purified SWI/SNF complex. As substrate we used mononucleosomes which contained core histones and a 161-bp DNA segment with or without a GRE positioned in a defined nucleosomal context by use of a synthetic DNA-bending sequence, the TG motif (54). Nucleosome remodeling was monitored by a DNase I footprinting assay (12). We obtained SWI/SNF complex from either human HeLa cells or rat liver nuclear extract. Our results show that in the presence of GR a 2.5- to 3.8-fold-lower concentration of either the human or the rat SWI/SNF preparation is required to obtain a similarly perturbed nucleosome than is required in the absence of GR and that this effect requires the presence of a GRE. Another transcription factor, nuclear factor 1 (NF1), was unable to stimulate SWI/SNF-dependent nucleosome remodeling of mononucleosomes which contained an NF1 binding site. We conclude that binding of GR to a GRE in a nucleosome enhances SWI/SNF activity. We were not able to detect any augmentation by the SWI/SNF complex of the binding of GR to the nucleosomal GRE. Conversely, the binding of NF1 to its nucleosomal binding site was noticeably increased by the activity of SWI/SNF. The results are discussed with special reference to GR-mediated targeting of the SWI/SNF complex.

MATERIALS AND METHODS

A polyclonal antiserum against rat BRG1. Oligonucleotides complementary to the 3′ part of the known sequence of the human *BRG1* gene (GenBank accession number U29175), for which the corresponding amino acid sequence was different from that of the human brahma protein, were used to clone a 416-bp fragment of the rat *BRG1* gene by PCR (coding oligonucleotide, CACGAGGAGCAGG ATGAGGAG; noncoding oligonucleotide, GGTGGTCGGGGTGGAGGAGC C). The cDNAs used in PCR were prepared from total RNA preparations of liver, kidney, and spleen tissues from rats (Sprague-Dawley) by reverse transcriptase (Moloney murine leukemia virus H-RT; Bethesda Research Laboratories). PCR products identical in size, about 420 bp on agarose gel electrophoresis, were obtained from all three cDNA preparations. DNA sequencing of the fragment from rat spleen cDNA revealed a 416-bp PCR product. The fragment was ligated into a pGex3 expression vector (Pharmacia Biotechnology) and expressed as a glutathione *S*-transferase (GST) fusion protein in *Escherichia coli*. Antiserum was raised against the GST fusion protein in a rabbit after purification on a glutathione-Sepharose column according to the manufacturer's recommendations. The antiserum was purified on a protein A-Sepharose column (Pharmacia Biotechnology).

Purification of the SWI/SNF complex from rat liver tissue. Eight rats were killed by cervical dislocation, and their livers were homogenized in an ice-cold solution consisting of 2.1 M sucrose, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.6), 2 mM EDTA, 0.5 mM dithiothreitol (DTT), and a protease inhibitor cocktail containing 0.7 μg of leupeptin per ml, 0.7 μg of pepstatin per ml, 1.6 μg of aprotinin (Boehringer) per ml, 0.15 mM spermine, 0.5 mM spermidine (both from Sigma), and 0.5 mM phenylmethylsulfonyl fluoride (Sigma). The homogenization was done by two three-stroke cycles with a loose-fitting Teflon-glass Potter-Elvehjem homogenizer. The protease cocktail was included in all of the following purification steps unless otherwise indicated. The nuclei were pelleted through a 2.1 M sucrose cushion at 22,000 rpm in an SW27 rotor for 45 min at 4°C. The nuclei were lysed in 10 to 20 ml of a solution containing 0.5 M KCl, 20 mM HEPES (pH 7.6), 0.2 mM EDTA, and 10% glycerol and then centrifuged at 13,000 × *g* for 30 min at 4°C. The extract was diluted 2.5-fold with 20 mM Na_2HPO_4 (pH 7.0) before being applied to a P-11 phosphocellulose (Whatman, Maidstone, United Kingdom) column (2.5 by 2 cm) equilibrated with 20 mM Na_2HPO_4. The column was washed with three column volumes of 20 mM Na_2HPO_4 (pH 7.0) containing 0.3 M KCl, and then the SWI/SNF protein complex was eluted with 20 mM Na_2HPO_4 (pH 7.0) containing 0.8 M KCl. A 60-ml Sephadex G-25 column (Pharmacia Biotechnology) was then used to desalt and change the buffer of the sample to 20 mM HEPES (pH 8.0) with 0.1 M KCl, 10% glycerol, and 0.1 mM EDTA. The sample was applied to a 1-ml Mono Q column (Pharmacia Biotechnology) and eluted with a linear 0.1 to 0.55 M KCl gradient in 20 mM HEPES buffer (pH 8.0) with 10% glycerol and 0.1 mM EDTA at a flow rate of 0.5 ml/min; 0.6-ml fractions were collected. The total volume of the gradient was 7.5 ml. Samples of the peak fractions of the SWI/SNF activity (eluted at approximately 0.35 M KCl) were then fractionated on a Superose HR 6 column (Pharmacia Biotechnology) equilibrated with a buffer consisting of 20 mM HEPES (pH 8.0), 0.1 M KCl, 10% glycerol, 0.5 mM DTT, 8 μg of aprotinin per ml, and 0.3 mM phenylmethylsulfonyl fluoride at a flow rate of 0.2 ml/min; 0.6-ml fractions were collected. The samples were stored in 20% glycerol at −110°C.

Preparation of the human SWI/SNF complex which contained BRG1. The purification from HeLa cells of human SWI/SNF was performed as described by Kwon et al. (28). A partially purified preparation obtained after the phosphocellulose chromatography, complex B (28), was used in this study.

Preparation of GR. The GR-[^{3}H]triamcinolone acetonide complex was purified from rat liver tissue as previously described (42).

Preparation of NF1. A recombinant NF1 preparation was prepared from HeLa cells infected with vaccinia virus which contained a full-length clone with

six histidines fused to the N terminus (20). The purification was performed as previously described (4).

DNA constructs. The plasmids pGo4, pNo4, and pNi4 harbor either a GRE or an NF1 binding site in an *Eco*RI/*Hin*dIII insert of 161 bp. This insert also contains a 95-bp synthetic DNA-bending sequence, the TG motif (54), which directs the rotational positioning of the binding site of the transcription factor. Thus, the Go4 insert, when reconstituted in vitro onto a histone octamer, will position its single GRE so that its two consecutive major grooves are facing the periphery of the nucleosome and are translationally positioned 40 bp from the nucleosome dyad (31). No4 has a single NF1 site in a similar position 50 bp from the nucleosome dyad, while the NF1 site of Ni4 is facing inward and is translationally positioned 45 bp from the nucleosome dyad (4). The plasmid p5TG has a 151-bp *Eco*RI/*Hin*dIII insert containing five 20-bp TG motifs but no binding sites for GR or NF1 (4).

Nucleosome reconstitution and DNase I footprinting. Nucleosomes were reconstituted onto a 161-bp DNA fragment for Go4, No4, and Ni4 and onto a 151-bp fragment for 5TG. The fragments were 5′ end labeled with [γ-^{32}P]ATP (NEN Dupont; specific radioactivity, 6,000 Ci/mmol) by use of T4 polynucleotide kinase (New England Biolabs). Go4 and 5TG were labeled at the *Eco*RI site, and No4 and Ni4 were labeled at the *Hin*dIII site. Nucleosomes were reconstituted by a modified high-salt exchange method (34) using long fragments of histone H1-depleted chromatin (36) from purified rat liver nuclei (19). The histones were dissociated from the long chromatin by using a high salt concentration in the presence of the end-labeled fragment of DNA, and this mixture was then diluted stepwise with a buffer containing no salt for several hours at room temperature to a final NaCl concentration of 0.15 M. The mononucleosomes were purified on a 5 to 30% glycerol gradient as previously described (42). The DNase I digestion was performed on mononucleosomes (10,000 cpm, 4 pg/μl) incubated for 1 h with either GR or NF1 in the presence or absence of human or rat SWI/SNF protein complex in a buffer containing 20 mM Tris-HCl (pH 7.8), 10% glycerol, 35 mM KCl, 3.8 mM ATP, 1.9 mM $MgCl_2$, 5 mM DTT, and 0.1 mg of pork insulin (a kind gift from Novo Nordisk) per ml in a total volume of 45 μl at 25°C. DNase I digestion was performed as previously described (42), and the samples were analyzed on denaturing 6% polyacrylamide gels.

Quantitation of the SWI/SNF nucleosome disruption activity. Quantitation of the SWI/SNF activity, detected by DNase I footprinting, was performed by PhosphorImager analysis using ImageQuant software (Molecular Dynamics). The SWI/SNF activity in the presence of GR was compared with the activity in the absence of GR by measuring the integrated counts of sites which were altered in an SWI/SNF-dependent manner. The counts obtained were corrected for the background, which was measured at the same positions in control lanes. The control lanes contained only nucleosomes for the series without GR and nucleosomes incubated with only GR for the series in the presence of GR. The background-corrected counts of the SWI/SNF-dependent site were plotted as a function of the amount of SWI/SNF complex which had been added. This generated two curves for each quantitated SWI/SNF-dependent site, one for the SWI/SNF activity in the presence of GR and once for the activity in the absence of GR. The amount of SWI/SNF complex required to give the same level of nucleosome disruption (i.e., the same counts), namely, approximately 50% of the maximal effect, in each particular experiment was determined. GR-induced stimulation was expressed as the ratio of the SWI/SNF concentration in the absence of GR to the concentration in the presence of GR. The average of several experiments was then calculated for two different sites for which the largest SWI/SNF effects were seen (see Fig. 4A and C and 5). The effect of NF1 on the SWI/SNF activity when a nucleosome containing an NF1 binding site was used was quantitated in the same way (see Fig. 6A). A reference band not affected by the process to be quantitated is often used to normalize for the variations in sample loading and DNase I activity. This was not possible here since the SWI/SNF activity affected all bands and the use of a reference band would have introduced a systematic error. By PhosphorImager analysis of the full-length probe we estimated the variation in the amount loaded in each lane to be $\pm$15%. There was no systematic error introduced by the variation in loading and hence no significant effect on the fold GR stimulation of the SWI/SNF activity obtained. Quantitation of the full-length probe and the sum of DNase I-cut fragments by PhosphorImager analysis gave assurance that at least 69% of the DNA remained undigested and hence that a single DNase I cleavage per DNA strand was maintained in the footprinting reactions (6).

DMS methylation protection. Samples were incubated as described for the DNase I footprinting assay. Dimethyl sulfate (DMS) methylation protection was performed as previously described (32) and analyzed on denaturing 6% polyacrylamide gels. Quantitation of GR (32) and NF1 (4) binding was performed by PhosphorImager analysis.

SDS-PAGE and Western blotting. Samples were precipitated in 30% trichloroacetic acid and separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE). The proteins were transferred to an Immobilon polyvinylidene difluoride transfer membrane (Millipore) by using a semidry blotter (Sartorius) in a transfer buffer containing 30 mM glycine-glycine buffer (Sigma), 10 mM imidazole, and 0.1% SDS with 4% methanol. The membrane was incubated overnight with polyclonal rabbit anti-rat BRG1 antiserum in 20 mM Tris-HCl (pH 8.0) with 0.05% Tween 20 and 150 mM NaCl. Before development of the Western blot, it was first incubated with a secondary goat anti-rabbit antibody conjugated to alkaline phosphatase (Promega); it was then developed with a mixture of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-1-phosphate at pH 9 according to the manufacturer's recommendations (Promega).

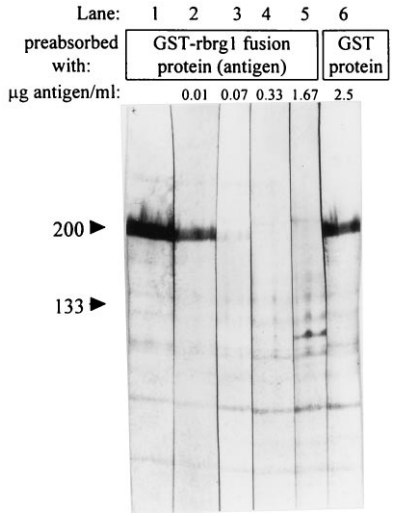

FIG. 1. Immunoblot of an SDS-polyacrylamide gel of rat liver nuclear extract, using polyclonal anti-rat BRG1 (rbrg1) antibodies. Rat liver nuclear extract (5 μg/lane) was separated by SDS–7% PAGE and transferred to a membrane which was subsequently cut into strips. These strips were probed with protein A-purified anti-rat BRG1 antibodies (5.6 μg/ml) which had been preabsorbed with increasing amounts of antigen (purified GST-BRG1 fragment expressed in *E. coli*). Rat liver nuclear extract was incubated with the antibodies alone (lane 1), with antibodies which had been preabsorbed with the indicated concentrations of antigen (lanes 2 to 5), and with antibodies which had been preabsorbed with bacterially expressed GST (2.5 μg/ml) (lane 6). Size markers (in kilodaltons) are indicated to the left.

Protein determination. The protein concentration was determined by the Bradford protein assay (Bio-Rad) with a standard curve based on bovine serum albumin (fraction V).

Nucleotide sequence accession number. The nucleotide sequence of the rat *BRG1* gene fragment used in the preparation of anti-BRG1 antiserum has been deposited in the EMBL database and given accession number X99723.

RESULTS

Characterization of a polyclonal anti-rat BRG1 antiserum. We raised antibodies against the protein product of the rat homolog of human *BRG1* so that we could purify the rat SWI/SNF complex and study its chromatin opening activity. To obtain an antigen, we produced oligonucleotides complementary to the bromodomain in the 3′ end of the known sequence for the human *BRG1* gene. The oligonucleotides were used in a PCR with total cDNA of rat spleen tissue as the template. The product of this PCR was a 416-bp DNA fragment which was also obtained with cDNA from kidney and liver tissues. Its DNA sequence corresponded to positions 3829 to 4250 of the published human *BRG1* sequence (accession number U29175) (26). In-frame deletions, corresponding to one amino acid in the human gene and three amino acids in the rat gene, made the rat clone 6 bp shorter than the corresponding human cDNA. The rat and the human *BRG1* genes had 90% nucleotide sequence homology in this segment. At the amino acid level, 98.5% identity and 99% similarity was observed. The rat *BRG1* fragment was expressed as a GST fusion protein in *E. coli*. A rabbit antiserum to the whole fusion protein was raised. The antiserum recognized a main band with a relative molecular mass (M_r) of 200 kDa in rat liver nuclear extract immunoblots (Fig. 1, lane 1). Several weak bands were also recognized. Parallel immunoblotting with preimmune serum from the same rabbit did not reveal any bands (data not shown). The 200-kDa protein signal selectively disappeared when the anti-

serum was preabsorbed with the purified GST-BRG1 fusion protein prior to Western blotting (Fig. 1, lanes 2 to 5) but was not decreased when the antiserum was preabsorbed with GST protein alone (Fig. 1, lane 6). The M_r of 200 kDa agrees with the size which has been reported for the human BRG1 protein (26). Furthermore, an antiserum raised against human BRG1, kindly provided by G. Crabtree (26), also revealed a band with an M_r of 200 kDa in the rat extract (data not shown). In addition, immunoblotting with our rat BRG1 antiserum revealed a single band with an M_r of 190 kDa in a *Xenopus* oocyte extract, and such extracts from oocytes injected with the human BRG1 expression vector pBJ5 (26) resulted in a double band of 190 and 200 kDa (60a). We conclude that our polyclonal antiserum recognizes a major component of rat liver extract whose size is identical to that of human BRG1 and that cross-reacts with human BRG1 protein. We assume that the endogenous component with an M_r of 190 kDa which was found in *Xenopus* oocyte extracts represents an endogenous frog homolog of BRG1.

Partial purification of the rat SWI/SNF protein complex containing BRG1. Immunoblotting with the anti-rat BRG1 antiserum showed that most of the rat liver BRG1 antigen was found in the supernatant after extraction of purified rat liver nuclei in HEPES buffer containing 0.5 M KCl (data not shown). However, we detected no nucleosome-disrupting activity in this fraction when aliquots were incubated with mononucleosomes which had been reconstituted in vitro and subsequently analyzed by DNase I footprinting (12). This analysis is referred to as the SWI/SNF activity assay. The 0.5 M KCl nuclear extract was applied to a phosphocellulose column. Most of the BRG1 antigen was eluted at 0.8 M KCl, a fraction that also contained detectable SWI/SNF activity (data not shown). The second column, a Mono Q ion exchanger, was eluted with a linear KCl gradient. Immunoblotting of the Mono Q fractions showed that the rat BRG1 protein eluted at approximately 0.35 M KCl, and the assay for SWI/SNF activity showed that the fractions which contained BRG1 also contained SWI/SNF activity (data not shown). We purified the extract further by gel filtration on Superose HR 6. The BRG1 protein eluted in a fraction corresponding to an M_r of greater than 670 kDa (Fig. 2A). The fractions which contained rat BRG1 antigen (Fig. 2B) corresponded to the fractions which contained SWI/SNF nucleosome-disrupting activity, as was shown by the SWI/SNF activity assay (Fig. 2C, fractions 18 to 20). The large size of the rat BRG1 complex, which we could only estimate here due to the lack of M_r standards larger than 670 kDa, is consistent with the M_r found for the human SWI/SNF complex (28, 45). We sometimes found bands of BRG1 antigenic activity with lower molecular weights in later fractions of the Superose HR 6 chromatogram. These bands, which became stronger when protease inhibitors were not included in all steps, are believed to be products of BRG1 proteolysis. In addition, a weak nucleosome-disrupting activity around fractions 24 to 25 was repeatedly observed, corresponding to an M_r of approximately 555 kDa (Fig. 2A and C). These fractions did not have any detectable BRG1 antigenic activity and may thus represent another nucleosome-disrupting activity.

To investigate whether any major components were lost during the three-step chromatography of the rat SWI/SNF preparation, we applied a whole nuclear extract from rat liver tissue directly onto the Superose HR 6 column. The rat BRG1 antigen and the nucleosome-disrupting SWI/SNF activity eluted at the same position as they did for the purified SWI/SNF preparation (data not shown), suggesting that the complex was stable throughout the purification.

Both the yeast and human SWI/SNF complexes require hydrolyzable ATP in order to disrupt nucleosomes (12, 26, 28). Figure 3A shows that the rat SWI/SNF complex also required hydrolyzable ATP for nucleosome-disrupting activity. No detectable nucleosome disruption occurred when ATP was replaced by the nonhydrolyzable adenosine 5′-*O*-(3-thiotriphosphate) (γ-S-ATP) (Fig. 3A, compare lanes 2 to 4 with lanes 5 to 7). The nucleosomal 10-bp ladder of DNase I cuts remained essentially unaltered upon DNase I footprinting in the absence of hydrolyzable ATP. No effect of the rat SWI/SNF preparation could be detected on naked DNA, either with ATP or with γ-S-ATP (Fig. 3B).

Binding of GR to a nucleosomal GRE enhances SWI/SNF-dependent nucleosome disruption. We reconstituted a mononucleosome with a DNA fragment (Go4) which contained a GRE in order to study whether the binding of GR to this nucleosome would affect the SWI/SNF activity. The GRE in this nucleosome binds GR with about 1.5-fold-lower affinity than in naked DNA (32). The nucleosome-disruptive effect of increasing concentrations of SWI/SNF complex was evaluated by DNase I footprinting in the presence and the absence of purified GR. The GR concentration, about 7 nM active GR monomer, rendered a specific, albeit weak, footprint at the nucleosomal GRE (Fig. 4A, lanes 4 and 5; Fig. 4C, lane 4). The footprint is weak due to the protection from DNase I cutting by the rotationally positioned nucleosomal DNA.

In the presence of GR, lower concentrations of both the human (Fig. 4A) and rat SWI/SNF complexes (Fig. 4C) were required to achieve an altered DNase I cleavage pattern signifying SWI/SNF activity. This DNase I cleavage pattern was not seen when GR was bound to the Go4 nucleosome in the absence of SWI/SNF complex (Fig. 4A, lanes 4 and 5). We quantitated the SWI/SNF activity by measuring the integrated counts of such SWI/SNF-induced sites with a PhosphorImager. SWI/SNF-dependent alterations can be observed at many positions along the DNA fragment, as seen in Fig. 4A and C. Two sites particularly sensitive to SWI/SNF activity were chosen for quantitation. These sites, referred to as α and β, are located 23 to 26 bp and 24 bp on either side of the nucleosome dyad, respectively. The α site is located close to the GRE (Fig. 4A and C). The SWI/SNF activity was plotted as a function of the amount of SWI/SNF complex added (Fig. 4B). The maximum SWI/SNF effect varied from experiment to experiment, but the trend was always the same. More SWI/SNF complex was required to disrupt nucleosomes in the absence of GR than was required in the presence of GR. The GR-dependent SWI/SNF stimulation also varied with the nucleosomal site chosen for quantitation. For the α site, there was a 3.8-fold ± 1.3-fold ($n = 7$) stimulation by the human SWI/SNF complex, while for the β site, there was a 2.7-fold ± 0.5-fold ($n = 7$) stimulation. The rat SWI/SNF complex was stimulated by GR at the corresponding α and β sites 3.2-fold ± 1.2-fold ($n = 5$) and 2.5-fold ± 0.4-fold ($n = 6$), respectively. In order to address whether this effect required binding of GR to the nucleosome, we used a DNA fragment, 5TG, which lacked a GRE but was otherwise similar to Go4. No GR-dependent stimulation of SWI/SNF activity was seen for this construct on either of the quantitated sites, α or β, which are located 32 to 35 bp and 15 bp on either side of the nucleosome dyad, respectively (Fig. 5). In addition, GR-dependent stimulation was not obtained when nucleosomal No4 was used (data not shown), a construct in which the GRE has been exchanged for a binding site for the transcription factor NF1 (4).

We then tested whether stimulation of the SWI/SNF nucleosome disruption would occur with another DNA-binding transcription factor. We incubated partially purified NF1 protein and the SWI/SNF complex together with mononucleosomal

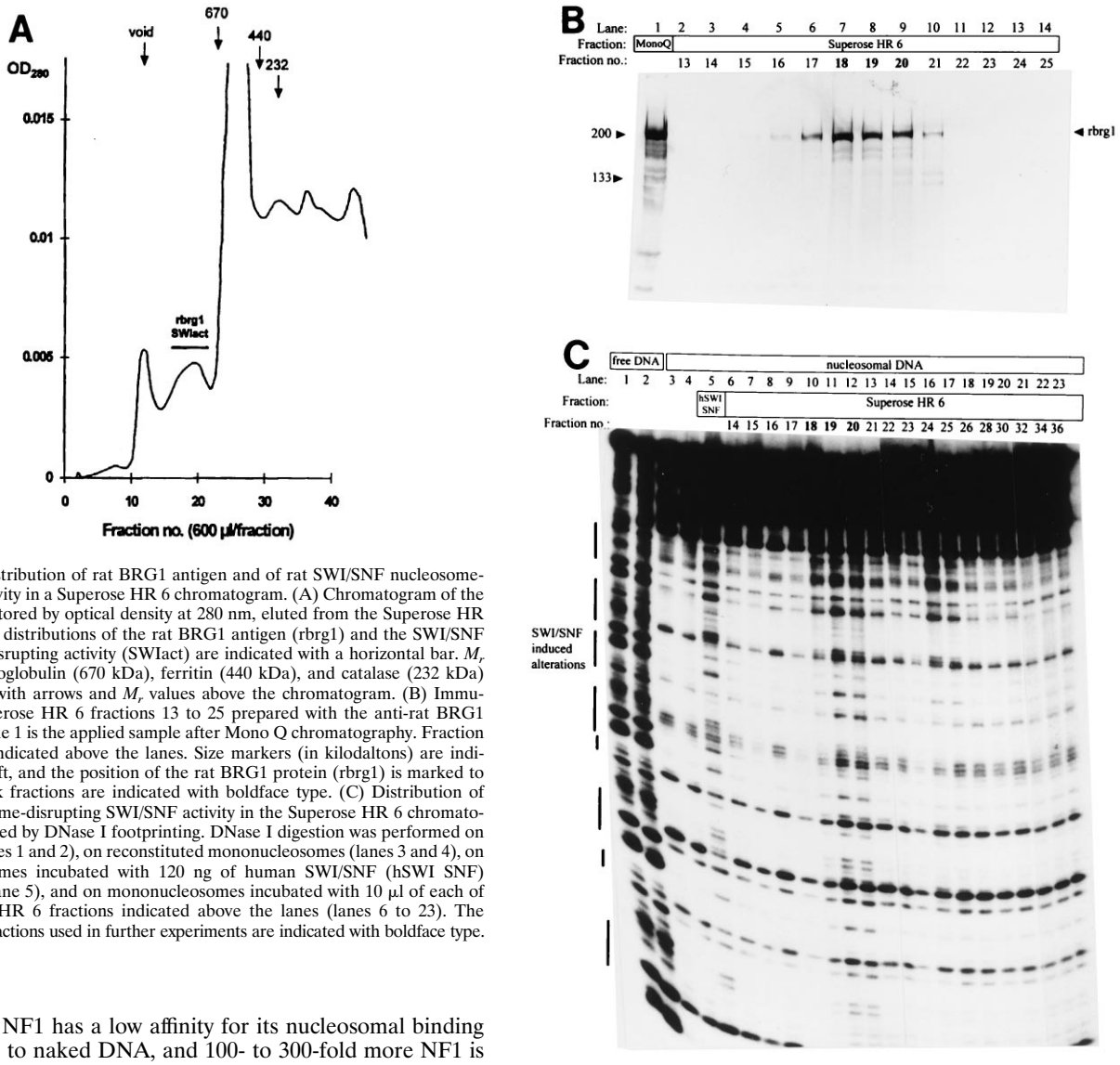

FIG. 2. Distribution of rat BRG1 antigen and of rat SWI/SNF nucleosome-disrupting activity in a Superose HR 6 chromatogram. (A) Chromatogram of the proteins, monitored by optical density at 280 nm, eluted from the Superose HR 6 column. The distributions of the rat BRG1 antigen (rbrg1) and the SWI/SNF nucleosome-disrupting activity (SWIact) are indicated with a horizontal bar. M_r standards thyroglobulin (670 kDa), ferritin (440 kDa), and catalase (232 kDa) are indicated with arrows and M_r values above the chromatogram. (B) Immunoblot of Superose HR 6 fractions 13 to 25 prepared with the anti-rat BRG1 antiserum. Lane 1 is the applied sample after Mono Q chromatography. Fraction numbers are indicated above the lanes. Size markers (in kilodaltons) are indicated to the left, and the position of the rat BRG1 protein (rbrg1) is marked to the right. Peak fractions are indicated with boldface type. (C) Distribution of mononucleosome-disrupting SWI/SNF activity in the Superose HR 6 chromatogram as analyzed by DNase I footprinting. DNase I digestion was performed on free DNA (lanes 1 and 2), on reconstituted mononucleosomes (lanes 3 and 4), on mononucleosomes incubated with 120 ng of human SWI/SNF (hSWI SNF) preparation (lane 5), and on mononucleosomes incubated with 10 μl of each of the Superose HR 6 fractions indicated above the lanes (lanes 6 to 23). The pooled peak fractions used in further experiments are indicated with boldface type.

No4 DNA. NF1 has a low affinity for its nucleosomal binding site relative to naked DNA, and 100- to 300-fold more NF1 is required to obtain a footprint over the nucleosomal binding site than is required for naked DNA (4). Such a high concentration of NF1 leads to nonspecific binding to the nucleosomal DNA, and alterations of the DNase I digestion pattern were observed with NF1 alone throughout the entire DNA fragment. Nevertheless, distinct SWI/SNF-induced alterations of the DNase I cleavage pattern were observed. Quantitation of the indicated α and β sites located 27 to 32 and 26 bp, respectively, on either side of the nucleosome dyad (4) (Fig. 6A) gave a 1.5-fold ± 0.7-fold ($n = 3$) and a 1.2-fold ± 0.2-fold ($n = 4$) stimulation of SWI/SNF activity, respectively, in the presence of NF1 (Fig. 6). The stimulation of the SWI/SNF activity which was induced by GR was significantly different ($P < 0.02$, Student's t test) from the small effect induced by NF1 with the exception of the rat α site ($P = 0.1$, Student's t test). Also, the graphs obtained by plotting SWI/SNF activity in the presence and absence of NF1 on nucleosomal No4 essentially follow each other, in contrast to those with GR and nucleosomal Go4 (compare Fig. 4B and 6B). We conclude that GR, but not NF1, when bound to its DNA binding site, is able to stimulate SWI/SNF nucleosome disruption.

The effect of SWI/SNF activity on the access of GR and NF1 to their nucleosomal DNA-binding sites. When nucleosomes have been disrupted by the yeast and human SWI/SNF complexes, binding of GAL4 derivatives and TBP to their cognate binding sites is facilitated (12, 24, 28). These transcription factors have considerably lower affinities for their nucleosomal binding sites than for the same sites on free DNA. This is in sharp contrast to GR, which binds almost as strongly to its cognate site in a nucleosome as in free DNA (32).

We observed that the GR footprint over the GRE vanished with increasing amounts of the SWI/SNF preparations (Fig. 4A and C) and that this depended on the presence of ATP (data not shown). Thus, DNase I cleavage sites in the nucleosomal GRE, which were protected by GR binding in the absence of the SWI/SNF complex, reappeared in the presence of the SWI/SNF activity. This gave the impression that the SWI/SNF activity triggered dissociation of GR from the GRE. However, cleavage at these DNase I sites in the GRE was also increased by SWI/SNF activity in the absence of GR (Fig. 4A, lanes 12 to 17), suggesting that disruption of histone-DNA contacts causes this effect. To examine whether GR had dissociated from the nucleosomal GRE, we carried out DMS methylation protec-

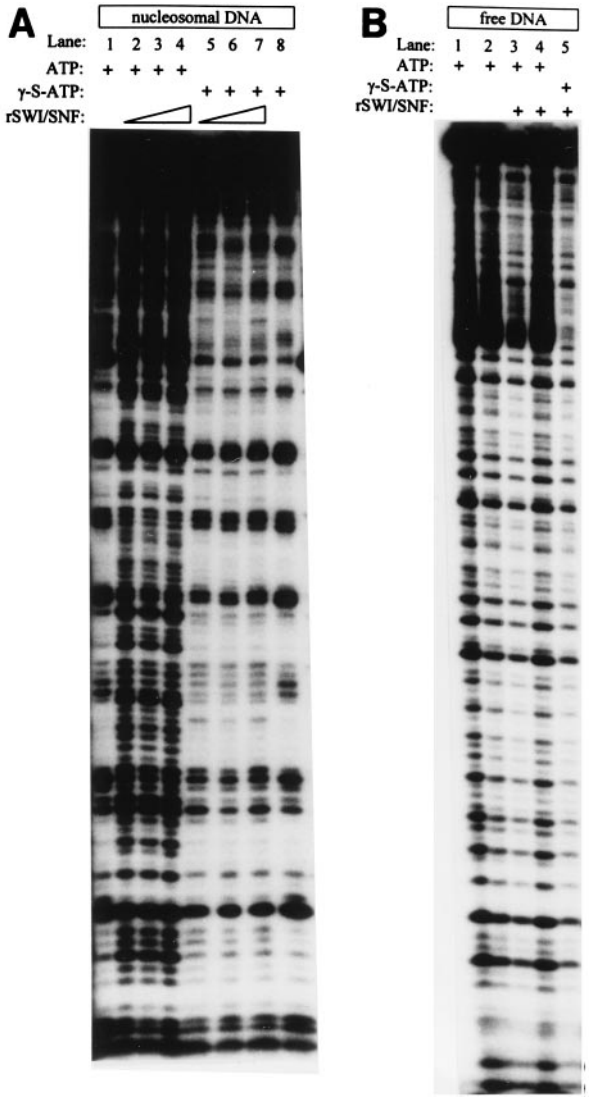

FIG. 3. The rat SWI/SNF protein complex requires hydrolyzable ATP for nucleosome disruption. (A) DNase I footprinting of mononucleosomes in the presence of 3.8 mM ATP–1.9 mM $MgCl_2$ (lanes 1 to 4) and increasing amounts of rat SWI/SNF (rSWI/SNF) preparation which had been purified on Superose HR 6 (60, 120, and 240 ng in lanes 2 and 5, 3 and 6, and 4 and 7, respectively). In lanes 5 to 8, ATP was replaced by 3.8 mM γ-S-ATP. (B) The rat SWI/SNF complex has no effect on naked DNA. Shown are the results of DNase I digestion of naked DNA in the presence of 3.8 mM ATP–1.9 mM $MgCl_2$ (lanes 1 to 4) or in the presence of 3.8 mM γ-S-ATP–1.9 mM $MgCl_2$ (lane 5). In lanes 3 to 5, 200 ng of rat SWI/SNF (rSWI/SNF) preparation was added.

tion analysis when GR was bound to a nucleosome in the presence and absence of the SWI/SNF complex. This showed that the GR-GRE contacts remained intact in the presence of the SWI/SNF complex, and we therefore conclude that the GR was still bound to the GRE (Fig. 7). These experiments also indicated that SWI/SNF activity did not enhance the binding of GR to its nucleosomal site. The fact that GR has almost as high an affinity for its site in the Go4 nucleosome as for the site in free DNA probably explains this result.

The affinity of NF1 for its binding site within a nucleosome is low regardless of the rotational phase and translational positioning relative to the histone octamer (4). Since the SWI/SNF complex can facilitate the binding of other factors with lower affinities for their nucleosomal binding sites, we thought it interesting to investigate the influence of the SWI/SNF complex on the binding of NF1 to mononucleosomes. DNase I footprinting experiments indicated that the human and rat SWI/SNF preparations facilitated NF1 binding, although the results were difficult to quantitate due to interference between NF1 binding and SWI/SNF activity (Fig. 8A). However, we were able to quantitate the specific NF1 binding in the presence and absence of SWI/SNF activity by using DMS methylation protection (Fig. 8B). In our hands, this method is not influenced by nonspecific protein-DNA interaction (4). The results showed that the rat SWI/SNF complex facilitated the binding of NF1 to the nucleosomal site, especially at low concentrations of NF1, at which the difference in binding site occupancy was around 10-fold. Interestingly, there was no detectable difference between SWI/SNF-dependent NF1 binding to a nucleosome in which the NF1 site was oriented with its major grooves toward the histone octamer (Ni4) and its binding to a nucleosome in which the NF1 binding site was rotated 180° and faced the periphery of the nucleosome (No4) (Fig. 8B).

DISCUSSION

GR- and GRE-dependent enhancement of SWI/SNF-mediated nucleosome disruption. We have shown that GR bound to a nucleosomal GRE enhances the ATP-dependent nucleosome disruption caused by the SWI/SNF complex. The nucleosome disruption, as revealed by DNase I, occurs over the entire nucleosome, but some sites are more sensitive than others. We have chosen two sites particularly sensitive to SWI/SNF activity for quantitation. These sites are located fairly symmetrically with respect to the nucleosome dyad on the GRE-containing nucleosome Go4. There is a tendency for a stronger GR stimulation of the SWI/SNF activity close to the GRE. The stimulatory effect by GR is 2.5- to 3.8-fold. This enhancement is not seen when NF1 is used in such experiments. One explanation for these results is that GR, when bound to a GRE in a nucleosome, attracts the SWI/SNF complex by direct protein-protein interaction and increases the local SWI/SNF concentration around this nucleosome. Our demonstration of a functional GR-SWI/SNF interaction corroborates previous in vitro results obtained by Yoshinaga et al. (65). They found that the yeast SWI/SNF complex, assayed by immunoblotting with an SWI3 antiserum, coprecipitated with the DNA-binding domain of GR (150 amino acids long) and with the activating domain from the N-terminal half of GR fused to the DNA-binding domain (213 amino acids long).

Another explanation for the observed enhancement of nucleosome disruption by GR is that GR binding as such decreases nucleosome stability. The histone-DNA interaction of a given nucleosome defines an energy threshold which must be overcome in order to disrupt the nucleosome. If the binding of GR decreased that threshold by destabilizing the nucleosome, the SWI/SNF concentration required to disrupt the nucleosome would also be decreased. Our results do not rule out this possibility. However, the small difference in GR-GRE affinity for free and nucleosomal DNA, only 1.5-fold in nucleosome Go4 (32), and the lack of any structural effects of GR binding on the nucleosomal DNA, as evaluated by DNase I footprinting, argue against this explanation. Whether GR binding decreases the stability of a nucleosome or it serves as a physical targeting factor, we conclude that the result of GR binding is a stimulation of the SWI/SNF-mediated nucleosome disruption. To the best of our knowledge, this is the first report demonstrating a targeting of SWI/SNF activity. A recent report by Owen-Hughes et al. (40) describes a related phenomenon,

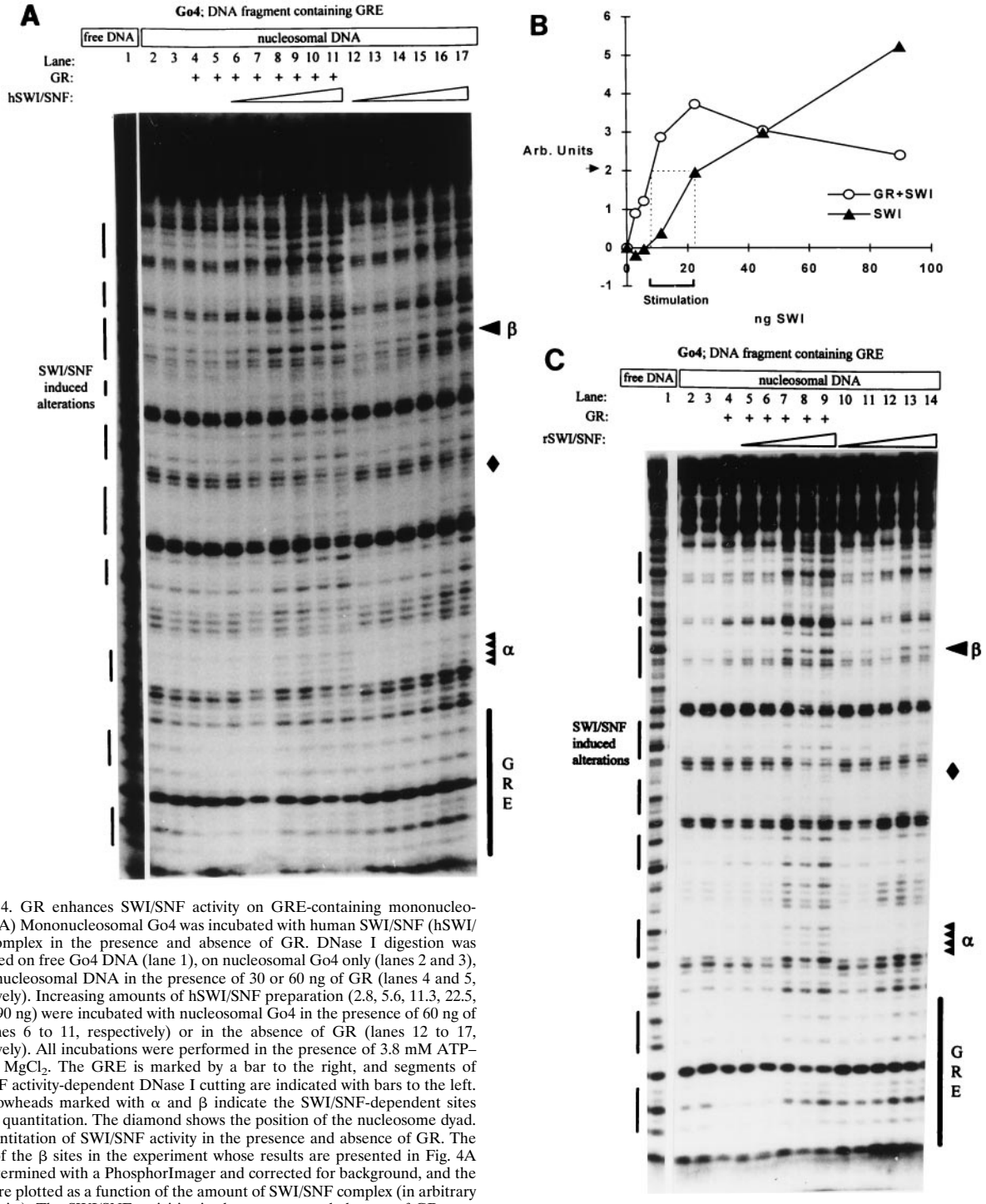

FIG. 4. GR enhances SWI/SNF activity on GRE-containing mononucleosomes. (A) Mononucleosomal Go4 was incubated with human SWI/SNF (hSWI/SNF) complex in the presence and absence of GR. DNase I digestion was performed on free Go4 DNA (lane 1), on nucleosomal Go4 only (lanes 2 and 3), and on nucleosomal DNA in the presence of 30 or 60 ng of GR (lanes 4 and 5, respectively). Increasing amounts of hSWI/SNF preparation (2.8, 5.6, 11.3, 22.5, 45, and 90 ng) were incubated with nucleosomal Go4 in the presence of 60 ng of GR (lanes 6 to 11, respectively) or in the absence of GR (lanes 12 to 17, respectively). All incubations were performed in the presence of 3.8 mM ATP–1.9 mM $MgCl_2$. The GRE is marked by a bar to the right, and segments of SWI/SNF activity-dependent DNase I cutting are indicated with bars to the left. The arrowheads marked with α and β indicate the SWI/SNF-dependent sites used for quantitation. The diamond shows the position of the nucleosome dyad. (B) Quantitation of SWI/SNF activity in the presence and absence of GR. The counts of the β sites in the experiment whose results are presented in Fig. 4A were determined with a PhosphorImager and corrected for background, and the results are plotted as a function of the amount of SWI/SNF complex (in arbitrary [Arb.] units). The SWI/SNF activities in the presence and absence of GR were compared at approximately 50% of the maximal effect, marked with an arrow. The ratio between the amounts of SWI/SNF complex added in the presence and in the absence of GR was then calculated (dotted lines). (C) Mononucleosomal Go4 was incubated with rat SWI/SNF complex (rSWI/SNF) in the presence and absence of GR. DNase I digestion was performed on free Go4 DNA (lane 1), on nucleosomal Go4 only (lanes 2 and 3), and on nucleosomal Go4 in the presence of 60 ng of GR (lane 4). Increasing amounts (12.5, 25, 50, 100, and 200 ng) of the Superose HR 6-purified rat SWI/SNF were incubated with 60 ng of GR (lanes 5 to 9, respectively) or without GR (lanes 10 to 14, respectively). See the legend to Fig. 4A for further details.

the site-specific remodeling of one nucleosome positioned in the center of an array of 11 nucleosomes and containing five GAL4 binding sites. In that study, the combined action of five GAL4 protein dimers and SWI/SNF activity resulted in a persistent and site-specific chromatin disruption. However, the site-specific effect was detected only after removal of SWI/SNF and GAL4 by competition.

The stimulating effect of GR on SWI/SNF activity is weak, only a factor of 2.5 to 3.8 under the in vitro conditions used here. We can speculate that other factors contribute in vivo to the strength of the effect, factors which are lacking in vitro. Furthermore, other, later steps leading to transcription may increase the stimulating effect in vivo. It has been reported that the yeast SWI/SNF complex is a component of the SRB complex, which is part of the RNA polymerase II holoenzyme (61) tightly associated with the C-terminal domain repeat. This

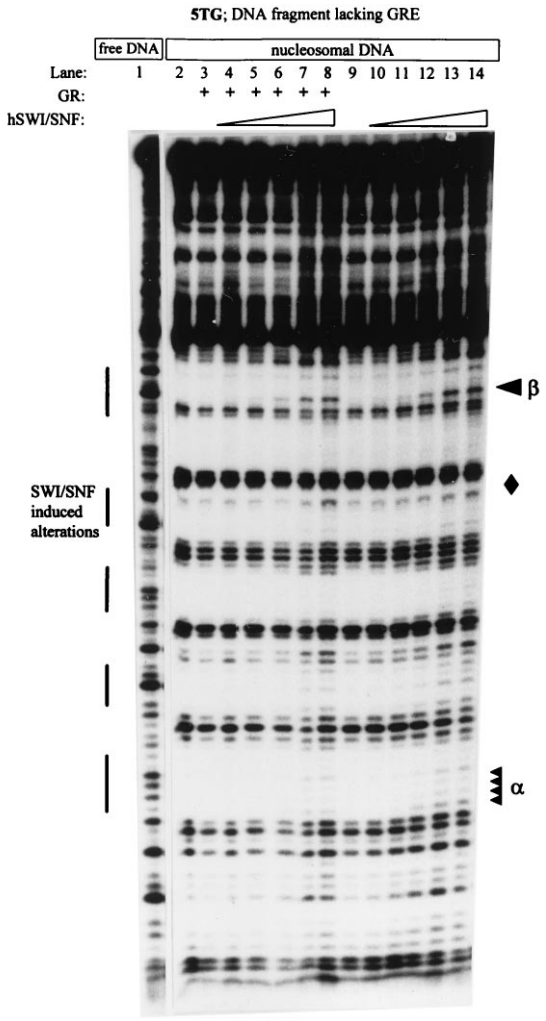

FIG. 5. GR has no stimulating effect on SWI/SNF activity on mononucleosomes lacking a GRE. DNase I digestion was performed on free 5TG (lane 1), on nucleosomal 5TG only (lanes 2 and 9), and on nucleosomal 5TG in the presence of 60 ng of GR (lane 3). Increasing amounts of human SWI/SNF preparation (hSWI/SNF) (5.6, 11.3, 22.5, 45, and 90 ng) were incubated in the presence of 60 ng of GR (lanes 4 to 8, respectively) or in the absence of GR (lanes 10 to 14, respectively). See the legend to Fig. 4A for further details.

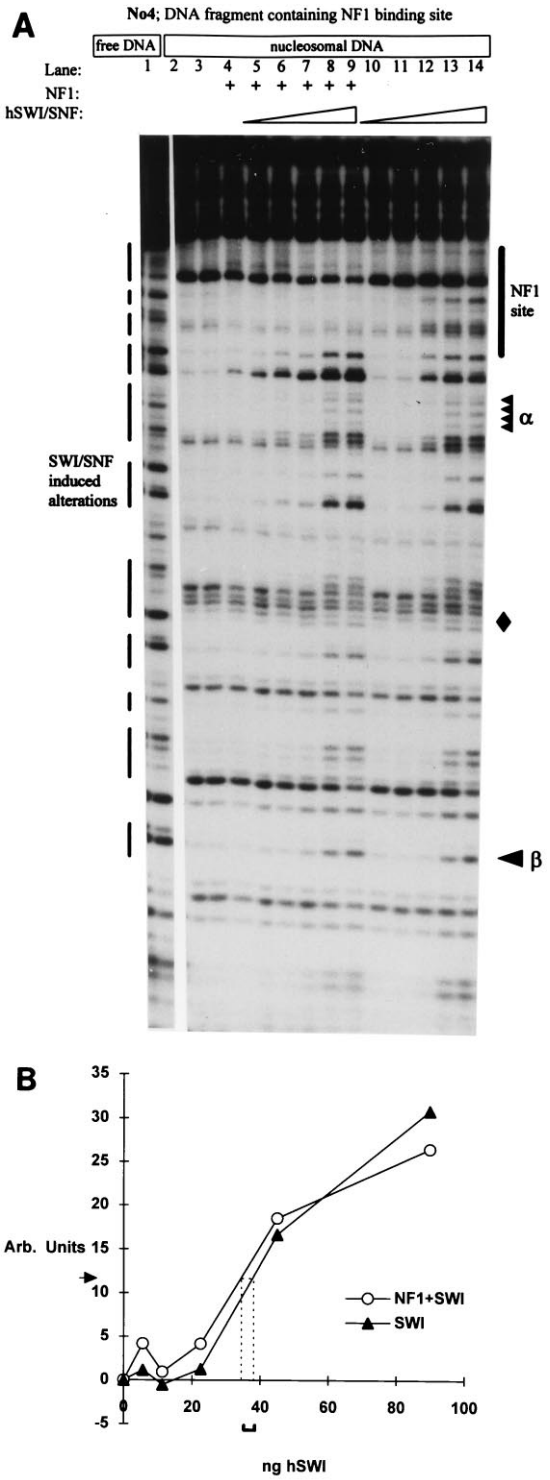

FIG. 6. NF1 binding does not stimulate SWI/SNF activity on mononucleosomes containing an NF1 site. (A) Mononucleosomal No4 was incubated with SWI/SNF complex in the presence and absence of NF1. DNase I digestion was performed on free No4 DNA (lane 1), on nucleosomal No4 only (lanes 2 and 3), and on nucleosomal No4 in the presence of 1 μl of NF1 (lane 4). Increasing amounts of human SWI/SNF preparation (hSWI/SNF) (5.6, 11.3, 22.5, 45, and 90 ng) were incubated with 1 μl of NF1 (lanes 5 to 9, respectively) or without NF1 (lanes 10 to 14, respectively). The NF1 binding site is indicated with a bar to the right, and SWI/SNF-induced alterations are marked with bars to the left. The arrowheads marked with α and β indicate the SWI/SNF-dependent sites used for quantitation. The diamond shows the position of the nucleosome dyad. (B) Quantitation of SWI/SNF activity in the presence and absence of NF1. Data from the β site in the experiment presented in panel A are processed as described in the legend to Fig. 4B. Arb., arbitrary.

suggests that the SWI/SNF complex is targeted to the promoter together with RNA polymerase II. We speculate that in promoters which require nucleosome remodeling for transcription, gene-specific activators form protein contacts with components from the SWI/SNF complex and with other subunits of the RNA polymerase II holoenzyme as well. This would result in a stronger targeting specificity. We have used immunoblots probed with monoclonal antibodies against the C-terminal domain repeat of RNA polymerase II (Promega) to address whether our rat SWI/SNF preparation contains RNA polymerase II. On a Superose HR 6 column, the bulk of the polymerase was eluted just prior to the peak fractions of the SWI/SNF complex, but we were unable to separate these two complexes (39a). These results neither prove nor disprove that a subfraction of the RNA polymerase II is physically associated with the SWI/SNF complex. Furthermore, we have not been able to link the GR-dependent stimulation of nucleosome disruption to any specific component of the SWI/SNF-containing complex.

The identification of additional nucleosome remodeling protein complexes, such as the ATP-dependent NURF purified

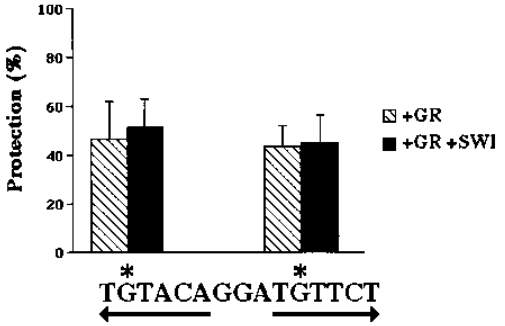

FIG. 7. DMS methylation protection assay assessing the GR-GRE contacts. Two G residues (marked with asterisks), one in each of the palindromic half-sites in GRE (indicated by the arrows below the GRE DNA sequence), were quantitated for DMS methylation protection upon binding of 60 ng of GR in the absence (hatched bars) and presence (solid bars) of 230 ng of rat SWI/SNF preparation. The standard deviations are marked on the bars (n = 8).

from *Drosophila melanogaster* (58), makes another explanation plausible. There may be several other chromatin-disrupting complexes in the cell which remain to be investigated (44). If they have different functions or act together with different gene-specific factors, then the low-level stimulation by GR may be due to the fact that, in vivo, GR functions with a related complex. Alternatively, our SWI/SNF preparation may contain a mixture of several different complexes. If this explanation is valid, then these different complexes have similar chromatographic properties, including similar overall sizes. A recent report by Wang et al. details the occurrence of heterogeneous and cell-type-specific SWI/SNF complexes (60).

Nucleosome stability. We must consider the question of nucleosome stability since the SWI/SNF activity assay was based on nucleosome disruption. The substrate in these reactions was in vitro-reconstituted mononucleosomes purified by glycerol gradient centrifugation. This purification generated an 11S peak which, together with the induced 10-bp DNase I ladder, confirmed that the mononucleosomes were intact. We routinely used a low concentration of in vitro-reconstituted mononucleosomes in these experiments, about 4 pg/μl. Recently, Godde and Wolffe demonstrated that mononucleosomes are less stable at lower concentrations (18). We did not experience the problem of spontaneous nucleosome disruption, either from incubations containing column chromatographic fractions flanking the eluted immunoreactive BRG1 complex (Fig. 2C) or from the binding of only GR to nucleosomes in the absence of SWI/SNF (Fig. 4A). Our nucleosomal DNAs contain 95 bp of a synthetic DNA-bending sequence, the TG motif, which is known to bind the histone octamer with high affinity (54), and this may be the explanation for the stability of our nucleosomes even at low concentrations. We conducted a series of experiments with nucleosomes at five times the concentration (20 pg/μl) usually used in order to examine the effect of nucleosome concentration on SWI/SNF-mediated nucleosome disruption. About five times as much SWI/SNF complex was needed to obtain a comparable degree of nucleosome disruption as was needed at the lower concentration (39a). However, the relative requirements for SWI/SNF complex and for hydrolyzable ATP were not altered, and we conclude that spontaneous nucleosomal disruption cannot explain our results.

SWI/SNF helps NF1, but not GR, bind to a nucleosomal site. In vitro experiments show that NF1 has a relatively low affinity for its binding site in a nucleosome compared with its very high affinity for its binding site on free DNA (3, 4, 49). Here we have

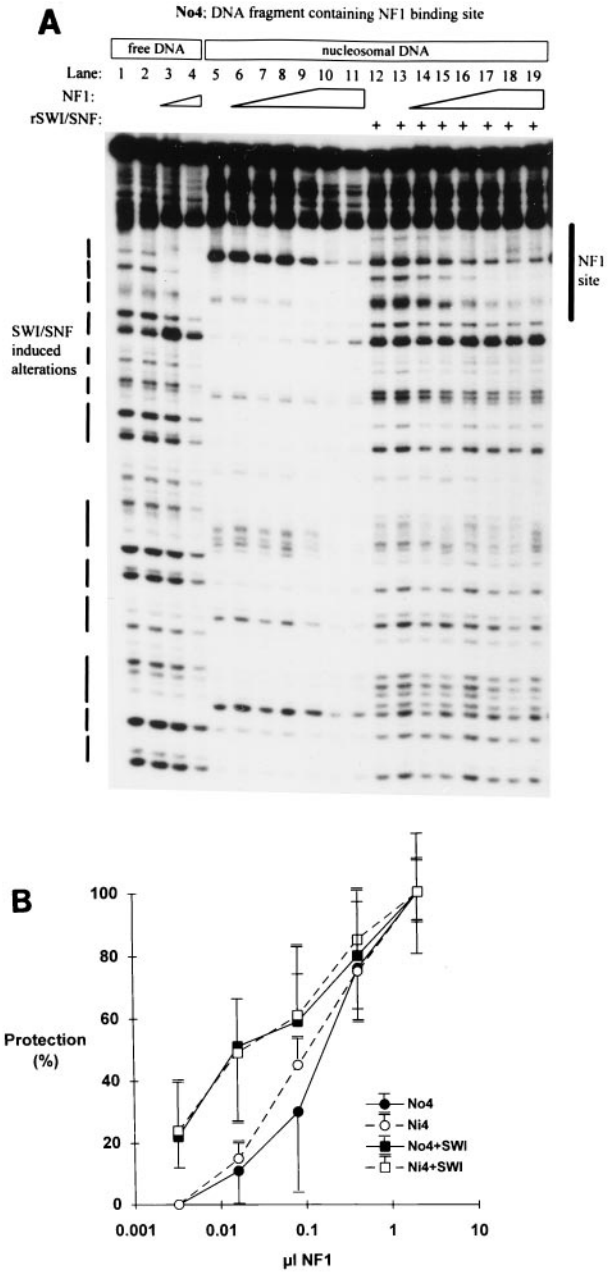

FIG. 8. Binding of NF1 to its nucleosomal binding site, rotated toward the periphery (No4) or toward the histone octamer (Ni4), is increased in the presence of rat SWI/SNF complex (rSWI/SNF). (A) Mononucleosomal No4 was incubated with NF1 in the presence and absence of SWI/SNF complex. DNase I footprinting analysis was performed on free No4 DNA only (lanes 1 and 2), on free DNA in the presence of 0.016 and 0.08 μl of NF1 (lanes 3 and 4, respectively), on mononucleosomal No4 only (lane 5), on mononucleosomal No4 with increasing amounts (0.0032, 0.016, 0.08, 0.4, 2, and 2 μl) of NF1 in the absence of SWI/SNF complex (lanes 6 to 11, respectively) or in the presence (lanes 14 to 19, respectively) of 230 ng of rat SWI/SNF preparation, and on nucleosomal No4 with SWI/SNF complex only (lanes 12 and 13). For further details, see the legend to Fig. 6A. (B) DMS methylation protection of the NF1 binding of the same type of incubation as described for panel A. Closed circles and squares indicate NF1 binding to nucleosomal No4 (in which the major grooves in the NF1 site are facing the periphery of the nucleosome) at increasing concentrations in the absence and in the presence of rat SWI/SNF protein complex, respectively. Open circles and squares indicate NF1 binding to nucleosomal Ni4 (in which the major grooves of the binding site are facing the histone octamer) in the absence and in the presence of SWI/SNF complex, respectively. The methylation protection obtained with 2 μl of NF1 was set to 100%, and the smaller volumes of NF1 were adjusted accordingly. The standard deviations are indicated for each point by error bars (n = 3).

shown that the binding of NF1 to its nucleosomal binding site is facilitated by the SWI/SNF complex at low NF1 concentrations (Fig. 8B). This is similar to the effect of the SWI/SNF complex on the binding of GAL4 derivatives and TBP (12, 24). The increase of NF1 binding at low concentrations caused by SWI/SNF activity may be of physiological interest because NF1 is excluded from its binding site in the uninduced MMTV promoter in vivo (11).

In contrast to the binding of NF1, there was no indication that the binding of GR to its nucleosomal GRE was facilitated by SWI/SNF activity (Fig. 7). The affinity of GR for its GRE in free DNA is only 1.5 times higher than its affinity for the GRE in a nucleosome when the GRE is positioned 40 bp from the nucleosome dyad with the major grooves facing outward (32). Consequently, any increase in binding with SWI/SNF would be difficult to detect. Not even a different nucleosomal positioning of the GRE, in which the GRE was rotated 180° and the affinity between GR and the GRE was reduced sixfold compared to naked DNA (32), resulted in an SWI/SNF-dependent stimulation of GR binding (39a).

The SWI/SNF-induced enhancement of DNase I cutting inside the GRE, which occurs both in the presence and in the absence of GR, may be caused by alterations of the histone-DNA contacts by the SWI/SNF activity. GR binds DNA via the major groove (35), while DNase I cleaves DNA via the minor groove (55). DNA which is rotationally positioned on a nucleosome shows a strong nucleosomal 10-bp ladder of enhanced cleavage with intervening segments of DNase I protection. Binding of GR to a rotationally positioned nucleosomal DNA does not seem to change the access of DNase I to the minor groove more than marginally, and hence the nucleosomal GR-induced footprint is usually weak (cf. Fig. 4A and C). SWI/SNF disrupts histone-DNA contacts, and it is conceivable that this will increase access to DNA for DNase I in the minor groove while GR remains bound to the major groove on the opposite side of the DNA axis. This would explain the SWI/SNF-induced masking of the GR footprint as illustrated in Fig. 4A and C. DMS methylates guanine at the N-7 position, which is located in the major groove of DNA. The GR-dependent protection from DMS methylation in the GRE demonstrates that GR binding persists in spite of the SWI/SNF-induced masking of the DNase I footprint.

A model for GR-mediated chromatin disruption and gene regulation. A role of chromatin may be to restrict transcription factor access and in this way prevent promoter leakage at inducible genes. Both NF1 and TBP are constitutively present in the nucleus, and therefore their access to inducible promoters needs to be restricted. As noted by Cordingley et al. (11), NF1 and TFIID cannot gain access to their DNA binding sites in the MMTV promoter in vivo until after treatment with glucocorticoid hormone. These authors suggested that GR triggers increased access of NF1 and TFIID at this promoter. The appearance of the DNase I-hypersensitive site in the nucleosome organizing the GRE and the NF1 binding site occurs within minutes of hormone treatment (53, 66). Consequently, the rearrangement of the nucleosomal structure upon hormone treatment is probably a prerequisite for binding of NF1 and TFIID in this promoter. A similar mechanism seems to operate in the regulation of the tyrosine aminotransferase gene in response to GR (9, 52) and to the dioxin receptor-mediated induction of the *Cyp1A1* promoter (39). Our results suggest that GR functionally targets SWI/SNF to the promoter and thereby triggers nucleosome disruption.

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