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Specific glucocorticoid receptor binding to DNA reconstituted in a nucleosome

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We have reconstituted a nucleosome with core histones from rat liver using a restriction fragment containing a sequence from the mouse mammary tumour virus (MTV) long terminal repeat (LTR). This sequence harbours glucocorticoid responsive elements (GREs) which mediate glucocorticoid hormone induction of transcription from the MTV promoter via glucocorticoid receptor (GR) binding. Exonuclease III and DNase I footprinting demonstrated that the reconstituted nucleosome was specifically located between positions −219 and −76. A nucleosome was previously shown to be located at a similar or identical position in the MTV promoter *in situ* and to be structurally altered upon glucocorticoid hormone induction. We demonstrated, by DNase I footprinting, that GR is able to bind sequence specifically to the DNA in the *in vitro* assembled nucleosome. No evidence for unfolding of the nucleosome was obtained, but the DNase I footprinting pattern demonstrated GR induced local alterations in the DNA.

Key words: glucocorticoid receptor/MTV LTR/nucleosome reconstitution/specific DNA binding

Introduction

The glucocorticoid receptor (GR) is a soluble intracellular protein which is involved in transcriptional regulation (for review, see Yamamoto, 1985). The action of GR involves binding to specific DNA segments in the vicinity of a target promoter, e.g. the long terminal repeat (LTR) of the mouse mammary tumour virus (MTV) (Chandler *et al.*, 1983; Buetti and Kühnel, 1986). These sequences, defined as glucocorticoid responsive elements (GRE), coincide with a hormone dependent DNase I hypersensitive site (Zaret and Yamamoto, 1984). Richard-Foy and Hager (1987) demonstrated positioning of nucleosomes within the MTV promoter cloned into episomal bovine papilloma virus (BPV) vectors. One nucleosome, positioned between −250 and −60 upstream of the transcription start site, contains the GRE (Buetti and Kühnel, 1986). In the presence of hormone the organization of nucleosomes remained unaltered except for the region between −250 and −60, which became more sensitive to the chemical methydiumpropyl-EDTA-Fe[II] (MPE-Fe[II]). Thus, a hormone dependent local alteration of the chromatin structure is induced by GR binding. In a previous study using an *in situ* exonuclease III protection assay Cordingley *et al.* (1987) showed that nuclear factor 1 (NF1) binding to its cognate sequence in the MTV promoter was hormone dependent. It might suggest that GR alters the chromatin conformation in order to allow binding of NF1.

In many promoters, protein:DNA interaction results in the appearance of DNase I hypersensitive sites (Zaret and Yamamoto, 1984; Becker *et al.*, 1984; Emerson and Felsenfeld, 1984) detectable *in situ* in the chromatin of isolated nuclei. It has been suggested that these sites represent an altered chromatin conformation or histone free regions. Although the nature of these sites remain unclear, they may indicate a possible need to alter the chromatin conformation in these regions in order to establish transcription from a nearby promoter.

Lorch *et al.* (1987) reported that prokaryotic and eukaryotic RNA polymerases are able to transcribe through a nucleosome assembled on a restriction fragment which contains a promoter located outside of the nucleosome, but that the initiation was inhibited if the start site of transcription was included in the nucleosome.

In this paper we have asked whether DNA organized in a nucleosome allows the formation of a specific complex with GR, and if so, if this binding induces displacement of histones or other alterations in the nucleosome structure. We have reconstituted nucleosomes *in vitro* carrying the GRE from the MTV LTR. The position of the nucleosome on the DNA fragment was investigated by DNase I and exonuclease III protection experiments (Ramsay, 1986; Rhodes, 1985). This strategy demonstrated that the reconstituted nucleosome was located at a similar or identical position as *in vivo* (Richard-Foy and Hager, 1987). DNase I footprinting demonstrated that GR can indeed bind to a GRE organized in a nucleosome. The binding resulted in local structural changes of the DNA organized into a nucleosome.

Results

Nucleosome reconstitution

Two DNA fragments, MTV wt and MTV−181/−169, were used for nucleosome reconstitution. These fragments contain segments from MTV LTR which previously have been shown to bind GR *in vitro* (Payvar *et al.*, 1983; Scheidereit *et al.*, 1983) and confer GR inducibility to both the homologous promoter and a heterologous promoter (Buetti and Kühnel, 1986; Chandler *et al.*, 1983). MTV wt, 199 bp in length, harbours the sequence from MTV LTR between −198 to −58 relative to the transcription start site, in addition to 57 bp from the pGEM-1™ cloning vector (see Materials and methods and Figure 5). MTV−181/−169, a 196 bp fragment, is similar to MTV wt but carries a linker scanning mutation at −181/−169, which has been shown to drastically reduce GR inducibility in transfected cells (Buetti and Kühnel, 1986).

Richard-Foy and Hager (1987), have demonstrated nucleosome phasing within the MTV promoter *in situ*. One nucleosome was located between −250 and −60. The two

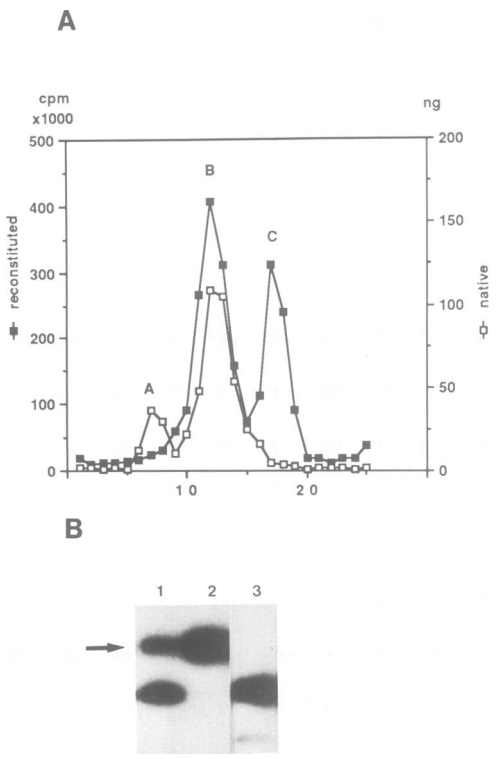

Fig. 1. A. Centrifugation of the reconstituted MTV wt material in a 5–30% glycerol gradient. Native micrococcal nuclease digested chromatin served as internal reference. The ^{32}P-labelled reconstituted material was measured as Cerenkov counts (solid squares) and the unlabelled native nucleosomal DNA as absorbance at 260 nm (open squares). ^{32}P end labelled DNA fragments, 186 bp and 2295 bp in length were used as external sedimentation rate standards (6.5S and 12.1S, respectively, Wrange *et al.*, 1986). Peak B and C sedimented at 11S and 6.5S, respectively. Peak A is likely to correspond to the dinucleosomes as predicted from analysis of deproteinized reference nucleosomes with agarose gel electrophoresis. **B.** Autoradiogram of a low ionic strength polyacrylamide gel loaded with reconstituted material prior to purification in a glycerol gradient and an aliquot of the 11S peak (**lanes 1** and **2**, respectively) (peak B in part A). Naked DNA was included in **lane 3**. The arrow indicates the position of native mononucleosomes as judged by ethidium bromide staining of the gel.

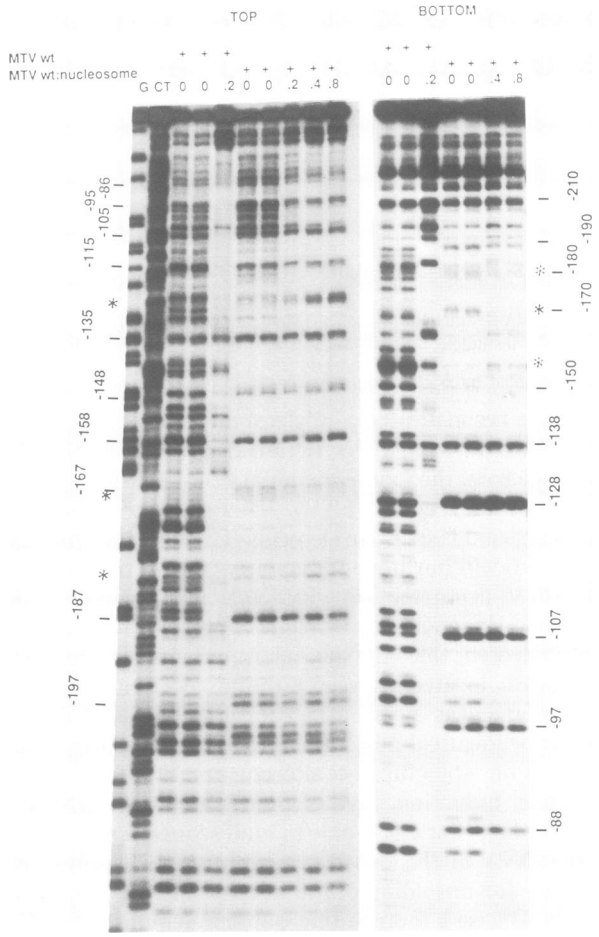

Fig. 2. DNase I footprints of the top and bottom strand of the MTV wt fragment. Naked DNA (**lanes 3–5** of the top strand and **1–3** of the bottom strand), or DNA associated with histone octamers (**lanes 6–10** of the top strand and **lanes 4–7** of the bottom strand) was digested with DNase I. GR was added in the pmol amounts indicated above each lane. G and CT markers were included in **lanes 1** and **2**, respectively. The nucleosome specific patterns of DNase I-sensitive sites are indicated with bars and the position relative to the CAP site. The nucleosome specific GR-induced effects are indicated with stars.

restriction fragments used in this study for nucleosome reconstitution overlap with this sequence and were chosen in order to try to obtain a similar positioning *in vitro*.

Nucleosomes were reconstituted by a high salt exchange method (Losa and Brown, 1987). Prior to reconstitution the 5′ termini of either MTV wt or MTV −181/−169 were end labelled with [^{32}P]ATP using T4 polynucleotide kinase. Long pieces of soluble H1 depleted chromatin, derived by limited micrococcal nuclease digestion of isolated rat liver nuclei (Lutter, 1978), were mixed with the ^{32}P-labelled DNA fragment at 1 M NaCl followed by stepwise dilution to 0.1 M NaCl. Reconstituted nucleosomes were separated from larger chromatin entities and protein free [^{32}P]DNA by glycerol gradient centrifugation, where mononucleosomes form a peak at 11S (Lorch *et al.*, 1987).

In order to analyse the ^{32}P-labelled reconstituted material, reference native nucleosomes were derived from rat liver H1 depleted chromatin by extensive digestion with micrococcal nuclease. This produced mainly mono- and dinucleosomes as judged by agarose gel electrophoresis of deproteinized samples (Ramsay, 1986; data not shown), and they appeared as two distinct peaks in a glycerol gradient centrifugation (Figure 1A, peak A and B).

The reconstituted material also appeared as two peaks in glycerol gradients; one peak which sedimented identically to native mononucleosomes (peak B) and peak C, corresponding to naked DNA fragment. The reconstituted nucleosomes were also analysed by low ionic strength polyacrylamide gel electrophoresis. In such a gel the migration of DNA is reduced when associated with a histone octamer (Lorch *et al.*, 1987). The reconstituted material appeared as two bands on the autoradiogram. The upper band comigrated with native mononucleosomes, detected by ethidium bromide staining (see arrow in Figure 1B), and the lower band comigrated with naked DNA. Thus, reconstituted DNA behave identically to native mononucleosomes as judged by sedimentation in glycerol gradients as well as migration in low ionic strength polyacrylamide gel electrophoresis. This is in agreement with previous reports in which the same technique for nucleosome reconstitution was used (Losa and Brown, 1987).

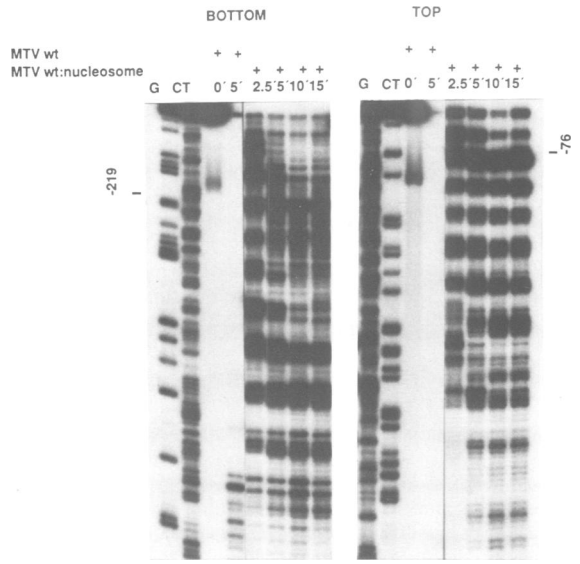

Fig. 3. Exonuclease III protection of the top and bottom strands of MTV wt. The DNA was digested with exonuclease III either naked (**lanes 3** and **4** of the bottom and top strand) or complexed with histones (**lanes 5–8** of the bottom and top strand) for the time in minutes indicated above each lane. The first two lanes in both the bottom strand and the top strand footprints were marker G and CT lanes. The positions of the first persistent stops of the exonuclease III progression are indicated on the sides.

Nucleosome boundaries

The position of the histone octamer on the reconstituted DNA fragment was determined by DNase I and exonuclease III footprinting experiments. Figure 2 shows a DNase I footprint of the top and bottom strand of MTV wt labelled at the *Pvu*II site and the *Bam*HI site, respectively. DNA organized in a nucleosome core displays a distinct pattern of alternating nuclease sensitivity and protection compared to naked DNA. The sites susceptible to nuclease cleavage are separated by ~10 bp which is typical for a DNA helix attached to the surface of a nucleosome core (Drew and Travers, 1985; Lutter, 1978). It reflects the specificity of DNase I which binds to the minor groove of double-stranded DNA and cuts phosphodiester bonds on either of the two DNA strands. Consequently, the minor groove will be exposed only when facing outwards on the surface of a histone octamer. In several footprinting experiments the nucleosome typical cleavage pattern was clearly detectable from approximately position −200 to −75 on the top strand and from −210 to −90 on the bottom strand. A stretch of at least 135 bp on both strands displays a nucleosome like DNase I pattern.

The nucleosome boundaries were further examined by exonuclease III protection of both DNA strands of the reconstituted MTV wt fragment (Ramsay, 1986). The first persistent exonuclease III protection at position −76 in the top strand and position −219 in the bottom strand (Figure 3) correlates well with the DNA segment showing an altered DNase I cutting pattern, approximately −200/−75 and −210/−90 in the top and bottom strand, respectively. The 144 bp defined by the exonuclease III boundaries and the overlapping 135 bp partially protected in a nucleosome specific manner by DNase I demonstrates the position of the DNA on the histone octamer. The same length of DNA, within 2 bp, was protected on isolated native mononucleosomes as well as on reconstituted nucleosomes (Ramsay, 1986; Lutter, 1978; Drew and Travers, 1985). This finding together with the observed nucleosome specific cleavage pattern strongly suggests that one complete histone octamer is directed to a unique site on the MTV wt fragment. The DNase I protection between roughly the positions −210 and −75 and the nucleosome boundaries at positions −219 and −76 suggest the nucleosome dyad to be located around position −147/−148.

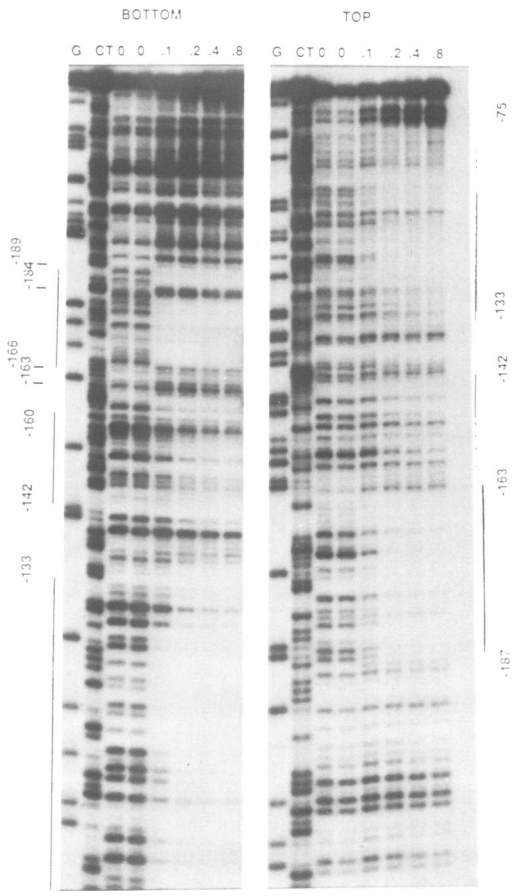

Fig. 4. DNase I footprints of bottom and the top strands of MTV wt. The amounts of added GR in pmol are indicated above each lane. The first two lanes of each footprint were G and CT markers. The GR-induced footprints are indicated (solid lines). Diffuse footprint borders are indicated with dotted lines and approximate positions of footprint borders are indicated.

GR binds to the MTV nucleosome

DNase I footprinting was used to test whether GR is able to bind to its specific DNA sequences when organized into a nucleosome. In such experiments GR was incubated with naked DNA or nucleosome reconstituted DNA prior to DNase I digestion. GR induces three footprints on naked MTV wt sequence (Payvar *et al.*, 1983). This is shown for the bottom and the top strand (Figure 4). Two distinct footprints at −189/−166 and −133/−75 and a less distinct footprint at −160/−142 can be seen. Enhanced cleavage occurs in the bottom strand at positions −184 and −163 (Figure 4).

GR induced several differences in the cleavage pattern of reconstituted nucleosomes. In the downstream receptor

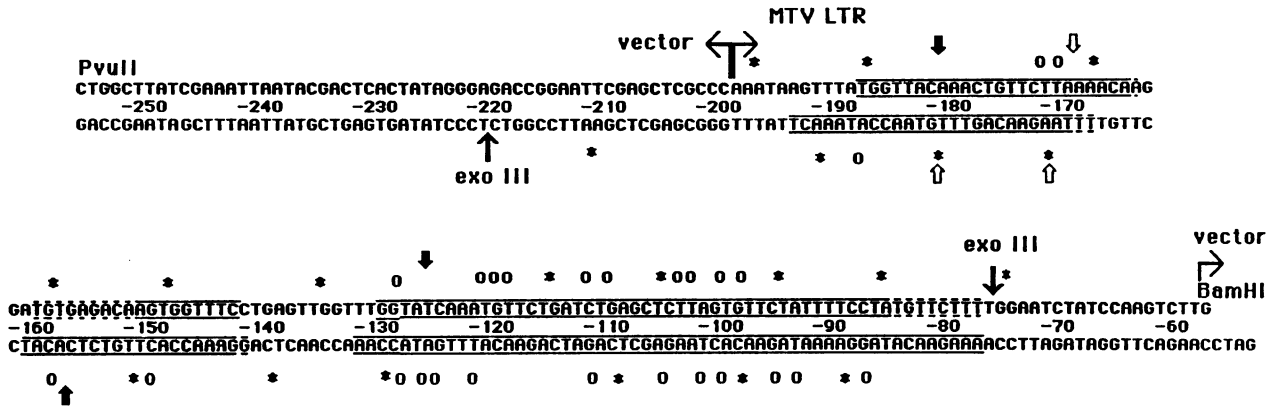

Fig. 5. Summary of the DNase I and exonuclease III protection patterns derived from the top and the bottom strands of MTV wt. The vector part and the MTV LTR part of the fragment is indicated. The GR-induced DNase I footprints in naked DNA are indicated by lines immediately above and below each strand; dotted lines indicated unclear borders. Stars indicate the DNase I-sensitive sites repeated approximately every 10 bp, typical for nucleosomal DNA. Solid arrows marked 'exo III' idicate the first strong exonuclease III stop on each strand of nucleosome reconstituted DNA. Open as well as solid thick arrows indicate nucleosome specific DNase I protections and hypersensitivities, respectively, induced by GR. GR-induced nuclease protection in nucleosomal DNA that is not nucleosome specific is indicated by open circles for each protected base on either strand.

binding site (see Figure 2, top strand), a pronounced hypersensitivity was induced in the presence of GR at position −126. Furthermore, protection is detectable in the somewhat less prominent nucleosome specific cutting region between positions −120 and −76. The protection occurs between the 10 bp repeated nuclease sensitive sites, leaving the nucleosome typical DNase I sensitive sites unaltered. In the upstream binding site one protection is seen at position −169 and a weak enhancement of cleavage at position −181.

GR induced similar effects on the DNase I pattern of the bottom strand of the MTV wt nucleosome. The position −170 is protected from nuclease cleavage in the upstream binding site. A less prominent protection is seen at position −180. Also weak enhancement of cleavage can be observed at positions −184, −163 and −156. In the downstream region protection is observed between the nucleosome typical DNase I sites from position −125 to −80 (Figure 2).

In conclusion, GR induces (i) effects similar to those observed in naked DNA and (ii) several effects which are specific for the nucleosome reconstituted DNA and serve as evidence for nucleosome−GR interaction (stars in Figure 2). These are further discussed below. A summary of GR effects in the DNase I footprinting patterns on naked and reconstituted fragments are shown in Figure 5. The nucleosome specific GR effecs are indicated with thick arrows.

In order to check the specificity of the complex formation between GR and the nucleosomes, MTV −181/−169, a fragment similar to MTV wt but harbouring a mutation in the −189/−165 footprint, was reconstituted. This mutation results in complete loss of the −189/−165 footprint while leaving the downstream protections unaltered. Furthermore, it drastically reduced glucocorticoid inducibility in transfected cells (Buetti and Kühnel, 1986). The histone octamer was positioned at the same unique site on the MTV −181/−169 fragment as MTV wt. GR induced the same changes in the nuclease cleavage pattern corresponding to the intact downstream footprints, but no GR effects were detected at the mutated site (Figure 6).

Although the results presented thus far demonstrate that GR binds to the specific binding sites and induces local alterations of the DNA organized in a nucleosome, we have no evidence for exclusion or dissociation of histones under these *in vitro* conditions.

Discussion

The histone octamer was positioned at one preferred site on the DNA fragment. This is based on the first strong exonuclease III stop on each strand, separated by 144 bp and in agreement with the nucleosome specific DNase I protection. We cannot exclude that a small amount of the DNA is differently positioned. However, the strong preference for one distinct position is evident from the exonuclease III experiments (c.f. Figure 3). Nucleosome reconstitution with other DNA fragments has also shown strong positioning (Ramsay, 1986; Rhodes, 1985; Lorch *et al.*, 1987; Losa and Brown, 1987). Such a positioning is probably determined by the bendability of the DNA sequence, influencing the energy required for the tight wrapping of the DNA double helix around a histone core (Drew and Travers, 1985; Nelson *et al.*, 1987).

Richard-Foy and Hager (1987) reported on a nucleosome located approximately between −250 and −60 *in situ*, which is similar or identical to the nucleosome position −219/−76 reported here. The significance of this similarity is however uncertain. The position of the *in vitro* reconstituted nucleosome could for example be influenced by fragment length or the presence of vector sequences (Figure 5). *In vivo/in vitro* correlations of nucleosome positioning have previously been described for the 5S RNA genes in *Xenopus* and sea urchin (Rhodes, 1985; Simpson and Stafford, 1983; Thoma and Simpson, 1985).

We have demonstrated that GR has the capacity to bind specifically to DNA, even when associated with a histone octamer. GR-induced effects on the nucleosome specific DNase I pattern could be detected at several positions on both DNA strands and were distributed in the protected regions defined as footprints on naked DNA. Both strands display GR effects which in some positions are qualitatively similar and in others different to the effects seen on naked DNA. Due to extensive nucleosome specific protection in the upstream region, GR-induced protections were mainly

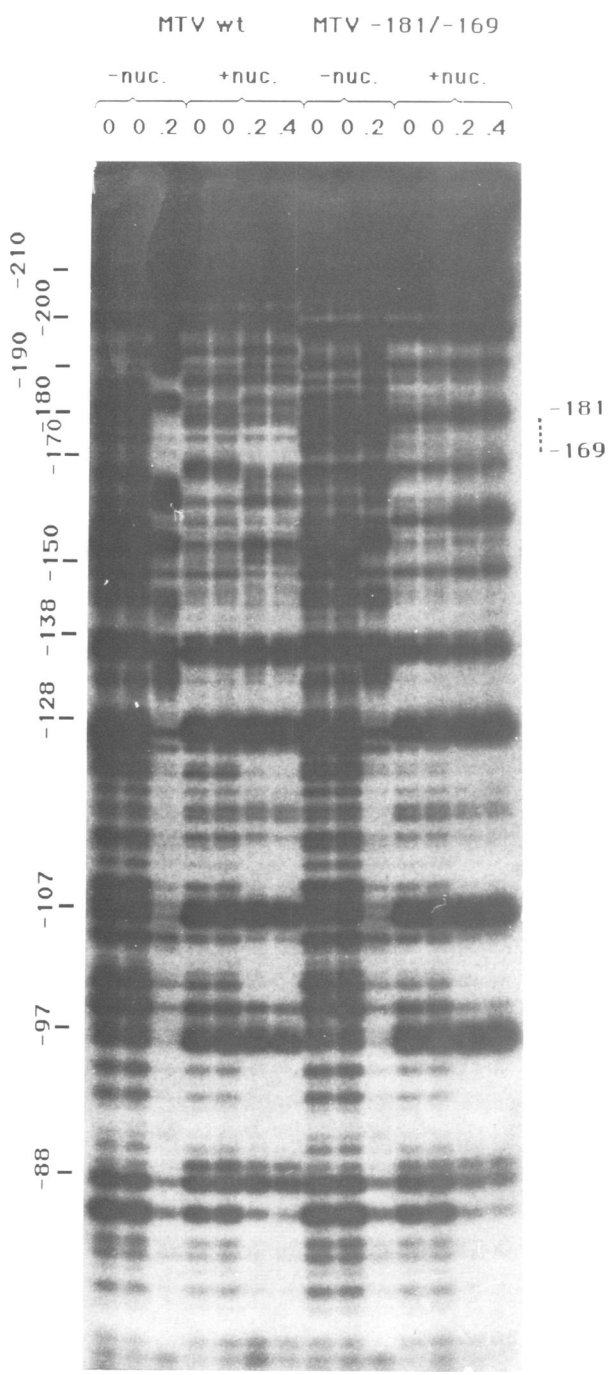

Fig. 6. DNase I footprint of the bottom strand of MTV wt and MTV−181/−169 fragments. Naked DNA (**lanes 1−3** of MTV wt and **1−3** of MTV−181/−169), or associated with histone octamers (**lanes 4−7** of MTV wt and **4−7** of MTV−181/−169) were digested with DNase I. GR was added in the pmol amounts indicated above each lane. The nucleosome specific patterns of DNase I-sensitive sites are indicated with bars for MTV wt. The mutated site in MTV−181/−169 is indicated with a broken line.

observed in the downstream region, around −125/−80 (open circles, Figure 5). The qualitatively different GR effects are observed on both the top and bottom strand and indicated with stars in Figure 2 and with thick arrows in Figure 5. These nucleosome specific GR effects serve as evidence for GR binding to the nucleosomal DNA; the alterations cannot originate from contaminating free DNA

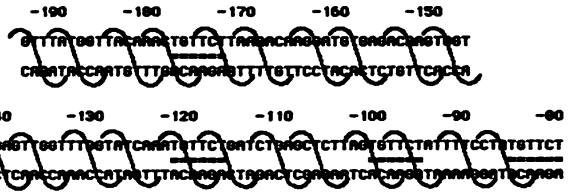

Fig. 7. Sequence diagram showing the rotational setting of MTV wt DNA on the nucleosome. The sequence in relation to the major and minor grooves of the DNA double helix is seen from the outside looking towards the surface of the nucleosome. The four different GR recognition sequences TGTTCT are indicated. The rotational setting of the DNA is based on nucleosome specific DNase I pattern with sensitivities repeated approximately every 10 bp. These are assumed to be cuts in the minor groove facing out from the histone surface (Lutter, 1978; Drew and Travers, 1985).

since this DNA, if present in detectable amounts, would give rise to a cutting pattern indistinguishable from the control, incubated with GR and naked DNA. However, also the qualitatively similar GR-induced protections around −125/−80 argue for GR−nucleosome interaction from a quantitative standpoint, since strong bands on the autoradiogram disappear upon GR addition while leaving the nucleosome specific DNase I pattern intact (Figure 2). Furthermore, the reconstituted nucleosome appeared to be very stable since no detectable amounts of free DNA were observed in low ionic strength polyacrylamide gels (Figure 1B, lane 2).

Evidence for sequence specificity in the GR binding was also provided by the observation that the GR-induced differences in the upstream binding site were absent in the reconstituted mutant, MTV −181/−169 (Figure 6).

DNase I and dimethyl sulphate (DMS) protection and interference data have revealed several features of the specific GR−DNA complex (Scheidereit and Beato, 1984). Comparison of the binding sequences from a number of GR regulated genes have shown that GR binds to an imperfect inverted repeat present both in the upstream and in the downstream region of MTV LTR. An important sequence motif is the hexanucleotide TGTTCT present in an identical or closely related form upstream of many of the studied genes (Klock *et al.*, 1987; Scheidereit *et al.*, 1986). Furthermore, DMS protection and interference have suggested that GR approaches the DNA through the major groove with important DNA contacts separated by ~10 bp (Scheidereit and Beato, 1984) suggesting that binding occurs in two consecutive major grooves on the same face of the DNA helix.

DNase I cleaves the DNA bound to the surface of a nucleosome in a specific manner, i.e. an alternating pattern is produced with sensitivities where the minor groove is faced out and protections where the minor groove is faced in towards the histone surface. It results in a nucleosome specific DNase I pattern where the nuclease sites are staggered on the two strands (Figure 5; Lutter, 1978; Drew and Travers, 1985). Consequently, the rotational setting of the DNA on the reconstituted MTV wt nucleosome can be determined with respect to the DNA sequence. Figure 7 illustrates the rotational setting of the MTV wt sequence on the surface of a nucleosome. The major and minor groove distribution along the sequence is seen from the outside looking towards the nucleosome surface. Since GR probably

binds through two major grooves separated by one turn of the DNA helix, the rotational setting of the MTV wt sequence in the −189/−166 footprint, with the TGTTCT motif in the major groove facing away from the histone surface, would be readily available for GR binding. The upper part of the −133/−80 fooprint harbours a rotational setting placing the recognition motif TGTTCT in the major groove facing towards the histone surface. This recognition sequence is in a more closed configuration but may still be accessible from the side. The prominent GR-induced hypersensitive site appearing at position −126 could be due to a conformational change or tension in the DNA as a result of such a binding. Further downstream the recognition sequences are less conserved but contain two TGTTCT motifs, the first one, at position −99, with a rotational setting placing the TGTTCT in the major groove facing in and the second one, at position −84, placing TGTTCT in the major groove facing out.

GR binding to DNA is a crucial step in the induction process (Payvar *et al.*, 1983; Buetti and Kühnel, 1986). Half maximal induction is achieved within 8−9 min after hormone administration (Ucker and Yamamoto, 1986), and a parallel and reversible appearance of a DNase I hypersensitive site occurs at the GRE during induction (Zaret and Yamamoto, 1984). Such hormone induced DNase I hypersensitive sites have been described in several other genes, e.g. in the 5′ region of the tyrosine aminotransferase gene (TAT) (Becker *et al.*, 1984).

A binding site for nuclear factor 1 (NF1) overlaps the 3′ border of the GRE in the MTV promoter (Novock *et al.*, 1985). Mutations within the NF1 recognition sequence strongly impair hormone induction (Buetti and Kühnel, 1986; Miksicek *et al.*, 1987). Another example of a similar functional relationship between GR and a transcription factor has been observed in the tryptophan oxygenase gene (TO) (Schüle *et al.*, 1988). *In vivo* footprinting of the GRE in the 5′ flanking region of TAT also indicates the binding of additional factors close to the GRE in a hormone dependent manner (Becker *et al.*, 1986). Cordingley *et al.* (1987), using episomal BPV:MTV promoter constructs and *in situ* exonuclease III protection, have demonstrated that NF1 occupied its cognate binding site as a result of hormone treatment. A similar experimental strategy showed that nucleosomes within the MTV promoter are specifically positioned (Richard-Foy and Hager, 1987). One nucleosome, located at −250/−60 was dissociated or structurally altered after administration of hormone as judged by the increased DNA degradation by MPE-Fe (II). This may suggest a role for GR in creating an altered nucleosome structure which could be the basis for the hormone dependent DNase I hypersensitive sites in the 5′ region of glucocorticoid regulated genes as well as for the above mentioned functional cooperativity between GR and other transcription factors. The cooperative effect of two closely positioned GR footprints described, e.g. in the MTV and TAT genes may also have a related explanation based on a stronger destabilizing effect on nucleosomal structure by two GR binding sites located within the same nucleosome (Buetti and Kühnel, 1986; Jantzen *et al.*, 1987). We observed discrete GR induced alterations of the nucleosomal DNA, as for example the prominent hypersensitivity at position −126 on the top strand. The relation between these effects and the apparently more drastic structural changes observed as DNase I-hypersensitive sites *in situ* remain unclear. It is possible that the small local changes in DNase I sensitivity upon GR binding constitutes the basis for the DNase I-sensitive site found *in situ* (Zaret and Yamamoto, 1984). We had however anticipated more drastic GR-induced effects in order to explain the DNase I-hypersensitive site. One possible explanation for this discrepancy could be that the particular nucleosome studied here has a modified structure *in vivo*, rendering it labile on binding of GR or that another factor(s) may be required which is not present in our *in vitro* system.

Materials and methods

Plasmids

The DNA fragment MTV wt was derived from the plasmid pMTV wt. This plasmid contains sequences from the MTV LTR, cloned in pLS wild-type, previously described by Buetti and Kühnel (1986). It was constructed by isolating a fragment from pLS wild-type, carrying LTR sequences from a *Sst*I site at position −104 relative to the transcription start site, to a *Aha*III site at position −198. This fragment was ligated into *Sma*I, *Bam*HI cut Gemini I vector (Promega Biotec) by use of two complementary oligonucleotides harbouring the wild-type MTV sequence from the *Sst*I site at position −104 to position −58 where a *Bam*HI site was introduced. The DNA fragment MTVT −181/−169 was derived from a plasmid constructed identically to pMTVT wt, but using a linker scanning (LS) mutant MTV LTR fragment from pLS−181/−169 instead of pLS wild-type (Buetti and Kühnel, 1986).

The constructed plasmids were first cut with either *Bam*HI or *Pvu*II and treated with calf intestinal alkaline phosphatase. Either end was then selectively labelled, using [^{32}P]ATP and T4 polynucleotide kinase (Maniatis *et al.*, 1982) before cleavage with the second enzyme. The fragments were separated on 5% polyacrylamide gels, localized by autoradiography and purified on Schleicher & Schuell DEAE membrane by electroelution according to the procedure recommended by the manufacturer.

Reconstitution of nucleosomes

Nucleosomes were reconstituted by a high salt exchange method which was similar to the reconstitution method described by Losa and Brown (1987) using long pieces of chromatin as a histone source. The chromatin was prepared as described by Lutter (1978), from rat liver nuclei (Gorski and Schibler, 1986). This preparation yields long pieces of H1 depleted chromatin, approximately 30−60 nucleosomes in length. End labelled restriction fragment was mixed with donor chromatin to a final concentration of 1.0 absorbance units/ml ($A_{260\ nm}$) in a buffer containing 1 M NaCl, 15 mM Tris−HCl pH 7.5, 0.2 mM Na_2EDTA and 0.2 mM phenylmethylsulphonyl fluoride. The mixture was incubated for 20 min at 37°C before stepwise dilution during several hours in room temperature, with buffer containing no NaCl, to a final NaCl concentration of 0.1 M. After completed dilution the reconstituted nucleosomes were purified by sedimentation in a 5−30% glycerol gradient containing 50 mM Tris−HCl pH 7.5, 1 mM Na_2EDTA and 0.1 mg/ml pork insulin (a gift from Kabi Vitrum), in a Beckman SW 50.1 rotor at 35 000 r.p.m. for 15 h at 4°C.

The purification and the electrophoretic mobility of the reconstituted material was checked on 5% polyacrylamide gels (Lorch *et al.*, 1987).

GR preparation

For the binding experiments, rat liver glucocorticoid receptor was purified as previously described (Wrange *et al.*, 1986) with the exception that the last DEAE Sepharose step was replaced by chromatography on a 5 ml FPLC Mono Q™ column (Pharmacia, Uppsala) (Eriksson and Wrange unpublished; Carlstedt-Duke and Wrange, unpublished). The column was equilibrated in ETG 7.8 (20 mM Tris−HCl pH 7.8; 1 mM Na_2EDTA; 10% (v/v) glycerol; 2 mM DTT) and eluted with a linear salt gradient of 0−0.3 M NaCl at a flow rate of 1 ml/min and a total gradient volume of 25 ml. GR was eluted at 0.16 M NaCl and was detected by bound [^{3}H]triamcinolone acetonide (2.25 Ci/mmol). GR-containing fractions, 80−95% pure according to SDS gel electrophoresis (Eriksson and Wrange, unpublished), were pooled and insulin, DTT and glycerol added to final concentrations of 0.1 mg/ml, 10 mM and 40%, respectively and stored at −80°C. GR was quantitated assuming one ^{3}H ligand per 90 kd GR polypeptide.

GR binding and nuclease protection

The typical binding reaction contained 20 000 c.p.m. of naked or reconstituted DNA fragment in a total volume of 100 μl, in ETG 7.8 also

containing 5 mM DTT, 50 mM NaCl and 0.1 mg/ml pork insulin. The final DNA concentration varied between 20 and 100 pg/ml. GR was added, with the amounts in pmol as indicated in Figures 2, 4 and 6, and incubated for 30 min at 25°C.

DNase I treatment was performed as described by Wrange *et al.* (1986), except that reconstituted DNA was treated with double concentration of DNase I. After DNase I treatment the DNA was analysed on denaturing 6% polyacrylamide gels.

The nucleosome reconstituted MTV wt and MTV−181/−169 was digested with exonuclease III in order to determine the nucleosome boundaries (Ramsay, 1986). The reconstituted DNA fragment was treated with 100 units of exonuclease III (Pharmacia, Uppsala) in 100 μl GR binding buffer containing 3 mM $MgCl_2$ for the times indicated in Figure 3. The reaction was stopped by adding 17 μl of a stop solution containing 1% SDS and 100 mM Na_2EDTA, extracted with an equal volume of phenol:$CHCl_3$(2:1) followed by ethanol precipitation and analysis on 6% denaturing polyacrylamide gels.

G and C,T sequence marker lanes were prepared as described by Maxam and Gilbert (1977).

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