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Glucocorticoid receptors recognize DNA sequences in and around murine mammary tumour virus DNA

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In several rodent cell lines, glucocorticoids increase the transcription of murine mammary tumour virus (MMTV) proviral DNA in a process mediated by the glucocorticoid receptor. To investigate whether a direct interaction between the receptor and specific sequences on the induced genes can be implicated in the hormonal regulation of transcription, filter binding studies were performed with partially purified glucocorticoid receptor of rat liver and eight cloned MMTV proviral probes. Both the 40 000 and the 90 00 mol. wt. forms of the receptor do bind preferentially to restriction fragments containing the right 400–500 nucleotides of the MMTV long terminal repeat units (LTR). Using LTR deletion mutants, we confirm that the right end of the LTR contains at least one binding site for the glucocorticoid receptor. In addition, the receptor binds preferentially to the mouse genomic sequences flanking at least three endogenous proviral copies, and to sequences within the *env* genes in some of the endogenous and exogenous proviruses. These findings are compatible with the hypothesis that steroid hormones regulate specific gene expression through a direct interaction of the hormone-receptor complex with DNA sequences in and around the induced genes.

Key words: steroid hormone receptors/protein-DNA interaction/retrovirus expression

Introduction

Since the discovery that glucocorticoid hormones are able to induce the expression of mouse mammary tumour virus (MMTV) in homologous and heterologous cell lines, this system has been studied extensively. It has been shown that the induction is mediated by glucocorticoid receptors, takes place without an apparent lag phase, and cannot be blocked by cycloheximide (Ringold, 1979). Recently, several groups have succeeded in transferring cloned fragments of MMTV DNA into heterologous cells and have shown that proviral transcription in these transformants is enhanced by glucocorticoids (Buetti and Diggelmann, 1981; Huang *et al.*, 1981; Hynes *et al.*, 1981; Lee *et al.*, 1981). These and more recent reports (Fassel *et al.*, 1982; Groner *et al.*, 1982) support the hypothesis that the information required for the hormonal regulation is located within the long terminal repeats (LTR) of the proviral DNA.

By analogy to procaryotic systems, one can ask whether the hormonal regulation of transcription is mediated by a direct interaction of the hormone receptor with specific DNA sequences located within or in the neighbourhood of the regulated genes. The availability of partially purified glucocorticoid receptor (Wrange *et al.*, 1979; Westphal and Beato, 1980) and of cloned endogenous and exogenous MMTV proviral DNA (Groner *et al.*, 1980; Hynes *et al.*, 1981; Herrlich *et al.*, 1981) has allowed us to address this question *in vitro*, using nitrocellulose filter binding assays (Riggs *et al.*, 1970). The results show that the receptor recognizes and binds preferentially to DNA fragments located in the regulatory region of the proviral LTR as well as to other regions of the proviral DNA and flanking mouse genomic DNA.

Results

Purity of the receptor preparations and quantitative binding parameters

An electrophoretic analysis of representative preparations of the 40 000 and 90 000 mol. wt. forms of the glucocorticoid receptor is shown in Figure 1. The prominent bands were identified as the glucocorticoid receptors by photoaffinity labelling as previously described (Gronemeyer and Pongs, 1980; Westphal *et al.*, 1981).

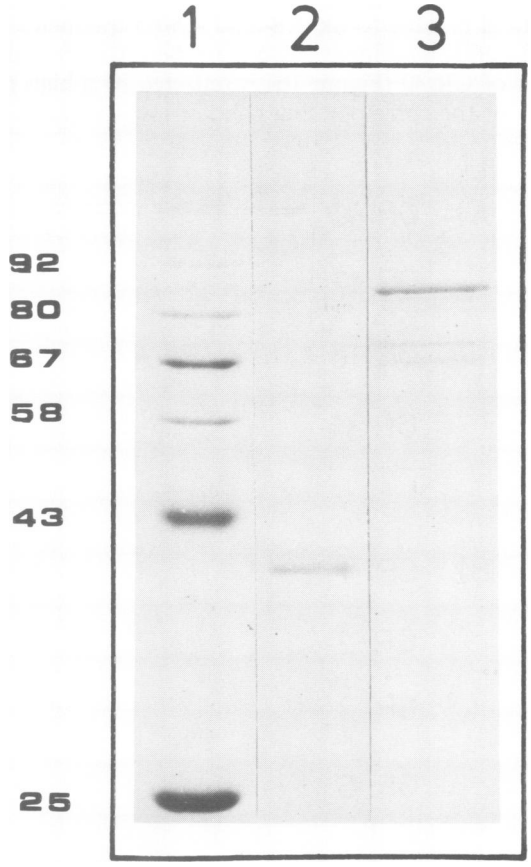

Fig. 1. Gel electrophoresis of the purified glucocorticoid receptors. Gels containing 10% polyacrylamide and 0.1% SDS were run and stained with Coomassie brilliant blue. **Lane 1**, mol. wt. markers: phosphorylase b, transferrin, BSA, catalase, ovalbumin, and chymotrypsinogen A. (The mol. wt. in kilodaltons is indicated on the left). **Lane 2**, 1.5 μg of the 40 000 receptor. **Lane 3**, 4 μg of the 90 000 receptor after the second DNA-cellulose chromatography (Wrange *et al.*, 1979).

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Incubation of labelled DNA with increasing concentrations of receptors resulted in increasing retention of DNA on the filters until, at a receptor molar excess of 5- to 10-fold, 90–100% of the labelled DNA was retained. With both forms of receptor the binding curves thus obtained were sigmoidal, suggesting that more than one receptor molecule is required for the retention of a DNA fragment. The optimal conditions for binding were found to be: 25°C for 45 min, in 60 mM KCl, 1 mM $MgOAc_2$, 0.1 mM EDTA Na_2, 0.1 mg/ml bovine serum albumin (BSA) and 2–4% glycerol. The effect of increasing the KCl concentration was a decrease in the total amount of DNA retained on the filters. At the same time, the specific retention of some of the labelled DNA fragments was increased. A similar effect was obtained when native unlabelled calf thymus DNA was added to the labelled DNA fragments before incubation with the receptor.

Endogenous proviral clones

Four endogenous proviral probes were used, of which one, GR 40, represents a complete copy of the proviral genome including its flanking sequences (Figure 2). The other three probes include only the right half of the proviral genomes and the corresponding mouse flanking sequences. GR 17 corresponds to the 3′ half of the genetic locus *mtv-2* that is responsible for tumour development (Michaelides *et al.*, 1981).

As an example of the type of data obtained, Figure 3 summarises an experiment carried out with the two halves of GR 40 and increasing concentrations of the 40 000 receptor. Preferential binding to the 1.8- and 4.5-kb fragments on the left half of GR 40 (Figure 3A) and to the 2.1-kb fragment on the right of GR 40 (Figure 3B) is reflected by a high relative intensity of the corresponding band at low receptor concentration. At high receptor concentrations, the relative intensity of all fragments converges to one because quantitative retention of all fragments is reached. If, however, calf thymus DNA is added, total DNA retention is decreased, but the relative intensity of those bands that bind preferentially increases. With this type of assay only the 1.8-kb fragment on the left half of GR 40 and the 2.1-kb fragment on the right half show preferential binding, whereas the 4.5-kb fragment is competed effectively by calf thymus DNA (Figure 3C and D). The preferential retention of this fragment observed in Figure 3A could be due to its relatively large size, which facilitates its retention on the filter even in the absence of specific binding. This artefactual retention of large fragments can be prevented by the addition of calf thymus DNA (Figure 3C) or by increasing the KCl concentration of the binding reaction above 0.1 M (see below). In all later experiments, binding reactions with calf thymus DNA and/or high concentrations of KCl were included.

To investigate the influence of DNA fragment size on filter retention, we carried out an experiment with the 90 000 receptor and subcloned fragments of the left half of GR 40, in which the vector DNA was left attached to the cloned fragments (Figure 4). When the plasmid containing the 1.8-kb *Eco*RI-*Pst*I fragment was compared to the vector and to the plasmids containing the 0.9-kb and 0.6-kb *Pst*I fragments, we observed preferential binding of the receptor only to the plasmids containing the 1.8-kb and the 0.6-kb inserts. Binding to this latter fragment was, however, not detected in the experiments shown in Figure 3A and C. From this observation and similar experiments with other small fragments we concluded that a minimal fragment size of ~0.8 kb is a prerequisite for detecting preferential retention in this type of

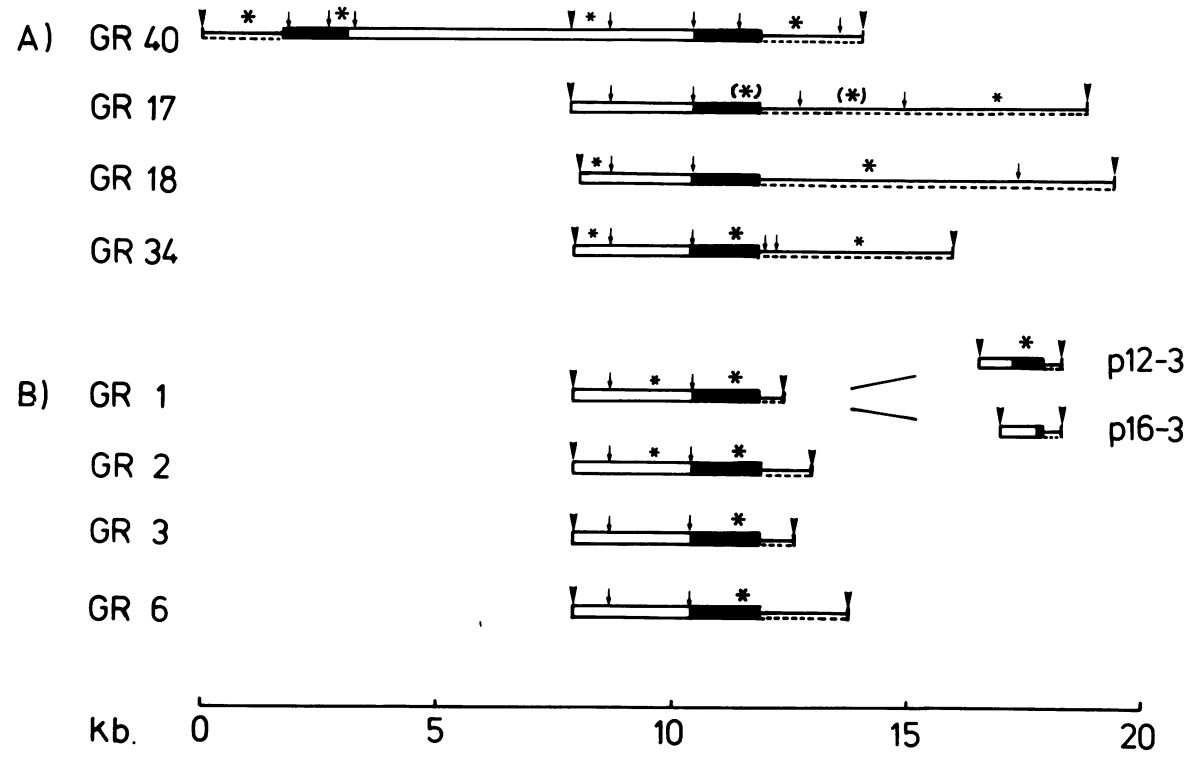

Fig. 2. Endogenous **(A)** and exogenous **(B)** proviral MMTV clones. Dotted lines (– – – –) denote mouse genomic sequences, solid bars (▬) the viral LTR and open bars (▭) the proviral coding region. Restriction sites for *Eco*RI (▼) and *Pst*I (↓) are indicated. Restriction fragments which exhibit strong preferential binding of glucocorticoid receptors are marked by large asterisks, and those fragments showing less consistent preferential binding by small asterisks. The asterisks in brackets in GR 17 indicate the uncertainty in assigning preferential binding (see text).

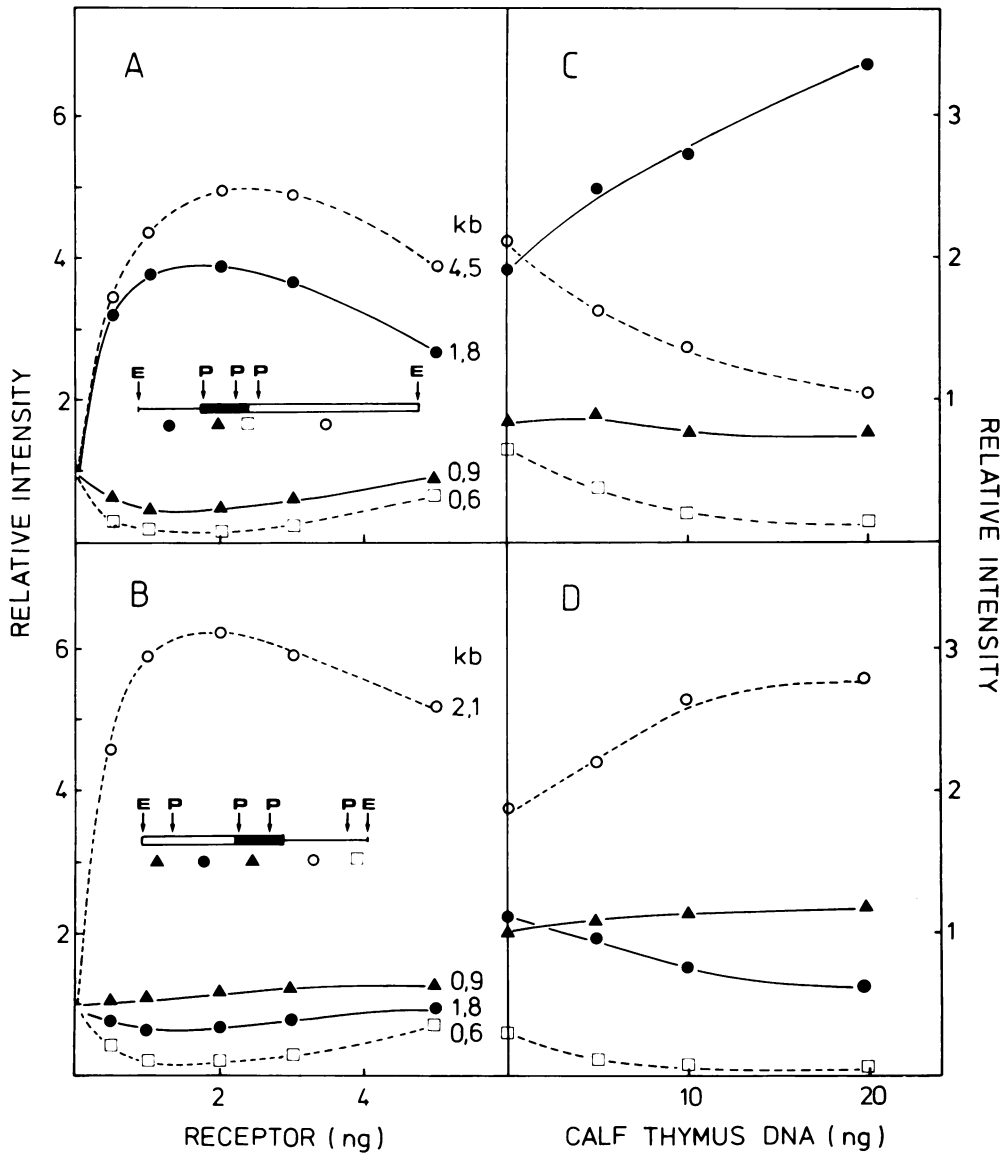

Fig. 3. Binding of the 40 000 receptor to fragments of the left and right halves of GR 40 proviral DNA. Aliquots (5 ng) of labelled DNA fragments from the left half (**A,C**) and the right half (**B,D**) of GR 40 were assayed for receptor binding in a final volume of 25 μl. The DNA retained on the filters was eluted, electrophoresed in 1% agarose gels, and autoradiographed. In order to obtain the 'relative' intensities, the fractional intensities of the bands in each lane were measured and divided by those of the corresponding bands in the input DNA. **A,B**: incubation with increasing receptor concentrations. **C,D**: incubation with 10 ng receptor and increasing concentrations of unlabelled calf thymus DNA. Note that in the right half of GR 40 there are two fragments of equal size (0.9 kb, **B** and **D**).

assay.

An example of the results obtained with the *Pst*I restriction fragments of GR 34 and GR 17 cloned in pM2 is shown in the autoradiogram of Figure 5. In the case of GR 34 both forms of the receptor bind preferentially to the 1.45-kb fragment containing the LTR (Figure 5A, lanes 3 and 5), and to a lesser extent to the 1.05-kb fragment containing the left end of the *env* gene region, and to the 4.3-kb fragment containing mouse flanking sequences and vector DNA (Figure 5, lanes 4 and 6). With GR 17, preferential binding of both forms of the receptor to a 2.25-kb band is observed (Figure 5B, lanes 3, 5, and 6). Since this band contains two restriction fragments of equal size, receptor binding cannot be unequivocally assigned (Figure 2A). At high receptor concentration, a weak preference for the 4.3-kb fragment containing the right end of the mouse flanking sequences is also observed (Figure 5B, lane 4).

The results of binding experiments with both forms of the receptor to *Pst*I restriction fragments of all four endogenous proviral clones are summarized in Figure 2A. The uncertainty in the LTR region of GR 17 arises from the fact (see above) that the *Pst*I digestion yields two fragments of 2.25 kb, one containing the LTR and the other mouse flanking sequences. With this limitation, all restriction fragments containing the right third of the LTR bind both forms of the receptor preferentially. In addition, there are preferential binding sites in fragments containing mouse flanking sequences (GR 40, GR 17) and in fragments containing *env* gene sequences (GR 40, GR 18, GR 34).

Exogenous proviral clones

As an example of the data obtained with exogenous proviral probes Figure 6A shows the results of an experiment with the GR 1 clone and the 40 000 receptor. The 2.3-kb *Pst*I

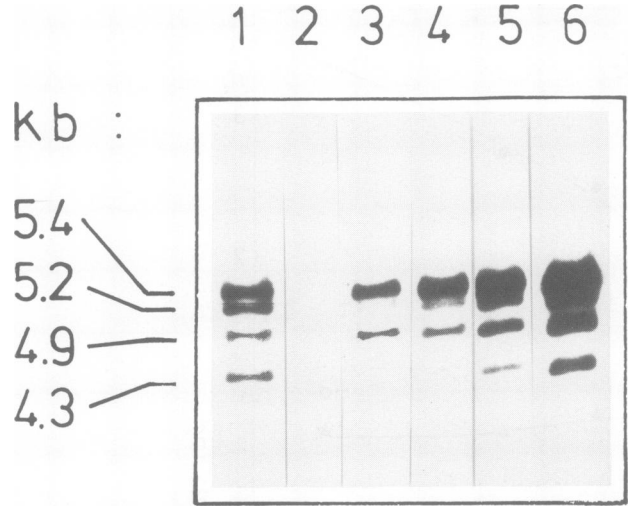

Fig. 4. Binding of the 90 000 receptor to subcloned fragments of the left half of GR 40. The 1.8-kb *Eco*RI-*Pst*I fragment containing the flanking sequences at the 5′ end of GR 40 was subcloned between the *Eco*RI and the *Pst*I sites of pBR322 (final size 5.4 kb). The 0.9-kb and the 0.6-kb *Pst*I frament of the left LTR of GR 40 were subcloned in the *Pst*I site of pBR322 (final sizes 5.2 and 4.9 kb, respectively). These three plasmids were mixed with pBR322 (4.3 kb), linearized with *Pvu*II and end-labelled for filter binding experiments. The figure represents an autoradiogram of the electrophoretically separated DNA probes. **Lane 1**. Input DNA. **Lane 2**. DNA retained on the filter in the absence of receptor. **Lanes 3–6**. DNA bound by 1, 2, 4, and 10 ng of receptor.

fragment containing the LTR, 0.2 kb of mouse flanking DNA, and 0.7 kb of vector DNA shows a clear preferential binding. Similar results were obtained with 90 000 receptor. When the same clone was digested with *Pst*I and *Eco*RI a preferential binding to the 1.9-kb *Pst*I fragment in the *env* gene region was observed. An example of the discrepancy between the results obtained with single and double digestion of clone GR 2 is shown in Figure 7. The preferential binding to the 5.9-kb fragment containing the LTR is very evident in the single *Pst*I digest (Figure 7A). In the *Pst*I/*Eco*RI double digest, the LTR is located in a 2.3-kb fragment, but weak preferential binding is observed to the 1.9-kb fragment in the *env* gene region (Figure 7B). This is the only fragment with cohesive ends in the double digest.

The results of extensive binding experiments with restriction fragments of the exogenous clones GR 1, GR 2, GR 3, and GR 6 are summarized in Figure 2B. As with the endogenous probes, preferential binding to the fragments containing the LTR was always observed. In two cases (GR 1, GR 2) a less pronounced preference for the 1.9-kb fragment in the *env* gene region was observed in double digestion experiments.

Deletion mutants

In an attempt to narrow down the region of the LTR that preferentially binds the receptor we took advantage of the LTR deletion plasmids, p12-3 and p16-3, (Figure 2B). These are derived from a chimeric plasmid, p2.6, that contains part

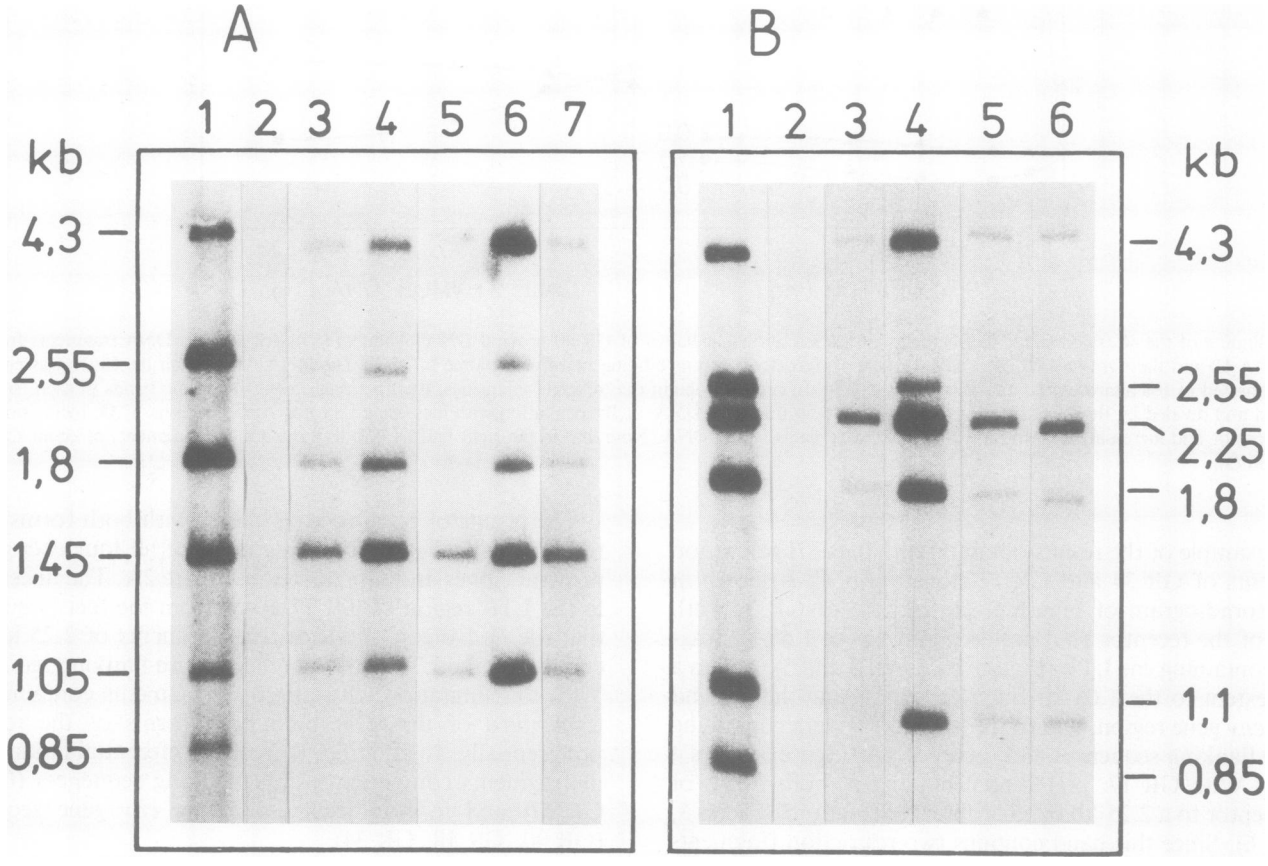

Fig. 5. Binding of receptor to restriction fragments of GR 34 **(A)** and GR 17 **(B)**. Plasmids containing the GR 34 and GR 17 proviral DNA and their flanking sequences (subcloned in the chimeric plasmid M2) were digested with *Pst*I and end-labelled for binding studies. **Lane 1**: input DNA. **Lane 2**: DNA retained in the absence of receptors. **Lane 3** and **4**: DNA bound by 6 and 12 ng, respectively, of the 90 000 receptor. **A, Lane 5** and **6**: DNA bound by 3 and 6 ng of the 40 000 receptor. **Lane 7**: DNA bound by 6 ng of the 40 000 receptor in the presence of 50 ng of calf thymus DNA. **B, Lane 5,6**: DNA bound by 6 ng of the 40 000 receptor in the presence of 50 ng of calf thymus DNA or 120 mM KCl respectively.

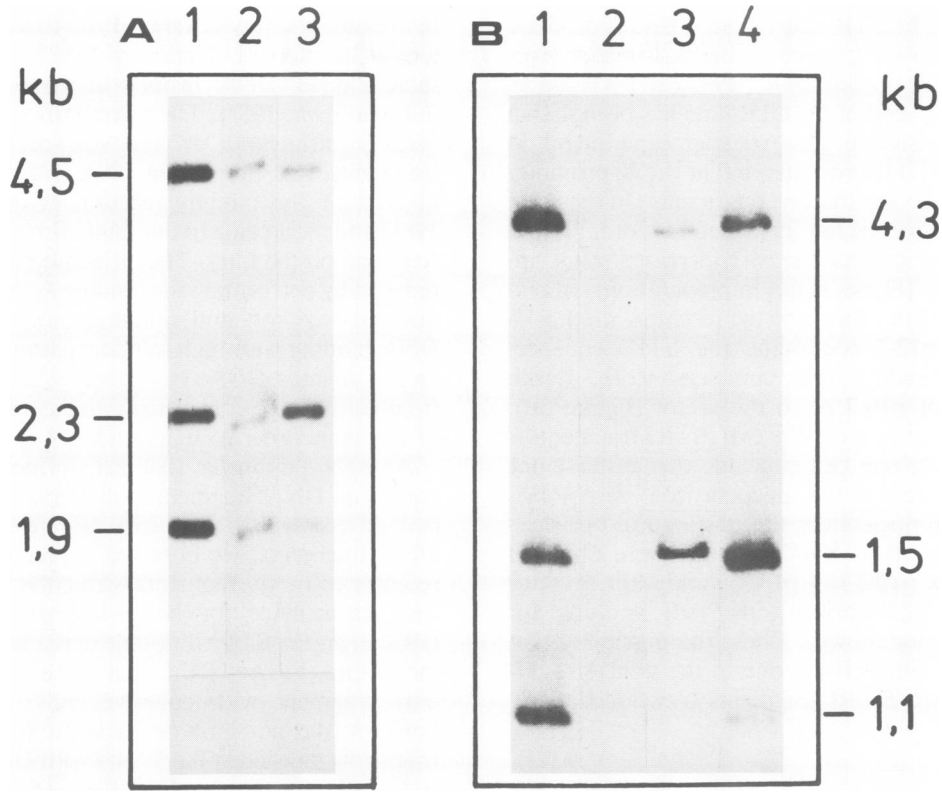

Fig. 6. Binding of receptors to restriction fragments of GR 1 **(A)** and of p12-3/p16-3 **(B)**. Prior to labelling, GR 1 was digested with *Pst*I, whereas p12-3 and p16-3 were mixed and digested with *Eco*RI. Therefore, the 4.3-kb vector band in the input DNA (right) is present in 2-fold the concentration of the 1.5-kb and the 1.1-kb inserts. **Lane 1**: input DNA. **Lane 2**: DNA retained in the absence of receptors. **Lane 3**: DNA bound by the 40 000 receptor. **Lane 4**: DNA bound by the 90 000 receptor.

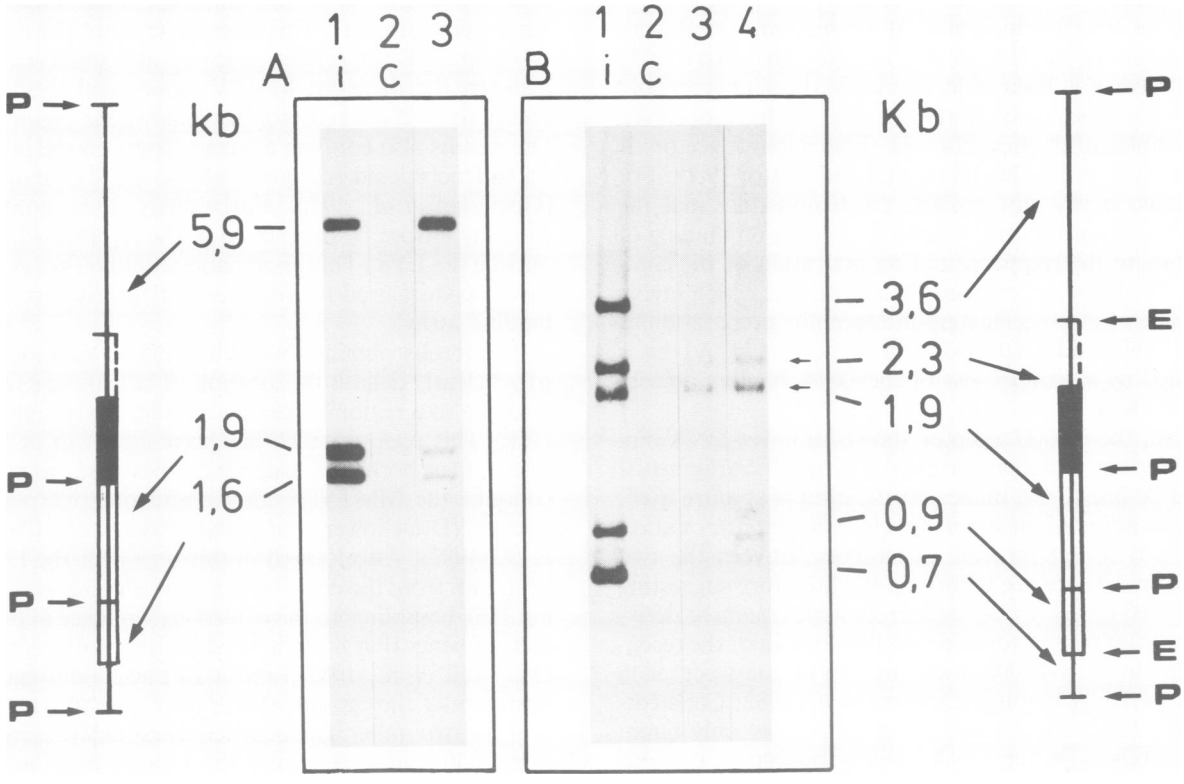

Fig. 7. Binding of the 40 000 receptor to restriction fragments of GR 2: **A**: GR 2 DNA (10 ng) was digested with *Pst*I and labelled at the 3′ ends with [α^{32}P]dCTP and the large fragment of DNA polymerase I. **Lane 1**, input DNA. **Lane 2**, DNA retained on the filter in the absence of receptor. **Lane 3**, DNA bound by 0.8 ng of receptor. **B**: GR 2 DNA (10 ng) was digested with *Eco*RI and *Pst*I, treated with alkaline phosphatase and 5′ end-labelled with [γ^{32}P]ATP and T4 polynucleotide kinase. **Lane 1**, input DNA. **Lane 2**, DNA retained on the filter in the absence of receptor. **Lane 3**, DNA bound by 0.6 ng of receptor. **Lane 4**, DNA bound by 6 ng of receptor in the presence of 50 ng of calf thymus DNA. The restriction sites used for labelling are shown. E: *Eco*RI sites. P = *Pst*I sites. Other symbols are as described in the legend to Figure 2.

of the *env* gene, the right LTR and flanking region of GR 1 and the thymidine kinase *(tk)* gene of herpes simplex virus (HSV) cloned in pBR322 (Groner *et al.*, 1982). p12-3 contains 585 nucleotides at the 3′ end of the LTR, and has been shown to be hormone responsive in gene transfer experiments in which initiation at the LTR promoter and at the *tk* promoter are both increased by dexamethasone treatment. On the other hand, p16-3 only contains the last 29 nucleotides of LTR and does not respond to dexamethasone with increased transcription of the *tk* promoter (Hynes *et al.*, in preparation). In binding experiments with both forms of the receptor, the 1.5-kb *Eco*RI fragment of p12-3 containing the LTR sequences shows a preferential binding when compared to the 1.1-kb equivalent fragment of p16-3 or to the vector (Figure 6B). Since the only difference between the two *Eco*RI fragments is the length of the LTR, one can conclude that at least one preferential binding site for the glucocorticoid receptor is located within the 556 nucleotides that distinguish one deletion mutant from the other. Similar results were obtained when the plasmids p12-3, p16-3 and the vector (pBR322 + HSV-*tk*) were linearized with *Pvu*II, and used for binding studies (data not shown). Thus, the possibility that the results shown in Figure 6B are due to the small size difference between the two *Eco*RI fragments is excluded.

Discussion

The results reported above are compatible with the notion that the glucocorticoid receptor recognizes specific DNA sequences near the glucocorticoid-induced MMTV proviral genes. This interpretation depends on the assumption that the binding protein is actually the receptor. This assumption is supported by the stoichiometry of binding observed with some preparations of the 40 000 receptor that were 90% pure. We have not been able to purify the glucocorticoid receptor in the absence of hormone, and we have observed that the complex of the receptor with triamcinolone acetonide is activated and very stable (Westphal and Beato, 1980). For these reasons, it was not possible to demonstrate that the DNA binding reported here is really dependent on binding of the hormone to the receptor, and on activation of the complex. There is, however, circumstantial evidence which favors this sequence of events. Heating the receptor preparation at 45°C for 15 min leads to dissociation of the steroid and this is accompanied by a parallel loss of the DNA binding activity (data not shown).

In general, the 90 000 receptor showed a lower DNA binding capacity but a higher selectivity for specific fragments. This could be due to the milder purification procedure used, (i.e., without ammonium sulfate precipitation steps), or alternatively could reflect intrinsic differences between the two receptor forms. There are reports in the literature suggesting that the 40 000 receptor may be a proteolytic fragment derived from the 90 000 receptor and that the domain of the receptor missing in the 40 000 mol. wt. form may act as a modulator of DNA binding (Dellweg *et al.*, 1982; Carlstedt-Duke *et al.*, 1982). Nevertheless, our data show only quantitative differences in the DNA-binding properties of both receptor forms.

The extent of preferential binding detected with the different DNA probes varies with different receptor preparations, but preference for the LTR-containing fragments was between 3- and 10-fold with an average at ~5-fold. These figures are not very different from those measured for the extent of hormonal induction of MMTV transcription with the same cloned DNA probes in gene transfer experiments (Groner *et al.*, 1982). The quantitative analysis of the binding data is influenced by the size of the DNA restriction fragments. It is known that DNA fragment size influences the kinetics of the binding reaction (Winter and von Hippel, 1981) and, according to our data, also affects the efficiency of retention on the filter. The influence of fragment size may be reduced by performing the binding assay with high concentrations of receptor, and simultaneously preventing unspecific DNA binding with high salt concentrations ($\geq$100 mM KCl) or calf thymus DNA. Nevertheless, a comparison of several fragments can be considered reliable only when their sizes are of the same order of magnitude.

Another parameter that can influence the results is the nature of DNA fragment ends. In some experiments where two different restriction enzymes were used to prepare the DNA fragments, we observed a preferential binding of the receptor to those fragments with cohesive ends. This was the case, for instance, for the 1.9-kb *Pst*I fragment of GR 1 and GR 2 in an *Eco*RI/*Pst*I double digest labelled subsequently at the 5′ end (Figure 7B). Under these conditions, this was the only fragment with cohesive ends. The same fragment, however, did not show any specific binding when only *Pst*I fragments labelled at the 3′ end with DNA polymerase (blunt ends) were used for binding (Figure 6A and 7A). Interestingly, it is a similar fragment containing part of the *env* gene that was recently found to be bound preferentially by a 90 000 mol. wt. form of the glucocorticoid receptor (Payvar *et al.*, 1981). Since, however, these authors used a labelling procedure that should yield blunt ends, their results are not in agreement with ours. This discrepancy could be due to the use of different DNA probes, since we also observed differences between the different MMTV clones with respect to their ability to bind the receptor at the *env* gene region (Figure 2). Our results also confirm a recent report on the interaction of the glucocorticoid receptor with the LTR of cloned MMTV (Govindan *et al.*, 1982). Surprisingly, these authors do not find any unspecific interaction of the receptor with sequences outside the LTR. This discrepancy could reflect differences in the procedure used for receptor purification, or for the filter binding assay.

The results obtained with the deletion mutants p12-3 and p16-3 clearly delimit the location of at least one receptor binding site to a region of 556 nucleotides at the right end of the LTR. This region, which has been shown to be biologically relevant for hormone response (Hynes *et al.*, in preparation), contains the MMTV promoter and putative regulatory sequences (Donehower *et al.*, 1981; Fasel *et al.*, 1982; Kennedy *et al.*, 1982). Also located in this region of the LTR (105 bp upstream from the cap site) is a 19-nucleotide sequence showing 75% homology to the consensus sequence in the egg white protein genes that is recognized by the progesterone receptor (Mulvihill *et al.*, 1982), and a 25-nucleotide region (396 bp upstream of the cap site) that is also present in a similar position in two other glucocorticoid-responsive genes, the rat growth hormone gene and the human propiomelanocortin gene (Cochet *et al.*, 1982).

In addition to the site(s) located at the LTR, there are other sites for receptor binding in the mouse flanking sequences, for instance, at the 5′ end of GR 40, and sites with lower affinities for the receptor in the region of the *env* gene. It re-

mains to be established whether these sites are biologically relevant by testing their functional activity. We would like to point out that the *Ltk*$^-$ cells used for gene transfer experiments have endogenous MMTV proviral copies that do not respond to glucocorticoids (Hynes *et al.*, 1981). Thus, although the interaction of the receptor-hormone complex with specific DNA sequences may be involved in the induction process, other regulatory mechanisms must control the accessibility of individual proviral copies to the hormonal regulation.

Materials and methods

Preparation of receptors

Receptor was purified from the liver cytosol of adrenalectomized male Wistar II rats labelled with [^{3}H]triamcinolone acetonide (4 Ci/mmol; 1 Ci = 3.7 x 10^{10} becquerels). Two published purification procedures were used. The first yields a 40 000 mol. wt. form of the receptor that is 80–90% pure (Westphal and Beato, 1980), and the second a 90 000 mol. wt. form with an average purity of 50% (Wrange *et al.*, 1979). Both forms of the receptor were stored frozen at −85°C in the presence of purified BSA (0.1 mg/ml). Since the 90 000 receptor contains 10 mM pyridoxal 5′-phosphate, prior to use, it was passed through a small column of Sephadex G-100 previously equilibrated with 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM dithoerythritol, 10% glycerol and 0.1 mg/ml BSA.

Quantitation of the receptor content in the final preparations was carried out by the dextran-coated charcoal technique (Westphal and Beato, 1980), assuming one steroid binding site per receptor molecule. Protein concentration was determined by a filter method (Schaffner and Weissman, 1973), using BSA as standard.

DNA probes

The endogenous MMTV proviral clones were isolated from the liver DNA of a GR mouse, and the exogenous MMTV proviral DNA sequences from the DNA of the GR tumour cell line (Groner and Hynes, 1980; Groner *et al.*, 1980; Herrlich *et al.*, 1981). Both proviral types were cloned into Charon 4A phages, starting from the corresponding *Eco*RI-digested DNA. With the exception of clone GR 40, which contains a complete proviral genome with its flanking sequences, all the other clones used in this study extend from the central *Eco*RI site to *Eco*RI sites located at different distances 3′ from the right proviral LTR (see Figure 2). For the binding experiments reported here the exogenous proviral probes and the two halves of GR 40 were subcloned into the *Eco*RI site of the plasmid pBR322. The endogenous proviral probes GR 17, GR 18, and GR34 were subcloned in the chimeric plasmid M2 that contains sequences from pBR322 and the *tk* gene of HSV (Groner *et al.*, 1982). In addition, several subgenomic *Pst*I fragments were also subcloned into the *Pst*I site of pBR322. The deletion mutants p12-3 and p16-3, were generated from a hybrid MMTV-LTR-*tk* chimeric plasmid, p2-6, that itself was constructed with the exogenous clone GR 1 and the *tk* gene of HSV (Groner *et al.*, 1982).

The cloning experiments were performed under the biological and physical containment conditions specified by the 'Richtlinien zum Schutz vor Gefahren durch *in vitro* rekombinierte Nukleinsäuren' of the B.F.T. of the Federal Republic of Germany.

Filter binding assay

For the filter binding assay, the DNA probes were digested with the appropriate restriction enzymes and 3′ end-labelled with the Klenow fragment of *Escherichia coli* DNA polymerase I and the corresponding [α^{32}P]dNTP. The specific activity of the labelled DNA was 1–5 x 10^6 c.p.m./μg. The labelled DNA fragments were incubated with the partially purified receptors at 25°C for 45 min in 'binding buffer' (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA Na_2, 1 mM $MgOAc_2$, 0.1 mM dithioerythritol, 0.1 mg/ml BSA, and 60 mM KCl). The final volumes were 25–50 μl and the molar ratio of receptor to DNA varied between 0.1 and 10. In some cases KCl was added to final concentrations up to 200 mM. For competition experiments, native calf thymus DNA was added together with the labelled DNA fragments at concentrations varying between 0.5 and 5 ng/μl.

At the end of the incubation the samples were filtered at room temperature through nitrocellulose filters (Gelman GN6, 0.45 μg, 6 mm diameter) as previously described (Westphal and Beato, 1981). The filters were then washed twice with 100 μl binding buffer, and the DNAs retained on the filters were eluted in 200 μl of 10 mM Tris/HCl, pH 7.5, 1 mM EDTA Na_2, 0.1% SDS, and 50 μg/ml yeast tRNA. The DNA fragments were precipitated with 0.3 M NaCl and 3 volumes of ethanol at −70°C, and electrophoresed through 1% agarose gels. The gels were dried on a sheet of DEAE-paper (DE-81, Scheicher & Schüll) and autoradiographed with Kodak X-omat AR film with or without intensifying screen (Cronex Quanta II, DuPont). The fractional intensity of the individual bands in the autoradiograms was measured by scanning with a microdensitometer (Quick Scan, Desaga), and compared to the fractional intensity of the same bands in input DNA.

Acknowledgements

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