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Nuclear factor I acts as a transcription factor on the MMTV promoter but competes with steroid hormone receptors for DNA binding

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Several steroid hormones induce transcription of the mouse mammary tumor virus (MMTV) promoter, through an interaction of their respective receptors with the hormone responsive elements (HREs) in the long terminal repeat (LTR) region. The molecular mechanism underlying transcriptional activation is not known, but binding of nuclear factor I (NFI) to a site adjacent to the HRE appears to be required for efficient transcription of the MMTV promoter. In JEG-3 choriocarcinoma cells the MMTV promoter is transcribed inefficiently, even after transfection of the receptor cDNA and treatment with glucocorticoids or progestins. These cells contain low levels of NFI as cotransfection of NFI cDNA enhances MMTV transcription and this effect is inhibited by mutation of the NFI binding site. In DNA binding experiments with purified NFI from pig liver, the glucocorticoid and progesterone receptors do not co-operate but rather compete with NFI for binding to their respective sites on the LTR. Similar results are obtained with a functional recombinant NFI synthesized *in vitro*. Competition for DNA binding is probably due to steric hindrance as the DNase I footprints of the hormone receptors and NFI do overlap. These results suggest that, though NFI acts as a transcription factor on the MMTV promoter, transcriptional activation does not take place through a direct facilitation of DNA binding of NFI by steroid hormone receptors.

Key words: glucocorticoid receptor/progesterone receptor/transcriptional control/MMTV-LTR/NFI

Introduction

Transcriptional regulation by steroid hormones is accomplished by mechanisms that involve direct binding of the hormone receptors to regulatory DNA sequences in the vicinity of the target promoter. Such regulatory sequences have been identified in a variety of genes and share common structural features that have led to the definition of a consensus 15-mer, the hormone responsive element (HRE), able to confer hormone inducibility to heterologous promoters (for a review see Beato, 1989). A homodimer of the hormone receptor is the functional entity that binds to the HRE and, by an unknown mechanism, activates transcription from adjacent promoters (Scheidereit *et al.*, 1983; Chalepakis *et al.*, 1988b; Kumar and Chambon, 1988; Tsai *et al.*, 1988).

In the long terminal repeat (LTR) region of the mouse mammary tumor virus (MMTV), several HREs have been identified that are able to bind the receptors for glucocorticoids and progestins (Scheidereit *et al.*, 1983; Chalepakis *et al.*, 1988a), and to mediate transcriptional activation by a variety of steroid hormones (Cato *et al.*, 1986; Arriza *et al.*, 1987; Cato *et al.*, 1987; Cato and Weinmann, 1988; Chalepakis *et al.*, 1988a; Ham *et al.*, 1988). The exact molecular mechanism by which binding of the homone receptor to the LTR-HREs activates transcription is unknown. However, in the LTR region, immediately adjacent to the most promoter proximal HRE, there is a binding site for the transcription factor nuclear factor I (NFI) that seems to play an important role in transcriptional activation of the MMTV promoter (Nowock *et al.*, 1985; Buetti and Kühnel, 1986; Miksicek *et al.*, 1987). Mutations of this NFI binding site that hinder binding of NFI *in vitro* strongly impair glucocorticoid-induced transcription (Miksicek *et al.*, 1987; Cato and Weimann, 1988; Buetti *et al.*, 1989).

In cells carrying minichromosomes containing the MMTV-LTR, induction of transcription by glucocorticoids is accompanied by *in vivo* binding of the NFI to its cognate sequence near the promoter (Cordingley *et al.*, 1987). Prior to hormone administration, no bound NFI can be detected on the LTR promoter by the exonuclease III mapping technique. As the amount of NFI activity in the nuclear extracts of these cells is not affected by glucocorticoid treatment (Cordingley and Hager, 1988), and NFI exhibits very high affinity for its cognate sequences in naked DNA, the site on the MMTV-LTR appears to be inaccessible for NFI binding in hormone naive cells (Cordingley *et al.*, 1987). After hormone administration, NFI is found complexed to the promoter, suggesting that binding of the hormone receptor to the MMTV-HRE facilitates binding of NFI. This could be accomplished by direct protein–protein interaction as postulated for other transcription regulatory proteins (Ptashne, 1988). However, no evidence for a ternary complex including the hormone receptor and NFI has been obtained in exonuclease III digestions of minichromosomes from induced cells (Cordingley *et al.*, 1987).

To analyze further the interactions taking place on the MMTV promoter, we have directly addressed the question of whether NFI acts as a transcription factor of the MMTV promoter and whether binding of the hormone receptors to the HRE stabilizes NFI binding to its cognate site or *vice versa*. Using a choriocarcinoma cell line deficient in MMTV transcription, we show that cloned NFI can act as a transcription factor on the MMTV promoter. In DNA binding experiments with NFI purified from pig liver (Meisterernst *et al.*, 1988a) or with cloned NFI (Meisterernst *et al.*, 1988b), we find that they compete with hormone receptors for binding to their respective sites on the MMTV-LTR.

Results

Transfected NFI cDNA stimulates MMTV induction by glucocorticoids and progestins in JEG-3 cells

In previous gene transfer experiments with the human choriocarcinoma cell line JEG-3, we observed that the MMTV promoter was inefficiently transcribed even after cotransfection of the glucocorticoid receptor (GR) cDNA and hormone treatment. As the same cells are able to use the MMTV-HRE linked to the thymidine kinase promoter of herpes simplex virus (Akerblom *et al.*, 1988), we reasoned that factors for the transcription of the MMTV promoter may be absent or present at low concentration in JEG-3 cells. We tested this hypothesis by analyzing the influence of cotransfecting an expression vector containing the cDNA for NFI (Meisterernst *et al.*, 1988b) on the transcription of the MMTV promoter. Both glucocorticoid and progesterone stimulation of the MMTV promoter were considerably enhanced when NFI cDNA was cotransfected into JEG-3 cells (Figure 1A). In an average of three experiments, glucocorticoid induction was enhanced 20-fold and progesterone induction 17-fold (Figure 1).

To ensure that the observed effect was due to binding of NFI to the MMTV promoter, we analyzed the influence of mutations in the NFI binding site. For this purpose we used the mutants pOX and pS1 (Figure 6 and Miksicek *et al.*, 1987). In pOX, the palindromic NFI binding site has been mutated by insertion of 8 bp into the *Hin*fI site and, as a consequence, binding of NFI has been shown to be severely impaired (Miksicek *et al.*, 1987). Using this mutant, the influence of cotransfected NFI cDNA on MMTV expression was also dramatically reduced (Figure 1B). In pS1 an oligonucleotide has been introduced that regenerates a binding site for NFI albeit a few nucleotides further downstream (Figure 6). This mutant responded efficiently to cotransfection of NFI cDNA, demonstrating that binding of NFI to the MMTV promoter is responsible for the observed stimulation of MMTV transcription (Figure 1C).

These experiments clearly show that, upon hormonal induction, NFI acts as a transcription factor on the MMTV promoter. However, none of the constructions used was stimulated by cotransfection of NFI cDNA in the absence of hormone (Figure 1), suggesting that binding of the hormone–receptor complex to the MMTV-HRE is a prerequisite for NFI action.

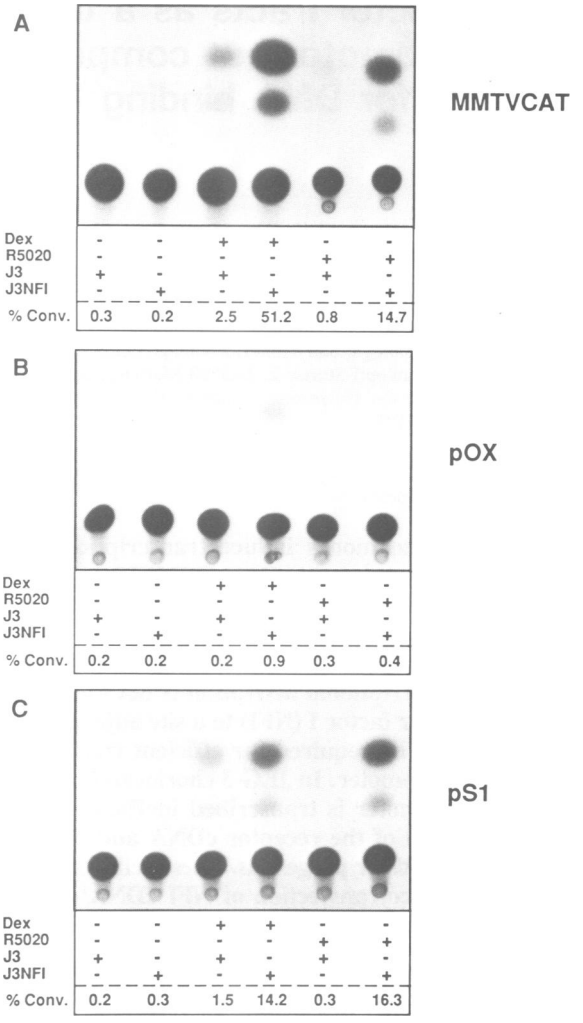

Fig. 1. Influence of cotransfected NFI cDNA on the expression of the MMTV promoter in JEG-3 cells. JEG-3 cells were transfected with MMTVCAT (**A**), pOX (**B**) or pS1 (**C**) and cotransfected with J3 vector (lanes 1, 3 and 5) or J3NFI (lanes 2, 4 and 6). In lanes 3 and 4 the cells were induced by dexamethasone and in lanes 5 and 6 by R5020. The percentage of CAT conversion is indicated and represents an average of three independent experiments.

Influence of the progesterone receptor on DNA binding of purified NFI

To study a possible direct interaction between progesterone receptor (PR) and NFI we used DNase I footprinting experiments with a 722 bp DNA fragment containing the relevant region of the MMTV promoter. Binding of purified NFI to this DNA fragment was detected at low protein concentration and led to the protection of the previously identified region between −80 and −60 (Figure 2, lanes 15–18, and Nowock *et al.*, 1985). Partially purified PR from rabbit uterus yielded a footprint covering the regions between −190 and −162 and between −138 and −72 (Figure 2, lanes 6–10, and Chalepakis *et al.*, 1988a). In the presence of PR, weak binding of NFI was detected (Figure 2, compare lanes 11–13 with 15–17). Similarly, in the presence of NFI, binding of the PR to its cognate sequences was reduced (Figure 2, compare lanes 1 and 2 with lanes 6 and 7). The inhibitory effect of NFI on receptor binding is not dramatic and is more obvious over the promoter proximal receptor binding sites near the NFI recognition sequence.

These results show that the PR and NFI do not co-operate for binding to linear DNA *in vitro*. In particular, binding of the PR does not facilitate the interaction of the NFI with its cognate sequence. On the contrary, a competition between the two proteins for DNA binding was observed.

Influence of the glucocorticoid receptor on DNA binding of purified NFI

Experiments similar to those reported for the PR were conducted with the glucocorticoid receptor (GR) and a 208 bp DNA fragment of the MMTV promoter. The GR generated the expected four DNase I footprints between −190 and −70 (Figure 3B, lanes 1–4, and Scheidereit *et al.*, 1983). In the presence of NFI, the expected footprint between −80 and −60 was observed, but almost no binding of the GR could be detected (Figure 3B, compare lanes 5–8

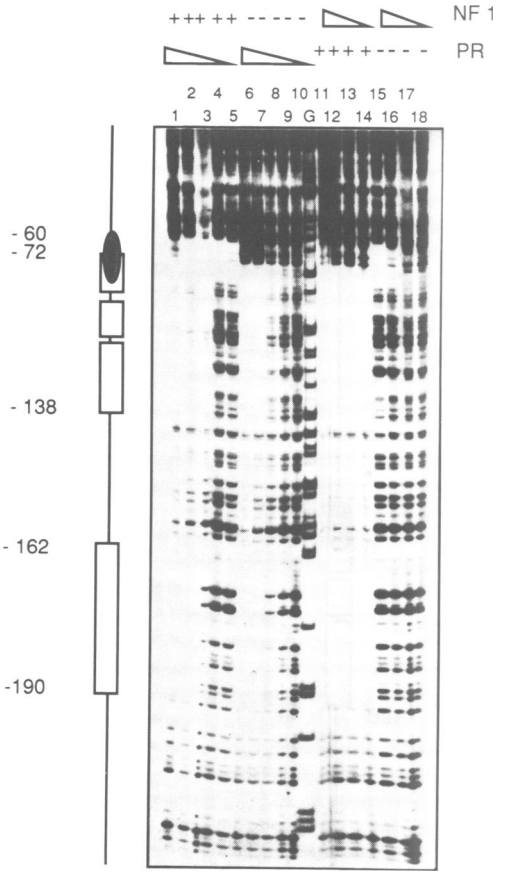

Fig. 2. Influence of purified NFI on binding of the PR to the MMTV promoter in a linear DNA fragment. A 722 bp *Bam*HI−*Bgl*II fragment of p1313 was labeled with [^{32}P]ATP in the *Bam*HI site at position −236. This fragment was preincubated in the presence (lanes 1−5) or absence (lanes 6−10) of 200 fmol of purified NFI. In a second step the samples were incubated with 200 ng (lanes 1 and 6), 100 ng (lanes 2 and 7), 50 ng (lanes 3 and 8), 25 ng (lanes 4 and 9) or 0 ng (lanes 5 and 10) of purified PR. In the reverse experiment the fragment was preincubated in the presence (lanes 11−14) or absence (lanes 15−18) of 100 ng PR, followed by a second incubation with 200 fmol (lanes 11 and 15), 100 fmol (lanes 12 and 16), 50 fmol (lanes 13 and 17) or 0 fmol (lanes 14 and 18) NFI. Receptor binding sites are indicated by open rectangles and the NFI binding site by a dashed oval.

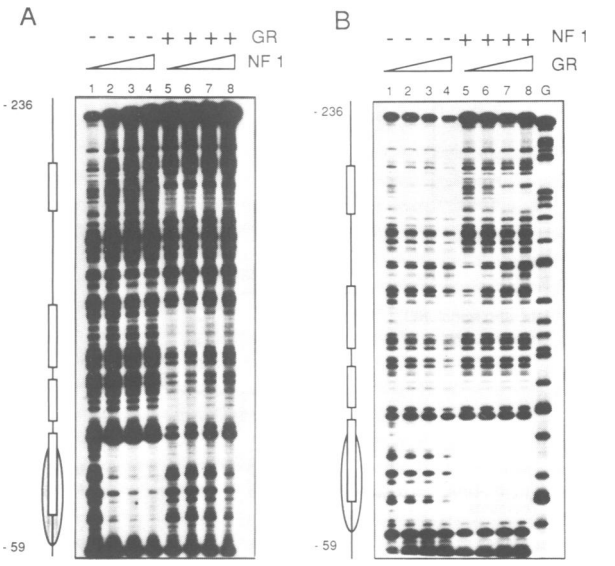

Fig. 3. Influence of GR on binding of purified NFI to the MMTV promoter in a linear DNA fragment. (**A**) A 208 bp *Bam*HI−*Nco*I fragment of pNuc was labeled in the *Nco*I site with [^{32}P]ATP and preincubated in the absence (lanes 1−4) or presence (lanes 5−8) of 500 ng GR, followed by a second incubation with 0 fmol (lanes 1 and 5), 25 fmol (lanes 2 and 6), 50 fmol (lanes 3 and 7) or 100 fmol (lanes 4 and 8) NFI. (**B**) In the reverse experiment, the fragment was preincubated in the absence (lanes 1−4) or presence (lanes 5−8) of 100 fmol of purified NFI. In a second step the samples were incubated with 0 ng (lanes 1 and 5), 100 ng (lanes 2 and 6), 200 ng (lanes 3 and 7) or 400 ng (lanes 4 and 8) of purified GR.

with lanes 1−4). In this case, NFI inhibited binding of the GR not only to sites adjacent to the NFI recognition sequence, but also to the promoter distal receptor sites between −190 and −160.

In the reverse experiments, the labeled DNA fragment was preincubated with or without GR, followed by the addition of increasing concentrations of NFI (Figure 3A). Here again, there was no indication of binding co-operativity. Rather, binding of the GR markedly inhibited subsequent binding of NFI to the −80 to 60 region (Figure 2A, compare lanes 5−8 with lanes 1−4).

Influence of hormone receptors on DNA binding of cloned NFI

The binding experiments mentioned above were performed with a highly purified form of NFI from pig liver (Meisterernst *et al.*, 1988a). As it has been shown that there are several proteins with equivalent binding behavior, it could be argued that the particular member of the NFI gene family we have used is not involved in MMTV-LTR transcription *in vivo*. In an attempt to explore this possibility we performed similar experiments with the same cloned NFI that we have shown to be active on the MMTV promoter in gene transfer experiments (see above). This cDNA was cloned into an SP6 expression vector, and used for *in vitro* transcription/translation experiments. The product of this assay was a 49 kd polypeptide that corresponds to the expected size of the NFI protein (Figure 4A). The *in vitro* synthesized NFI was used in band retardation assays with oligonucleotides corresponding to either the MMTV-NFI binding site or the NFI binding site of the adenovirus (Figure 4B). In both cases a specific retarded complex was observed that could be competed off by addition of 10 ng cold specific competitor oligonucleotide. With unprogrammed lysate no complex could be observed (data not shown). Thus, the *in vitro* translated NFI bound to MMTV promoter with the expected specificity.

For analyzing the interaction between *in vitro* synthesized NFI and hormone receptors, we used the exonuclease III protection assay (Shalloway *et al.*, 1980; von der Ahe *et al.*, 1985). In the absence of receptors, addition of NFI generated the expected stop at position −56 (Cordingley *et al.*, 1987, and Figure 4C). In the presence of either GR or PR, the intensity of the NFI signal at −56 was not enhanced but was, rather, reduced by a factor of 2−4 (compare lanes 1−4 with lanes 5−8 and 9−12 in Figure 4C). These results confirm our observations with purified NFI from pig liver and show that receptors and a functional NFI compete rather than act synergistically for binding to the MMTV promoter.

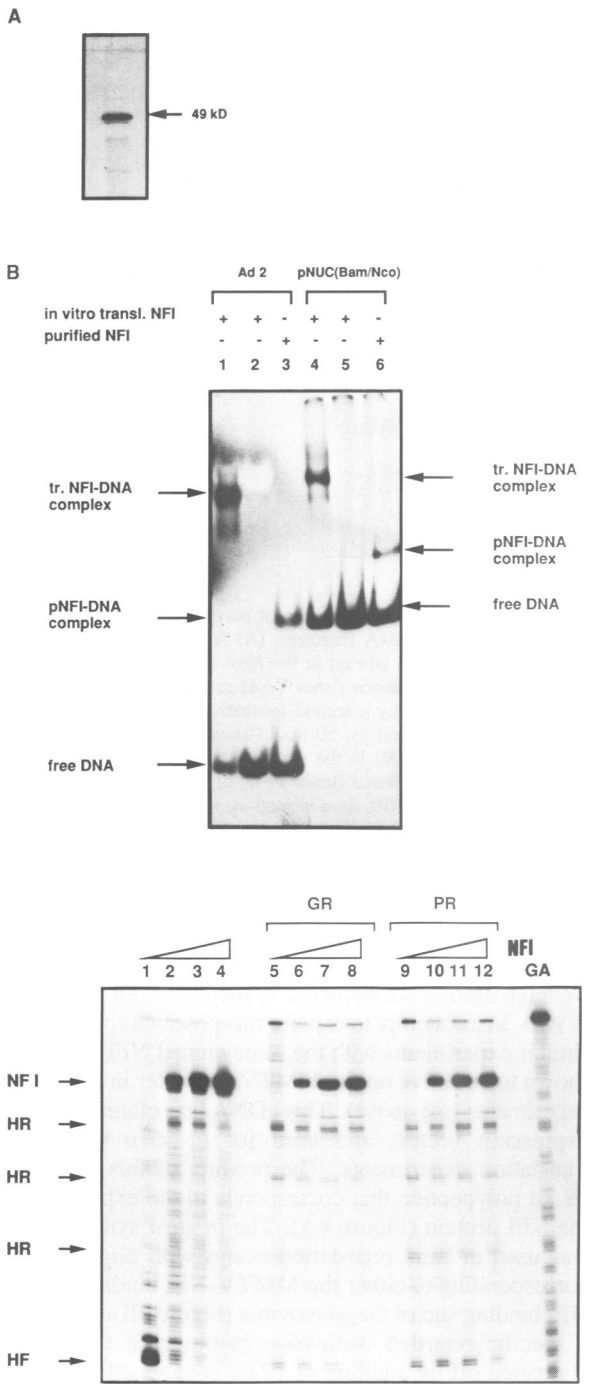

Fig. 4. Experiments with cloned NFI-cDNA. (**A**) 2 μg of NFI mRNA were translated in a reticulocyte lysate system in the presence of [^{35}S]methionine. 2 μl of this mixture were analyzed on 10% SDS–PAGE. (**B**) 10 000 cpm of an adenovirus NFI binding site oligonucleotide (lanes 1–3) or of a 208 bp *Bam*HI–*Nco*I fragment of pNuc (lanes 4–6) were incubated with 1 μl of lysate containing NFI (lanes 1, 2, 4 and 5) or with purified NFI from pig liver (lanes 3 and 6). In lanes 2 and 5 the complexes were competed with 10 ng of cold adenovirus oligonucleotide. (**C**) A 208 bp *Bam*HI–*Nco*I fragment of pNuc was labeled at the *Bam*HI site at position −236. The fragment was preincubated in the absence of receptors (lanes 1–4) or in the presence of either GR (lanes 5–8) or PR (lanes 9–12). In a second step the samples were incubated with 0 (lanes 1, 5 and 9), 0.5 (lanes 2, 6 and 10), 1 (lanes 3, 7 and 11) or 2 μl (lanes 4, 8 and 12) of NFI-containing lysate. After *Exo*III digestion the samples were analyzed on a sequence gel. *Exo*III stops of NFI and HR are shown, as is the half size fragment (HF).

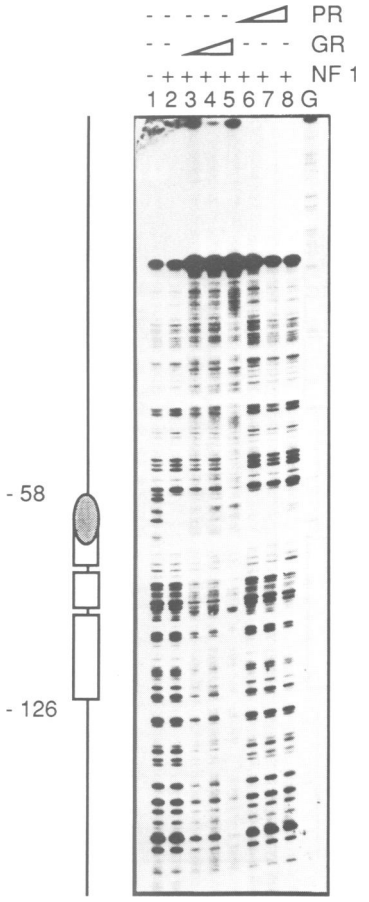

Fig. 5. Influence of hormone receptors on NFI binding to the MMTV promoter in a closed circular supercoiled DNA. A 722 bp *Bam*HI–*Bgl*II fragment of p1313 was labeled at the *Bam*HI site at position −236 and ligated in the presence of 1.5 μg/ml ethidium bromide. After removal of the ethidium bromide the supercoiled minicircles were separated from linear and nicked material on a preparative agarose gel and recovered from the gel by electroelution. In lanes 2–8 this minicircle was preincubated with 100 fmol of NFI. Lane 1 shows a control without NFI. In a second step the samples were incubated with 100, 200 or 400 ng GR (lanes 3–5) or with 50, 100 or 200 ng of PR. After the DNase I digestion the minicircles were linearized with *Xho*II and analyzed on a sequencing gel.

Interaction between hormone receptors and purified NFI on supercoiled DNA

Next we wanted to know whether a similar interaction between steroid hormone receptors and NFI is found in closed circular supercoiled DNA. This is important, since short linear DNA fragments represent a DNA structure free of some of the constraints found in genomic DNA. To analyze this question, we generated minicircles of 722 bp that contained the relevant region of the MMTV-LTR and carried a ^{32}P label at a single position. After binding of receptors or NFI, the DNA was digested with DNase I, extracted with phenol and restricted at an appropriate site, before electrophoresis in a sequencing gel. The results of a representative experiment are shown in Figure 5. Though the concentration of PR used for these experiments was low, no indication of co-operation between the receptor and NFI was observed; instead, competition was seen. The NFI footprint was weakened in the presence of increasing

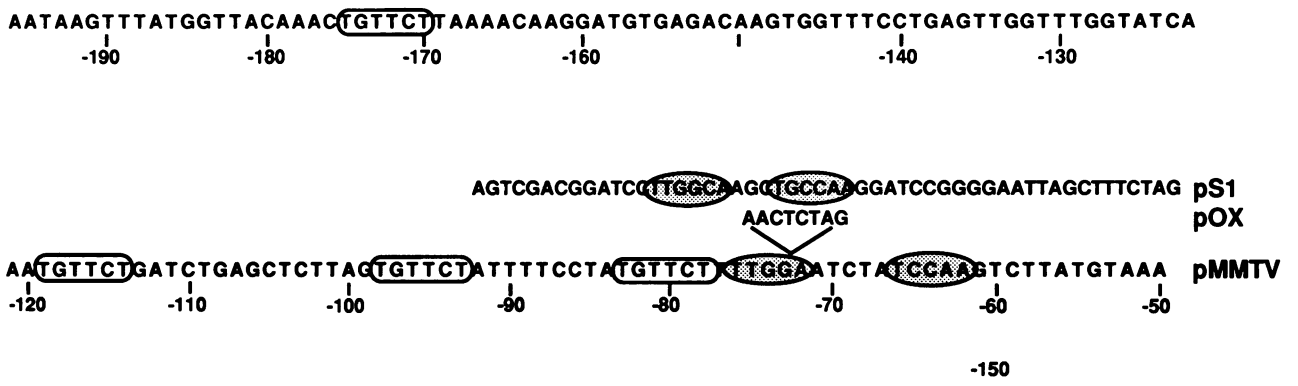

Fig. 6. Nucleotide sequence of the MMTV promoter around the HRE and the binding site for NFI. The nucleotide sequence of sense strand between positions −196 and −50 of the MMTV promoter is shown. The 4 hexanucleotide motifs are indicated by open rectangles and the two half sites of the NFI binding site by dashed ovals. In the mutants pOX and pS1 the indicated sequences were inserted into the *Hin*dI site of the MMTV promoter (described in Miksicek *et al.*, 1987).

concentrations of either GR or PR (compare Figure 5 lane 2 with lanes 5 and 8). Thus, independently of DNA topology, hormone receptors and NFI do not act synergistically but rather compete for binding to the MMTV promoter.

Discussion

NFI acts as a transcription factor on the MMTV promoter

A role for NFI in transcription of the MMTV promoter has been repeatedly formulated in the past (Nowock *et al.*, 1985; Buetti and Kühnel, 1986; Miksicek *et al.*, 1987; Cordingley *et al.*, 1987; Gowland and Buetti, 1989), but a direct demonstration in a complementation assay has not been reported. Here we have shown for the first time that cloned NFI can complement a mammalian cell line which is deficient in NFI and utilizes the MMTV promoter inefficiently, thus proving that NFI indeed acts as a transcription factor on the MMTV promoter *in vivo*. NFI is important for expression of the MMTV promoter after hormonal treatment with either glucocorticoids or progestins, but is inactive in the absence of hormone. Therefore there is a requirement for binding of the hormone−receptor complex to the HRE in order for NFI to show functional activity (Cordingley *et al.*, 1987; Piña *et al.*, 1990). The residual activity detected with the mutant pOX after glucocorticoid treatment may reflect the fact that only the spacing of the two palindromic NFI binding sites has been changed (Figure 6). We have preliminary evidence indicating that this mutant is still able to bind purified NFI albeit with much lower affinity than the wild-type MMTV promoter (U.Brüggemeier and M.Beato, unpublished).

That NFI acts as transcription factor for the MMTV promoter has also been recently shown in a cell-free transcription assay. Mutation of the half palindrome in the NFI binding site in the MMTV promoter reduces its transcriptional efficiency in a nuclear extract from HeLa cells by 10 to 15-fold (Kalff *et al.*, 1990). These results correlate well with those obtained in gene transfer experiments with JEG-3 cells and suggest that a similar mechanism is operating *in vivo* and *in vitro*.

NFI and hormone receptors compete rather than cooperate for binding to the MMTV promoter

The results of DNA binding experiments clearly show that there is no co-operation between GR or PR and NFI for binding to their cognate sequences on the MMTV promoter. Rather a competition is found, that is particularly evident with the GR, and takes place on both linear as well as on circular DNA molecules. These experiments also provide circumstantial evidence for co-operativity among GR molecules bound to the different receptor binding sites within HRE, as NFI interferes not only with GR binding to the adjacent receptor binding site but also to the distal site at −190/−160 (Scheidereit and Beato, 1984).

Though these results were obtained with a purified form of NFI that may lack part of the coding information present in the full length protein (Meisterernst *et al.*, 1988b), very similar observations were made using a cloned NFI that was shown to be functional *in vivo*. This cloned NFI, when expressed *in vitro*, generated a full length polypeptide chain that bound very efficiently to the MMTV promoter. Here again no co-operation but rather a competition between NFI and hormone receptors was observed in terms of their affinity for the MMTV promoter.

To exclude the possibility that other members of the NFI family may be responsible for the functional synergistic interaction with the hormone receptors, we performed experiments with crude extract from rat liver nuclei that should contain a mixture of NFI polypeptides (Scheidereit *et al.*, 1989). The limits of the exonuclease III footprint observed with the crude nuclear extract were identical to those found with purified NFI and indistinguishable from those reported for binding of NFI to the MMTV promoter *in vivo* (Cordingley *et al.*, 1987). Using the crude extract, a competition between NFI and hormone receptor for binding to the MMTV promoter was observed (Scheidereit *et al.*, 1989). Thus, it seems improbable that the observed lack of co-operativity between receptors and NFI is due to the use of a member of the NFI family that is not involved in MMTV transcription *in vivo*. However, in the absence of a functional assay for purified NFI, this possibility cannot be formally excluded. The results with the crude extract also tend to exclude the possibility that the interaction between receptors and NFI is mediated by an adaptor or bridging molecule, as this entity should be present in the nuclear extract of responsive cells.

Inspection of the nucleotide sequence of the MMTV promoter shows that the binding sites for steroid hormone receptors and NFI are very close together (Figure 6). There are only two nucleotides between the proximal TGTTCT

motif recognized by the hormone receptors and the distal TGGA motif recognized by NFI. The reported DNase I footprints observed with the GR and the PR completely cover the distal TGGAA motif (Figure 2 and 3, and Scheidereit *et al.*, 1983; Chalepakis *et al.*, 1988a), whereas the nuclease footprint of NFI covers part of the proximal TGTTCT motif (see also Nowock *et al.*, 1985; Miksicek *et al.*, 1987). These observations already suggest that the hormone receptor and NFI could hinder each other in terms of the DNA binding. Our findings do not exclude the possibility that when placed at an appropriate distance from each other, receptors and NFI could act synergistically in terms of DNA binding. In fact, reports consistent with this idea have been obtained with artificial constructions (Schüle *et al.*, 1988; Strähle *et al.*, 1988).

How could hormone administration facilitate NFI binding in vivo?

Our finding that hormone receptors and NFI compete rather than co-operate for binding to the MMTV promoter *in vitro* is in apparent conflict with the observation that hormone administration is a prerequisite for NFI binding *in vivo* (Cordingley *et al.*, 1987). There are several possibilities to explain this conflict. One would be to assume that the hormone receptor is bound to the MMTV-HRE prior to hormone administration, thus preventing NFI from interacting with its cognate sequences. Our finding that GR (Willmann and Beato, 1985) and PR (Schauer *et al.*, 1989) can bind to the MMTV-HRE *in vitro* with high affinity and specificity is compatible with this interpretation. As the rate of dissociation of the receptor from the MMTV-HRE is enhanced upon binding of the hormone (Schauer *et al.*, 1989), one could postulate that hormone administration leads to dissociation of the receptor, and thus facilitates NFI binding. Though this is an interesting possibility that could explain some of the observations in the MMTV system, it is in disagreement with experimental measurements of HRE occupancy *in vivo*. In rat liver cells, the HRE of the tyrosine amino transferase gene is only occupied by the GR after administration of dexamethasone (Becker *et al.*, 1986). However, no indication of the presence of GR bound to the MMTV-HRE has been obtained from *in vivo* experiments either prior to or following hormone administration (Cordingley *et al.*, 1987). Thus, there is no sufficient experimental support for the occlusion of the MMTV promoter by the hormone-free receptor.

An alternative explanation could be provided by the chromatin structure of the MMTV promoter. In minichromosomes carrying the MMTV-LTR, several nucleosomes are found located in precise positions along the DNA (Richard-Foy and Hager, 1987). In particular, a nucleosome-like structure covers the region between −230 and −60 that encompasses the binding sites for GR and NFI (Richard-Foy and Hager, 1987). No DNA bound NFI is detected prior to hormone treatment, but upon addition of dexamethasone, this region becomes hypersensitive to DNase I and NFI is found complexed to its binding site (Cordingley *et al.*, 1987). Thus it seems plausible that binding of the GR to the nucleosomally organized MMTV promoter leads to a change in chromatin structure that makes the promoter accessible for NFI binding. However, in these experiments, no signal for DNA bound receptor was detected before or after hormone administration. It is therefore possible that binding of NFI leads to dissociation of the GR from the HRE.

We favor a role of chromatin structure in determining MMTV transcription, in view of the recent finding that the MMTV promoter can be organized into precisely positioned nucleosomes *in vitro* (Perlmann and Wrange, 1988; Piña *et al.*, 1990). Purified hormone receptors can bind to such nucleosomally organized MMTV-HRE with relatively high affinity whereas NFI is unable to bind to the MMTV promoter in nucleosomes (Piña *et al.*, 1990). Therefore, the precise positioning of the double helix on the surface of the histone octamer could determine the accessibility of the recognition sequences for transcription factors on the MMTV promoter. If binding of the hormone receptors alters the structure of the regulatory nucleosome (Piña *et al.*, 1990), it could influence the ability of NFI to interact with its cognate sequence to initiate transcription. This interesting hypothesis can now be tested experimentally.

Materials and methods

Plasmids

p1313 is a plasmid containing a genomic fragment of integrated MMTV that has been described previously (Hynes *et al.*, 1983). pMTV-Nuc is a derivative of p1313 where the sequences between positions 132 (*Sac*I) and 2199 (*Nco*I) have been replaced by a 78 bp synthetic DNA fragment. This fragment contains wild-type MMTV sequences from the *Sac*I site on the MMTV promoter (position −102 relative to the CAP site) to position −37, followed by an *Eco*RI site (−36) and an *Nco*I 5′-overhanging end). pNFI2 is a plasmid containing the pig liver NFI/pCTF2 cDNA in an SP6 expression vector (Meisterernst *et al.*, 1989). pJ3NFI was obtained by cloning the *Hin*dIII−*Eco*RI fragment of pNFI2 in the eukaryotic expression vector pJ3 (Leeds *et al.*, 1989). pOX and pS1 have been described by Miksicek *et al.* (1987). The oligonucleotide with the NFI binding site from the adenovirus 2 ITR used in band shift experiments has the sequence: AATTCCTTA-TTTTGGATTGAAGCCAATATGATAATGAGGG.

In vitro transcription and translation

Capped transcripts of the plasmid pNFI2 were synthesized *in vitro* in the presence of 500 μM m^7GpppG using SP6 polymerase (Promega) and translated in rabbit reticulocyte lysate (Promega) for 1 h at 30°C, according to the manufacturer's instructions. The translation products were analyzed in SDS−polyacrylamide gels.

Preparation of supercoiled DNA minicircles

The plasmid p1313 was kinased with [γ-^{32}P]ATP in the *Bam*HI site and restricted with *Bgl*II. The compatible ends of the 722 bp fragment were ligated in the presence of 1.5 μg/ml ethidium bromide at 20°C. Supercoiled minicircles were separated from linear and nicked forms on a 2% agarose gel (40 mM Tris−HCl, 20 mM sodium acetate, 1 mM EDTA, pH 7.9), recovered from the gel by electroelution and precipitated with ethanol.

Protein binding

GR was purified from rat liver (Scheidereit *et al.*, 1983) and PR from rabbit uterus (Chalepakis *et al.*, 1988a). NFI was isolated from pig liver as previously reported (Meisterernst *et al.*, 1988a). For binding studies, DNA was incubated with different amounts of GR, PR, NFI or *in vitro* translated NFI as indicated in the figures for 30 min at 25°C in buffer containing 20 mM Tris−HCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 100 μg/ml BSA and 100 mM NaCl pH 7.6 in 20−200 μl total sample volume. Samples incubated with *in vitro* translated NFI are supplemented with 1 μg of poly dI.dC. The extent of DNA binding was determined by the gel retardation assay as previously described (Schauer *et al.*, 1989).

DNase I and Exo III digestion

After the addition of 10 μl of $MgCl_2$ (100 mM) and 100 ng of poly dI.dC to the samples, DNA was digested with DNase I (2 U) or *Exo*III (10 U) for 30 s at 25°C. The reaction was stopped by the addition of 10 μl of 0.5 M EDTA and 5 μg of tRNA, followed by phenol−chloroform−isoamyl alcohol (25:25:1) extraction. The samples were ethanol precipitated and analyzed on a 6.5% sequencing gel. Supercoiled minicircles were linearized with *Xho*II prior to analysis on a sequencing gel.

Cell culture and transfections

JEG-3 cell (Keller and Bridson, 1971) were maintained in MEM medium with 10% fetal calf serum. 5×10^5 cells were transfected by the $CaPO_4^-$ method (Akerblom *et al.*, 1988) with 1 μg reporter plasmid, 0.5 μg RSVGR (Miesfeld *et al.*, 1986) or 1 μg of rPR (Loosfelt *et al.*, 1986) and 1 μg of pJ3NFI or pJ3.CT DNA was added to give a total of 5 μg DNA. After 4.5 h the cells were shocked with medium containing 8% glycerol. After 16 h the medium was changed and steroid hormones were added (10^{-7} M dexamethasone and 10^{-8} M R5020). 48 h later the cells were collected and harvested by 3 cycles of freezing and thawing in 100 μl of Tris−HCl, pH 7.8. Protein concentrations were determined by the Bradford assay (Bradford, 1976). CAT assays were performed as described elsewhere (Gorman *et al.*, 1982) using 100 μg of protein. The percentage of CAT conversion was determined by a Hewlett Packard System 200 Imaging Scanner.

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