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Identification of two transactivation domains in the mouse oestrogen receptor

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ABSTRACT

We have identified two discrete transactivation domains within the mouse oestrogen receptor whose relative activities vary according to the target promoter. One domain lies within the N-terminal region and is active in the absence of oestradiol. The second domain is contained within the C-terminal portion of the protein and depends upon oestrogen binding for its activity. The location and oestrogen dependence of this domain has been confirmed using chimaeric receptors containing the Lex A DNA binding domain. Although transactivation by the C-terminal domain is dependent upon ligand binding the analysis of receptor deletion mutants has demonstrated that these two functions are not entirely coincident.

INTRODUCTION

Steroid hormones influence a wide variety of cellular processes by regulating gene expression. Their effects are mediated by receptor proteins which recognize and bind to sequence specific enhancers, termed steroid response elements, to regulate the rate of transcription of hormone responsive genes. To date the response elements identified appear to contain inverted repeat sequences which correspond to one of two motifs. The motif TGTTCT mediates the responses to glucocorticoids, progestins and androgens (1,2), while the response element for oestrogens (3,4) is based on the consensus TGACC. The steroid receptors are members of a family of ligand inducible transcription factors that also includes receptors for thyroid hormone, a number of vitamins and several whose ligands have yet to be identified (5). On the basis of the degree of sequence homology the receptors have been divided into six regions (A−F) (6). Region C, which is highly conserved throughout the family, contains two zinc fingers and is responsible for sequence specific DNA binding (5). The C-terminal receptor region (E/F) is hydrophobic and contains the ligand binding domain (5).

There appear to be multiple transcriptional activation domains within receptors as judged by analysing the activity of receptor mutants. Within both the oestrogen and glucocorticoid receptors transactivation domains have been mapped in the N-terminal region (7−9), in the hormone binding region (10,11) and associated with the DNA binding domain (12−14). In our studies of the mouse oestrogen receptor we have investigated the organization of functional domains by analysing the activity of a number of deletion mutants and chimaeric receptors. We have identified two discrete transactivation domains whose relative activities vary according to the target promoter.

MATERIALS AND METHODS

Receptor deletion mutants

Deletion mutants are all named according to the amino acid residues which they retain. The construction of deletion mutants MOR 121-599, MOR 121-569, MOR 121-552, MOR 121-538, MOR 121-507 and MOR 121-498 has previously been described (15). Mutants MOR 121-428, MOR 121-403, MOR 121-384, MOR 121-339 and MOR 121-315 were all generated from MOR 121-599 by digestion with the unique enzymes Bgl II, Sma I, Xho I, Xba I and Pvu II respectively, blunted and Eco RI linkers ligated. These inserts were then excised with Hind III and Eco RI and transferred to the expression vector pJ3TERM (see below) containing an in-frame termination codon. MOR 1-339 was generated by subcloning a Hind III-Xba I fragment from the wild-type receptor construct pJ3MOR (16) into the vector pJ3TERM. MOR 89-599 was also cloned from pJ3MOR by excising a Not I-Eco RI fragment and cloning into the vector pJ3ORF2 (see below) to allow expression in the correct open reading frame. The N- and C-terminal sequences of constructs were confirmed by single-stranded dideoxy-sequencing in M13mp18 and mp19 (17).

Expression vectors

The eukaryotic expression vector pJ3Ω, provided by J. Morgenstern, contains the SV40 early promoter and enhancer, small-t intervening sequence and large-T polyadenylation signal. The vectors pJ3ORF1, 2 and 3 and pJ3TERM have been generated from this vector to allow in-frame initiation and termination of deletion mutants.

Chimaeric receptor fusions

The chimaeric receptor fusion plasmids were constructed using the vector psvβLEX which was kindly provided by R. Treisman. This contains the SV40 enhancer and β-globin promoter linked to sequences encoding the N-terminal 87 amino acids of the bacterial repressor Lex A. Fragments containing either the N- or C-terminus of the receptor linked to the SV40 small-t intervening sequence and large-T polyadenylation signal were transferred from pJ3TERM into this vector using Hind III linkers to maintain the correct open reading frame (as confirmed by dideoxy-sequencing). The resultant fusion constructs pLex-MOR N180 and pLex-MOR C315 contained linking sequences between the Lex A and receptor coding sequences of VDLQPKLGLPLT and VDLQPKL respectively.

Reporter plasmids

The reporter pERE-BLCAT was constructed by cloning an oligonucleotide corresponding to an oestrogen response element derived from the vitellogenin A2 promoter of *Xenopus leavis* (−331 to −295) into the Xba I site of pBLCAT2 (18). This contains the marker gene chloramphenicol acetyl transferase (CAT) driven by the Herpes simplex virus thymidine kinase (TK) promoter sequences from −105 to +51. pERE-TK-CAT is of similar construction but contains the TK promoter sequences from −242 to +8 (16). pA2(−821/+14) which contains the vitellogenin promoter sequences from −821 to +14 cloned upstream of CAT was kindly provided by G. Ryffel. pLex OP-BLCAT was constructed by cloning an oligonucleotide corresponding to the Lex A operator (TACTGTATGTACATACAGTA) into the Xba I site of pBLCAT2.

Cell culture and transient transfection

NIH 3T3 D4 cells were routinely cultured in DMEM containing 10% foetal calf serum (Gibco, Paisley, Scotland). Cells were seeded for transient transfection at a density of 1×10^5 per 5 cm dish in 4 ml of DMEM containing 10% foetal calf serum and 10^{-6}M tamoxifen. After 24 hr the cells were refed and transfected by calcium phosphate co-

precipitation with a total of 10 μg of DNA which included 0.5 μg of the plasmid pJ3βgal (constructed by R. White). The β-galactosidase activity arising from this plasmid was used as an internal control of transfection efficiency. After 6 hr the cells were washed three times with phenol red-free DMEM and then fed with phenol red-free DMEM containing 10% dextran-charcoal treated serum, 10^{-6}M tamoxifen in the presence or absence of 10^{-7}M oestradiol. Cells were harvested between 24 and 48 hr after feeding and the cell suspensions divided to allow measurement of both β-galactosidase, according to the method of Norton and Coffin (19), and CAT activity, as previously described (16).

Capped RNA synthesis and in vitro translation

MOR 89-599 and MOR 121-599 were transferred from their eukaryotic expression vector to pSP64 using Hind III-Eco RI fragments. All other mutants were transferred similarly using Hind III and Bgl II. The resultant plasmids were linearised for cRNA synthesis using either Sst I, (and filled in with Klenow) or Eco RI as appropriate. RNA synthesis was as described by Melton *et al.* (20) with the following modifications. Templates were transcribed with 0.5 mM ATP, UTP and CTP, 50 μM GTP and 0.5 mM RNA cap structure analogue [m^7G(5′)ppp(5′)G] (Biolabs, Bishops Stortford, U.K.). The RNA was translated in a rabbit reticulocyte lysate (Amersham International, U.K.) as described by Fawell *et al.* (15).

Ligand binding assay

Aliquots of *in vitro* translated receptor were assayed for their ability to bind [16α-^{125}I]-iodoestradiol (Amersham International, U.K; 2,000 Ci/mmol) using the method of Coffer *et al.* (21) as modified by Fawell *et al.* (15).

Streptavidin biotin-conjugated DNA-binding assay

DNA binding was assayed essentially as described by Waterman *et al.* (14) using receptor translated *in vitro* in the presence of [^{35}S]-methionine (approximately 200,000 cpm per binding reaction). Receptor mutants were incubated with 5 pmol of a double-stranded oligonucleotide probe corresponding to an ERE (5′-BIOTIN-CATTAACTTTGATCA*GGTCA*CTG*TGACC*TCTAG-3′) or an unrelated control sequence of similar length (5′-BIOTIN-GGCCGAGGTGACACCGTGGGCCTGCCGGGC-3′). Each probe was labelled with biotinamidocaproate N-hydroxysuccinimide ester at the 5′ end distal to the receptor binding site. Specific binding was assessed as radioactivity recovered by streptavidin agarose beads corrected for binding to the control oligonucleotide.

Electrophoretic mobility shift assay

1−5 μl of *in vitro* translated receptor was pre-incubated for 15 min at room temperature in 20 μl of binding buffer (10 mM HEPES pH 7.4, 50 mM KCl, 1 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.1 mM $ZnCl_2$, 20% glycerol) containing 1 μg poly-dI.dC:poly-dI.dC, 1 μg tRNA and 0.1 mg BSA, with hormone or antihormone added as indicated. After addition of radio-labelled oligonucleotide probe (see below) the samples were incubated for a further 30 min at room temperature followed by 30 min at 4°C. Samples were applied directly to pre-run 6.0% polyacrylamide (30% acrylamide, 0.8% *bis*-acrylamide stock), 0.5×TBE gels, and electrophoresed in 0.5×TBE at 200V for 90 min. Gels were fixed for 15 min in 10% acetic acid, 30% methanol, dried and autoradiographed.

The probes were prepared by annealing oligonucleotides containing either the ERE (5′-CTAGAAAGTCA*GGTCA*CAG*TGACC*TGATCAAT-3′) or a control sequence (5′CTAGAAAGTC*AGAACA*CAG*TGTTCT*GATCAAT-3′) corresponding to a glucocorticoid response element (GRE), and then labelled by filling in 5′ overhanging ends with [^{32}P]-dCTP and 'cold' dNTP's.

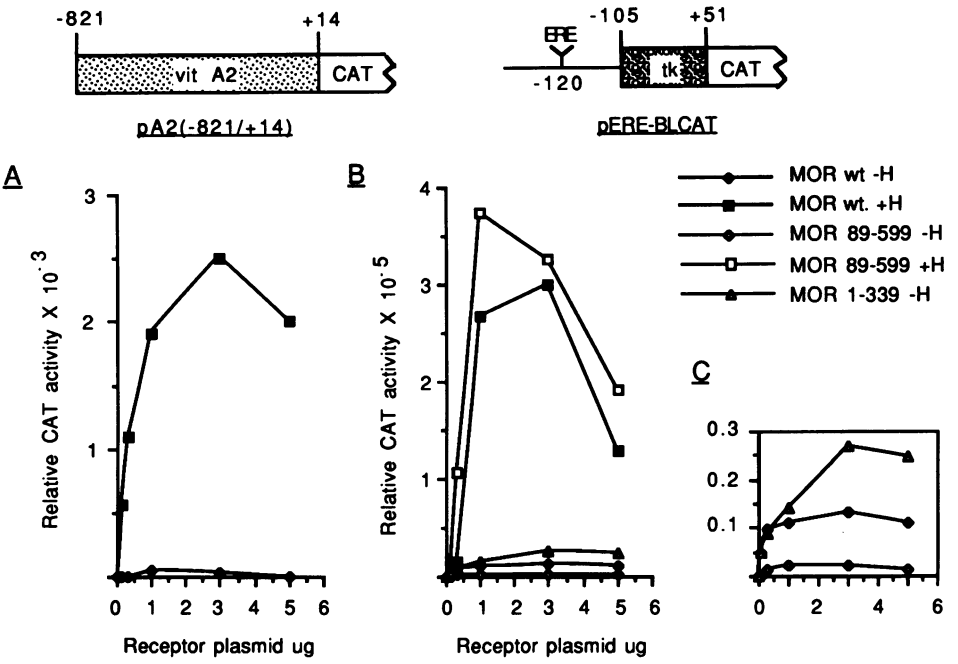

Fig.1 *Reporter response to input plasmid levels*
The response of the reporters pA2(−821/+14) [panel *A*] and pERE-BLCAT [panel *B*] to input receptor plasmid levels has been assessed in NIH 3T3 D4 cells. These were co-transfected with 5 μg of reporter, 0.5 μg pJ3βgal, receptor expression vector as indicated and with pJ3Ω added to a total of 10.5 μg/dish. Relative CAT activity refers to unit CAT activity normalised for the β galactosidase activity of each cell extract. Panel *C* shows the oestradiol independent stimulation of pERE-BLCAT from panel *B* on an extended scale.

Antibody preparation
The peptide NH_2-CQQVPYYLENEPSA-COOH corresponding to residues 130−142 of the mouse oestrogen receptor was coupled via the N-terminal Cys to thyroglobulin and used to immunise rabbits as described by Fawell and Higgins (22). The resultant polyclonal antiserum, MP16, was then characterized by western blotting and shown to specifically recognise both mouse and human oestrogen receptors (S.E.F.—data not shown).

RESULTS

We have mapped transactivation domains within the mouse oestrogen receptor by testing the ability of deletion mutants to stimulate a number of responsive promoters. Previously we had tested the wild-type receptor by transient transfection into COS-1 cells using the chimaeric reporter pERE-TK-CAT (16) in which the TK promoter extends from −250 to +8 relative to the cap site. A large proportion of the receptor activity in these cells arose in the absence of exogenous oestradiol and could not be abolished by the addition of the antioestrogen tamoxifen. Subsequently we have found that pERE-BLCAT (Fig. 1) in which the TK promoter extends from −105 to +51 gave rise to similar levels of oestrogen-independent activity but the total activity of the receptor in the presence of oestradiol was significantly increased (data not shown). Therefore pERE-BLCAT has been

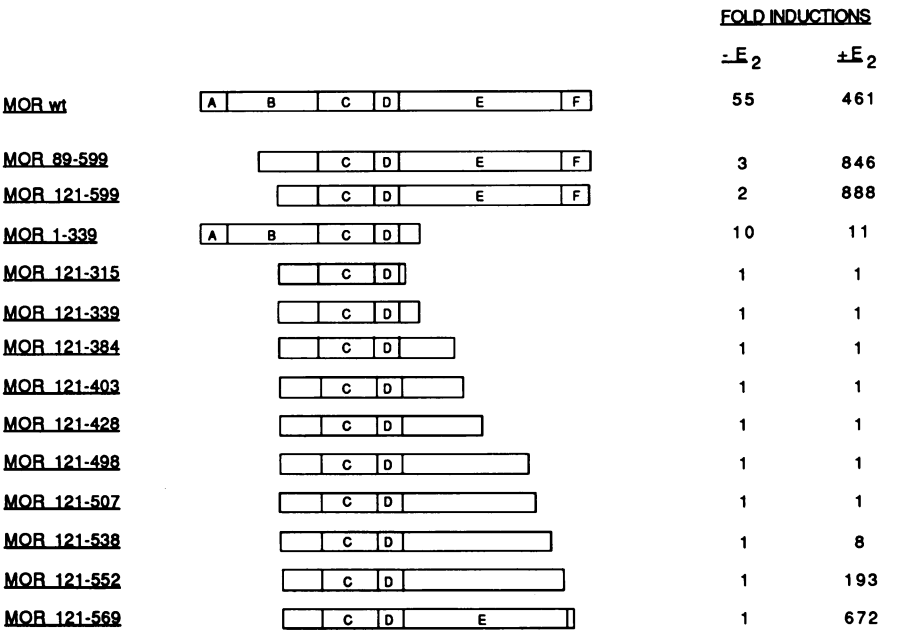

Fig. 2 *Transactivation of pERE-BLCAT by the receptor mutants*
The receptor mutants shown are named according to the amino acid residues which they retain. NIH 3T3 D4 cells were co-transfected with 5 μg of pERE-BLCAT, 0.5 μg pJ3βgal, 0.3 μg pJ3Ω/receptor mutant construct and 4.2 μg pJ3Ω. The cells were harvested after 48 h incubation in either 10^{-6}M tamoxifen ($-E_2$) or 10^{-6}M tamoxifen with 10^{-7}M oestradiol ($+E_2$). Fold inductions represent CAT activity normalised for β galactosidase levels over the relative basal activity of pERE-BLCAT. The Figure shows typical values, the experiment being repeated four times with similar results.

routinely used as the reporter. These distinct ligand independent and dependent receptor activities have been detected in all cell lines examined (Rat 1, NIH 3T3 D4, HeLa and BT20 cells) and were sufficiently large in the NIH 3T3 D4 cells to allow the mapping of receptor sequences responsible for both of these functions. In order to ensure that the CAT activity expressed by the reporter plasmids reflects the activity of receptor we have examined CAT activity with input receptor plasmids ranging from 0.1−5 μg/dish with a fixed amount of reporter (5 μg/dish). On the basis of these titration curves (Fig. 1) the mutant receptors were tested at input concentrations of 0.3 μg/dish with pERE-BLCAT and 1.0 μg/dish with the vitellogenin promoter pA2(−821/+14).

Mapping of transactivation domains with pERE-BLCAT

The wild-type receptor was able to transactivate pERE-BLCAT by up to 500-fold. Although 5- to 50-fold inductions were obtained in the absence of oestradiol addition of hormone consistently increased the receptor activity by a further 10-fold (Fig. 2). Deletion of the N-terminal sequences of the receptor (mutants MOR 89-599 and MOR 121-599) abolished the oestradiol independent activity of the receptor but had no effect on the total receptor activity in the presence of oestradiol. Deletion of C-terminal sequences, leaving only 34 amino acids of region E, (MOR 1-339) resulted in a hormone-independent activity of between 2−10% of total wild-type levels. Transactivation by deletion mutants MOR 89-599

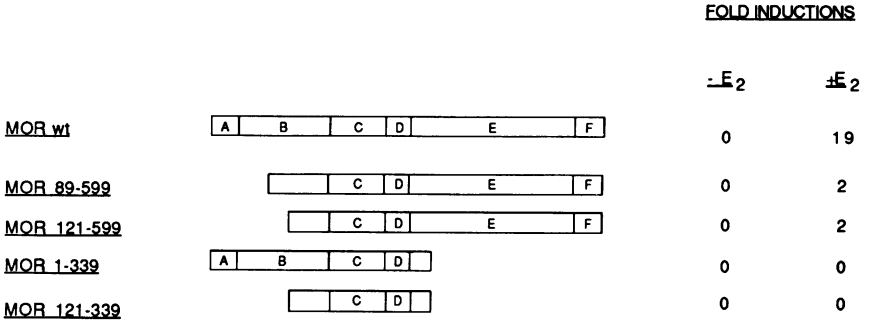

Fig. 3 *Transactivation of pA2(−821/+14) by the receptor mutants*
Cells were co-transfected with 5 μg of pA2(−821/+14), 0.5 μg pJ3βgal, 1 μg pJ3Ω/receptor mutant construct and 3.5 μg pJ3Ω. Cells were cultured for 48 h in either 10^{-6}M tamoxifen ($-E_2$) or 10^{-6}M tamoxifen with 10^{-7}M oestradiol ($+E_2$).

and MOR 1-339 has been examined over the full range of input receptor plasmid levels (Fig. 1B and 1C). In these experiments, the activity of N- and C- terminal sequences were sufficient to account for the hormone independent and dependent activities of the wild-receptor respectively. When both of the N- and C-terminal sequences were deleted (MOR 121-339) we detect no residual activity. We have examined the hormone dependent activity of the receptor in more detail by generating mutants containing progressive C-terminal deletions of the mutant MOR 121-599. When the majority of region F is deleted (MOR 121-569) the mutant functions with a similar total activity to that of the wild-type receptor. Mutants MOR 121-552 and MOR 121-538 gave rise to inductions of 193-fold and 8-fold respectively indicating that the 14 residues between the C-terminal boundaries of these two deletions contain amino acids which are important for the transactivation function of the receptor. Further C-terminal deletions (MOR 121-507 to MOR 121-315) completely abolish any detectable transactivation (Fig. 2).

These data therefore suggest that the receptor contains two discrete transactivation domains. The major domain lies within the C-terminal region and requires oestradiol for its activity whilst the second, N-terminal, domain functions constitutively in the absence of ligand.

Mapping of transactivation domains with the vitellogenin promoter

We have also examined a number of the receptor mutants with the vitellogenin promoter pA2(−821/+14) as reporter. Wild-type receptor stimulates this promoter about 20-fold with transactivation observed only in the presence of oestradiol (Fig. 3). The N-terminus of the receptor (MOR 1-339) fails to give any detectable transactivation of the vitellogenin promoter, consistent with the lack of hormone independent activity of the wild-type receptor. However, deletion of the N-terminal 88 (MOR 89-599) or 120 (MOR 121-599) residues of the receptor reduces the level of hormone dependent transactivation by 10-fold, demonstrating the importance of these N-terminal sequences in the stimulation of the vitellogenin A2 promoter.

Analysis of chimaeric receptors

To examine the transactivation domains more directly, we have constructed chimaeric receptor fusion proteins containing the N-terminal 87 amino acids of the bacterial repressor

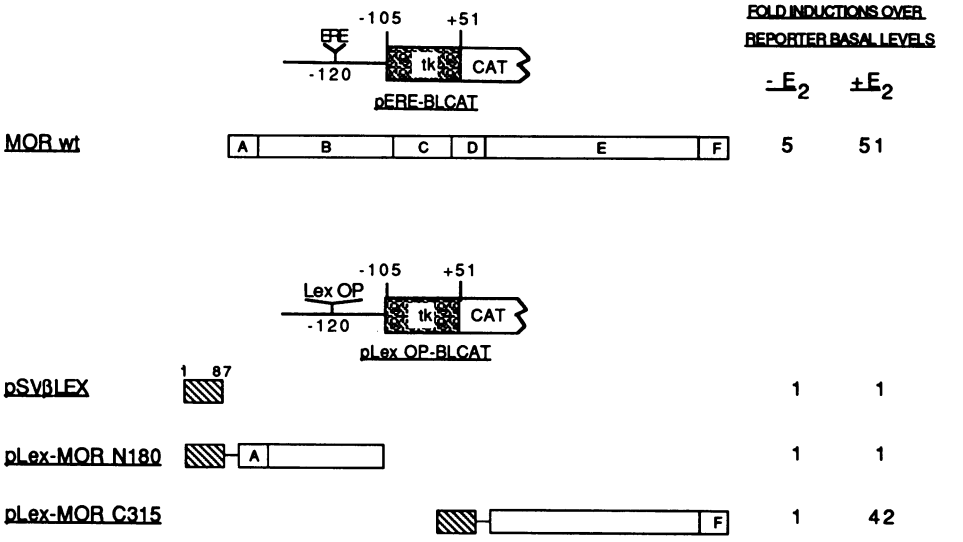

Fig. 4 *Analysis of the chimaeric receptor proteins*
The chimaeric receptors contain the N-terminal 87 amino acids of Lex A linked to the N-terminal 180 residues (pLex-MOR N180) or C-terminal 285 residues (pLex-MOR C315) of the receptor. These constructs were tested alongside the wild-type receptor. Cells were transfected with 0.5 μg pJ3βgal, 4.1 μg pJ3Ω and either 5 μg of pERE-BLCAT with 0.3 μg pJ3Ω/pJ3MOR or 5 μg of pLex OP-BLCAT with 0.3 μg pSVβLEX/chimaeric receptor plasmids. Cells were harvested after 48 h incubation in either 10^{-6}M tamoxifen ($-E_2$) or 10^{-6}M tamoxifen with 10^{-7}M oestradiol ($+E_2$).

Lex A. These have been shown to contain sequences sufficient to allow both weak dimerisation of Lex A and specific binding to the Lex A operator (23, and references therein). We have generated constructs in which sequences encoding this DNA binding domain have been fused to sequences encoding either the N-terminal 180 amino acids or C-terminal 285 amino acids of the mouse oestrogen receptor. The ability of these fusion proteins to transactivate has been assessed by co-transfection with pLex OP-BLCAT, a reporter construct in which the ERE of pERE-BLCAT has been replaced by the consensus sequence for the Lex A operator (Fig. 4). The C-terminal sequences of the receptor were able to transactivate at a level (42-fold) which is similar to that of the wild-type receptor when tested with pERE-BLCAT (51-fold) in the same experiment. This transactivation is entirely dependent on the presence of oestradiol. In contrast, we have been unable to detect any activity arising from the N-terminal fusion protein.

Ligand binding

Our analysis of deletion mutants and chimaeric receptor proteins establishes that the MOR contains an oestrogen dependent transactivation domain within region E. Sequences between residues 538 and 552 appear to be particularly important since MOR 121-552 gave rise to 193-fold induction whereas MOR 121-538 gave rise to only an 8-fold stimulation. However, as this domain is active only in the presence of oestradiol, we established whether the loss of transactivation was caused by the loss of ligand binding. Scatchard analysis indicates that both mutants bind oestradiol with high affinity (Fig. 5) and that although the Kds of both MOR 121-538 (0.3 nM) and MOR 121-552 (0.12 nM) are somewhat

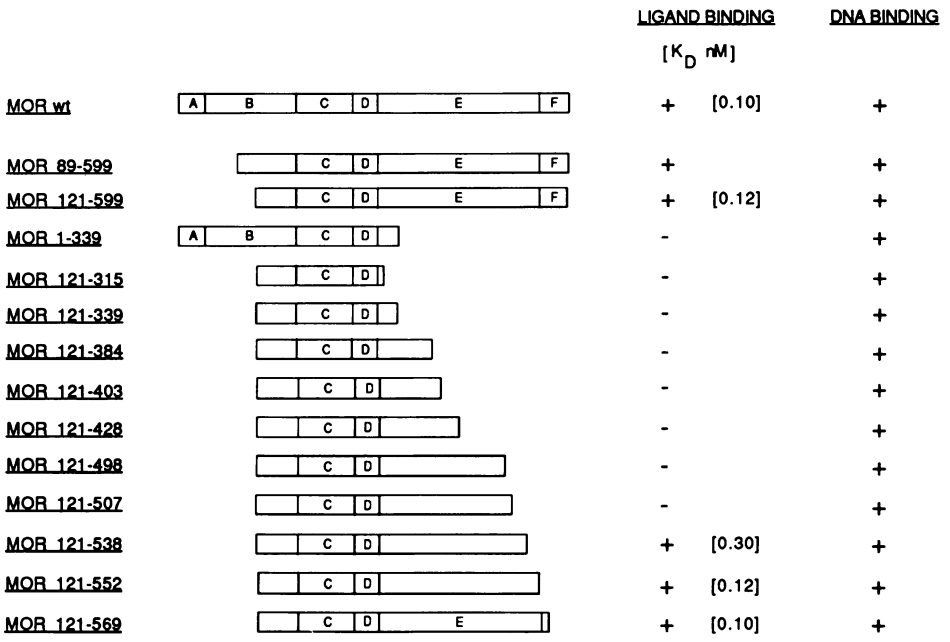

Fig. 5 *Ligand and DNA binding of receptor mutants*
Ligand binding was assayed by the dextran-coated charcoal method using [16α-^{125}I]-iodoestradiol at a concentration of 1 nM, corrected for non-specific binding in the presence of excess diethylstilboestrol. The dissociation constant values shown were derived from a Scatchard analysis described previously (15). [^{35}S]-methionine labelled *in vitro* translated receptor mutants (200,000 cpm) were assayed for specific binding to ERE containing biotinylated oligonucleotides (5 pmol in a final volume of 50 μl) by precipitating with streptavidin agarose beads and counting by liquid scintillation. Data were corrected for binding to non-specific sequences and was typically 10,−100,000 cpm greater than background levels (3,−10,000 cpm).

increased relative to that of the wild-type receptor (0.10 nM), these mutant receptors would be fully occupied by oestradiol in our transient transfection experiments. In contrast, since MOR 121-507 shows no specific binding, even at a ligand concentration of 50 nM, the inability of this mutant to transactivate may still reflect the loss of ligand binding activity.

DNA binding

We have examined the ability of the receptor mutants to recognise and bind specifically to an ERE using biotinylated oligonucleotides. When translated *in vitro* all the receptor mutants bound to the biotinylated ERE and could be competed off by addition of non-biotinylated ERE. The amount of binding was approximately 3−4 fold greater than that binding to a control biotinylated oligonucleotide (Fig. 5).

To examine the effect of oestradiol on DNA binding, we have used a mobility shift assay. *In vitro* translated receptor gave rise to an ERE-receptor complex even in the absence of added ligand (Fig. 6A, lane 1). The complex could be specifically competed with an excess of unlabelled ERE oligonucleotide (Fig. 6A, lane 6) but not by an unrelated sequence (data not shown) and was shown to be due to receptor since addition of an anti-oestrogen receptor antibody (MP16) resulted in a 'supershift' of the complex (Fig. 6A, lane 7). Addition of either oestradiol or the agonist diethylstilboestrol had no effect on the intensity

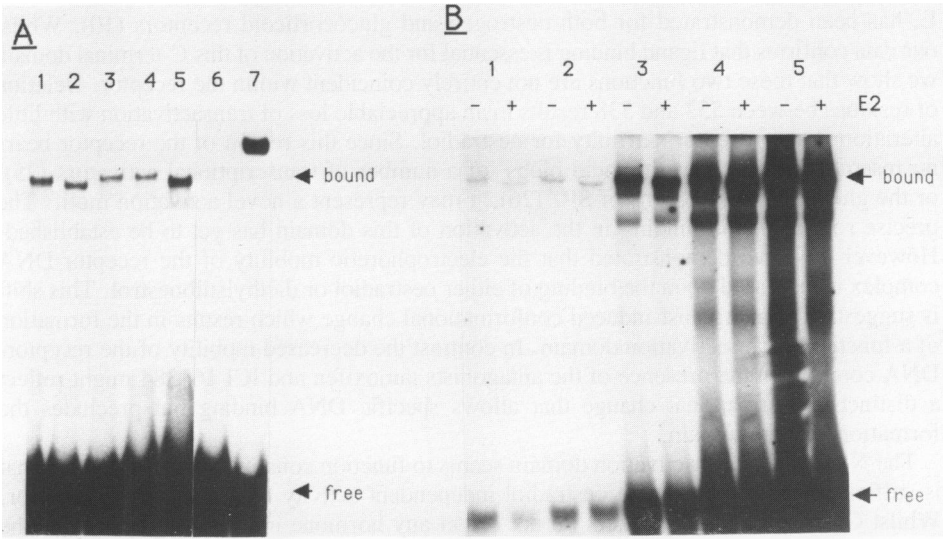

Fig. 6 *Effect of ligand upon the binding of the mouse oestrogen receptor to its response element*
The binding of *in vitro* translated MOR to an ERE containing oligonucleotide was assessed using an electrophoretic mobility shift assay as described in the methods. Panel *A*. Binding reactions were carried out after preincubation with: lane 1, no added hormone; lane 2, 10^{-8}M oestradiol; lane 3, 10^{-6}M tamoxifen; lane 4, 10^{-7}M ICI 164384; lane 5, 10^{-7}M diethylstilboestrol; lane 6, 20 ng unlabelled ERE; lane 7, 2 μl antiserum MP16. Panel *B*. The binding of *in vitro* translated receptor in the presence and absence of 10^{-8}M oestradiol with ERE probe concentrations of lane 1, 2.5 fmol; lane 2, 5 fmol; lane 3, 25 fmol; lane 4, 100 fmol; lane 5, 250 fmol.

of the shifted complex but resulted in a reproducibly faster migrating species (Fig. 6A, lanes 2 and 5 respectively). The antagonists, tamoxifen (Fig. A, lane 3) and ICI 164384 (Fig. 6A, lane 4), also gave complexes of similar intensity, but these migrated reproducibly slower than the unliganded receptor.

Since the single concentration of probe used in these experiments (2.5 nM) may not have detected an effect of ligand on the receptor's affinity for DNA, a range of probe concentrations were examined. At all concentrations tested (0.125−12.5 nM) the unliganded and oestradiol-complexed receptor shifted equivalent amounts of probe DNA (Fig. 6B).

DISCUSSION

We have shown that the mouse oestrogen receptor contains two distinct transactivation domains whose activities vary according to the responsive promoter. One domain is located in the C-terminal region of the receptor and is only active in the presence of oestradiol. The second, N-terminal domain, gives rise to low level constitutive transactivation. Whilst these results are consistent with the data of Chambon and co-workers (7,24) they contradict the proposal of Waterman *et al.* (14) that a relatively small DNA binding domain of the oestrogen receptor is sufficient for transactivation. Since this proposal was based upon the observation that deletion of either the N- or C-terminus of the receptor fails to abolish transactivation we suggest that their data is in fact also consistent with the existence of two transactivation domains.

The co-localisation of ligand binding and inducible transactivation domains within region

E, has been demonstrated for both oestrogen and glucocorticoid receptors (10). Whilst our data confirms that ligand binding is essential for the activation of this C-terminal domain we show that these two functions are not entirely coincident within the receptor. Deletion of residues between 552 and 538 results in an appreciable loss of transactivation with little alteration in the receptor's affinity for oestradiol. Since this region of the receptor bears no resemblance to either the 'acid blobs' of a number of transcriptional activators (25), or the glutamine rich regions of SP1 (26), it may represent a novel activation motif. The precise role of ligand binding in the activation of this domain has yet to be established. However we have demonstrated that the electrophoretic mobility of the receptor-DNA complex is increased upon the binding of either oestradiol or diethylstilboestrol. This shift is suggestive of an agonist-induced conformational change which results in the formation of a functional transactivation domain. In contrast the decreased mobility of the receptor-DNA complex in the presence of the antagonists tamoxifen and ICI 164384 might reflect a distinct conformational change that allows specific DNA binding but precludes the formation of this domain.

The N-terminal transactivation domain seems to function constitutively in a manner that is sufficient to account for the oestradiol independent activity of the wild-type receptor. Whilst Chambon and co-workers do not detect any hormone independent activity of the human oestrogen receptor in HeLa or CV1 cells, they have also demonstrated that C-terminal deletion mutants retain a low level constitutive activity (24). The hormone independent activation of pERE-BLCAT implies that the mouse oestrogen receptor interacts with this reporter *in vivo* in the absence of oestradiol binding. Since radioimmunoassay failed to detect any oestradiol in the steroid-depleted media and the hormone independent activity is not affected by the addition of the antagonist tamoxifen, it is unlikely that this arises from low levels of endogenous oestradiol. Since we have also observed hormone independent activity of the mouse oestrogen receptor in the absence or presence of tamoxifen in HeLa cells (J. Ham and C. Emmas personal communication) the differences in the activity of the mouse and human oestrogen receptors are not determined by the cell type in which they have been tested. This suggests that real differences may exist between the mouse oestrogen receptor and the human oestrogen receptor from MCF-7 cells. In this respect it is interesting to note that the MCF-7 breast cancer oestrogen receptor contains a Gly to Val point mutation within the hormone binding domain at a residue which is conserved between all species of oestrogen receptor so far examined (27).

We have demonstrated that a number of the receptor deletion mutants stimulate the two target genes pERE-BLCAT and pA2(−821/+14) differentially. In particular, our results suggest that a cooperative interaction between the N- and C-terminal domains is required for maximal stimulation of the vitellogenin promoter of pA2(−821/+14). Differences in the response of the rat prolactin (14), pS2 and the chimaeric vit-tk-CAT (7) promoters to human oestrogen receptor mutants raised the possibility that transactivation is modulated by the nature of the ERE. Since pERE-BLCAT and pA2(−821/+14) contain an identical ERE we conclude that the varying responses observed are the result of the differential specificities of the two transactivation domains for the transcription factors of a particular responsive promoter. In support of this hypothesis the reporter vit-tk-CAT, containing the vitellogenin A2 promoter sequences from −331 to −87 linked to the TK promoter from −105 to +51, respond to receptor deletion mutants (7) in a similar manner to pERE-BLCAT. In addition, whilst both the A and B forms of the chicken progesterone receptor can stimulate the mouse mammary tumour virus LTR only the A form, which lacks the

N-terminal sequences, can activate the ovalbumin promoter (28). A number of groups have shown that the glucocorticoid receptor is able to cooperate more efficiently with some transcription factors than with others and that the relative activities of these factors are modulated by the distance and stereospecific alignment of binding sites (29,30).

Multiple transactivation domains may be a general feature of the steroid receptors. Although the precise location of most of these domains has not been determined the sequences do not appear to be conserved between different classes of receptor. Whilst the N-terminal transactivation domain of the glucocorticoid receptor is acidic in nature and can be replaced by an amphipathic α-helix (11) the corresponding region in the oestrogen receptor contains no such motif. The existence of multiple and diverse transactivation domains within the steroid receptors may facilitate cell and steroid specific activation of gene expression and contribute to the complex multi-hormone regulation of some genes.

The classical model of steroid hormone action suggests that the role of ligand is to induce specific DNA binding of the receptor. This model is supported by genomic footprinting experiments on the tyrosine amino-transferase gene (31) which indicate that steroid is required for binding of the glucocorticoid receptor *in vivo*. In contrast, *in vitro* footprinting experiments with purified glucocorticoid (32) and progesterone (33) receptor show specific DNA binding in the absence of ligand. Beato has suggested that these discrepancies might involve the heat shock protein hsp 90 (34). It is proposed (35−37) that hsp 90 may interact with the steroid-free receptor *in vivo* and that one role of hormone is to dissociate the complex to allow specific DNA binding. However, this is unlikely to provide the complete explanation since we present circumstantial evidence that the mouse oestrogen receptor can recognise and bind to an ERE *in vivo* in the absence of any ligand. In addition we have demonstrated that oestrogen receptor translated *in vitro* binds to DNA in a hormone independent manner. This is consistent with the ligand independent DNA binding of the rabbit progesterone receptor in crude cytosols (38). In contrast, DNA binding by the human oestrogen receptor in cytosols (39) and rat glucocorticoid receptor translated *in vitro* (40) was enhanced although not dependent upon ligand binding. At present there is no obvious explanation for the differences in the ligand dependence of receptor-DNA binding *in vitro*.

While the activity of the mouse oestrogen receptor is increased by oestradiol we have shown that it can stimulate pERE-BLCAT in its absence. This hormone independent activity is intriguing and raises the possibility that steroid receptors may interact with other target promoters *in vivo* in the absence of ligand to play a physiological role in the maintenance of basal levels of gene expression.

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