Effect of a negative regulatory element (NRE) on the human *CYP1A1* gene expression in breast carcinoma MCF-7 and hepatoma HepG2 cells

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Abstract The expression of the cytochrome P4501A1 gene, *CYP1A1*, is induced by e.g. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) mainly by transcriptional mechanisms. The inducers mediate their effect upon binding and activation of the aryl hydrocarbon receptor (AHR) transcription-factor complex. Utilizing chimeric *CYP1A1*/CAT constructs transient gene expression experiments indicate that the putative negative regulatory element (NRE) of *CYP1A1* influence the relative TCDD induced CAT activity in HepG2 cells, whereas this effect was not observed in MCF-7 cells. Differences in the formation of cell-specific protein–DNA complexes were demonstrated by gel retardation assays suggesting a functional difference of NRE in these two cell lines.

Key words: P450IA1; Gene transcription; Negative regulatory element; Cell specificity; Human cell

1. Introduction

The cytochrome P450 enzymes are a superfamily of isoenzymes involved in the oxidative metabolism of a variety of endogenous compounds, including steroids, fatty acids, prostaglandins, leukotrines and biogenic amines. Moreover, these enzymes play an important role in biotransformation of xenobiotica [1,2], e.g. the metabolism of polycyclic aromatic hydrocarbons (PAH) into their ultimate carcinogenic forms [3–5]. The P4501A family includes two genes, *CYP1A1* and *CYP1A2*, and their expression is inducible by PAH and dioxins [2]. The most active form in the metabolism of PAH is the *CYP1A1* gene product, exhibiting aryl hydrocarbon hydroxylase (AHH) activity [6,7]. In the human population a large variation in *CYP1A1* inducibility has been observed [8–10]. Using human lymphoblast cultures approximately 10% were shown to exhibit a high AHH-inducibility to exposure of PAH, and this phenotype was correlated to an increased risk of bronchogenic carcinoma [11]. Furthermore, moderate to high constitutive *CYP1A1* expression has been shown to correlate with poor prognosis in human breast cancer [12,13]. Studies have demonstrated that different human breast cancer cell lines vary greatly with respect to the basal and TCDD induced expression of *CYP1A1* [14,15]. Interindividual differences in regulation of *CYP1A1* seem to be related to differences in susceptibility to carcinogens and it is therefore important to understand the cellular distribution and regulation of *CYP1A1* contributing to activation of these compounds at the target site.

The induction of this gene by e.g. 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD) is regulated mainly at the transcriptional level via the aryl hydrocarbon receptor (AHR) [16,17]. As a ligand-activated transcription factor, the AHR activates the transcription of a large number of genes [2,18–20], of which *CYP1A1* is the most extensively studied. The induction response is a complex sequence of events initiated by binding of the agonist to the AHR resulting in dissociation of 90-kDa heat shock protein, receptor activation/transformation, nuclear translocation and DNA-binding of the activated AHR complex to specific genomic sequences termed xenobiotic or dioxin responsive elements (XREs, DREs). The activated nuclear AHR transcription factor complex is a heterodimer consisting of two basic region/helix-loop-helix proteins, the AHR and the AHR nuclear translocator (ARNT) protein [16,17,21]. Additional factors seem to be involved in tissue- and cell-specific modifications of the induction response. These include the protein kinase-C dependent phosphorylation as an essential step for the DNA-binding activity of the AHR-transcription-complex [22,23], AHR level and *CYP1A1* expression [24, 25]. Another *cis*-acting DNA element BTE (basic transcriptional element) located immediately upstream the transcription start site is required for maximal inducible expression of the rat *CYP1A1* gene [26]. In vitro analyses of the mouse *cyp1a1* and the human *CYP1A1* gene have indicated that the AHR-mediated activating processes are regulated by negative regulatory mechanisms, and an autoregulatory effect of the endogenous mouse CYP1A1 protein has been suggested [17]. Deletion analyses of the human *CYP1A1* promotor region have indicated the presence of a negative regulatory element (NRE) located between −833 and −558 bp [27]. This element is shown capable of down-regulating a heterologous promotor/enhancer involving specific nuclear protein binding to a 21-bp palindrome (−794 to −774) [28].

The objective of this study was to investigate a possible cell specific effect(s) of the NRE on its natural promotor/enhancer (*CYP1A1*) activity in human hepatoma HepG2 and breast carcinoma MCF-7 cells. The HepG2 cells were selected as prototype for the study since *CYP1A1* expression is highly inducible and previous NRE studies were carried out in this cell line [27]. MCF-7 was used as model for breast cancer cells. The effects of NRE on constitutive and TCDD induced activity of the

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHR, aryl hydrocarbon receptor; CAT, chloramphenicol acetyl transferase; NRE, negative regulatory element; PAH, polycyclic aromatic hydrocarbons; AHH, aryl hydrocarbon hydroxylase; XRE or DRE, xenobiotic or dioxin response element; ARNT, AHR nuclear translocator; EMSA, electrophoretic mobility shift assay; SV40, simian virus 40; *β-gal*, β-galactosidase; FCS, fetal bovine serum; DMEM-PH, Dulbecco's Modified Medium without Phenol red; DMSO, dimethylsulfoxide; 1-deoxy^{14}C-Cm, 1-deoxy[dichloroacetyl-1-^{14}C]chloramphenicol; DTT, dithiothreitol; RT, room temperature; HSV tk, herpes simplex virus thymidine kinase.

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CYP1A1 promotor activity were analyzed by transient gene expression assays using chimeric *CYP1A1*-reporter gene constructs, and cell-specific DNA-binding activity of nuclear extracts and analyzed by electrophoretic mobility shift assays (EMSA) using the 21-bp palindrome of NRE as probe.

2. Materials and methods

2.1. Materials, plasmids, and cells

All chemicals were obtained at the highest purity available from Sigma, Boehringer or Pharmacia. Restriction endonuclease and DNA modification enzymes were purchased from either New England Biolabs or Life Technologies A/S, mammalian cell culture media, fetal calf serum and lipofectin from GIBCO BRL. 1-Deoxy[dichloroacetyl-1-^{14}C]chloramphenicol (1-deoxy^{14}C-Cm) (55 Ci/mmol) in 0.25M Tris-HCl, pH 7.5, [α-^{32}P] and [γ-^{32}P]deoxyribonucleotide triphosphates, 3,000 Ci and 5,000 Ci/mmol, respectively, were acquired from Amersham Corp., TCDD was obtained from Wellington Laboratories, Lenexa, Kansas, and oligonucleotides from DNA technology, Science Park, Aarhus, Denmark. The reporter plasmids used in this study are shown in Fig. 1. pRNH11c was provided by Dr. R.N. Hines, Detroit, MI. pdN11c was constructed by deletion of the proposed NRE (−833 to −558) by digestion of pRNH11c with *ApaI*/*Sac*II enzymes, blunt-ending by T_4 DNA polymerase treatment and religation. pSdN11c was constructed by substitution of the NRE with the unrelated 275 bp *Bam*HI/*Sal*I fragment (Stuff$_{275}$) of the *tet*^{R} gene in pBR327. pRSV*βgal* (encoding the *β*-galactosidase enzyme) was kindly provided by Dr. R. Toftgaard, Karolinska Institute, Huddinge, Sweden. pC$_{10}$*cat* (carrying the *Bgl*II/*Eco*RI *cat* gene cartridge from pA$_{10}$*cat*$_2$ [29]) was kindly provided by Dr. R.N. Hines, Detroit, MI, pSV0*cat* (derived from pSV2*cat* [30] by deletion of the SV40 early promotor/enhancer region) was provided by Dr. P. Jørgensen, University of Aarhus, Denmark. pNRE$_{105}$, and pNRE$_{170}$ were constructed by use of the intact 275 bp NRE (NRE$_{275}$) fragment isolated after *ApaI*/*Sac*II digestion of the pRNH11c. The NRE$_{275}$ was digested with *Bst*NI, and the two NRE subfragments were isolated, blunt-ended using T_4 DNA polymerase and cloned into the *Sma*I site of pUC19. The plasmid constructs were verified by restriction endonuclease digestions and/or DNA sequence analysis [31]. The human hepatoblastoma cell line HepG2 (passage 76), purchased from American Type Culture Collection (Rockville, MD), was cultured in Dulbecco's Modified Eagle Medium (DMEM) with glutamax-1 supplemented with 10% fetal bovine serum (FCS), 64 μg/ml garamycin^R and incubated in a humidified incubator at 37°C, 5% CO_2. The cells (passage 77–87) were subcultured (1:6) every 3–4 days. The human breast carcinoma cell lines MCF-7 was obtained from the Breast Cancer Task Force Cell Culture Bank, Mason Research Institute (Worcester), used at passage 298–310, propagated in DMEM without Phenol red (DMEM-PH) supplemented with 1% FCS, 64 μg/ml garamycin^R, 2.5 mM glutamine, 6 μg/l insulin, and incubated as described for HepG2. The cells were subcultured (1:10) every 3–4 days, after trypsination DMEM-PH supplemented with 10% charcoal-stripped FCS was added for 24 h.

2.2. Transient expression assays

Transfections were done in triplicate in 60-mm-diameter dishes. The optimal DNA amount and ratio between reporter plasmid(s) and pRSV*βgal* (internal standard for transfection efficiency) were determined by titration experiments. The HepG2 and MCF-7 cells followed different schedules. HepG2: (i) 5×10^5 HepG2 cells were plated 24 h before transfection with 6 μg *CYP*1A1/*cat* reporter gene plasmid and 0.6 μg pRSV*βgal* using 24 μg lipofectin in Opti-MEM Reduced Serum Medium; (ii) After 22 h the media was replaced with growth media containing 0.1% DMSO (solvent control) or 10 nM TCDD (induction); (iii) After another 26 h the cells were harvested. MCF-7: (i) 3.5×10^5 cells plated in growth media containing 10% charcoal-stripped FCS; (ii) Media replacement (DMEM-PH, 1% FCS) 24 h later; (iii) Transfection as described for HepG2 for 5 h followed by, (iv) treatment with DMSO (0.1%) or TCDD (10 nM) for 21 h and then the cells were harvested. Cell harvest and CAT assays were performed essentially as described [15] with following modifications. Cells were washed in ice-cold TNE (50 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA), and TNE + 5% glycerol was used for harvest and cell-extract preparation. CAT activity was determined using 0.25 μCi 1-deoxy^{14}C-Cm and 1 mM acetyl coenzyme A as substrates. Cell extracts containing 10 μg protein was analyzed in parallel with control CAT (0.05 U; Pharmacia, Biotech). CAT activities were always measured in substrate excess ensuring the reactions within the linear phase. After separation of 1-deoxy^{14}C-Cm and 3-acetyl,1-deoxy^{14}C-Cm by thin-layer chromatography, the% acetylated^{14}C-Cm was quantitated by phosphorimage analyses (Molecular Dynamics, KEBO). The protein concentration was quantitated using Micro BCA Protein Assay Reagent (Pierce), and the *β*-gal enzyme content was determined using an ELISA-*β*-galactosidase kit (GIBCO BRL). CAT activity was normalized to transfection efficiency (*β*-gal) and protein content.

2.3. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts of HepG2 and MCF-7 were prepared as described in [32] after treatment of the cells with DMSO (0.1%) or TCDD (2 nM) in serum free growth media for 3 h. Protease inhibitors (2.5 μg/ml of antipain, aprotinin, leupeptin, and pepstatin and 0.2 mM phenyl-methylsulfonyl fluoride) and 5 mM dithiothreitol (DTT) was added to all buffers. The nuclei were extracted in high salt buffer (0.4 M KCl) for 45 min, and dialyzed for 12 h (0.1 M KCl). Protein concentration was determined by the Bradford dye-binding procedure (Bio-Rad). For EMSA, complementary oligonucleotides, carrying 5′*Eco*RI/3′*Bam*HI ends, corresponding to the 21-bp palindrome (−794 to −774, 5′-GTGCTCTGCCAATCAAAGCAC-3′), identified in NRE$_{105}$ [28], were annealed (NRE$_{pal}$) and labeled with [γ-^{32}P]ATP using T_4 polynucleotide kinase. Labeled probe was purified by Nick-Spin columns (Pharmacia). Unlabeled competing DNA included the NRE$_{275}$, the NRE$_{105}$ and NRE$_{170}$ fragments gel purified after digestion with *Eco*RI/*Bam*HI of their respective plasmids, or the unrelated *Bam*HI/*Sal*I Stuff$_{275}$ fragment of pBR327. Gel retardation assays were performed essentially according to the instructions of the BandShift kit (Pharmacia). Nuclear extract (10 μg of protein), 10 μg of poly(dI-dC), 10 μg acetylated BSA, and competing DNA were incubated for 5 min at room temperature (RT) in the presence of 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.5 mM DDT, 6.25 mM $MgCl_2$, 0.05% NP40, and 1 mM EDTA, then radiolabeled probe (0.13 ng of DNA, 10–12,000 cpm) was added, and the reaction mixture (20 μl) was incubated for 20 min at RT before fractionation on a 5% polyacrylamide gel in low-ionic-strength buffer (7 mM Tris-HCl, pH 7.5, 3 mM sodium acetate, 1 mM EDTA) at constant current (200 V) with recirculation of running buffer and cooling. The gels were fixed (10% CH_3COOH, 2% glycerol and rinsed in 2% glycerol), dried at 80°C, visualized, and quantitated by phosphorimage analyses.

3. Results and discussion

The possible cell specific effects of NRE on basal as well as induced expression of *CYP1A1* were examined in human breast and hepatoma cells. Chimeric *CYP1A1*-reporter gene plasmids were constructed and analyzed in transient gene expression assays. Deletion analyses of a gene promotor may cause posi-

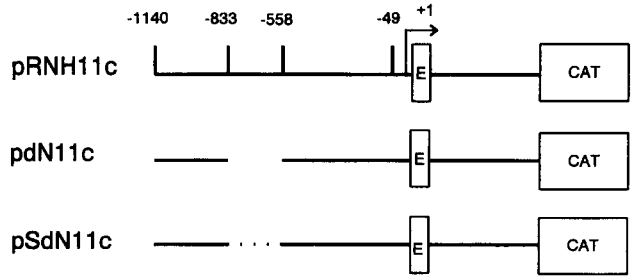


Fig. 1. Structure of *CYP1A1*/*cat* expression vectors. The parent plasmid pRNH11c: Contains −1140 to +2434 sequences of *CYP1A1* including 5′flanking sequences, exon 1 and intron 1 in front of the *cat* gene [27]. pdN11c: Deletion of the NRE fragment (−831 to −560) by digestion of pRNH11c with *ApaI*/*Sac*II enzymes, blunt-ending and religation. pSdN11c: Substitution of the NRE with an unrelated fragment (Stuff$_{275}$), the 275 bp *Bam*HI/*Sal*I from the *tet*^{R} gene in pBR327. The position of the inserted Stuff$_{275}$ fragment is shown by dotted lines.

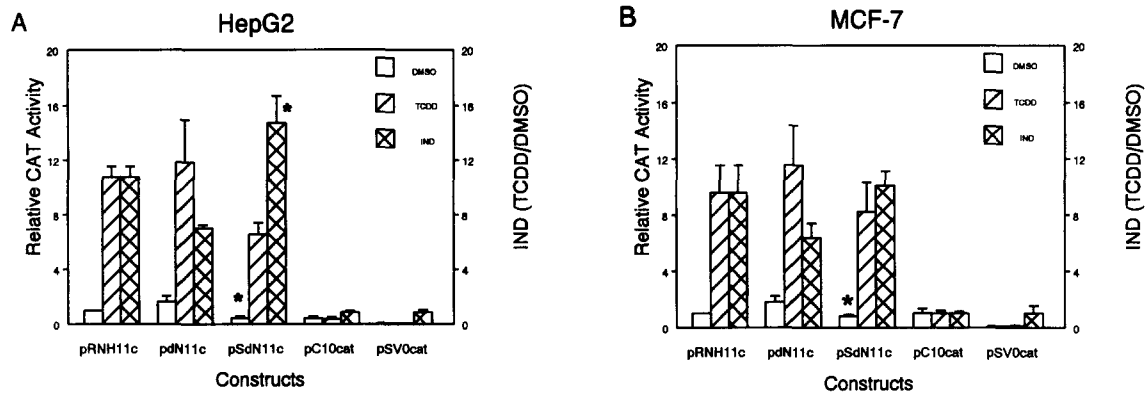


Fig. 2. Relative CAT activity and fold induction upon TCDD treatment in HepG2 (A) and MCF-7 (B) cells. Transfection and CAT assays were performed as described in section 2. The data were corrected for transfection efficiency (β-gal), and normalized to the basal CAT activity of pRNH11c (DMSO), which was set to 1. Fold induction was obtained by the ratio of TCDD induced CAT activity over basal activity. *Significantly different from corresponding pRNH11c values. pC10*cat* and pSV0*cat* were used as control plasmids. Values given as mean ± S.D., $n = 3$–4.

tion effects and influence the transcription activity [33], and thus result in misinterpretation. Therefore, plasmids carrying DNA segments harboring intact 5′ regulatory sequences (pRNH11c) were analyzed in parallel with constructs, where NRE was deleted (pdN11c) or substituted with unrelated DNA sequences (pSdN11c) cloned upstream of the *cat* gene (Fig. 1). Promotor strength of these chimeric plasmids were analyzed by transfection into MCF-7 and HepG2 cells using the pRSVβgal as an internal standard for transfection efficiency. The results in Fig. 2 show the relative CAT activity in HepG2 or MCF-7 cells treated with DMSO (solvent control) or 10 nM TCDD upon parallel transfection of the plasmid pRNH11c, pdN11c, pSdN11c, and the control plasmids pC_{10}*cat* and pSV0*cat*. The basal CAT activity of the parent plasmid pRNH11c was set to 1 in both cell lines. In HepG2 (Fig. 2A) and MCF-7 cells (Fig. 2B) deletion of the 275 bp NRE fragment (pdN11c) resulted in an almost 2-fold increase in the constitutive activity of the *CYP1A1* promotor, whereas substitution of the NRE with an unrelated DNA fragment of same size (pSdN11c) resulted in a significant decrease of the constitutive CAT activity compared with pRNH11c (MCF-7: 0.81 $P < 0.05$, and HepG2: 0.45, $P < 0.05$). Treatment of cells with 10 nM TCDD caused an approximately 10-fold induction of the pRNH11c encoded CAT activity in both cells lines. The TCDD induced CAT activity upon deletion of NRE (pdN11c) was similar to that observed for pRNH11c, however, the fold-induced activity was approximately 65% compared to pRNH11c in both cell lines (Fig. 2). In HepG2, pSdN11c caused a significant 1.4- and 2.1-fold ($P = 0.03$ and $P = 0.02$) higher inducibility than the induced parent plasmid or upon deletion of NRE alone, respectively (Fig. 2A). In contrast, in MCF-7 neither the induced CAT activity nor the fold-induction of pSdN11c were different from the parent plasmid (Fig. 2B). Consistent with the data in this study a 10-fold induction of the pRNH11c CAT activity upon inducer treatment of HepG2 was previously reported [27]. However, an 8-fold increase in the constitutive activity and a 5-fold reduction of the induced activity of the *CYP1A1* promotor were observed upon deletion of NRE [27]. This discrepancy concerning deletion of NRE between the previous reported results and this study is unclear, but may be explained by differences in transfection design or cell passage number. The findings in the present study indicate that deletion of the NRE fragment alone causes position effects modulating the basal activity of the intact promotor, possibly by favoring the interaction between upstream transcription factors and the basic transcription machinery as previously suggested [33]. The lower fold-induced activity of the pdN11c promotor may be explained by a disturbed stereospecific alignment of protein binding DNA-elements (e.g. XREs) on the DNA helix. A requirement for stereospecific positioning of enhancer elements for inducible transcription was reported for the human proenkephalin gene [34], between the SV40 enhancer and early promotor elements [33], and between glucocorticoid receptor binding sites [35]. Moreover, analysis of the *CYP1A1* enhancer in intact mouse hepatoma cells has suggested that nucleosome positioning over the enhancer influence AHR-DRE interactions implying a spacing dependence of DREs for maximal enhancer activity [36]. In addition, substitution of NRE (pSdN11c) indicates that the NRE possess DNA sequences essential for maximal constitutive activity of *CYP1A1* in both HepG2 and MCF-7 cells. The increase in fold-induced CAT activity upon substitution of NRE (pSdN11c) in HepG2 indicates that a negative regulative effect is exerted by the NRE segment on TCDD induced *CYP1A1* expression in HepG2 cells, whereas it has no effect in MCF-7 cells. This different effect of NRE in HepG2 and MCF-7 suggests that cell specific components may be involved.

Specific binding of nuclear extract protein(s) of HepG2 to the intact NRE_{275} and the subfragments NRE_{105} (−833 to −728) and NRE_{170} (−728 to −558) has been demonstrated, each of which exerting a negative regulatory activity on a simian virus 40 (SV40) enhancer/herpes simplex virus thymidine kinase (HSV tk) promotor activity [28]. The nuclear protein(s) recognized similar sites on both NRE subfragments, however, a 21-bp palindrome (−794 to −774) of NRE_{105} was the most important sequence for protein binding and negative regulatory activity [28]. In the present study EMSA was used to explore whether differences in putative regulatory proteins binding to the 21-bp palindrome could explain the different role of NRE on induced *CYP1A1* expression in HepG2 and MCF-7. Nuclear extracts from the cells lines, treated with either DMSO or 2nM TCDD for 3 h, were incubated with the ^{32}P-labeled,

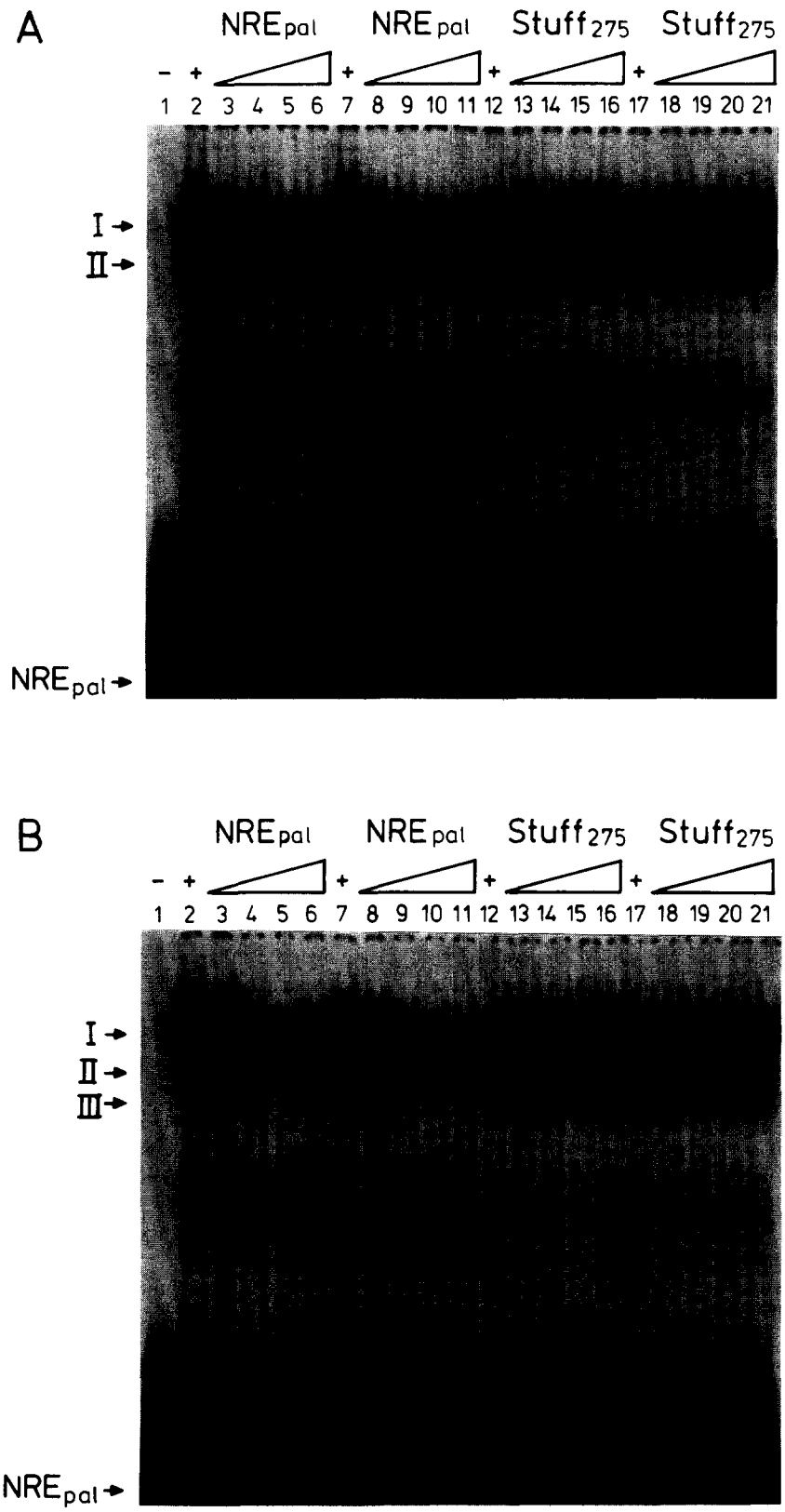
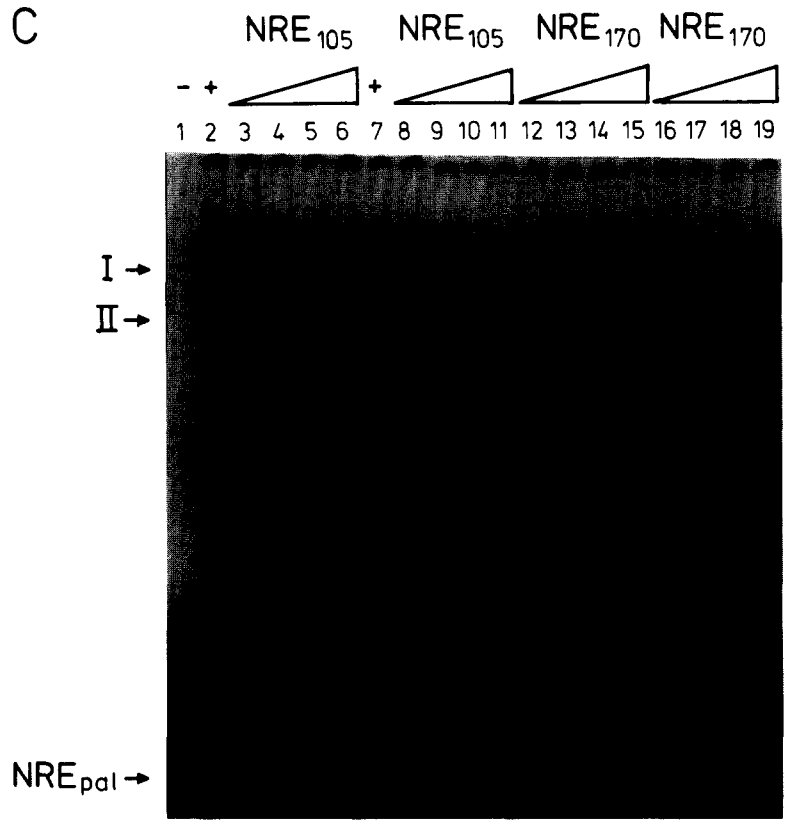


Fig. 3. Specific binding of nuclear extract proteins of HepG2 (A, C, E) and MCF-7 (B, D, F) cells. –, represents ^{32}P-NRE$_{pal}$ probe alone; +, represents the presence of nuclear extract without competitor. The specific complexes are indicated with an arrow (I, II, III). In A and B lanes 2–6 and lanes 12–16 are nuclear extracts from DMSO, and lanes 7–11 and lanes 17–21 from TCDD treated cells. In C and D lanes 2–6 and 12–15 are DMSO extracts, and lanes 7–11 and 16–19 TCDD extracts. In E and F lanes 2–6 are from DMSO and lanes 7–11 are from TCDD treated cells. Competing DNA was present at 20-, 40-, 80-, or 100-fold molar excess. All reactions were carried out using 10 μg nuclear protein, 0.13 ng of DNA (10–12,000 cpm)/20 μl. Reactions were repeated at least twice to establish reproducibility.



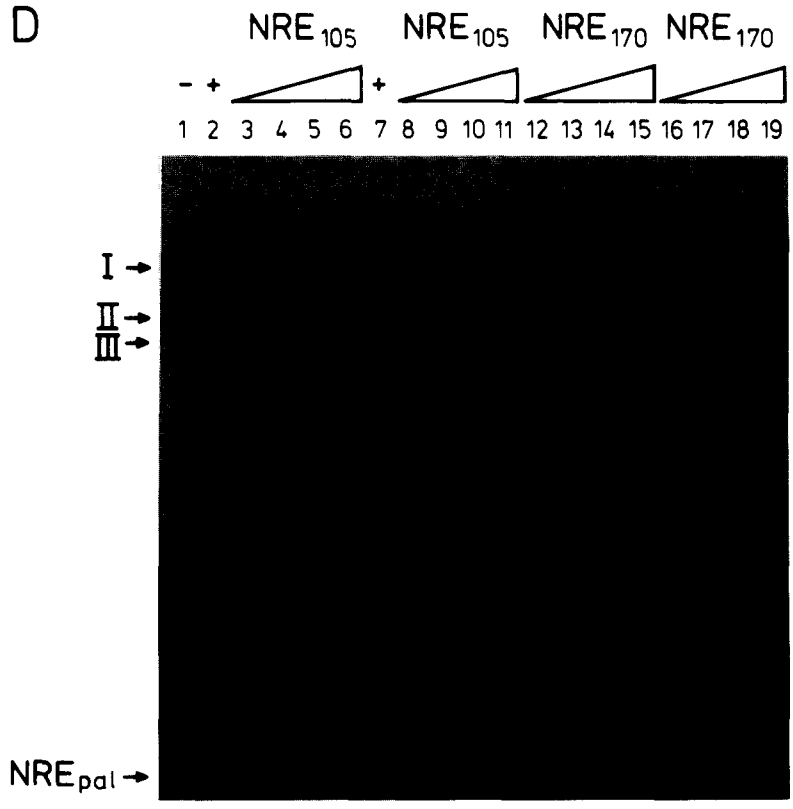


Fig. 3 (*continued*).

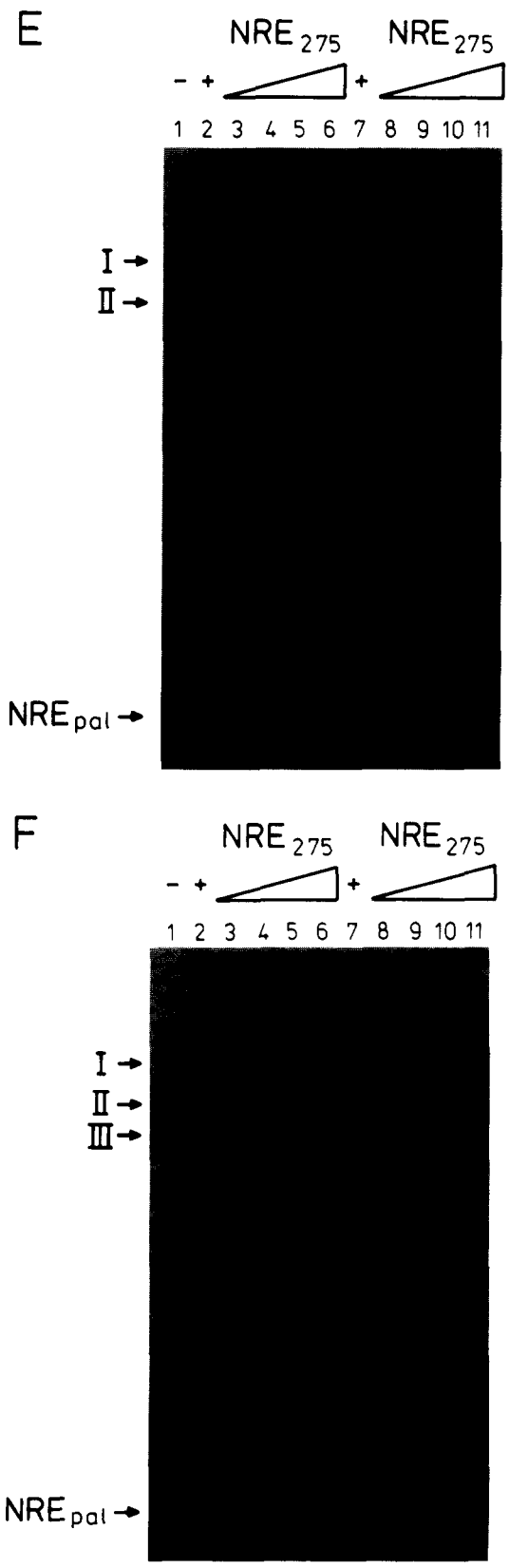


Fig. 3 (*continued*).

double stranded oligonucleotide containing the 21-bp palindrome (^{32}P-NRE$_{pal}$). Specific DNA-binding factors are constitutively expressed at similar levels in both cell lines (arrows, Fig. 3A–F), and the expression is neither enhanced by TCDD treatment (compare e.g. lane 2 vs. lane 7 in Fig. 3A,B) nor does TCDD seem to increase the affinity of DNA-binding activity specific for ^{32}P-NRE$_{pal}$ in the cell lines (Fig. 3A,B lanes 2–11). The results suggest that an additional specific protein (III) binds to the NRE$_{pal}$ probe in MCF-7 cells compared to HepG2. Competition mobility shift analyses show the presence of two NRE$_{pal}$ specific nuclear HepG2 proteins (Fig. 3A, I and II, lanes 2–11), whereas three specific DNA-binding proteins were identified in MCF-7 (Fig. 3B, I, II, and III, lanes 2–11). These factors are highly specific since 100-fold molar excess of unlabeled NRE$_{pal}$ competed approximately 90% for binding with the ^{32}P-NRE$_{pal}$ in nuclear extract of both cell lines, while equivalent molar excess of the unrelated Stuff$_{275}$ fragment did not (Fig. 3A,B lanes 12–21). The relative mobilities of the I and the II complex in the two cell lines appeared alike, suggesting that similar or identical proteins are involved in the binding to the NRE$_{pal}$ probe. In accordance with the results of Boucher et al. [28] the DNA sequences of the intact NRE$_{275}$ and the two NRE subfragments NRE$_{105}$ and NRE$_{170}$ had the capacity to compete the binding of proteins specific for ^{32}P-NRE$_{pal}$ (Fig. 3C–F). All three NRE fragments competed for ^{32}P-NRE$_{pal}$-protein complexes in both cell lines, however, as expected the NRE$_{105}$, which harbor the 21-bp NRE$_{pal}$ palindrome, competed more strongly for binding with the ^{32}P-labeled NRE$_{pal}$ probe (Fig. 3C,D lanes 2–11). Examination of the competition studies with the three NRE DNA fragments imply that the NRE$_{170}$ only carries sequences recognized by complex II (HepG2) or complex II and III (MCF-7) (Fig. 3C,D lanes 12–19), still, a similar competition pattern was observed when the intact NRE$_{275}$ fragment was used (Fig. 3E,F) suggesting a less specific competition reaction with larger DNA fragments. Since EMSA analyses, using NRE$_{105}$ or NRE$_{170}$ as labeled probe, did not imply additional differences between the two cell lines in protein-binding to the NRE (results not shown) it is hypothesized that the protein complex III plays a role with respect to the impaired effect of NRE on TCDD induced *CYP1A1* promotor activity in MCF-7 cells. Presently, the identity and role of proteins interacting with NRE$_{pal}$ are unknown. Possibly, distinct protein(s) binding to the NRE plays a role in the basal activity of the promotor/enhancer, and upon TCDD treatment interaction of this protein(s) with other factor(s) may modulate its action. The protein complex III may be a modified version of either complex I or complex II causing a modulation of its function, i.e. the study of viral immediate early (IE) proteins provides evidence that both positive and negative regulation may be controlled by the modification of factors that interact with the promotor and enhancer [37]. Moreover, different spliced forms of the Oct-2 transcription factor exert cell specific functions e.g. as an activator in B-lymphocytes or as a repressor in neuronal cells [38]. Since no XREs are identified in the human NRE, and treatment with TCDD does neither activate nor enhance NRE-binding proteins, the results do not support the proposed models for negative regulation through inhibition of the AHR function [39–43]. Furthermore, the suggested autoregulatory effect of endogenous murine *cyp1a1* [17] exerts its effect in a NRE independent manner in the human system (manuscript in preparation). Together, these studies implies that either post-translational modifications (e.g. phosphorylation), different splicing forms or protein–protein interactions of factors acting via the NRE may determine the cell-and tissue-specific action of this

element. Interestingly, recent published results demonstrated the binding of an additional specific DNA–protein complex to the NRE_{105} in nuclear extract of rodent hepatoma cells compared with HepG2 extract. However, in contrast to the human cells, this element was unable to down-regulate SV40 enhancer/HSV tk promotor activity in rodent hepatoma cells [44]. Taken together with the present observations, these data suggest that an additional NRE-specific-binding complex could be related to intertissue as well as interspecies lack of NRE function. The results presented here emphasize the need for a careful examination of the proteins binding to the NRE to establish a functional correlation between factor binding and repressing activity. Identification of the DNA elements and transcription factors which receive and mediate signals upon environmental xenobiotica exposure will increase the knowledge of these mechanisms and further the understanding of interindividual and intertissue susceptibility to xenobiotica exposure.

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References

[1] Nelson, D.R, Kamataki, T., Waxman, D.J., Guengerich, F.P., Estabrook, R.W., Feyereisen, R., Gonzales, F.J., Coon, M.J., Gunsalus, I.C., Gotoh, O., Okuda, K. and Nebert, D.W. (1993) DNA Cell Biol. 12, 1–51.
[2] Gonzales, F.J. (1989) Pharmcol. Rev. 40, 243–288.
[3] Sims, P. and Grover, P.L. (1981) in: Polycyclic Hydrocarbons and Cancer (Gelboin, H.V. and Tso, P.O.P. Eds.), Vol. 3, pp. 117–181, Academic Press, Inc., New York.
[4] Cooper, C.S., Grover, P.L. and Sims, P. (1983) in: Progress in Drug Metabolism (Bridges, J.W. and Chasseaud, L.F. Eds.) vol. 7, pp. 295–395, Wiley, New York.
[5] Jones, C.A., Santella, R.M., Huberman, E. Selkirk, J.K. and Grunberger, D. (1983) Carcinogenesis 4, 1351–1357.
[6] Ioannides, C. and Parker, D.V. (1990) Drug Metab. Rev. 22, 1–85.
[7] Wilson, N.M., Christou, M., Turner, C.R., Wrighton, S.A. and Jefcoate, C.R. (1984) Carcinogenesis 5, 1475–1483.
[8] Hayashi, S., Watanabe, J., Nakachi, K., Eguchi, H. Gotoh, O. and Kawajiri, K. (1994) Carcinogenesis 15, 801–806.
[9] Fujino, T., Gottlieb, K., Manchester, D.K., Park, S.S., West, D., Gurtoo, H.L., Tarone, R.E. and Gelboin, H.V. (1984) Cancer Res. 44, 3916–3923.
[10] Crofts, F., Tailo, E., Trachman, J., Cosma, G.N., Currie, D., Toniolo, P. and Garte, S.J. (1994) Carcinogenesis 15, 2961–2963.
[11] Nebert, D.W., Petersen, D.D. and Puga A. (1991) Pharmacogenetics 1, 68–78.
[12] Pyykkö, K., Tuimala, R., Aalto, L. and Perkiö, T. (1991) Br. J. Cancer 63, 596–600.
[13] Murray, G.I., Foster, C.O., Barnes, T.S., Weaver, R.J., Ewen, S.W.B., Melvin, W.T. and Burke, M.D. (1991) Br. J. Cancer 63, 1021–1023.
[14] Pasanen, M., Stacey, S., Lykkesfeldt, A., Briand, P., Hines, R. and Autrup, H. (1988) Chem.-Biol. Interact. 66, 223–232.
[15] Thomsen, J.S., Nissen, L., Stacey, S.N., Hines, R.N. and Autrup, H. (1991) Eur. J. Biochem. 197, 577–582.
[16] Swanson, H.I. and Bradfield, C.A. (1993) Pharmacogenetics 3, 213–230.
[17] Nebert, D.W., Puga, A. and Vasiliou, V. (1993) Ann. NY Acad. Sci. 685, 624–640.
[18] Nebert, D.W. (1989) CRC Crit. Rev. Toxicol. 20, 153–174.
[19] Sutter, T.R. and Greenlee, W.F. (1992) Chemosphere 25, 223–226.
[20] Okey, A.B., Riddick, D.S. and Harper, P.A. (1994) Toxicol. Lett. 70, 1–22.
[21] Whitelaw, M., Pongratz, I., Wilhelmsson, A., Gustafsson, J.-A.A. and Poellinger L. (1993) Mol. Cell. Biol. 13, 2504–2514.
[22] Carrier, F., Owens, R.A., Nebert, D.W. and Puga, A. (1992) Mol. Cell. Biol. 12, 1856–1863.
[23] Berghard, A., Gradin, K., Pongratz, I., Whitelaw, M. and Poellinger, L. (1993) Mol. Cell. Biol. 13, 677–689.
[24] Singh, S.S. and Perdew, G.H. (1993) Arch. Biochem. Biophys. 305, 170–175.
[25] Moore, M., Narasimhan, T.R., Steinberg, M.A., Wang, X. and Safe, S. (1993) Arch. Biochem. Biophys. 305, 483–488.
[26] Yanagida, A., Sogawa, K., Yasumoto, K.-I. and Fuji-Kuriyama, Y. (1990) Mol. Cell. Biol. 10, 1470–1475.
[27] Hines, R.N., Mathis, J.M. and Jacob, C.S. (1988) Carcinogenesis 9, 1599–1605.
[28] Boucher, P.D., Ruch, R.J. and Hines, R.N. (1993) J. Biol. Chem. 268, 17384–17391.
[29] Rosenthal, N., Kress, M. Gruss, P. and Khoury, G. (1983) Science 222, 749–755.
[30] Gorman, C. (1985) in: DNA Cloning Vol. II, Glover, D.M., Ed., Oxford, England, IRL Press, 143–176.
[31] Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[32] Abmayr, S.M. and Workman, J.L. (1993) in: Current Protocols in Molecular Biology (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. Eds.) pp. 12.0.3–12.1.9, Greene Publishing Associates and J. Wiley Interscience, USA.
[33] Sawadogo, M. and A. Sentenac (1990) Annu. Rev. Biochem. 59, 711–754.
[34] Comb, M., Mermod, N., Hyman, S.E., Pearlberg, J., Ross, M.E. and Goodman, H.M. (1988) EMBO J. 7, 3793–3805.
[35] Jantzen, H.-M., Strahle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. and Schütz, G. (1987) Cell 49, 29–38.
[36] Wu, L. and Whitlock Jr., J.P. (1993) Nucleic Acids Res. 21, 119–125.
[37] Maniatis, T., Goodbourn, S. and Fischer, J.A. (1987) Science 236, 1237–1245.
[38] Lillycrop, K.A., Dawson, S.J., Estridge, J.K., Gerster, T., Matthias, P. and Latchman, D.S. (1994) Mol. Cell. Biol. 14, 7633–7642.
[39] Arellano, L.O., Wang, X. and Safe, S. (1993) Carcinogenesis 14, 219–222.
[40] Watson, A.J., Weir-Brown, K.I., Bannister, R.M., Chu, F.-F., Reisz-Porszasz, S., Fuji-Kuriyama, Y., Sogawa, K. and Hankinson, O. (1992) Mol. Cell. Biol. 12, 2115–2123.
[41] Lusska, A., Wu, L. and Whitlock, J.P. (1992) J. Biol. Chem. 267, 15146–15151.
[42] Reick, M., Robertson, R.W., Pasco, D.S. and Fagan, J.B. (1994) Mol. Cell. Biol. 14, 5653–5660.
[43] Gradin, K., Wilhelmsson, A., Poellinger, L. and Berghard, A. (1993) J. Biol. Chem. 268, 4061–4068.
[44] Boucher, P.D. and Hines, R.N. (1995) Carcinogenesis 16, 383–392.