Pergamon

0960-0760(93)E0030-B

J. Steroid Biochem. Molec. Biol. Vol. 48, No. 4, pp. 317–323, 1994

0960-0760/94 $6.00 + 0.00

Nuclear Extracts Enhance the Interaction of Fusion Proteins Containing the DNA-binding Domain of the Androgen and Glucocorticoid Receptor with Androgen and Glucocorticoid Response Elements

P. De Vos,[1] F. Claessens,[2] L. Celis,[1] B. Peeters,[2] W. Rombauts,[2] W. Heyns[1] and G. Verhoeven[1]*

[1]*LEGENDO, Campus Gasthuisberg, Herestraat 49, 3000 Leuven and* [2]*Department of Biochemistry, Catholic University of Leuven, Faculty of Medicine, Campus Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium*

Comparable fragments of the androgen receptor (AR) (amino acids 540–607) and of the glucocorticoid receptor (GR) (amino acids 412–515) were expressed in *E. coli* as fusion proteins with protein A. Both fusion proteins, denoted ARF1 and GRF1, contain the DNA-binding domain and some flanking amino acids. *In vitro* binding assays have shown that both fusion proteins interact with androgen/glucocorticoid response elements (ARE/GREs) in an intron fragment of the C3(1) gene of the androgen-regulated rat prostatic binding protein and in the typically glucocorticoid-responsive long terminal repeat (LTR) promoter of mouse mammary tumour virus. Present results indicate that the interaction of both ARF1 and GRF1 with the C3(1) as well as the LTR fragments is enhanced in the presence of nuclear extract. The factor that gives rise to this enhancement appears to be ubiquitous and sensitive to trypsin and temperature treatment. In the C3(1) fragment, the enhancing effect requires the presence of an intact functional ARE/GRE (Core II) as well as a region spanning the ARE/GRE half-site Core I.

J. Steroid Biochem. Molec. Biol., Vol. 48, No. 4, pp. 317–323, 1994

INTRODUCTION

Nuclear extract (NE) has been shown to enhance *in vitro* DNA-binding of several members of the steroid receptor superfamily. This was described for the glucocorticoid receptor (GR) [1], the progesterone receptor (PR) [2], the estrogen receptor (ER) [3, 4], the vitamin D receptor (VDR) [5–7], the retinoic acid receptor (RAR) [8, 9] and for the thyroid hormone receptor (TR) [10–15]. In general, the nuclear factors that enhance DNA-binding appear to be ubiquitous rather than restricted to specific cell types but in most cases they have been rather poorly characterized. In those cases where the size has been estimated, it falls in the range of 45 to 67 kDa—with the exception of the factor that enhances DNA-binding of the GR, which has an apparent M_w of 700–3000. In some instances, cross-linking experiments have demonstrated a direct interaction between the receptor and the nuclear factor and some reports describe an interaction of the factor with DNA.

We previously described the production of the DNA-binding domain (DBD) of the androgen receptor (AR) and of the GR as fusion products with protein A [16, 17]. *In vitro* experiments show that these DBD molecules specifically interact with androgen/glucocorticoid response elements (ARE/GREs) in the first intron of the C3(1) gene of rat prostatic binding protein (PBP) and in the mouse mammary tumour virus-long terminal repeat (MMTV-LTR). Our present results indicate that binding of ARF1 and GRF1 to both the C3(1) and the LTR fragments is enhanced upon addition of NE from different cell lines and tissues.

MATERIALS AND METHODS

Preparation, temperature inactivation and trypsin digestion of NE

NE from liver, kidney and prostate of Wistar albino rats was prepared according to the method of Gorski *et*

*Correspondence to G. Verhoeven.
Received 5 July 1993; accepted 8 Nov. 1993.

al. [18]. NE from the FTO-2B rat hepatoma cell line [19] and from HeLa cells was prepared according to the method of Dignam *et al.* [20]. Both tissue and cell NE was dialysed against 25 mM Hepes–KOH pH 7.6, 40 mM KCl, 0.1 mM EDTA, 10% glycerol and 1 mM dithiothreitol and stored in aliquots at −70°C.

For temperature inactivation experiments, the FTO-2B NE was heated at 37, 65 or 95°C for 5 min and centrifuged for 1 min in a microfuge. Control extract remained on ice until added to the gel retardation mixture.

A trypsin-sensitivity experiment was set up as follows. NE from FTO-2B cells was treated with trypsin (final concentration 0.02 mg/ml) for 5 min on melting ice. Soy-bean trypsin inhibitor was added (finally 0.2 mg/ml) and after 5 min the extract was added to the gel retardation mixture. As a negative control, trypsin was preincubated with trypsin inhibitor for 5 min and added to the NE. After 5 min the extract was added to the gel retardation mixture. Another control was included in which trypsin and trypsin inhibitor, preincubated for 5 min, were directly added to the gel retardation mixture without the addition of NE. The final volume and chemical composition of each sample were identical.

Gel retardation assay

The probe derived from MMTV-LTR was described previously [17], as well as the probe from the C3(1) gene of PBP [see also Fig. 5(A)]. The site-directed mutations of the C3(1) fragment [see Fig. 5(B), 1–5] were described by Celis *et al.* [21]. Fragments 6 and 10 were prepared by annealing of oligonucleotides and ^{32}P-labelled with Klenow polymerase. Fragments 7 and 8 were produced by polymerase chain reaction amplification. The primers used were ^{32}P-labelled with T4-polynucleotide kinase. Fragment 9 is a subfragment of fragment 8, obtained after digestion with TaqI. In addition to these labelled probes, two non-radioactive double stranded oligos were prepared for competition experiments. One contained the Core I element and the second contained an NF-1 recognition site. Fragment 10 in Fig. 5 was also used as unlabelled competitor.

For gel retardation 10,000 cpm of each probe was incubated with 100 ng IgG-Sepharose-purified ARF1 or GRF1 in the presence or absence of 10 μg NE from different cell lines or tissues. The incubation of the probe with fusion protein and NE and the subsequent separation of bound and free probe by polyacrylamide gel electrophoresis were carried out as already described [17]. For competition experiments, 5, 10 or 20 pmol of unlabelled oligonucleotide were added, as indicated in the legend of Fig. 6.

RESULTS

A ubiquitous temperature- and trypsin-sensitive nuclear factor has enhancing effects in gel retardation experiments with ARF1 and GRF1

We previously reported the production in *E. coli* of the AR- and the GR-DBD as fusion products with protein A (ARF1 and GRF1) [17]. *In vitro*

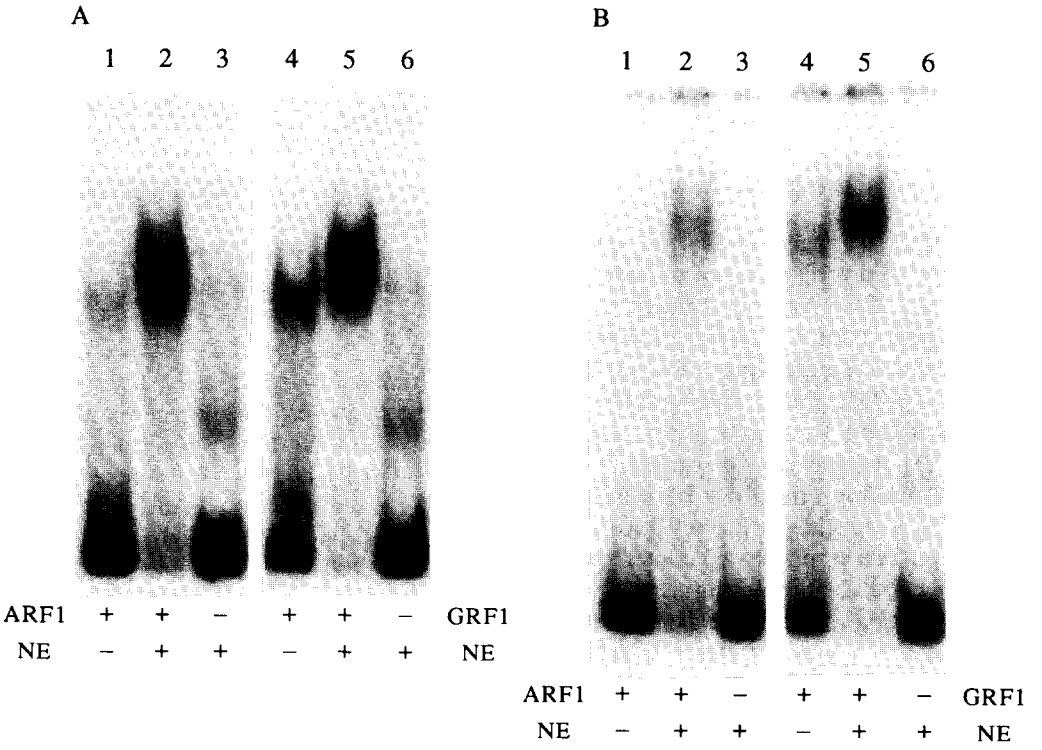


Fig. 1. Influence of NE on gel retardation. (A) The C3(1) fragment was subjected to gel retardation with the fusion proteins ARF1 (lanes 1–3) and GRF1 (lanes 4–6). Lanes 1 and 4 contain only fusion protein, lanes 2 and 5 contain fusion protein and NE of FTO-2B cells, lanes 3 and 6 contain FTO-2B NE. (B) Identical experiment with the MMTV-LTR fragment.

binding assays demonstrated that both fusion proteins interacted with DNA fragments derived from the first intron of the PBP C3(1) gene and from the MMTV-LTR. Both fragments can mediate androgen-, glucocorticoid- and progesterone responses [22, 23]. The C3(1) fragment contains 2 GRE-like sequences [see Fig. 5(A)], denoted Core I and II. Only Core II, which is more homologous to the GRE consensus than Core I, acts as a functional androgen, glucocorticoid and progesterone response element [22]. The LTR fragment contains 4 GRE-like sequences.

Figure 1 demonstrates that addition of FTO-2B NE to gel retardation assays increases the size as well as the intensity of the shifted complexes. This effect is seen for both the C3(1) [see Fig. 1(A)] and the LTR [see Fig. 1(B)] probes, the receptor protein being either ARF1 or GRF1. When NE is added alone, some smearing of the probes appears, but no bands can be detected. These data suggest that FTO-2B NE contains one or several factors that enhance retardation of DNA fragments by ARF1 and GRF1 in a cooperative rather than in an additive way.

For the sake of simplicity, only the C3(1) fragment and ARF1 were used for further investigation. As an initial characterization, the temperature-sensitivity of the nuclear factor was determined. NE from FTO-2B was either kept on ice or was heated to 37, 65 or 95°C and assayed in a gel retardation experiment with ARF1 and the C3(1) probe. Figure 2 shows that the factor withstands 37°C but is inactivated from 65°C on.

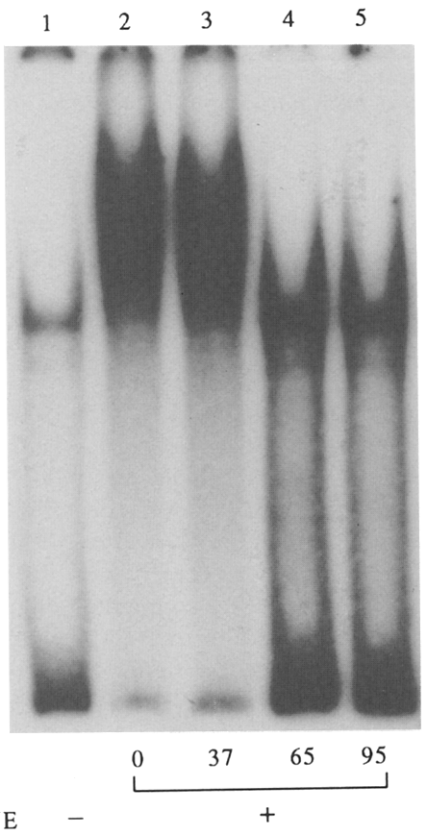


Fig. 2. Temperature-sensitivity of the nuclear factor. Gel retardation experiment with the C3(1) fragment and ARF1 in the absence of NE (lane 1) or with FTO-2B NE that was kept on ice (lane 2) or heated at 37, 65 or 95°C (lanes 3–5).

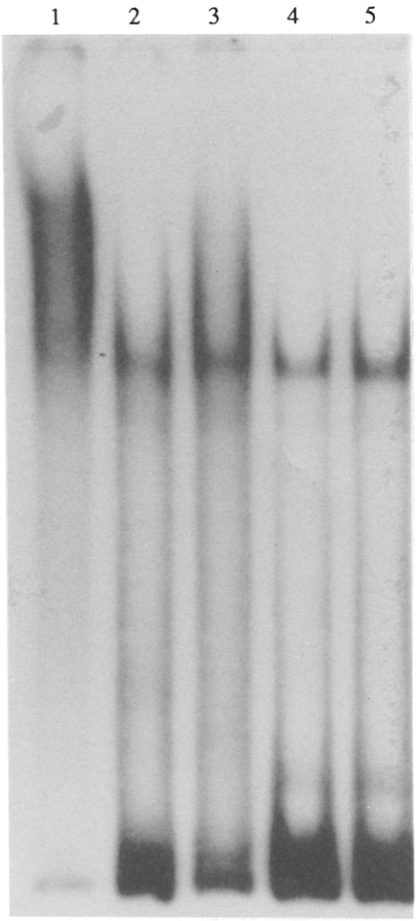


Fig. 3. Trypsin-sensitivity of the nuclear factor. Gel retardation experiment. Each lane contains the C3(1) fragment and ARF1. In lane 1, FTO-2B NE was added. In lane 2, the NE was first digested with trypsin, and then soy-bean trypsin inhibitor was added. As a negative control (lane 3), trypsin was preincubated with inhibitor before addition to the NE. In lane 4, trypsin was preincubated with inhibitor and added to the gel retardation mixture in the absence of NE. Lane 5 contains the C3(1) fragment and ARF1 only.

The factor is also sensitive to trypsin digestion, as indicated by Fig. 3. In lane 2, FTO-2B NE was treated with trypsin for 5 min. Soy-bean trypsin inhibitor was added and after 5 more min, the extract was added to a gel retardation mixture with ARF1 and the C3(1) probe. A negative control was included, in which trypsin was first incubated with trypsin inhibitor and then added to the NE (see Fig. 3, lane 3). This still results in a small loss of the effect, which reflects an incomplete inhibition of the tryptic activity. Therefore another control was included, in which trypsin and inhibitor were added to the gel retardation mixture in the absence of NE (lane 4). This did not result in a loss of retardation (as compared to lane 5, containing only ARF1), indicating that trypsin did not degrade ARF1. Both the heat inactivation and the trypsin digestion strongly suggest that the effect observed upon addition of FTO-2B NE is due to a protein factor.

In order to examine the tissue distribution of the factor, we then prepared NE from rat liver, kidney and

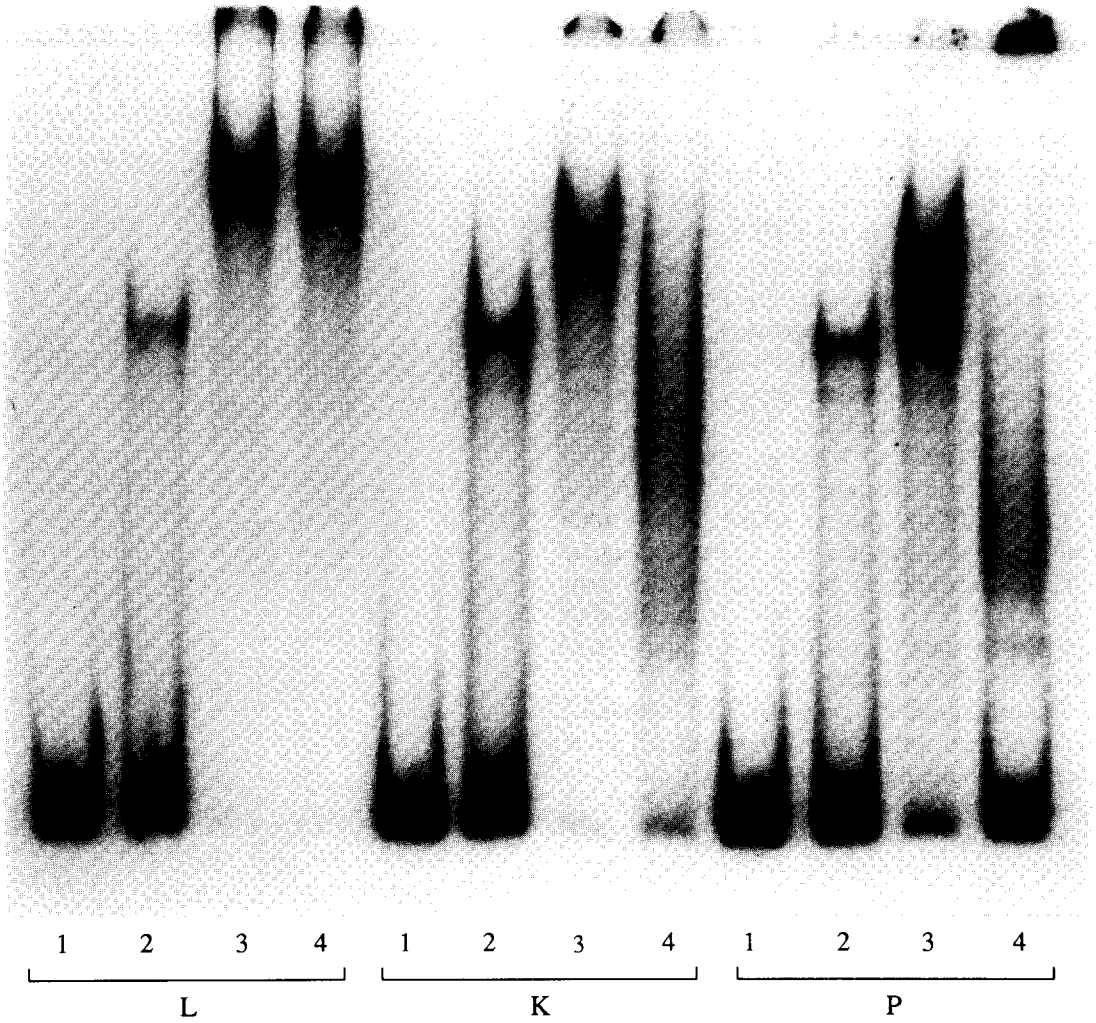


Fig. 4. Tissue distribution of the nuclear factor. Lane 1 only contains the C3(1) fragment. Lane 2 contains C3(1) and ARF1. Lane 3 additionally contains NE from liver (L), kidney (K) and prostate (P) as indicated. Lane 4 contains NE only.

prostate and tested them in a gel retardation assay with ARF1 and C3(1) (see Fig. 4). Prostate and kidney extracts, along with HeLa cell extracts (not shown), influence retardation in a way similar to FTO-2B. Addition of extract from liver results in a strong retarded complex in the absence of ARF1 and is therefore inconclusive. These results indicate that the factor is an ubiquitous protein, not restricted to the rat system, nor to specific androgen or glucocorticoid target tissues.

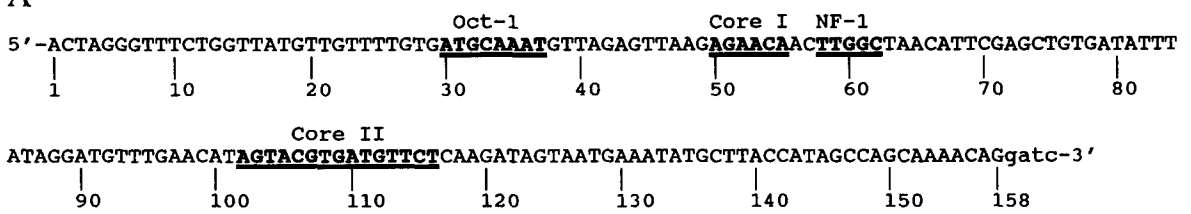
Mechanism of the enhancement of DNA-binding

In order to evaluate the contribution of particular regions in the C3(1) fragment to the enhancement of DNA-binding in the presence of NE, different mutant forms and subfragments of this probe were tested. The results are summarized in Fig. 5.

When the functional near-consensus ARE/GRE Core II is replaced by the non-functional ARE/GRE half-site Core I, binding of ARF1 is no longer observed, neither in the presence nor in the absence of NE [see Fig. 5(B), 2]. Binding is also prevented by addition of competitor oligonucleotide containing the Core II element (see Fig. 6). This indicates that basal binding of ARF1 requires the presence of Core II—or a perfect GRE sequence [see Fig. 5(B), 4]. Enhanced retardation (i.e. appearance of a larger and more intense shifted complex in gel retardation assays), on the other hand, requires an additional region, narrowed down to 24 nucleotides, around Core I [see Fig. 5(B), 8 and 9]. This region also contains a recognition half-site for the transcription factor Nuclear Factor 1 (NF-1) [24] and the DBD of NF-1 binds to this region with high affinity [21]. However, mutation of Core I or of the NF-1 site does not abolish the enhancement [see Fig. 5(B), 3 and 5], nor does addition of a competitor oligonucleotide containing an NF-1 site from the human growth hormone gene [25] or consisting of the mentioned 24 nucleotides (see Fig. 6).

The possibility was considered that the enhancement is not caused by binding of an additional factor but is rather due to modification of ARF1 by an enzyme in the NE. However, the fact that some DNA fragments are recognized by ARF1 but that the interaction fails to be enhanced in the presence of NE [see Fig. 5(B), 6, 9 and 10] strongly argues against such a mechanism.

A



B

	ARF1	ARF1 + NE
1. wild type	+	++
2. AGTACGTGATGTTCT (Core II) →→ AGTACGTGATTTTCT	-	-
3. AGAACA (Core I) →→ AGAAAA	+	++
4. AGTACGTGATGTTCT (Core II) →→ GGTACATGATGTTCT (GRE consensus)	+	++
5. TTGGC (NF-1) →→ AGATC	+	++
6. 98-151	+	+
7. 15-128	+	++
8. 46-128	+	++
9. 70-128	+	+
10. 5'-agcttACATAGTACGTGATGTTCTCAAGgagct-3' (contains Core II)	+	+

Fig. 5. Influence of mutations and truncations of the C3(1) fragment on the enhancement of DNA-binding. (A) Sequence of the C3(1) fragment. In bold and double underlined are the ARE/GRE half site Core I and the near-consensus ARE/GRE Core II. Also indicated are recognition sites for the transcription factors NF-1 and Oct-1. The lowercase letters indicate nucleotides that originate from subcloning. (B) Schematic representation of gel retardation with ARF1 and different mutants and subfragments of the C3(1) probe, in the presence or absence of FTO-2B NE. Line 2 : mutation in the Core II element. Line 3 : mutation in Core I. Line 4 : Core II is replaced by the GRE consensus. Line 5 : mutation of the NF-1 site. Lines 6–10 : subfragments as indicated. The lowercase letters in line 10 represent nucleotides that were added to allow sticky end ligation. A +sign in the first column indicates that ARF1 binds to the fragment. A +sign in the second column means that ARF1 binds to the fragment but no enhancement is seen in the presence of NE. A double +sign indicates that enhancement of retardation and formation of a larger complex is seen when both NE and ARF1 are added.

DISCUSSION

In recent years, several nuclear factors have been described that enhance the DNA-binding affinity of TR, RAR and VDR (see Introduction for references). At present it has become clear that many of them—if not all—correspond to retinoid X receptors (RXRs), which are also members of the steroid receptor superfamily (see Ref. [26] for review). RXRs can heterodimerize with TR, RAR and VDR, thereby increasing these receptors' affinity for their cognate REs [27–33]. As has been described for homodimerization of SRs, the heterodimer formation with RXRs occurs in the LBD of both partners.

In our experiments, enhancement of DNA-binding affinity is seen upon addition of NE to fusion proteins merely containing the DBD and small flanking regions of the AR and the GR. This sets our findings apart from the data described above. They are in line, however with another report describing increased DNA-binding affinity of the bacterially expressed DBD of RARα in the presence of NE [9]. Another distinction between our data and those obtained with TR, RAR and VDR, is that nuclear factors enhance the latter receptors' binding affinity for REs that are contained in oligonucleotides, whereas increased binding of the ARF1 and GRF1 fusion proteins requires longer probes. In this respect it may be noted that NE has been reported to enhance binding of the PR to an MMTV-LTR fragment [2].

Although the mechanism that underlies our observations is not fully understood, experiments with ARF1 and the C3(1) intronic fragment as well as mutated forms and subfragments of this probe give some valuable indications. Firstly, enhanced retardation is not seen unless the DNA fragment contains the intact Core II element or a consensus GRE, to which basal binding of ARF1 occurs. Secondly, a second region encompassing the Core I element is required for enhanced binding, which is not prevented, however, by addition of competitor oligonucleotide consisting of this region. Thirdly, binding of the nuclear factor and of ARF1 is cooperative, which implies that they must interact either directly or in an

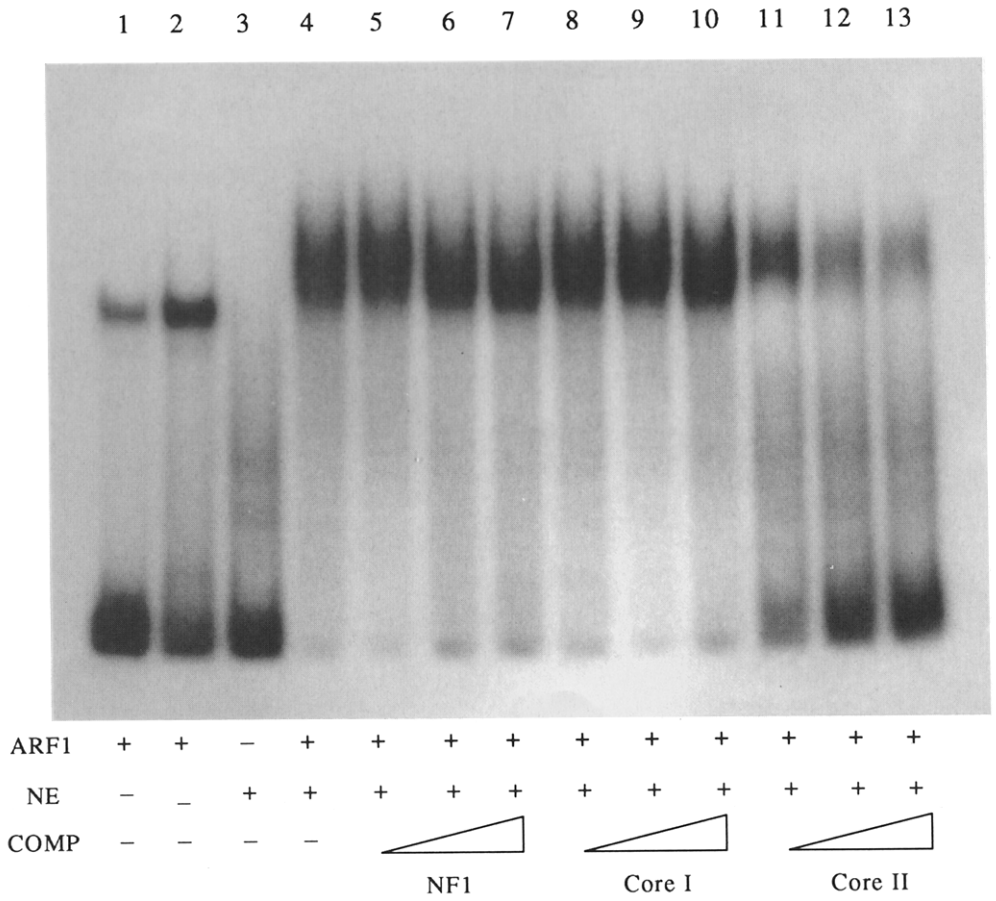


Fig. 6. The influence of competitor oligonucleotides on the enhancement of DNA-binding. Gel retardation experiment. All lanes except lane 3 contain ARF1. All lanes except 1 and 2 contain FTO-2B NE. In lanes 5–7, unlabeled oligonucleotide containing an NF-1 binding site was added (5, 10 and 20 pmol). Lanes 8–10 and 11–13 contain similarly increasing amounts of oligonucleotides containing Core I and II, respectively.

indirect way. The following mechanism may explain these observations. At any rate, a nuclear factor appears to interact with the region around Core I. However, the interaction is not affected by mutations in this region nor by addition of a large molar excess of a 24 nt.-long oligonucleotide consisting of this region. This may indicate that the factor binds to DNA in a non-sequence-specific way. Furthermore, the factor's binding site must at least be 25 nt. apart from the AR binding site Core II. This means that a direct interaction between the nuclear factor and the AR is unlikely and rather suggests that cooperativity is brought about by a third factor in the NE. In the case of the interaction between sequence-specific and general transcription factors, the existence of such bridging factors, often termed "coactivators" or "adaptors" has been well documented [34–37]. If a third factor is involved, this may also explain why attempts to purify the factor by chromatographic methods have so far resulted in loss of the enhancing activity.

Taken together, our data indicate that gel retardation of C3(1) and MMTV-LTR fragments with the fusion proteins ARF1 and GRF1 is markedly enhanced in the presence of NE from different cell lines and tissues. A more detailed study with mutants of the C3(1) fragment has shed some light on the mechanism underlying this enhancement and a model has been put forward. At this stage it is recommended to verify if the same results can be obtained with full-size AR. Unfortunately, purification of AR has so far proven impossible. To overcome this problem, we are currently engaged in a project that aims to produce full-size functional AR in a Vaccinia virus expression system.

Acknowledgements—We are grateful to H. Geeraerts for technical assistance and to V. Feytons for the synthesis of oligonucleotides. This work was supported by the Belgian National Incentive Program on Fundamental Research in Life Sciences initiated by the Belgian State-Prime Minister's Office Science Policy Programming, Grant 3.0015.88 from the Nationaal fonds voor Wetenschappelijk Onderzoek van België, and a grant from the Nationale Loterij.

REFERENCES

1. Cavanaugh A. H. and Simons S. S. Jr: Glucocorticoid receptor binding to calf thymus DNA. 1. Identification and characterization of a macromolecular factor involved in receptor-steroid complex binding to DNA. *Biochemistry* **29** (1990) 989–996.
2. Edwards D. P., Kühnel B., Estes P. A. and Nordeen S. K.: Human progesterone receptor binding to Mouse Mammary Tumour Virus DNA: dependence on hormone and nonreceptor nuclear factor(s). *Molec. Endocr.* 3 (1989) 381–382.
3. Feavers I. M., Jiricny J., Moncharmont B., Saluz H. P. and Jost J. P.: Interaction of two nonhistone proteins with the estradiol response element of the avian vitellogenin gene modulates the binding of estradiol-receptor complex. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 7453–7457.
4. Mukherjee R. and Chambon P.: A single-stranded DNA-binding protein promotes the binding of the purified oestrogen

receptor to its responsive element. *Nucleic Acids Res.* **18** (1990) 5713–5716
5. Liao J., Ozono K., Sone T., Mc Donnell D. P. and Pike J. W.: Vitamin D receptor interaction with specific DNA requires a nuclear protein and 1,25-dihydroxyvitamin D_3. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 9751–9755.
6. Ross T. K., Moss V. M., Prahl J. M. and De Luca H. F.: A nuclear protein essential for binding of rat 1,25-dihydroxyvitamin D_3 receptor to its response elements. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 256–260.
7. Sone T., Ozono K. and Pike J. W.: A 55-Kilodalton accessory factor facilitates Vitamin D receptor DNA binding. *Molec. Endocr.* 5 (1991) 1578–1586.
8. Glass C. K., Devary O. V. and Rosenfeld M. G.: Multiple cell type-specific proteins differentially regulate target sequence recognition by the α retinoic acid receptor. *Cell* **63** (1990) 729–738.
9. Yang N., Schüle R., Mangelsdorf D. J. and Evans R. M.: Characterization of DNA-binding and retinoic acid binding properties of retinoic acid receptor. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 3559–3563.
10. Beebe S. J., Darling D. S. and Chin W. W.: 3,5,3′-Triiodothyronine receptor auxiliary protein (TRAP) enhances receptor binding by interactions within the thyroid response element. *Molec. Endocr.* 5 (1991) 85–93.
11. Burnside J., Darling D. S. and Chin W. W.: A nuclear factor that enhances binding of thyroid hormone receptors to thyroid hormone response elements. *J. Biol. Chem.* **265** (1990) 2500–2504.
12. Murrat M. B. and Towle H. C.: Identification of nuclear factors that enhance binding of the thyroid hormone receptor to a thyroid hormone response element. *Molec. Endocr.* **3** (1989) 1434–1442.
13. O'Donnell A. L., Rosen E. D., Darling D. S. and Koenig R. J.: Thyroid hormone receptor mutations that interfere with transcriptional activation also interfere with receptor interaction with a nuclear protein. *Molec. Endocr.* 5 (1991) 94–99.
14. Thompson K. L., Santon J. B., Shephard L. B., Walton G. M. and Gill G. N.: A nuclear protein is required for thyroid hormone receptor binding to an inhibitory half-site in the epidermal growth factor receptor promoter. *Molec. Endocr.* **6** (1992) 627–635.
15. Zhang X. Tran P. B. and Phahl M.: DNA binding and dimerization determinants for thyroid hormone receptor α and its interaction with a nuclear protein. *Molec. Endocr.* 5 (1991) 1909–1920.
16. De Vos P., Claessens F., Winderickx J., Van Dijck P., Celis L., Peeters B., Rombauts W., Heyns W. and Verhoeven G.: Interaction of androgen response elements with the DNA-binding domain of the rat androgen receptor expressed in Escherichia coli. *J. Biol. Chem.* **266** (1991) 3439–3443.
17. De Vos P., Claessens F., Peeters B., Rombauts W., Heyns W. and Verhoeven G.: Interaction of androgen and glucocorticoid receptor DNA-binding domains with their response elements. *Molec. Cell. Endocr.* **90** (1993) R11–R16.
18. Gorski K., Carneiro M. and Schibler U.: Tissue-specific *in vitro* transcription from the mouse albumin promoter. *Cell* **47** (1986) 767–776.
19. Killary A. M. and Fournier R. E. K.: A genetic analysis of extinction: trans-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. *Cell* **38** (1984) 523–534.
20. Dignam J. D., Lebovitz R. M. and Roeder R. G.: Accurate transcription initiation by RNA polymerase II in a soluble extract from mammalian nuclei. *Nucleic Acids Res.* **61** (1983) 1475–1489.
21. Celis L., Claessens F., Peeters B., Heyns W. Verhoeven G. and Rombauts W.: Proteins interacting with an androgen-reponsive unit in the C3(1) intron. *Molec. Cell. Endocr.* **94**, 165–172.
22. Claessens F., Celis L., Peeters B., Heyns W., Verhoeven G. and Rombauts W.: Functional characterization of an androgen response element in the first intron of the C3(1) gene of rat prostatic binding protein. *Biochem. Biophys. Res. Commun.* **164** (1989) 833–840.
23. Cato A. C. B., Henderson D. and Ponta H.: The hormone response element of the mouse mammary tumour virus mediates the progesterone and androgen induction of transcription in the proviral long terminal repeat region. *EMBO Jl* **6** (1987) 363–368.
24. de Vries E., van Driel W., Sander J. L., van den Heuvel S. J. L. and van der Vliet P. C.: Contactpoint analysis of the HeLa nuclear factor I recognition site reveals symmetrical binding at one side of the DNA helix. *EMBO Jl* **6** (1987) 161–168.
25. Courtois S., Lafontaine D. A., Lemaigre F. P., Durviaux S. M. and Rousseau G. G.: Nuclear factor-I and activator protein-2 bind in a mutually exclusive way to overlapping promoter sequences and trans-activate the human growth hormone gene. *Nucleic Acids Res.* **18** (1990) 57–64.
26. Williams G. R. and Brent G. A.: Specificity of nuclear hormone receptor action: who conducts the orchestra? *J. Endocr.* **135** (1992) 191–194.
27. Bugge T. H., Pohl J., Lonnoy O. and Stunnenberg H. G.: RXRα, a promiscuous partner of retinoic acid and thyroid hormone receptors. *EMBO Jl* **11** (1992) 1409–1418.
28. Hallenbeck P. L., Marks M. S., Lippoldt R. E. Ozato K. and Nikodem V. M.: Heterodimerization of thyroid hormone receptor with H-2RIIBP (RXRβ) enhances DNA binding and TH-dependent transcriptional activation. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 5572–5576.
29. Kliewer S. A., Umesono K., Mangelsdorf D. J. and Evans R.: Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. *Nature* **355** (1992) 446–449.
30. Leid M., Kastner P., Lyons R., Nakshatri H., Saunders M., Zacharewski T., Cheng J.-Y., Staub A., Garnier J.-M., Mader S. and Chambon P.: Purification, cloning and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* **68** (1992) 377–395.
31. Marks M. S., Hallenbeck P. L., Nagata T., Segars J. H., Appella E., Nikodem V. M. and Ozato K.: H-2RIIBP (RXRβ) heterodimerization provides a mechanism for combinatorial diversity in the regulation of retinoic acid and thyroid hormone responsive sites. *EMBO Jl* **11** (1992) 1319–1435.
32. Yu V. C., Delsert C., Andersen B., Holloway J. M., Devary O. V., Näär A., Kim S. Y., Boutin J.-M., Glas C. K. and Rosenfeld M. G.: RXRβ: A coregulator that enhances binding of retinoic acid, thyroid hormone and vitamin D receptors to their cognate response elements. *Cell* **67** (1991) 1251–1266.
33. Zhang X.-k., Hoffmann B., Tran P. B. V., Graupner G. and Pfahl M.: Retinoid X receptor is an auxiliary protein for thyroid and retinoic acid receptors. *Nature* **355** (1992) 441–446.
34. Martin J. K.: The interactions of transcription factors and their adaptors, coactivators and accessory proteins. *BioEssays* **13** (1991) 499–503.
35. Prywes R. and Zhu H.: In vitro squelching of activated transcription by serum response factor: evidence for a common coactivator used by multiple transcriptional activators. *Nucleic Acids Res.* **20** (1992) 513–520.
36. Dynlacht B., Hoey T. and Tjian R.: Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **66** (1991) 563–576.
37. Bastian L. S. and Nordeen S. K.: Concerted stimulation of transcription by glucocorticoid receptors and basal tanscription factors: limited transcriptional synergism suggests mediation by coactivators/adaptors. *Molec. Endocr.* 5 (1991) 619–627.