The functional interaction between HMGA1 and the estrogen receptor requires either the N- or the C-terminal domain of the receptor

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Abstract **We have previously shown that HMGA1 enhances the transcriptional activity of promoters containing the estrogen response element (ERE) and increases binding of the estrogen receptor (ER) to ERE. Herein, we have assessed the transcriptional activity and ERE-binding ability of deleted ER fragments in absence or in presence of HMGA1. The HMGA1 protein stimulated binding and transcriptional activity by a factor of about 2-fold compared to the wild-type ER and both the N- and C-terminal ER deleted domains, but had no effect when both domains were deleted. These data show that HMGA1 cooperates with either the N- or the C-terminal transcriptional activation domain of the ER.**

Key words: High mobility group; Estrogen receptor domain; DNA binding; Transactivation

1. Introduction

The estrogen receptor (ER) is a member of a superfamily of nuclear receptors (NR3A) [1] that have common structural and functional domains. Each of these nuclear receptors is comprised of six functional domains (A–F) that have been evolutionarily conserved [2]. Two of the most highly conserved regions are: the central DNA-binding domain (DBD), domain C, and the C-terminal hormone-binding domain, domain E [3,4]. The DBD is responsible for the specific interaction of ER with the estrogen response element (ERE), a palindrome consisting of GGTCA half-sites separated by a linker sequence of 3 bp [5–7]. The hormone-binding domain (E) directs the specific interaction of the receptor with the hormone. The other regions, the amino-terminal A/B domain, the carboxyl-terminal F domain, and the centrally located region D domain, display considerable variation in their amino acid sequence. The ER contains of two transcriptional activation domains (TADs), the A/B and the E domains [8,9]. Numerous coregulatory proteins associate with these two TADs and mediate the estrogen response. They could be coactivators [10], corepressors [11,12] and accessory proteins [13]. Therefore, transcription of steroid hormone-responsive genes may be subject to the combined effects of activators and repressors that modulate the cellular response to the ER–estrogen complex.

The HMG protein family constitutes a class of conserved proteins involved in transcriptional regulation [14]. The HMGA1 proteins (previously designated HMG I(Y)) [15] are founding members of a class of non-histone nuclear proteins known as architectural transcriptional factors [16–18]. The structural feature that distinguishes HMGA1 from other HMG proteins is the presence of multiple DBDs called AT-hooks that preferentially bind to the minor groove of AT-rich regions of DNA [19–21]. The HMGA1 proteins recognize their DNA substrate's structure rather than nucleotide sequence, they have the ability to bend, unwind, and introduce supercoils into DNA substrates and to specifically interact with other proteins, many of which are transcription factors [18]. Because of this unusual characteristic, the HMGA1 protein is thought to play an important role in regulating the expression of many different genes in vivo (reviewed in [14]). The HMGA1 proteins are barely expressed in normal adult tissues in both rodents and humans [22,23] but their expression is increased in tumors. It has been suggested that alterations in the HMGA1 gene could play an important role in the generation of benign or malignant tumors (reviewed in [18,24–26]).

Recently we have demonstrated that HMGA1 enhances the transcriptional activity of promoters containing ERE and increases the binding of the ER to ERE [27]. It was unclear which domain of ER mediated the HMGA1 effect. The majority of the ERα-associated coregulatory proteins have been isolated on the basis of their ability to interact with the ERα-TAD (most commonly the LBD domain, domain E) rather than with the DBD [28–31]. Since HMGA1 stimulated the binding of ERα to DNA, it was of interest to determine which ERα domain was required for this effect. For this purpose, we assessed the effect of several ERα deleted fragments. The ability of HMGA1 to modulate ERE-mediated transcriptional activity and ER binding to DNA was examined. Our binding and transcriptional results are consistent; they showed that HMGA1 requires the A/B and/or the E domains to increase binding of ER and to enhance transcriptional activity of promoters containing ERE.

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2. Materials and methods

2.1. Materials

All reagents were of molecular biology grade and were purchased from Gibco. Oligonucleotides were purchased from Genset SA (Paris, France). Recombinant ER was purchased from PanVera Corporation (Madison, WI, USA). Antibodies directed against the N-terminal domain (SC-7207) or the C-terminal domain (SC-542) were purchased from Dako (France). The human recombinant HMGA1 protein used in these studies (specifically the HMGA1a isoform) was produced and purified by high performance liquid chromatography (HPLC) (>90%) as previously described [32]. For the reasons noted above, in this report, the HMGA1a recombinant protein is referred to simply as HMGA1. Transcription and translation coupled reticulocyte lysate system (TNT) was purchased from Promega. ^{32}P-radiolabeled nucleotides were from Amersham Pharmacia Biotechnology (France). [^{3}H]acetyl coenzyme A and Econofluor are NEN products (France).

2.2. Cell culture

The COS-7 and the HUH7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with phenol red and supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5 μg/ml fungizone. 48 h before the experiments, the medium was removed and replaced by DMEM without phenol red supplemented with 10% charcoal-treated calf serum (complete DMEM medium without phenol red).

2.3. Plasmids

Expression vectors for human wild-type ER (HE0) and ER deleted fragments (HE15, HE19 and HE70) were previously described [33,34]. HE0 and HE19 have been previously subcloned into the *Eco*R1 site of the pSG5 plasmid and HE15 and HE70 into the *Eco*R1 site of BSM13+. For the sake of homogeneity, we subcloned HE15 and HE70 into the *Eco*R1 site of the pSG5-Stratagene plasmid. The ERE element was cloned into the *Hin*dIII site of the *tk*-CAT plasmid (gift from Dr. C. Forest, Meudon, France) as previously described [35]. The pRc-CMV-HMGA1 expression vector was generated by subcloning the full-length human HMGA1a cDNA [36] into the pRc-CMV eukaryotic plasmid expression vector (Invitrogen).

2.4. Cell extract preparation

COS-7 cells were transfected with HE0, HE15, HE19 and HE70 as described by Massaad et al. [35]. For constructs containing the LBD (HE0 and HE19), transfection was performed with complete DMEM medium without phenol red. 48 h later and 1 h prior to harvesting, estradiol (10^{-7} M) was added to the cells.

Whole cell extracts was prepared by freezing cells at −80°C, thawing them on ice and centrifuging at 10 000×*g* for 15 min at 4°C. The supernatant was conserved at −80°C.

HE70 was prepared using a transcription and translation coupled reticulocyte lysate system (TNT, Promega). Briefly, 4 μg of pSG5-HE70 was incubated with the TNT lysate, T7 RNA polymerase, an amino acid mixture (1 mM), and a ribonuclease inhibitor (40 UI). The reaction was incubated for 2 h at 30°C.

2.5. Verification of plasmid expression

In order to verify the expression of HE0, HE15, HE19 and HE70, 'Western blot' analysis was performed in whole cell extract. Proteins (50 and 100 μg) and 200 ng of the human recombinant ER protein (PanVera) were separated on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels. To each sample, Laemmli buffer was added and then boiled for 2 min. Resolved protein was electrotransferred to nitrocellulose sheets, which were probed with (i) the human polyclonal ERα antibody directed against the amino acids 2–185, and (ii) the mouse polyclonal ERα antibody directed against a peptide mapping at the carboxyl-terminus. The detection was carried out using a peroxidase-conjugated anti-rabbit antibody. Immunoreactive bands were visualized using the enhanced chemiluminescence detection system according to the manufacturer's instructions (Amersham Corp.).

The HE70 translated proteins were loaded onto 15% SDS–PAGE gels, then gels were fixed (50% methanol, 10% glacial acetic acid) and dried. Bands were visualized by autoradiography.

2.6. Preparation of competitors and radiolabeled probes

The ERE and the binding site for HMGA1 (the distal negative regulatory element (DNRE)) sequences were synthesized and used as probes for this study (Table 1). Probes were obtained by annealing the coding and non-coding strands. The duplex molecules were labeled by the Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]deoxycytidine triphosphate (dCTP) (>3000 Ci/mmol; 1 Ci = 37 GBq).

2.7. Electrophoretic mobility shift assay (EMSA)

Binding reactions were carried out as described by Massade et al. [27]. To evaluate the binding of the recombinant HMGA1 protein to DNRE, and in order to verify that the HMGA1 protein was functional, increasing concentrations of HMGA1 were mixed with the binding reaction mix and with 0.1 ng of the DNRE probe.

The specificity of ERE binding to the HE0, HE15, HE19, HE70 proteins and the effect of HMGA1 on them was determined in the same EMSA.

The specificity of binding was assessed by competitive EMSA. Cell extracts of HE19, HE15, HE70 were incubated with the binding reaction mix and a 10-, 30- and 100-fold excess of homologous competitors ERE.

The effect of HMGA1 on ERE binding by the ER and its deleted fragments was evaluated by adding increasing concentrations of HMGA1 protein (20, 30 and 40 ng) to 90 ng of the ER recombinant protein or to 10 μl of whole extracts prepared with HE0, HE15, HE19 or HE70. ERE-containing complexes were quantified by PhosphoImager (Image Quant Software). The results were expressed as percent of control (which represents the sample that was not incubated with the HMGA1 protein).

2.8. Transfection experiments and CAT assay

They were performed by the calcium phosphate coprecipitation technique in HUH7 cells, a differentiated human liver cell line that did not express the ER and expressed the HMGA1 protein at low level. For each condition, the experiment was run in duplicate. The CAT activity was determined using the two-phase assay described by Massaad et al. [35]. Results were expressed in transcriptional activity (%) = [CAT activity (dpm) elicited by each sample×100]/[CAT activity (dpm) elicited by the ERE-*tk*-CAT plasmid]. Mann–Whitney *U*-test was used to compare the mean of transcriptional activity between the transfected cells with the HMGA1 expression vector and those transfected with the empty vector. All calculations were carried out using Sigma Stat Software. The level accepted as significant was $P < 0.05$.

3. Results

3.1. Expression of ER and its deleted domain

Fig. 1A describes the constructs used. HE0 represents the wild-type (595 amino acids), in HE19 the A/B domains were truncated (1–178 amino acids), HE15 lacks both E and F domains (285–595 amino acids) and in HE70 the A/B domain

Table 1
Nucleotides sequences of the probes

Element	Sequence	
ERE consensus	5′-gaaAGGTCATGGTGACCTac-3′	(strand I)
	3′-cttTCCAGTACCACTGGAtggcgg-5′	(strand II)
DNRE	5′-cagATTTAAGTCTAATTTAAAGTcgt-3′	(strand I)
	3′-gtcTAAATTCAGATTAAATTTCAgca-5′	(strand II)

Unrelated nucleotides are shown in lowercase.

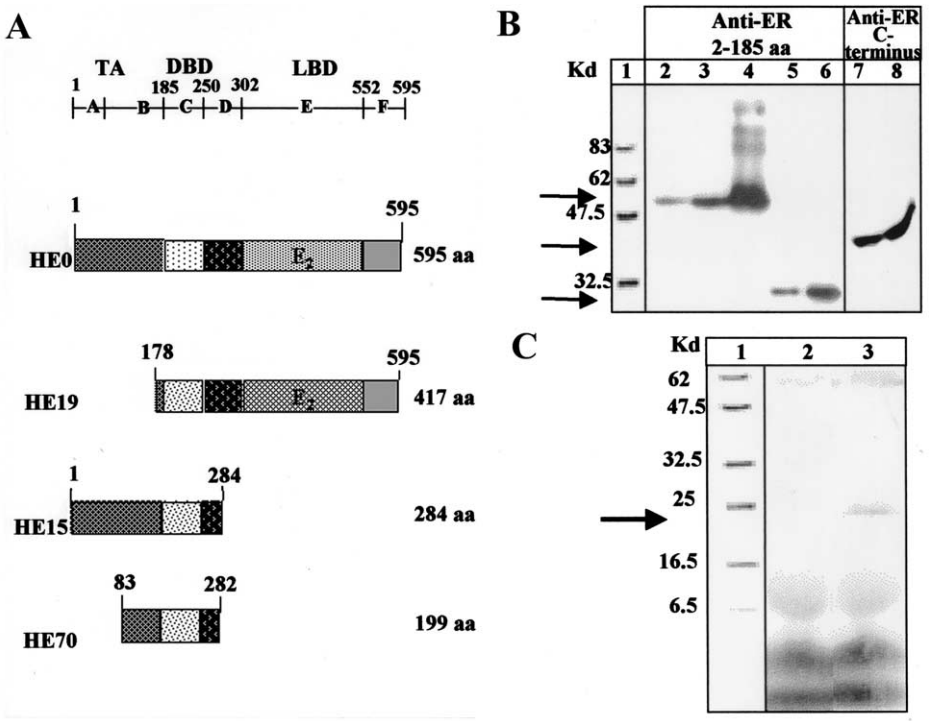

Fig. 1. Expression of the ER truncated fragments. A: Structure of wild-type ER and truncated fragments. B: COS-7 cells were transfected as described in Section 2 with 20 μg of the ER expression vectors (pSG5-HE0, pSG5-HE19, pSG5-HE15). Western blot was performed with 50 μg (lanes 2, 5, 7) and 100 μg (lanes 3, 6, 8) of protein. Lane 1 represents the molecular weight marker and lane 4 contains 200 ng of the ER recombinant protein commercialized by PanVera. The human polyclonal ERα antibody directed against the amino acids 2–185 was used. This antibody recognize the wild-type (HE0) and the recombinant proteins at 59.5 kDa (lanes 2, 3, 4) as well as the N-terminal truncated protein (HE15) at 28.4 kDa (lanes 5, 6). After stripping the membrane of human antibody, mouse polyclonal ERα antibody directed against a peptide located at the carboxyl-terminus was used. This antibody recognized HE0 and the C-terminal truncated protein (HE19) at 41.7 kDa (lanes 7, 8). C: HE70 was prepared by in vitro transcription and translation using the TNT kit (Promega). ^{35}S-labeled HE70 produced in vitro was analyzed by SDS–PAGE, after migration the gel was fixed and dried, then bands were visualized by autoradiography (lane 1, MWM; lane 2, the empty vector pSG5; lane 3, HE70 protein was produced in lysates programmed with plasmid PSG5-HE70). The arrow indicates the molecular weight of the HE70 translated fragment. This experiment was repeated four times for each preparation ($n = 4$).

was partially deleted (1–83 amino acids) as were both E and F domains (282–595 amino acids), it encodes amino acids 83–282.

Transient transfection was performed in COS-7 cell line for all constructs and 'Western blot' analysis was performed for each preparation ($n = 4$), using an ERα antibody directed against either the N-terminal part of the ERα protein, amino acids 2–185 (Fig. 1B, lanes 2–6) or a peptide located at the carboxy-terminal part (Fig. 1B, lanes 7, 8). The recombinant ERα protein was used as a control (lane 4). As shown in Fig. 1B the antibody directed against the N-terminal part of the protein recognizes the wild-type (HE0), the recombinant proteins as well as the C-terminal truncated protein (HE15). After stripping the membrane of the N-terminal antibody, the ERα antibody directed against the carboxy-terminal part of the protein was used. This antibody recognized the wild-type (HE0) and the N-terminal truncated protein (HE19).

HE70 was prepared both in the COS-7 cell line and by in vitro transcription and translation using the TNT kit (Promega). ^{35}S-labeled HE70 produced in vitro was analyzed by SDS–PAGE and autoradiography because no antibody against the DBD is available. As shown in Fig. 1C, the HE70 protein was produced in lysates programmed with plasmid PSG5-HE70 (Fig. 1C, lane 3) but not when the empty vector pSG5 was used (Fig. 1C, lane 2).

3.2. HMGA1 increases ER binding to ERE

Firstly we evaluated the binding of recombinant HMGA1 protein to its responsive element DNRE, by EMSA. As shown in Fig. 2A a shifted complex was observed, its intensity increased with the concentration of HMGA1 (Fig. 2A, lanes 2–5). However, when the ERE-containing oligonucleotides were incubated with increasing concentrations of the HMGA1 protein, no band shift was observed (data not shown). Then, we tested the effect of HMGA1 on the recombinant ER protein (Fig. 2B, lanes 1–4) and on the wild-type protein HE0 expressed in COS-7 cells (Fig. 2B, lanes 5–8). As expected, when both recombinant ER or wild-type HE0 and HMGA1 proteins were combined, an increase in the intensity of the shifted band was observed (Fig. 2B, lanes 2–4 and 5–8). These results were quantified and confirmed using several independent preparations ($n = 4$). The complex formed between HE0 and the ERE increased about 3-fold in presence of HMGA1 (Fig. 2B, right panel).

3.3. HMGA1 increases binding of the N- and C-truncated ER proteins to ERE

We established the specificity of binding of the truncated N- and C-terminal ER proteins to the consensus ERE by competition experiments. The ERE sequence was used as both a probe and a competitor. As can be seen in Fig. 2C and D, the

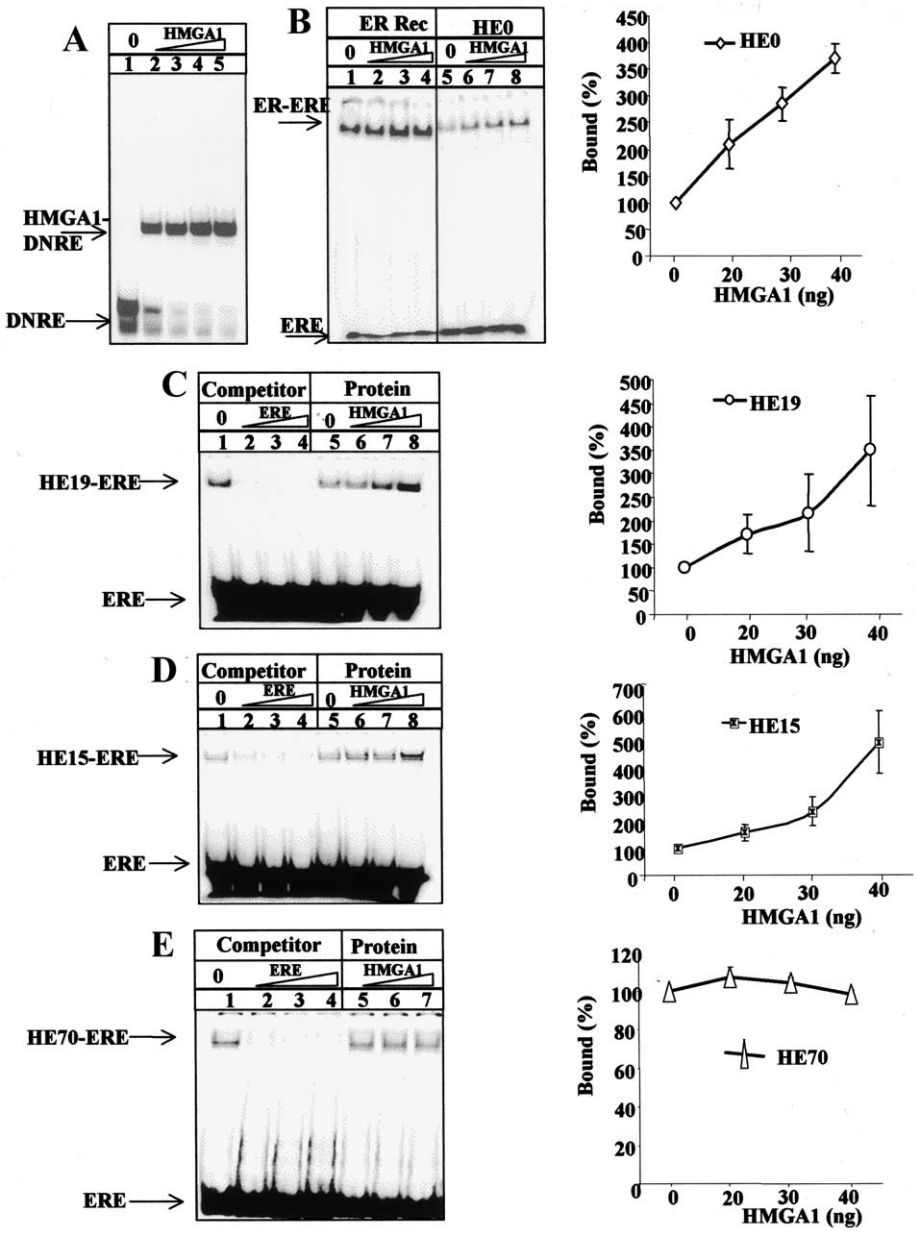

Fig. 2. HMGA1 increases binding of truncated ER containing A/B and E domain to ERE. A: In order to show a control of the HMGA1 protein, EMSA was performed using the response element of HMGA1 (DNRE) probe and increasing amounts of the human recombinant protein HMGA1 (0, 10, 20, 30, 40 ng) (lanes 1–5). B: The purified human commercial recombinant ER (PanVera) (lanes 1–4) or the expressed HE0 protein in COS-7 cells (lanes 5–8) were incubated with 0.1 ng of the ERE probe in the absence (lanes 1 and 5) or the presence (lanes 2–4 and lanes 6–8) of increasing amounts of purified human recombinant HMGA1 protein (20, 30 and 40 ng). C: On the same EMSA, we analyzed the specificity of HE19 binding to ERE (lanes 1–4) and the effect of HMGA1 on HE19 binding to ERE (lanes 5–8). Lanes 1 and 5 represent the HE19 binding to ERE. The specificity of binding was assessed by competitive EMSA using increasing amounts of unlabeled ERE (10-, 30-, 100-fold excess) (lanes 2, 3, 4 respectively). The effect of HMGA1 on the HE19 construct was determined by increasing amounts of the recombinant HMGA1 protein (20, 30, 40 ng) (lanes 6, 7, 8). D: The same experiment was performed with the C-terminal truncated protein (HE15). E: EMSA was performed using 0.1 ng of the ERE probe and the truncated HE70 in the absence (lane 1) or in the presence of increasing amounts of unlabeled ERE (10-, 30-, 100-fold excess) or with increasing amounts of the recombinant HMGA1 protein (20, 30, 40 ng) (lanes 5, 6, 7 respectively). The HMGA1 effect was quantified, the right-hand panels represent the quantification of the complexes by PhosphoImager. The percent of bound radioactivity in the ER (wild-type HE0 or truncated fragments HE15, HE19 and HE70)–ERE complexes was calculated. For every fragment, results were expressed as percent of control (0 ng of HMGA1). The results are the mean S.D. of four independent preparations with at least three assays for each preparation.

binding of both HE15 and HE19 to ERE decreased in the presence of an increasing amount of the homologous competitor (10- to 100-fold) (Fig. 2C and D, lanes 2–4).

To identify the region(s) that ERα requires to interact with HMGA1, we tested the ability of HMGA1 to activate the binding of N- and C-truncated proteins to DNA. When HMGA1 was combined with HE15 or HE19, an increase of the band intensity was observed (Fig. 2C and D, lanes 6–8) compared to the deleted ER alone (Fig. 2C and D, lane 5). The ratio of bound radioactivity in the complexes HE15–ERE and HE19–ERE increased by about 2- to 3-fold in presence of HMGA1 (Fig. 2C, D, right panel). However, in contrast with

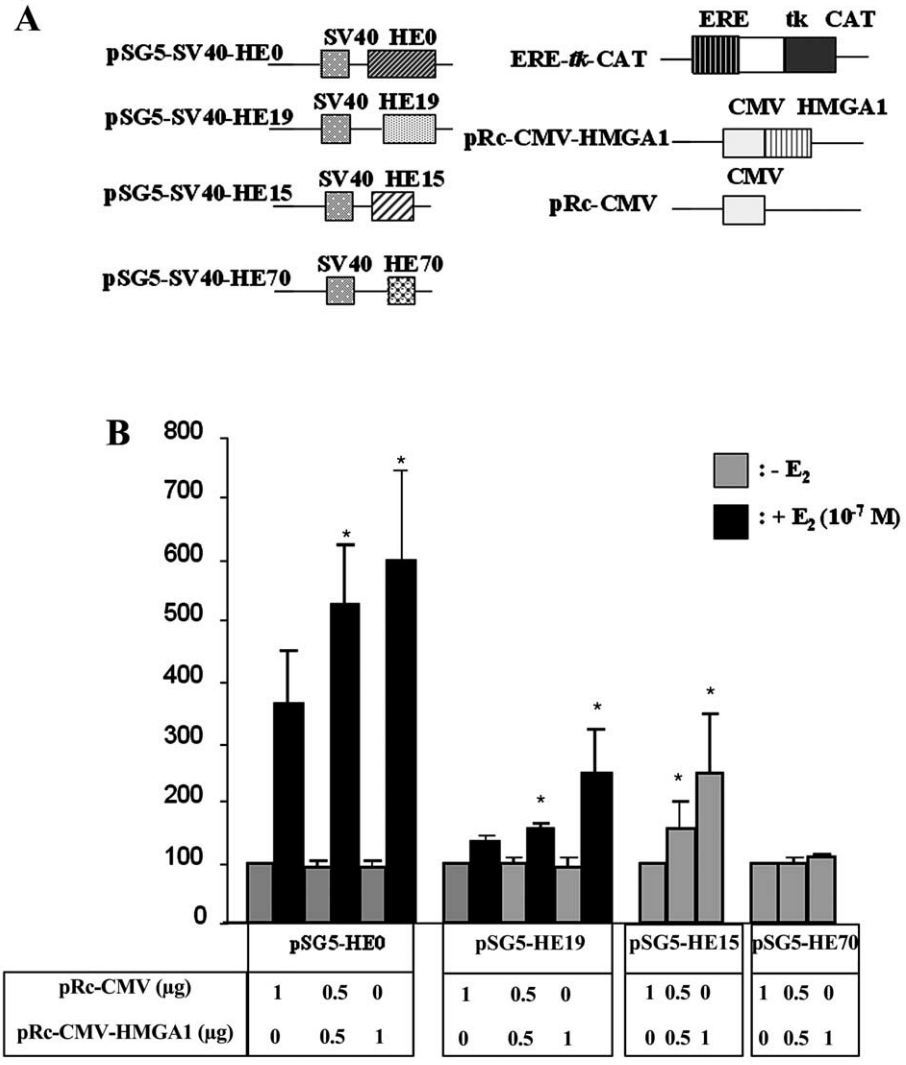

Fig. 3. HMGA1 enhances transcriptional activity of the ER in the HUH7 cell line. A: Structure of vectors. B: HUH7 cells were transfected with 1 μg of the ERE-*tk*-CAT plasmid, and 0.25 μg of each expression vector (pSG5-HE0, pSG5-HE15, pSG5-HE19 or pSG5-HE70) with or without 0.5 and 1 μg of the expression vectors pRc-CMV-HMGA1. E_2 was added at a final concentration of 10^{-7} M. The total transfected DNA amount was kept constant with the pRc-CMV plasmid. Results were expressed as percent of transcriptional activity = [CAT activity elicited by each sample × 100]/[CAT activity elicited by the ERE-*tk*-CAT plasmid alone]. The results are the mean S.D. of four independent experiments with for each point in duplicate. The *U*-Mann–Whitney test was used for statistical analysis, $^*P < 0.01$.

HE0, binding increased predominantly at the highest HMGA1 concentrations.

3.4. *HMGA1 does not modify the binding of the HE70 protein (N- and C-truncated domains) to ERE*

The same experiment was performed with HE70, which includes the DBD but in which both TADs were at least partially deleted. When HE70 was incubated with the radiolabeled ERE in presence of increasing concentrations of the ERE oligonucleotide, a specific competition was observed (Fig. 2E, compare lanes 2–4 to lane 1). In contrast with HE15 and HE19, HMGA1 did not increase the binding of HE70 to ERE (Fig. 2E, compare lanes 5–7 to lane 1, and right panel).

3.5. *The ERα A/B and E domains are required for activation by HMGA1*

We have tested the functional properties of the ER truncated fragments in presence of HMGA1. HUH7 cells were cotransfected with the reporter ERE-*tk*-CAT vector, the ER expression vectors (HE0, HE19, HE15) and increasing amounts of the HMGA1 expression vector (Fig. 3A). E_2 activated the transcription approximately 4-fold when the expression vector HE0 was transfected and 1.5-fold for HE19 (Fig. 3B). As expected, E_2 was ineffective for both HE15 and HE70 (data not shown). When the expression vector HMGA1 was cotransfected with these constructs, an enhancement of transcriptional activation was observed in induced cells for both HE0 and HE19, which elicited the same pattern of induction of about 2-fold but the basal activity remained unchanged. In contrast, HE15 displayed an increase in the basal activity of about 2-fold when the HMGA1 expression vector was added and HE70 showed a very weak transcriptional activity that was not enhanced by HMGA1 (Fig. 3B).

4. Discussion

A number of studies reported that purified steroid hormone

receptors bind poorly to their recognition sequences when compared to receptors associated with other cellular proteins. The addition of crude cellular extracts or other proteins restores the ability of purified receptors to bind to DNA. This suggests that nuclear receptors do not act alone but require the participation of other cellular proteins to bind to DNA [13,37–39]. In a previous study, we demonstrated that HMGA1 could increase ex vivo the transcriptional activity of ERE and in vitro the binding of ER to ERE without altering the mobility of the DNA–protein complex in gel mobility shift experiments. We also showed that this increase was probably due to a protein–protein interaction [27]. In this study, we investigated which of the ER domains was required for the HMGA1 protein-stimulated ER binding to DNA and transcriptional activity.

Wild-type ER, as well as the deleted receptors (HE0, HE15, HE19, HE70), were able to bind to ERE. HMGA1 alone could not bind to the ERE; however, addition of HMGA1 increased the binding of the complete ER protein (HE0) to the ERE in a dose-dependent manner. Moreover, when we tested the effect of HMGA1 on the truncated ER fragments (HE15, HE19) an increase of binding was observed in the presence of HMGA1. The effect of HMGA1 on HE0, HE19 and HE15 was similar at high concentrations. However, at lower amounts of HMGA1, its effect was more pronounced on HE0 than these deleted receptors. This suggests cooperation between the two domains to mediate the effect of HMGA1.

Several studies reported that putative coregulators interact in a hormone-dependent fashion with the C-terminal region of ER, most commonly the LBD domain [9]. In the present study, we have shown that HMGA1 enhanced the binding to DNA of HE0, HE19 and HE15 even in the absence of the LBD as in the case of HE15. This suggests that the effect of HMGA1 was not only dependent on the modification of the LBD structure induced by ligand binding.

These binding observations correlated well with the functional activity. In HUH7 cells, the ERE-containing promoter was activated 2-fold by treatment with E_2 for constructions containing the LBD domain (HE0 and HE19). Under the same condition, overexpression of HMGA1 significantly enhanced the induced transcriptional activity without affecting the basal activity of the reporter gene. This is due to the presence of the LBD in both HE0 and HE19. Indeed, LBD-containing ER is sequestered by various associated proteins and is unlikely to bind to HMGA1 and activate target genes. By interacting with this domain, E_2 leads to the dissociation of hsp90, hsp70 and other ER interacting proteins allowing its binding to DNA. Under these conditions, HMGA1 can interact with the ER. This hypothesis was supported by the fact that the basal activity remains unchanged in presence of the same concentrations of HMGA1. These observations suggest that HMGA1 can activate ER binding to DNA only when it is released from its inhibitory complex.

In contrast with HE0 and HE19, stimulation by HMGA1 of the transcriptional activity of the HE15 construct (amino acids 1–182), which lacks the ligand-binding domain, is not affected by the E_2 treatment. This mutant contains the DBD responsible for specific binding to ERE and the A/B region which is a hormone-independent TAD. Importantly, this truncated receptor is probably not associated with an inhibitory complex.

The HE70 truncated fragment lacks the amino acids 1–82 in the N-terminal part and the amino acids 283–594 in the C-terminal part. HMGA1 was unable to modulate ex vivo the transcriptional activity of ERE and in vitro the binding of HE70 to ERE. The regions deleted in HE70 contain activation functions (AFs) 1 and 2 (AF-1 and AF-2); the region between amino acids 41 and 150 is required for AF-1 activity and that between the amino acids 530 and 553 is necessary for the AF-2 activity. Previous deletion studies of ER showed that the activity of AF-2 is ligand induced, whereas AF-1 itself exhibits constitutive activity [40]. Furthermore, a coactivator-mediated functional synergism was reported between AF-1 and AF-2 to stimulate transcription [41]. It is not clear whether N- and C-terminal domains of ER were able to interact directly with each other; indeed, a 'bridging' coactivator able to interact with both regions was hypothesized to be required for an indirect interaction between the N- and C-termini [41]. Our working hypothesis is that HMGA1 can bind to both the AF-1 and/or AF-2 and, by an as yet unclear mechanism, increase the binding of the receptor to DNA. It is possible that the presence of both AFs improves the efficiency of HMGA1.

The role of HMGA1 could also be related to the importance of DNA topology in steroid receptor recognition of hormone response elements. In fact, the ER binding to the ERE results in a bend of the DNA toward the major groove. DNA bending is thought to facilitate interactions between components of the transcription complex bound to different sites and to promote DNA looping to allow single proteins to contact multiple DNA elements. HMGA1 participates in a wide variety of nuclear processes ranging from chromosome and chromatin mechanics to acting as an architectural transcription factor that regulates the expression of numerous genes in vivo [42–48]. A common feature of these processes is the assembly of higher order nucleoprotein complexes. Since endogenous hormone-responsive genes contain multiple transcription factor-binding sites that are separated by varying distances, HMGA1 could display a similar effect on the higher order nucleoprotein complexes formed on these genes in vivo.

In summary, we have shown that HMGA1 stimulates the transcriptional activation by ER probably by stimulating its binding to DNA. We also show that the effect of HMGA1 requires both the N-terminal and the C-terminal AFs of the ER. Given the high degree of conservation among the steroid receptor superfamily members, it could be proposed that the same domains in the presence of HMGA1 would also enhance the binding of other steroid hormone receptors.

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References

[1] Nomenclature, T.A.S.f.B.a.M.R.P.s.C.o. (1999) Cell 97, 161–163.
[2] Kumar, V., Green, S., Stack, G., Berry, M., Jin, J.R. and Chambon, P. (1987) Cell 51, 941–951.
[3] Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. and Chambon, P. (1989) Cell 59, 477–487.
[4] Gronemeyer, H. et al. (1992) J. Steroid Biochem. Mol. Biol. 41, 217–221.
[5] Klein-Hitpass, L., Ryffel, G.U., Heitlinger, E. and Cato, A.C. (1988) Nucleic Acids Res. 16, 647–663.
[6] Chusacultanachai, S., Glenn, K.A., Rodriguez, A.O., Read,

E.K., Gardner, J.F., Katzenellenbogen, B.S. and Shapiro, D.J. (1999) J. Biol. Chem. 274, 23591–23598.
[7] Schwabe, J.W., Chapman, L., Finch, J.T. and Rhodes, D. (1993) Cell 75, 567–578.
[8] Klinge, C.M. (2000) Steroids 65, 227–251.
[9] Klinge, C.M. (2001) Nucleic Acids Res. 29, 2905–2919.
[10] Smith, D.F. and Toft, D.O. (1993) Mol. Endocrinol. 7, 4–11.
[11] Chen, J.D. and Evans, R.M. (1995) Nature 377, 454–457.
[12] Horlein, A.J. et al. (1995) Nature 377, 397–404.
[13] Landel, C.C., Potthoff, S.J., Nardulli, A.M., Kushner, P.J. and Greene, G.L. (1997) J. Steroid Biochem. Mol. Biol. 63, 59–73.
[14] Reeves, R. (2001) Gene 277, 63–81.
[15] Bustin, M. (2001) Trends Biochem. Sci. 26, 431–437.
[16] Reeves, R. (2000) Environ. Health Perspect. 108, 803–809.
[17] Bianchi, M.E. and Beltrame, M. (1998) Am. J. Hum. Genet. 63, 1573–1577.
[18] Bustin, M. and Reeves, R. (1996) Prog. Nucleic Acid Res. Mol. Biol. 54, 35–100.
[19] Solomon, M.J., Strauss, F. and Varshavsky, A. (1986) Proc. Natl. Acad. Sci. USA 83, 1276–1280.
[20] Reeves, R. and Nissen, M.S. (1990) J. Biol. Chem. 265, 8573–8582.
[21] Huth, J.R., Bewley, C.A., Nissen, M.S., Evans, J.N., Reeves, R., Gronenborn, A.M. and Clore, G.M. (1997) Nat. Struct. Biol. 4, 657–665.
[22] Chiappetta, G. et al. (1996) Oncogene 13, 2439–2446.
[23] Giancotti, V. et al. (1993) Eur. J. Biochem. 213, 825–832.
[24] Tkachenko, A., Ashar, H.R., Meloni, A.M., Sandberg, A.A. and Chada, K.K. (1997) Cancer Res. 57, 2276–2280.
[25] Bianchi, M.E. and Beltrame, M. (2000) EMBO Rep. 1, 109–114.
[26] Reeves, R., Edberg, D.D. and Li, Y. (2001) Mol. Cell. Biol. 21, 575–594.
[27] Massaad-Massade, L., Navarro, S., Krummrei, U., Reeves, R., Beaune, P. and Barouki, R. (2002) Biochemistry 41, 2760–2768.
[28] Loven, M.A., Muster, N., Yates, J.R. and Nardulli, A.M. (2003) Mol. Endocrinol. 17, 67–78.
[29] Norris, J.D., Fan, D., Sherk, A. and McDonnell, D.P. (2002) Mol. Endocrinol. 16, 459–468.
[30] Zhou, D., Quach, K.M., Yang, C., Lee, S.Y., Pohajdak, B. and Chen, S. (2000) Mol. Endocrinol. 14, 986–998.
[31] An, J., Ribeiro, R.C., Webb, P., Gustafsson, J.A., Kushner, P.J., Baxter, J.D. and Leitman, D.C. (1999) Proc. Natl. Acad. Sci. USA 96, 15161–15166.
[32] Reeves, R. and Nissen, M.S. (1999) Methods Enzymol. 304, 155–188.
[33] Green, S. and Chambon, P. (1987) Nature 325, 75–78.
[34] Ylikomi, T., Bocquel, M.T., Berry, M., Gronemeyer, H. and Chambon, P. (1992) EMBO J. 11, 3681–3694.
[35] Massaad, C., Coumoul, X., Sabbah, M., Garlatti, M., Redeuilh, G. and Barouki, R. (1998) Biochemistry 37, 6023–6032.
[36] Johnson, K.R., Lehn, D.A. and Reeves, R. (1989) Mol. Cell. Biol. 9, 2114–2123.
[37] Romine, L.E., Wood, J.R., Lamia, L.A., Prendergast, P., Edwards, D.P. and Nardulli, A.M. (1998) Mol. Endocrinol. 12, 664–674.
[38] Onate, S.A., Prendergast, P., Wagner, J.P., Nissen, M., Reeves, R., Pettijohn, D.E. and Edwards, D.P. (1994) Mol. Cell. Biol. 14, 3376–3391.
[39] Prendergast, P., Pan, Z. and Edwards, D.P. (1996) Mol. Endocrinol. 10, 393–407.
[40] Warnmark, A., Treuter, E., Wright, A.P. and Gustafsson, J.A. (2003) Mol. Endocrinol. 17, 1901–1909.
[41] Metivier, R., Penot, G., Flouriot, G. and Pakdel, F. (2001) Mol. Endocrinol. 15, 1953–1970.
[42] Thanos, D. and Maniatis, T. (1992) Cell 71, 777–789.
[43] Lewis, H., Kaszubska, W., DeLamarter, J.F. and Whelan, J. (1994) Mol. Cell. Biol. 14, 5701–5709.
[44] John, S., Reeves, R.B., Lin, J.X., Child, R., Leiden, J.M., Thompson, C.B. and Leonard, W.J. (1995) Mol. Cell. Biol. 15, 1786–1796.
[45] Whitley, M.Z., Thanos, D., Read, M.A., Maniatis, T. and Collins, T. (1994) Mol. Cell. Biol. 14, 6464–6475.
[46] Lopez, S., Reeves, R., Island, M.L., Bandu, M.T., Christeff, N., Doly, J. and Navarro, S. (1997) J. Biol. Chem. 272, 22788–22799.
[47] Bonnefoy, E., Bandu, M.T. and Doly, J. (1999) Mol. Cell. Biol. 19, 2803–2816.
[48] Chuvpilo, S., Schomberg, C., Gerwig, R., Heinfling, A., Reeves, R., Grummt, F. and Serfling, E. (1993) Nucleic Acids Res. 21, 5694–5704.