Localization of an estrogen receptor binding site near the promoter of the uteroglobin gene

M.S. López de Haro, C. García and A. Nieto

Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

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By means of a DNA-cellulose competitive binding assay, we have studied the interaction of the estrogen receptor with genomic fragments of the estrogen responsive rabbit uteroglobin gene. The fragments spanned from 3255 bp upstream to 1754 bp downstream of the initiation site. Only a fragment (−396/+8) showed strong affinity for the receptor. Within this fragment a unique palindromic sequence (GGTCAccaTGCCC) was found which is very similar to the canonical consensus sequence for the estrogen receptor. A synthetic oligonucleotide of that structure specifically competed for the binding of the receptor to DNA-cellulose.

Steroid receptor; Estrogen; Uteroglobin; Gene expression

1. INTRODUCTION

Steroid hormones mediate their main biological response by binding to specific receptor proteins that, in turn, interact with short regions of the regulated genes and modulate their transcription [1]. These DNA regions, known as HRE, have been characterized by several methods and shown to contain specific consensus sequences for the hormonal receptors [2].

The steroid hormone regulation of the rabbit uteroglobin gene has received considerable attention, since this gene is subjected to tissue-specific regulation by several steroid hormones. Thus, uteroglobin is induced by progesterone and estradiol in uterus [3], by glucocorticoids in lung [4,5], by androgens in the epididymis [6] and by estrogens in the oviduct [7]. While HREs for glucocorticoids and progesterone have been observed in the uteroglobin gene [8], no data on the existence and localization of a HRE for estrogens are so far available. These data are important for further studies on the regulation of the expression of this gene. Here, we show evidence for an ER binding site near the promoter of the uteroglobin gene.

2. MATERIALS AND METHODS

[^{3}H]Estradiol (112 Ci/mmol) was obtained from Amersham International. DNA-cellulose (9 mg of DNA/g) was prepared as previously described [9]. The pUG5 clone, containing 5 kb of the uteroglobin gene [8], was a gift from Dr Beato, Marburg University, FRG. The DNA was digested with the appropriate restriction enzyme and the fragments were either subcloned in pUC18 or separated by electrophoresis on agarose gels.

Correspondence address: A. Nieto, Centro de Biología Molecular, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

Abbreviations: HRE, hormone regulatory element; ER, estrogen receptor

2.1. *Preparation of the ER*

Rabbit uterus was homogenized in 4 vols (w/v) of DTE (2 mM dithiothreitol, 1 mM EDTA, 40 mM Tris-HCl, pH 7.4). After centrifugation for 1 h at 150 000 × *g*, the supernatant was brought to 30% of $(NH_4)_2SO_4$ saturation, centrifuged for 15 min at 20 000 × *g* and the pellet was dissolved in DTE. This precipitation step served both to partially purify the receptor and to convert it into a DNA-binding form [10]. The receptor preparation was labelled by incubation for 2 h with 10 nM [^{3}H]estradiol and the free hormone was absorbed with dextran-coated charcoal [10]. All of the above operations were carried out at 0–4°C.

2.2. *DNA-cellulose binding assay*

This was performed essentially as described [11]. Briefly, 0.3–0.5 mg of DNA-cellulose was incubated at 0°C with the receptor preparation, with or without competing DNA. The binding buffer was DTE containing 10% glycerol and 50 mM NaCl and the final volume was 150 μl. After 45 min, the suspension was centrifuged, the pellet washed with binding buffer and its radioactivity was measured.

3. RESULTS

The schematic structure and the restriction fragments of the genomic clone pUG5 are shown in Fig.1. In a first approach, 4 large fragments were tested in the competitive binding assay. In this assay, useful in the localization of several HRE [11–13], DNA fragments compete with DNA-cellulose for the binding of the labelled receptor. As shown in Fig.2A, fragment c (−613/+437) was the most effective in the binding displacement. To delimit more precisely the binding

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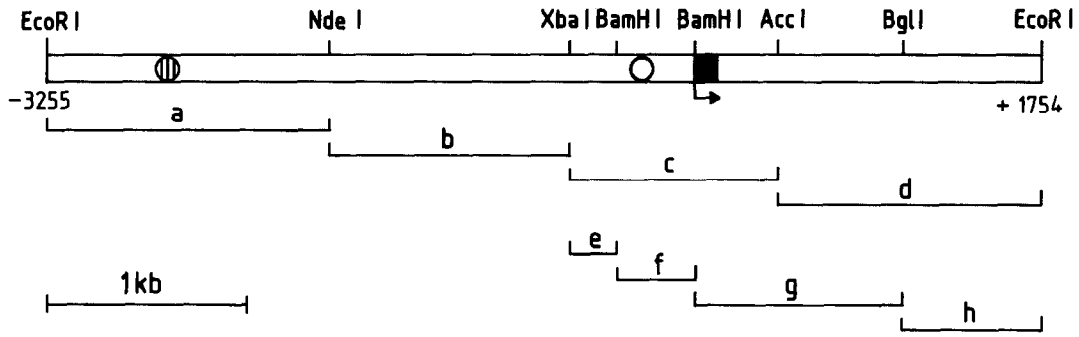

Fig.1. Schematic structure of the pUG5 clone. The black box represents the first exon. The numbering starts at the initiation site (arrow). The striped circle indicates the glucocorticoid/progesterone receptor binding site previously described [8]. The empty circle denotes the ER binding site observed in this study. Also shown are the restriction sites used to generate the fragments indicated by small letters.

site, smaller subfragments around this zone were probed (Fig.2B). The fragment f (−396/+8) strongly displaced the binding of the ER to DNA-cellulose.

Since the consensus sequence established for the estrogen HRE is the palindrome 5′-aGGTCAnnnTGACCt-3′ [2,13], we looked for similar sequences along the entire pUG5 DNA. A computer-based search indicated the existence of only two possibilities. The first one (cGGACAcggAGTCCt) was located at −2671/−2657, partially overlapping with the glucocorticoid/progesterone HRE detected previously ([8] and Fig.1). The second one (aGGTCAccaTGCCCt) was located at −266/−252, i.e. within fragment f. In the light of these findings, we obtained double-stranded synthetic oligonucleotides of either sequence and tested them in the competition assay (Fig.3). The first sequence was unable to displace the ER whereas the second oligonucleotide was shown to be very effective.

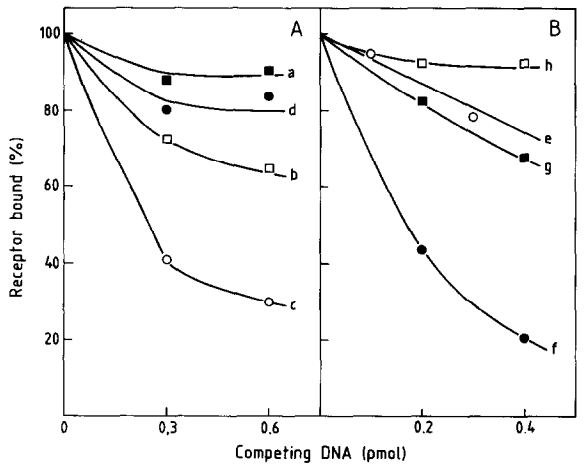

Fig.2. Competitive binding of uteroglobin genomic fragments to the estrogen receptor bound to DNA-cellulose. The percentage of labelled receptor bound to DNA-cellulose (100% in the absence of competitor) was plotted against increasing amounts of competing DNA. In A, the fragments used were: −3255/−1835 (a); −1834/−614 (b); −613/+437 (c); +438/+1754 (d). In B, the fragments were: −613/−397 (e); −396/+8 (f); +9/+1044 (g); +1045/+1754 (h).

4. DISCUSSION

In this work we present for the first time evidence for an ER binding site in the uteroglobin gene. The nucleotide sequence of this site appears to be the imperfect palindrome aGGTCAccaTGCCCt, differing only by one nucleotide (position 11) from the canonical consensus sequence [2,13] or from a functional HRE of the human pS2 gene [14]. The interaction of this sequence with the ER appears to be selective. No binding of the ER was observed with the glucocorticoid/progesterone receptor binding site of the uteroglobin gene. The receptor does not interact with other similar palindromic sequences (−2671/−2657) detected by the computer search. Weak interactions observed in the binding assay with some fragments (Fig.2) might be due to the presence of several GGTCA half-palindromic motifs interspersed throughout the uteroglobin gene. This motif is a functional HRE in the ovalbumin gene [15] and could interact, albeit weakly, with the ER.

The ER binding element of the uteroglobin gene is located in the promoter zone in contrast to the gluco-

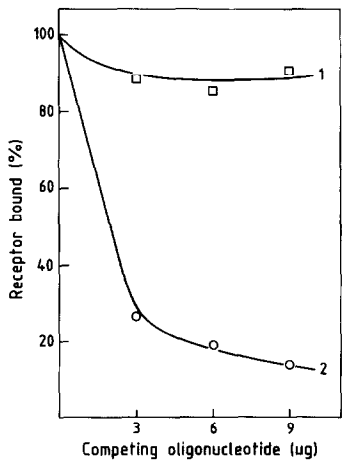

Fig.3. Competitive binding of synthetic oligonucleotides to the ER. The structures of the oligonucleotide upper strands were 5′-CGGACACGAGTCCT-3′ (1) and 5′-AGGTCACCATGCCCT-3′ (2). Conditions were as in Fig.2.

corticoid/progesterone binding sites which lay far upstream from the initiation site [8]. The localization of HREs on many different genes is quite variable [1,2], but the different HREs of the multihormonally regulated mouse mammary tumor virus are clustered or even overlapping [1,2]. It is possible that the different localizations of the HREs in the uteroglobin gene may play some role in the tissue-specific regulation of this gene.

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