Regulation of Endothelin-1 Gene Expression by Fos and Jun*

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The endothelin peptides constitute a family of potent vasoconstrictor molecules. Endothelin-1 (ET1) is secreted by vascular endothelial cells and may have a role in the regulation of vascular tone. To better understand the function of ET1, we have investigated the transcriptional regulation of the ET1 gene. Utilizing reporter gene transfection experiments, we have previously identified two promoter regions, located at base pairs −148 to −117 (Region A) and −117 to −98 (Region B) of the ET1 gene. Both regions are necessary for high level ET1 transcription in endothelial cells. A nuclear protein binding to the GATA motif in Region B has been identified and proven to be necessary for expression of the ET1 gene. However, the cis-acting sequences and their cognate binding proteins for Region B have not been investigated. To identify protein binding motifs in Region B we performed DNase I footprinting and gel mobility shift assays using a DNA fragment encoding base pairs −204 to −94 of the ET1 gene. Results from these studies indicated that the AP1 consensus sequence (GTGACTAA) in Region B as the only protein-binding motif. Site-directed mutagenesis of the ET1 AP1 site resulted in a 30-fold reduction in promoter activity, establishing the functional significance of this sequence. Additional experiments investigated the role of Jun and Fos in ET1 transcription. By employing antisera to Jun and Fos in gel mobility shift assays, both of these proteins were identified as endothelial cell nuclear proteins binding to the ET1 AP1 sequence. In trans-activation experiments, we showed that cotransfection of c-fos and c-jun expression plasmids markedly increased the transcription rate of chloramphenicol acetyltransferase reporter plasmids containing three synthetic ET1 AP1 sites. Taken together, these data indicate the importance of the AP1 recognition sequence, and the role of Fos and Jun proteins in the regulation of ET1 gene transcription.

Endothelin-1 (ET1) is a 21-amino acid peptide produced by vascular endothelium in culture and in vivo (1). ET1 is the most potent vasoconstrictor known. It also induces mitogenesis and increases the expression of proto-oncogenes in vascular smooth muscle cells, fibroblasts, and mesangial cells (2–5). Thus, ET1 may be involved in the regulation of vascular tone as well as in the pathogenesis of vasospasm, hypertension, and atherosclerosis. Knowledge of the regulation of ET1 gene expression is fundamental to understanding the role of the ET1 peptide in vascular disease. In addition, the ET1 gene provides a model system for studying the cell-specific mechanisms of gene transcription in endothelial cells.

Using regional deletion mutants of human ET1 5′-flanking sequence in reporter gene transfection experiments, we have identified two promoter regions which are necessary for constitutive transcriptional activity in bovine aortic endothelial cells (BAEC) in culture (6). Region A is located at base pairs (bp) −148 to −117 and Region B is located at bp −129 to −98 of the ET1 gene. When a 119-bp ET1 genomic fragment containing these two regions was joined to a heterologous promoter, this sequence was capable of directing endothelial cell-specific transcription (6). Wilson et al. (7) have also provided evidence for tissue-specific expression conferred by this region of the ET1 promoter.

Functional significance of Region A has been confirmed by reconstitution experiments and site-directed mutagenesis studies (6, 7). Sequence-specific protein binding to a GATA motif in this region has been documented (7). Wilson et al. (7) have also provided evidence that the GATA-binding protein in endothelial cells is present in a large variety of cell types and is different from the well-characterized erythroid-specific GATA-1 factor. It seems likely that one of the GATA factors recently described by Yamamoto et al. (8) is the protein responsible for regulation of ET1 transcription through this binding site.

In these studies we have investigated Region B of the ET1 gene. To identify the protein-binding sequences which might be important in the functional role of this region, we performed DNase I footprinting and gel mobility shift assays. These studies provided evidence for the binding of protein to an 8-bp sequence (GTGACTAA) at bp −109 to −102. This sequence is similar to the well-characterized AP1 site which has been shown to mediate hormone and growth factor responsiveness in association with other eukaryotic genes (reviewed in Refs. 9 and 10). Site-directed mutagenesis of this sequence abolished nuclear protein binding to Region B and markedly decreased the transcriptional activity of the ET1 promoter. The role of the ET1 AP1 site as the functional role of this region was thus confirmed. By using anti-Fos and anti-Jun antisera, we have demonstrated binding of members of the Fos and Jun nuclear protein families to the ET1 AP1 sequence. Finally, we have shown in trans-activation

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† The abbreviations used are: ET1, endothelin-1; BAEC, bovine aortic endothelial cells; bp, base pairs; GMSA, gel mobility shift assay; HEFES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

2 T. Quertermous, unpublished observations.
studies that c-fos and c-jun are capable of stimulating expression through the ET1 AP1 sequence, confirming that the DNA binding of Fos and Jun to the ET1 promoter reflects a functional interaction.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Nuclear Extracts—BAEC were isolated and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone), 600 μg of glutamine/ml, 100 units of penicillin/ml, and 100 μg of streptomycin/ml as described previously (6). BAEC were passaged every 3-5 days. Cells passage 6-10 were used for transfection experiments and for the preparation of nuclear extract. HeLa cells were obtained from the American Type Culture Collection and were cultured in conditions identical to those for BAEC. Nuclear extracts from BAEC and HeLa cells were prepared essentially as described by Dignam et al. (11).

**Plasmids—**All reporter fusion plasmids contained the prokaryotic chloramphenicol acetyltransferase gene in combination with either cloned ET1 5' promoter sequence or synthetic complementary oligonucleotides encoding a portion of the ET1 5' sequence. The plasmids pSV3GAL, pSPCAT, and pOCAT have been described previously (6). The plasmids -204CAT and -98CAT were constructed by cloning ET1 DNA fragments encoding bp -204 to +150 and bp -98 to +150, respectively, into pOCAT in the appropriate orientation. The plasmid AP1-MUT was identical to -204CAT except bp -108 and -107 were mutated (see Mutagenesis, below). The plasmid (3AP1)pSPCAT was generated by cloning two annealed complementary oligonucleotides encoding the sequence of the ET1 AP1 consensus sequence (TAGATCTGGTACTAAGAATCTAGGTGTTACTAACGGG) into the BglII site of pSPCAT. Two complementary oligonucleotides encoding the sequence of Region A (bp -148 to -117) were annealed and cloned to 5' to the 3 ET1 AP1 sites of (3AP1)pSPCAT to generate the plasmid (1A + 3AP1)pSPCAT. The authenticity of each fusion plasmid was confirmed by both restriction mapping and dideoxy chain termination sequencing. The Fos expression plasmid CMV-Fos, was a kind gift of Dr. T. Curran (Roche Institute of Molecular Biology, Nutley, NJ) and the Jun expression plasmid, RSV-Jun, was a kind gift of Dr. M. Karin (University of California San Diego, La Jolla, CA). Plasmid DNA was isolated by alkaline lysis followed by cesium chloride equilibrium centrifugation (12).

**Mutagenesis—**Site-directed mutagenesis of the ET1 AP1 sequence was performed by polymerase chain reaction according to the method of Higushi et al. (13). A DNA fragment encoding bp -204 to +150 was used as a template. The native DNA fragment was utilized to produce reporter plasmids -204CAT and gel mobility shift assay (GMSA) probe GS-1. The mutated promoter fragment was utilized to produce reporter plasmid AP1-MUT and gel mobility shift assay probe GS-2. The sequence GTGACTAA of the ET1 AP1 site was mutated to GGTACTAA by one set of mismatched primers: MG1 (GCTGTGTTGTTACTAATAAC) and MG2 (GTTATTAGTACCCACAGGC), and 1 set of primers flanking both ends of the templates. MG1 (CCCCGGGTACCCAGTTATCGACCTTGA) and MG4 (TGCAACTTAGGAGGTCCAAAGGCTTAC). The sequence of the mutated polymerase chain reaction fragments was confirmed by dideoxy chain termination sequencing.

**DNase I Footprinting—**DNase I footprinting was performed as described by Wilderman et al. (14). A DNA fragment containing bp -204 to -94 of the ET1 gene was labeled at the 5' end with [α-32P]dGTP using the Klenow fragment of DNA polymerase I. About 10,000 cpm (30 μg) of the labeled DNA fragment was incubated with nuclear extract and 1 μg of poly(d-l-dC)-poly(d-l-dC) on ice for 25 min and then 2 min at room temperature. Samples were then treated with DNase I (0.02 unit and 0.5 unit for samples without and with nuclear extract respectively) for 2 min at room temperature. Samples were then extracted with phenol/chloroform, ethanol precipitated, and analyzed by electrophoresis using an 8% polyacrylamide/urea gel. A G ladder of the same end-labeled DNA fragment was generated by Maxam and Gilbert technique (15).

**Gel Mobility Shift Assays—**GMSA were performed according to the method of Chboyok (16). The probes were made from either cloned DNA fragments of the ET1 gene or annealed synthetic complementary oligonucleotides encoding a portion of the ET1 gene. The DNA fragments were radiolabeled at the 5' end with [α-32P]dGTP using the Klenow fragment of DNA polymerase I. The oligonucleotides were labeled with γ-[32P]dATP with polynucleotide kinase before annealing. A typical binding reaction contained 10,000 cpm DNA probe, 2 μg of poly(d-l-dC)-poly(d-l-dC), 20 mM HEPES, pH 7.8, 60 mM KC1, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM EDTA, and 15 μg of nuclear protein extract. The samples were incubated at room temperature for 25 min and analyzed by 4% native polyacrylamide gel at pH 8.3 in running buffer (200 mM Tris, 20 mM magnesium chloride, 1.25 mM EDTA). Specificity of binding was ascertained by competition with a 100-fold molar excess of cold DNA fragments added to the reaction mixture. For supershift experiments, the nuclear extract was incubated with antibodies at 4°C for 2 h before addition to the reaction mixture. Anti-Fos antisera was kindly provided by Dr. T. Curran, and anti-Jun antisera by Dr. R. Tjian, and anti-c-Jun-antiplasmid by Dr. G. Reed (Massachusetts General Hospital, Boston, MA). The sequences of the competitors were as follows: AP1 (human collagenase gene), CTAGTGAGTGCTTACCGCCGTC; AP2, GATCAACTGACGCCCGGCGCCGT; AP3, CTAGTTGGAGCTTACCGCATG; SP1, GTAGTGAGCGCGCCGCGCATC; NFI, ATTTTGCGTTAACGCAATATG. DNA fragment ET-A1 encoded bp -148 to -117 (Region A); ET-A2 encoded bp -139 to -123 (Region A); and ET-B bp -109 to -102 (ET1 AP1) of the ET1 gene.

**Transfection and Chloramphenicol Acetyltransferase Assays—**BAEC cells were transfected with 20 μg of the appropriate chloramphenicol acetyltransferase construct by the calcium phosphate method (6). To correct for variability in transfection efficiency, 10 μg of pSV3GAL plasmid DNA was cotransfected in all experiments. For trans-activation experiments, 5 μg of expression plasmids for c-fos (CMV-Fos) and c-jun (RSV-Jun) or 10 μg of pCDM8 expression vector containing no insert was added to each dish in addition to the reporter chloramphenicol acetyltransferase construct and pSV3GAL. Twelve h before harvesting, the BAEC cells were changed to serum-free medium. Cell extracts were prepared 48 h after transfection. The chloramphenicol acetyltransferase and β-galactosidase assays were performed as previously described (6). The ratio of chloramphenicol acetyltransferase activity to β-galactosidase activity was expressed as relative chloramphenolic acetyltransferase activity. Each experiment was performed at least three times and each transfection was performed in triplicate. Since there was little variation between experiments, the data were presented as the mean of a triplicate transfection, for a single representative experiment.

**RESULTS**

GTGACTAA is the Binding Site for Transcriptional Factors in Region B—To identify the functional regulatory sequences in Region B, we first looked for evidence of protein binding to Region B by GMSA. The assays were performed with a 32P-labeled DNA fragment encoding bp -204 to -94 of the human ET1 gene (Fig. 1). Incubation of nuclear extract from BAEC with the radiolabeled DNA fragment retarded the mobility of the DNA and resulted in the formation of the band indicated by the arrow. A 100-fold molar excess of unlabeled oligonucleotides encoding either the classical AP1 sequence (human collagenase gene) or the human ET1 AP1

**FIG. 1. Binding of nuclear proteins to Region B.** The gel mobility shift assays were performed with a 32P-labeled DNA fragment encoding bp -204 to -94 of the ET1 gene with or without nuclear extract from bovine aortic endothelial cells. Only free probe was observed when no nuclear extract was incubated with the 32P-labeled ET1 genomic fragment (lane 1). Additional bands were obtained from the DNA fragment retarded by a free band as indicated by the arrow (lane 2). The retarded band was not observed when competitors encoding the human collagenase AP1 consensus sequence (AP1, lane 3) or the ET1 AP1 sequence (ET-B, lane 9) were included at 100-fold molar excess in the binding reaction.
sequence competed for the protein binding while oligonucleotides encoding unrelated sequences did not. Identical results were obtained with nuclear extract from HeLa cells (data not shown).

To further characterize the sequence of the binding site, we performed DNase I footprinting employing the same DNA fragment as used in the GMSA. In this case, the DNA was labeled to high specific activity only at the 5' end (anti-sense strand). Incubation of the DNA fragment with nuclear extracts from both BAEC and HeLa cells (Fig. 2) resulted in a protected region spanning bp −109 to −102 of the human ET1 gene. This sequence corresponds to the AP1 site of the human ET1 gene. Thus, the AP1 consensus sequence of Region B is the binding site for proteins present in nuclear extracts of both BAEC and HeLa cells.

The ET1 AP1 Sequence Is Necessary for High Level ET1 Gene Expression—To test whether the ET1 AP1 site is necessary for high level gene expression in BAEC, we introduced point mutations into the AP1 site of a DNA fragment encoding bp −204 to +150 of the human ET1 gene. The first two base pairs of the AP1 sequence (GTgactaa) were converted to TG by polymerase chain reaction (13). To verify that these mutations eliminated the binding activity of the AP1 site, GMSA was performed with both the 32P-labeled native DNA fragment GS-1 and the mutated DNA fragment GS-2. Incubation of BAEC nuclear extract with the native DNA fragment resulted in a retarded band consistent with the formation of a protein-DNA complex (Fig. 3, lane 3), which was absent when the mutated DNA fragment was used in the binding assay (lane 4). Formation of the protein-DNA complex was prevented by competitors encoding both the classical AP1 sequence and the ET1 AP1 sequence (lanes 5 and 7) but not by competitors encoding the Region A sequence (lane 6). These data provide further evidence that protein binding to this region of the ET1 promoter is mediated by the AP1 sequence and verify that the AP1 mutations disrupt normal binding activity.

\[ \text{FIG. 2. Characterization of GTGACTAA as the binding motif by DNase I. The 111-bp ET1 sequence including bp -204 to -94 was }^{32}\text{P}-\text{labeled at the 5'} \text{ end. Approximately 30 pg of the radioactive DNA fragment was incubated with 1 }\mu\text{g of poly(dI-dC) · poly(dI-dC), DNase I, and 25 }\mu\text{g of nuclear protein. The G sequence pattern of the ET1 fragment was generated by Maxam and Gilbert technique (lane 1). When compared with the unprotected DNase I digest of the ET1 fragment, (lane 2, no extract added), incubation with either BAEC (lane 3), or HeLa (lane 4) nuclear protein resulted in a protected region spanning bp -109 to -102 (labeled as GTGACTAA) of the ET1 gene.} \]

The ability of the native and the mutated DNA fragments to direct chloramphenicol acetyltransferase gene expression in BAEC was tested by transfection of fusion plasmids containing either the native ET1 promoter or the mutated promoter cloned into pOCAT. As shown previously (6), the plasmid −204CAT containing the native DNA fragment encoding bp −204 to +150 of the ET1 gene, directed high level chloramphenicol acetyltransferase gene expression. In contrast, plasmid AP1-MUT which differed from −204CAT by 2 mutated bp in the AP1 site had 30-fold lower activity. Plasmid −98CAT, containing an ET1 genomic fragment without Regions A and B, had only minimal promoter activity in BAEC.

The ability of the native and the mutated DNA fragments to direct chloramphenicol acetyltransferase gene expression in BAEC was tested by transfection of fusion plasmids containing either the native ET1 promoter or the mutated promoter cloned into pOCAT. As shown previously (6), the plasmid −204CAT containing the native DNA fragment directed high level of chloramphenicol acetyltransferase gene expression (Fig. 4). The plasmid AP1-MUT, although differing from −204CAT by only two nucleotides, was associated with 30-fold lower chloramphenicol acetyltransferase activity in transfected BAEC. The activity of the mutated promoter was similar to that of −98CAT, which contained an ET1 genomic fragment lacking both Regions A and B. These data demonstrate the functional significance of the ET1 AP1 site in directing high level constitutive expression of the ET1 gene in vitro.

\[ \text{Members of the Fos and Jun Families Bind to the AP1 Site} \]

To determine whether the DNA-protein complex observed in GMSA experiments included Fos and Jun, we as-

\[ \text{FIG. 3. Mutation of the AP1 site abolishes its ability to bind nuclear protein. Gel mobility shift assays were performed with probes GS-1 and GS-2 and nuclear extract from BAEC. The GS-1 probe was derived from a }^{32}\text{P}-\text{labeled DNA fragment encoding bp -204 to +150 of the ET1 gene. GS-2 is identical to GS-1, except 2 bp of the ET1 AP1 motif have been changed. Only free probe was observed when no nuclear extracts were added to the reaction (lanes 1 and 2). Incubation of GS-1 with BAEC nuclear extract resulted in a retarded band (lane 3). In contrast, incubation of GS-2 with BAEC nuclear extract failed to show the retarded band (lane 4). The DNA-protein complex was not observed in the presence of 100-fold molar excess of cold competitor encoding AP1 (ET-B, lane 5, and AP1, lane 7) but not by competitor encoding Region A sequence (ET-A1, lane 6).} \]

\[ \text{FIG. 4. Mutation of the AP1 site of the ET1 gene markedly decreases its ability to direct transcription. When transfected into BAEC, plasmid −204CAT, containing a native DNA fragment encoding bp −204 to +150 of the ET1 gene, directed high level chloramphenicol acetyltransferase gene expression. In contrast, plasmid AP1-MUT which differed from −204CAT by 2 mutated bp in the AP1 site had 30-fold lower activity. Plasmid −98CAT, containing an ET1 genomic fragment without Regions A and B, had only minimal promoter activity in BAEC.} \]
sessed the mobility of the DNA-protein complex following a binding reaction which included specific anti-Fos and anti-Jun antisera. Incubation of BAEC nuclear extract with the labeled ET1 AP1 probe resulted in retarded bands, consistent with binding of protein to the DNA fragment (Fig. 5, lane 2). Incubation of nuclear extract with Fos or Jun antisera generated supershifted bands (Fig. 5, lanes 3–6), representing antibody-Fos-Jun-DNA complexes. As a negative control, BAEC nuclear extract was incubated with an α2-antiplasmin antibody, and no supershifted band was observed. These data indicate that members of the Fos and Jun families present in BAEC nuclear extract bind to the ET1 AP1 site. It was not possible to shift or compete the entire protein-DNA complex with these antisera in these experiments (Fig. 5). Whether this represents binding to the AP1 sequence by members of the Fos and Jun families not recognized by these antibodies, the binding of unrelated proteins, or limitation of the experimental methodology is unclear.

**Fos and Jun Stimulate Expression Through the ET1 AP1 Sequence**—To determine whether binding to the ET1 AP1 consensus sequence by Fos and Jun reflected a functional interaction, we performed trans-activation experiments by cotransfecting chloramphenicol acetyltransferase reporter constructs and c-fos and c-jun expression vectors into BAEC (Fig. 6). Cotransfection of the expression vectors did not significantly increase the chloramphenicol acetyltransferase activity of the minimal SV40 promoter contained in pSPCAT. However, the chloramphenicol acetyltransferase activity of plasmid (3AP1)pSPCAT, which contained three synthetic ET1 AP1 sites fused to the SV40 promoter in pSPCAT, was increased 13-fold by c-fos and c-jun expression plasmids. To mimic the native arrangement of Regions A and B in the ET1 promoter, a synthetic DNA fragment encoding Region A was cloned 5′ to the 3 AP1 sites of (3AP1)pSPCAT and this reporter plasmid (1A + 3AP1)pSPCAT transfected into BAEC. Cotransfection of the c-fos and c-jun expression plasmids increased the expression of (1A + 3AP1)pSPCAT by 8-fold. When a native ET1 promoter was employed in the reporter construct (−204CAT), a 10-fold increase was observed in transcription rate in the presence of the c-fos and c-jun expression plasmids (data not shown). These data indicate a positive regulatory role for c-fos and c-jun in the high level ET1 gene transcription in BAEC in culture.

**DISCUSSION**

Reporter gene transfection experiments employing truncated and mutated ET1 promoters have implicated two regions as being involved in the high level expression of this gene in cells in culture (6, 7). In addition, a 119-bp fragment containing these two regions appears to increase transcription in an endothelial cell-specific fashion (6). The most 5′ region, Region A, contains a protein binding consensus sequence with a GATA motif (6, 7). Preliminary cloning and RNA blot data indicate that endothelial cells express a single member of this DNA-binding protein family, and that this protein is expressed in a wide variety of cell types. While this GATA protein is an important positive regulator of ET1 transcription, it cannot be primarily responsible for the restricted pattern of expression of this gene. In the experiments reported here, we have investigated Region B of the ET1 promoter. We have identified the sequence GTGACTAA as the only protein-binding site in this region and showed that it is a functional AP1 site. This sequence binds Jun and Fos, allows ET1 gene regulation by these trans-acting factors, and is necessary for the basal transcription of this gene in cells in culture.

AP1 sites have been found to be integral parts of promoters and enhancers associated with a number of eukaryotic genes (9, 17–23). This sequence was originally elucidated as the cis-acting sequence that allowed a transcriptional response to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (21, 24). The AP1 complex binding this DNA sequence was found to be a heterodimer composed of nuclear proteins encoded by c-jun and c-fos (9, 10, 21, 24–28). Recent studies have indicated increasing complexity in the assortment of proteins that can bind AP1 sites. Both the Fos family (c-fos, FosB, Fra-1, Fra-2) and the Jun family (c-jun, JunB, JunD) have been

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expanded with molecular cloning techniques (reviewed in Refs. 10 and 29). All members of these families are capable of binding to AP1 sites, although Fos members can bind only in association with a Jun family member. In addition, some members of the activation transcription factor and cyclic AMP-response-element-binding protein families of regulatory proteins can also bind to the AP1 consensus sequence and potentially modulate transcription of related genes (30, 31). One common feature of all these factors is a leucine zipper domain, believed to be important in heterodimer formation (32).

The AP1 site is generally considered to be a palindromic heptamer of sequence TGAG/CTCA. The role of each of these base pairs and various flanking sequences on the binding of Fos and Jun has been investigated (33). Risse et al. (33) could detect no binding of Fos and Jun to an AP1 site containing a core sequence similar to the ET1 AP1 site (TGAGTAA). It was thus important to investigate in a rigorous fashion the potential role of this sequence as a functional component of the ET1 promoter. The AP1 site studied in these experiments probably serves a number of roles in the regulation of the ET1 promoter. The AP1 site has classically been characterized as a hormone response element. In association with other genes, it has been shown to mediate a transcriptional response to 12-O-tetradecanoylphorbol-13-acetate, transforming growth factor-β, thrombin (24, 34–38). ET1 mRNA levels have been shown to be regulated by all of these factors, and this effect could be at the level of transcription, via the ET1 AP1 site (1, 40–42). In addition to its role in mediating hormone response, this AP1 site may be involved in regulating the basal level of transcription of the ET1 gene. Mutation of the AP1 site contained in a 350-bp segment of the ET1 promoter almost completely eliminated its ability to direct transcription in chloramphenicol acetyltransferase constructs (Fig. 4). Thus, AP1 is involved in determining the base-line rate of transcription of this gene in BAEC in culture. It is impossible to determine whether these findings reflect a true function of the AP1 site in vivo, or whether the base-line level of transcription of ET1 in cultured cells represents a response to stimulation by growth factors, hormones, and endotoxin. A transcriptional response to all of these factors could be mediated through the AP1 site. The rat stromelysin gene has been shown to mediate a transcriptional response to 12-0-tetradecanooylphorbol-13-acetate, hormone response, this AP1 site may be involved in regulating the base-line level of transcription of the ET1 gene, as evident from the recent characterization of a natural occurring mutant form of FosB (ΔFosB) (29). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cross-linked DNA-protein complexes will allow a more informative comparison of AP1-binding proteins present in different cell types.

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