

Interleukin-1 or phorbol induction of the stromelysin promoter requires an element that cooperates with AP-1

Karen Sirum-Connolly¹ and Constance E. Brinckerhoff^{1,2*}

Departments of ¹Biochemistry and ²Medicine, Dartmouth Medical School, Hanover, NH 03756, USA

Received September 24, 1990; Revised and Accepted December 7, 1990

ABSTRACT

Interleukin-1, a mediator of inflammation, or tumor promoting phorbol esters induce transcription of stromelysin, a metalloproteinase that degrades extracellular matrix molecules and that is overexpressed in diseases such as rheumatoid arthritis. Sequences required for induction of transcription of the human stromelysin promoter are contained on a 46 base pair fragment. This fragment contains a sequence with a high degree of similarity to the binding site for the transcription factor activator protein-1 (AP-1) and indeed, the AP-1 sequence of this fragment is necessary but not sufficient for the maximal response to phorbol 12-myristate 13-acetate (phorbol) or interleukin-1. Maximal induction requires functional cooperation between the AP-1 sequence and a neighboring upstream regulatory sequence (URS) of the stromelysin promoter which is also necessary but not sufficient. We demonstrate that both the AP-1 sequence and the URS bind phorbol or interleukin-1 induced nuclear proteins. Cooperation of the AP-1 sequence with another sequence present in the stromelysin promoter may be a general mechanism whereby the AP-1 element, which is found in many promoters, achieves a maximal and specific response to various stimuli.

INTRODUCTION

Interleukin-1 (IL-1) is an inflammatory mediator that is present in the synovial fluids from a variety of rheumatological diseases including rheumatoid arthritis (1). These diseases are characterized by the presence of excess amounts of collagenase and stromelysin, enzymes that degrade the extracellular matrix and that are produced predominantly by fibroblasts within the joint (2). We have studied mechanisms controlling the expression of stromelysin. Previously we reported that when cultures of normal human skin fibroblasts are treated with the tumor promoter phorbol 12-myristate 13-acetate (phorbol) or IL-1, expression of the mRNA for stromelysin is induced (3). We demonstrated that this induction is at the level of transcription: a 307 base pair (bp) fragment of the human stromelysin promoter linked to the reporter gene chloramphenicol acetyl transferase

(CAT), gave a 4 to 8 fold induction of CAT activity in response to phorbol or IL-1 β (3). This fragment contains a 9 bp sequence with 89% identity to the collagenase binding site for the transcription factor AP-1 (4), which is comprised of several proteins, some of which are the products of the fos and jun oncogene families (5). Others have reported that 3 repeats of an AP-1-like sequence can drive reporter gene expression in a mouse T lymphoma cell line in response to costimulation with IL-1 and phytohemagglutinin (PHA) (6), while another inflammatory mediator, tumor necrosis factor alpha, induces transcription through 5 tandem copies of the human collagenase AP-1 sequence in primary fibroblasts (7).

We have focused our studies on the native configuration of the human stromelysin promoter in which tandem repeating copies of the AP-1 sequence are not present (3). Here we report that a 46 bp fragment of the stromelysin promoter (designated 0.05S), which spans nucleotides -54 to -100 relative to the transcription start site (8), and which includes an AP-1 sequence is as responsive to phorbol or IL-1 as the longer promoter fragment when transiently transfected into normal human foreskin fibroblasts. Therefore, this fragment contains sequences important for IL-1 and phorbol regulation. We find that in normal fibroblasts, a single copy of the AP-1 sequence is not highly responsive to phorbol or IL-1 treatment: both the AP-1 sequence and additional sequences native to the stromelysin promoter are required for the maximal response. In addition, both regions have the ability to bind phorbol or IL-1 induced nuclear proteins.

MATERIALS AND METHODS

DNA Manipulations

The synthetic oligonucleotides (oligos) were made on a Biosearch Cyclone DNA synthesizer. All the oligos contained 5' Hind III and 3' Bam HI linkers and were cloned 5' to 3' relative to the thymidine kinase (TK) promoter driving chloramphenicol acetyl transferase (CAT) expression in the reporter plasmid pBLCAT2 (9). Cloning of synthetic oligos and DNA sequencing of the cloned oligos to confirm that the correct sequence was cloned was performed by standard techniques (10).

Cell Culture

Normal human foreskin fibroblasts were obtained from the Newborn Nursery at Dartmouth Hitchcock Hospital. Cells were

* To whom correspondence should be addressed

grown in Dulbecco's Modified Eagle's Medium with 10% fetal calf serum, were passaged 1:3 with trypsin, and were used between passages 6 and 13 as described (3).

DNA Transfection

Each plasmid used for transfection was purified through a cesium chloride gradient (10). For transfection, 10 μ g of plasmid DNA and 10 μ g of sheared calf thymus carrier DNA were transiently cotransfected into 5×10^5 fibroblasts in 60 millimeter (mm) dishes by the calcium phosphate coprecipitation method with glycerol shock as described (8, 11, 12). After a 24 hour recovery period in medium containing 10% fetal calf serum, the cells were washed to thoroughly remove the serum, transferred to serum free medium, and treated for 24 hours with 10^{-8} M phorbol or 400 U/mL human recombinant IL-1 β (a kind gift from Immunex Corp., Seattle, WA). Cells were harvested as described (13).

CAT Assay

CAT activity was measured by assaying percent incorporation of 14 C-chloramphenicol into the acetylated form by liquid scintillation counting of the appropriate regions of thin layer chromatography sheets as described (13). All assays were performed in the linear range and equal amounts of protein were used for each sample. Each plasmid, from at least two different plasmid preparations, was tested 3 to 10 times in cells from at least 3 different individuals. The fold induction of CAT activity upon treatment with 10^{-8} M phorbol or 400 U/mL or IL-1 β was normalized to the small degree of induction observed for the parental pBLCAT2 vector. Although we have observed that the magnitude of induction may vary from one batch of cells to the next, the relative activities of each of the constructs remains constant.

Nuclear Extracts

Nuclear extracts were prepared as described (14) from primary cultures of normal human foreskin fibroblasts. Cells were grown to subconfluence, placed in serum free medium for 16 hours prior to treatment for 2 hours with 10^{-8} M phorbol or 400 U/mL IL-1 β . Each set of extracts (untreated, phorbol, or IL-1 treated) shown in a figure was isolated from the same batch of fibroblasts at the same time to control for possible variations in levels of DNA binding proteins between different foreskins or between different passage numbers.

Gel Mobility Shift Assay

Five μ g of nuclear extract from untreated, phorbol, or IL-1 treated fibroblasts was incubated with approximately 1.5 ng of 32 P-labeled double stranded oligo probe (approximately 30,000 counts per minute, prepared using the Klenow fragment of DNA polymerase I), in the presence or absence of 5, 50 or 500 fold molar excess unlabeled oligo competitors as described (15,16). The unbound free probe was resolved from the DNA-protein complexes by electrophoresis through a 4% 29:1 nondenaturing acrylamide gel in a buffer containing 45 mM Tris base, 45 mM boric acid, and 1 mM ethylene diamine tetraacetic acid (EDTA), pH 8.5, as described (15). The gels were then exposed to Kodak XAR film at -70°C with intensifying screens for approximately 24 hours. Selected autoradiographs were quantitated by scanning with a densitometer (E-C Apparatus Corp.) and analyzed using a Hewlett-Packard 3390-A peak area integrator.

RESULTS AND DISCUSSION

Transcriptional Regulatory Regions of the Human Stromelysin Promoter

To further our understanding of how phorbol or IL-1 induce transcription, we sought to define the role of specific sequences contained on the human stromelysin promoter. We assayed the ability of fragments and mutants of the stromelysin promoter to drive reporter gene transcription in response to phorbol or IL-1. Figure 1 shows the sequence of a 46 bp fragment of the stromelysin promoter (designated 0.05S), spanning nucleotides -54 to -100 relative to the transcription start site (8). Also

A. HUMAN STROMELYSIN SEQUENCE ELEMENT SIMILARITIES

NIP like	PEA3 like	AP-1 like	
GTCTGCTGCCATTTGGATGAAAGCAAGGATGAGTCAAGCTGCGGGT			HuStrom 0.05S

GATCCTCTCACCTGCTGCCATGCTTCCCAT			NIP from Hu IL-3
*** *			
CAGGAAGT			PEA3 from polyoma virus enhancer

ATGAGTCAG			AP-1 from Hu Coll

B. OLIGONUCLEOTIDES

0.05S	GTCTGCTGCCATTTGGATGAAAGCAAGGATGAGTCAAGCTGCGGGT
mt0.05S	
Δ 1 AP-1	GTCTGCTGCCATTTGGATGAAAGCAAGGAGGAGTCAAGCTGCGGGT
mt0.05S	
Δ 3 URS	GTCTGCTGCCATTTTCTGAAAGCAAGGATGAGTCAAGCTGCGGGT
mt0.05S	
Δ 4 URS	GTCTTAGTCCATTTGGATGAAAGCAAGGATGAGTCAAGCTGCGGGT
URS	GTCTGCTGCCATTTGGATGAAA
mtURSA3	GTCTGCTGCCATTTTCTGAAA
mtURSA4	GTCTTAGTCCATTTGGATGAAA
SAP-1	GCAAGGATGAGTCAAGCTGCGGG
mtSAP-1	GCAAGGTGGAGTCAAGCTGCGGG

C. REPORTERS

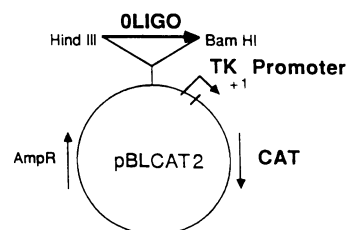


Figure 1a. DNA sequence of the 46 bp human stromelysin promoter fragment (HuStrom 0.05S), located -54 to -100 relative to the transcription start site (8), compared to sequences in the nuclear inhibitory protein DNA binding sequence (NIP) of the human IL-3 promoter (Hu IL-3) (23), the polyoma virus enhancer alpha domain element 3 (PEA 3) sequence (21), and the AP-1 binding sequence from the human collagenase gene (Hu coll) (4). Asterisk (*) indicates conserved nucleotides between the two sequences being compared. **b.** DNA sequences of the synthetic oligonucleotides (oligos) used in this study. The oligos were synthesized with Hind III and Bam HI linkers. The wild type 0.05S, URS and S AP-1 oligos are shown with the mutant oligos listed below. The bases changed in the mutants are underlined. **c.** Map of the reporter plasmid used to assay transcriptional activity of the stromelysin promoter fragments. Single copies of the oligos listed in Figure 1b were cloned 5' to 3' relative to the thymidine kinase (TK) promoter into the Hind III and Bam HI sites of the polylinker of pBLCAT2, a vector that contains the TK promoter in front of the chloramphenicol acetyl transferase (CAT) gene (9).

shown are the synthetic oligonucleotides (oligos) containing deletions and substitutions within this fragment, and the reporter constructs used to assay their activity.

Figure 2a compares the phorbol or IL-1 induced CAT activity of the full length 0.05S fragment to the activity of deletions of this fragment. When one copy of the 0.05S fragment is cloned in front of the thymidine kinase (TK) promoter, phorbol or IL-1 induces CAT activity of the 0.05S construct by an average of 5 fold relative to the TK CAT plasmid without any additional sequences (the parental pBLCAT2 plasmid is modestly responsive to phorbol or IL-1 giving a 1.5 to 2.0 fold induction therefore all the data presented are normalized to the induction of the TK promoter in the absence of additional sequences). Since the 5 fold induction of the 0.05S construct is the same degree of induction we previously reported for a 307 bp stromelysin promoter fragment (3), we conclude that this 46 bp fragment of the stromelysin promoter contains sequences important for phorbol or IL-1 induced transcription.

Within the 0.05S fragment is the AP-1-like element (see Figure 1). We made a 23 bp synthetic oligo, representing the 3' half of the 0.05S fragment which contains the 9 bp AP-1-like site and flanking nucleotides (S AP-1). Figure 2a shows that one copy of the S AP-1 DNA fragment is modestly responsive to phorbol or IL-1 (an average of 2–3 fold) but clearly indicates that the

additional sequences contained on the 0.05S fragment lead to a greater phorbol or IL-1 response. We term this additional region the upstream regulatory sequence (URS). Figure 2a also shows that the URS by itself, like the S AP-1 fragment, can confer a moderate degree of phorbol or IL-1 responsiveness on the TK promoter (an average of 3 fold), but is not as responsive as the 0.05S fragment. Therefore, in primary cultures of normal human fibroblasts, a single copy of the AP-1-like sequence is not highly responsive. Sequences in addition to the AP-1 sequence, native to the stromelysin promoter and in their normal single copy configuration, are necessary for the maximal response to phorbol or IL-1.

We further assayed the role of sequences contained on the 0.05S fragment by constructing mutations in the 0.05S fragment and by testing the activity of these mutants. Figure 2b shows the phorbol or IL-1 responsiveness of three mutant fragments relative to the activity of the wild type (wt) 0.05S fragment. A single base change in the AP-1-like element, which decreases AP-1 factor binding to the AP-1 binding site in the human collagenase promoter (17), diminished the IL-1 response of the 0.05S fragment to 35% of the wt activity and diminished the phorbol response to 55% of the wt activity. Thus the AP-1-like region is necessary for the maximal phorbol or IL-1 response of the 0.05S stromelysin promoter fragment.

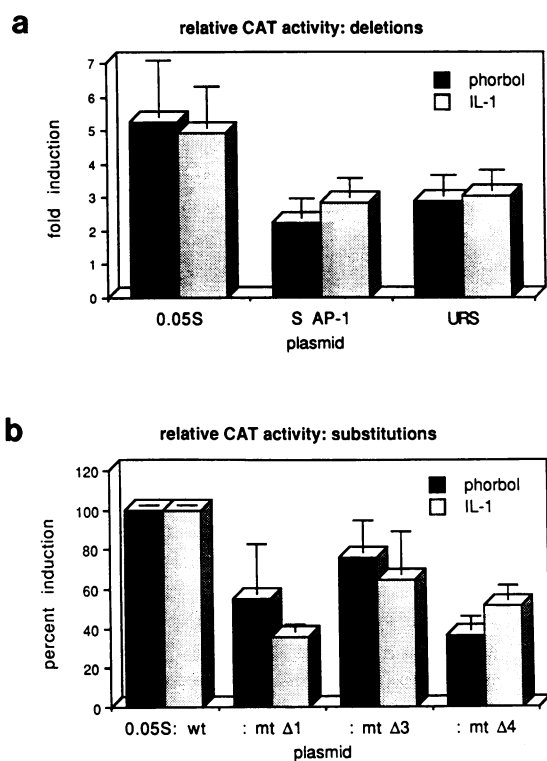


Figure 2a. Transcriptional activity of the human stromelysin 46 bp promoter fragment (0.05S) and of the deletions of this fragment. Normal human foreskin fibroblasts were transiently transfected with the indicated plasmid by the calcium phosphate method (see Materials and Methods for details). CAT activity was measured by assaying percent incorporation of ¹⁴C chloramphenicol into the acetylated form. The fold induction of CAT activity upon treatment with 10⁻⁸ M phorbol 12-myristate 13-acetate (phorbol) or 400 U/mL of human recombinant IL-1β was normalized to the small degree of induction observed for the parental pBLCAT2 vector. **b.** Transcriptional activity of 3 different mutant 0.05S stromelysin promoter fragments relative to the activity of the wild type fragment (0.05S). See the legend to Figure 2a for details.

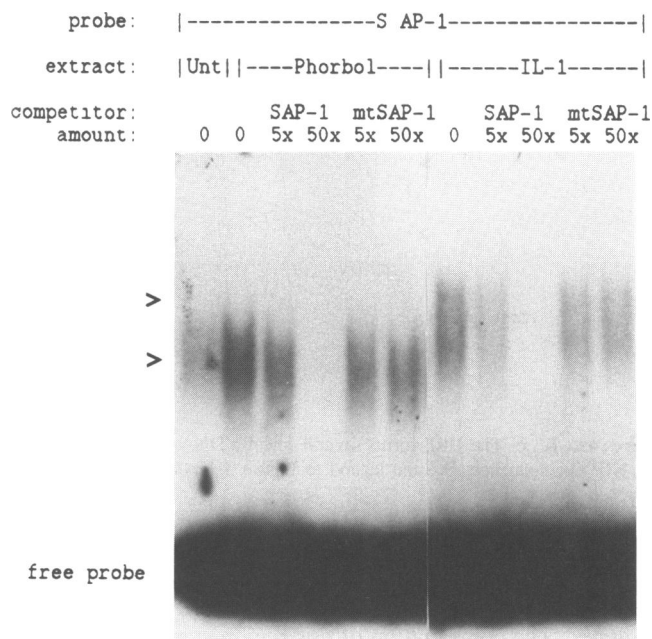
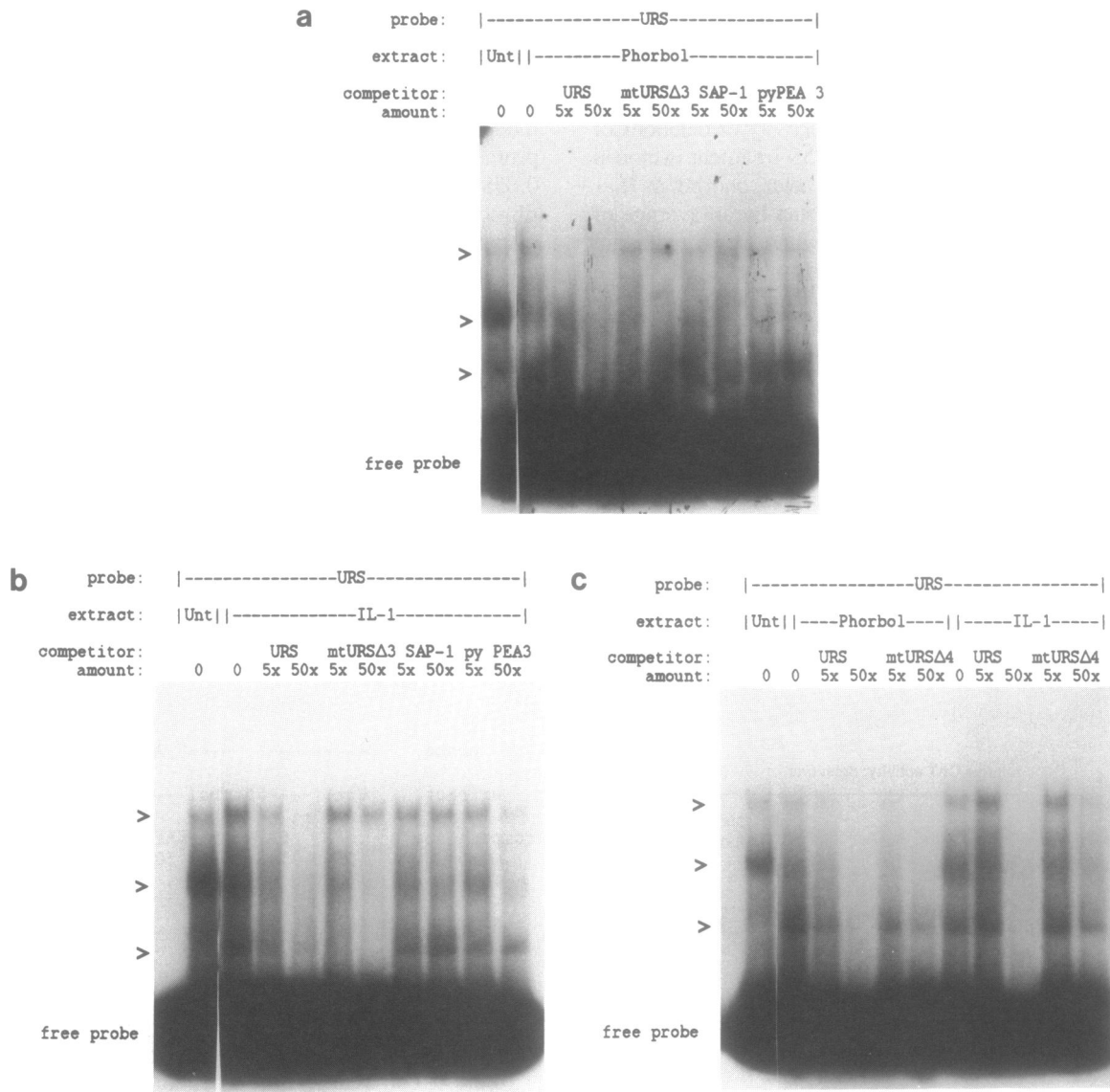


Figure 3. The S AP-1 sequence binds specific phorbol or IL-1 inducible nuclear proteins. Nuclear extracts were made from untreated human foreskin fibroblasts, or from cells that were treated with 10⁻⁸ M phorbol, or 400 U/mL IL-1 as indicated. Five μg of nuclear extract was incubated with ³²P-labeled double stranded oligo probe in the presence or absence of 5, 50 or 500 fold molar excess unlabeled oligo competitors. See Figure 1 for the names and sequences of all oligos used. The unbound free probe was resolved from the DNA-protein complexes by electrophoresis through a nondenaturing acrylamide gel. 'Unt' indicates nuclear extracts from cells that were processed in the same manner as those from phorbol or IL-1 treated cells except for the absence of either inducing agent. Arrowheads indicate specific complexes referred to in the text. Mobility shift experiments were performed on at least three different batches of nuclear extracts prepared from cells from at least two different individuals. Representative experiments are shown. In all batches of extract that demonstrated an increase in binding to the S AP-1 probe with phorbol or IL-1 treatment, we also saw the change in the binding pattern on the URS probe with phorbol or IL-1, as explained in the text.

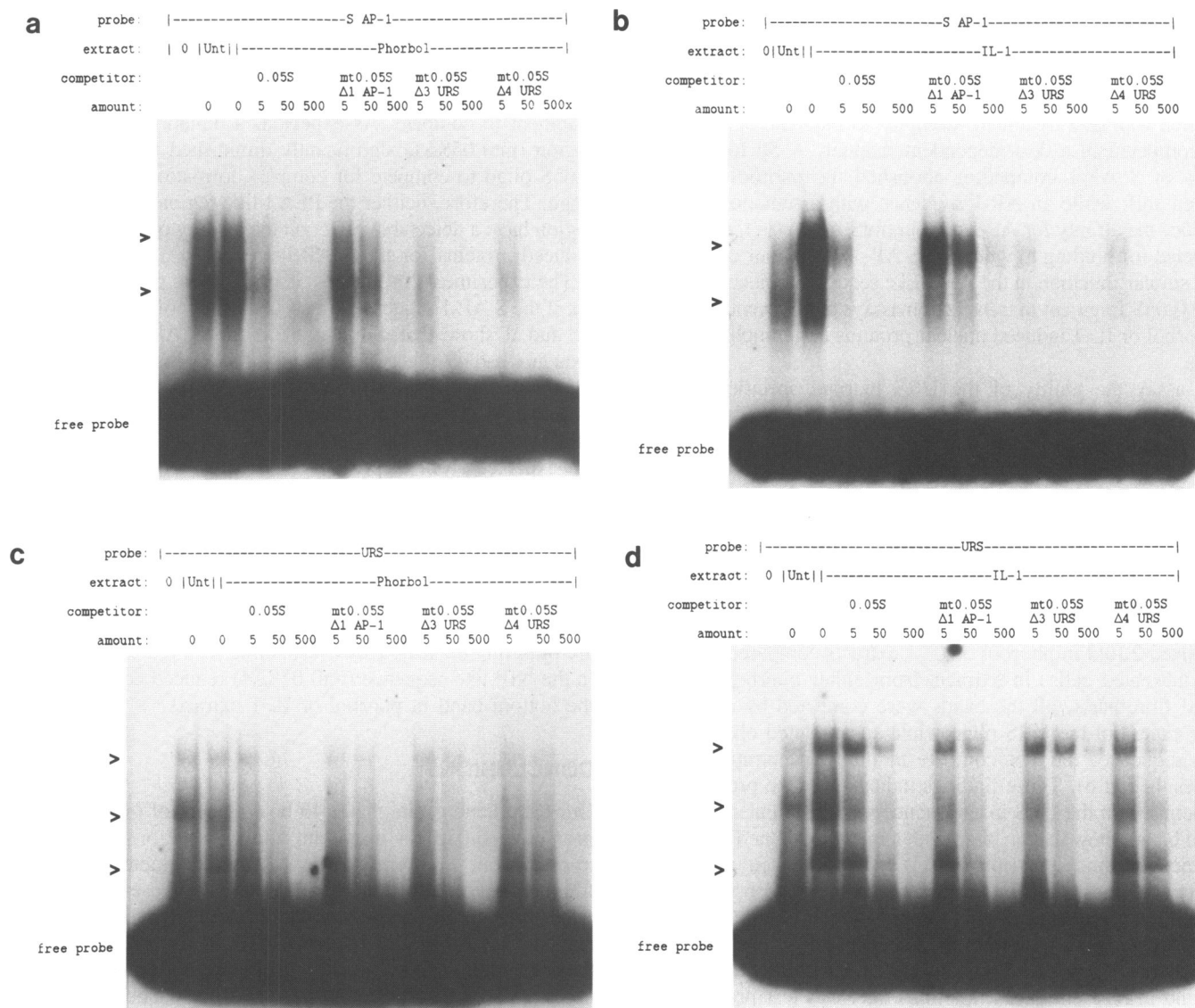


Figures 4a., b., c. The URS forms several specific DNA-protein complexes including a phorbol or IL-1 induced interaction dependent upon the presence of a wild type NIP-like sequence. See the legend to Figure 3 for details.

Upstream of the AP-1-like sequence, the URS region of 0.05S fragment contains a region conserved in the stromelysin and collagenase promoters of the human (4), rat (18), and rabbit (19, 20) genes. The conservation of this region suggests that it may have an important regulatory function in metalloproteinase gene expression and for this reason we undertook a mutational analysis of it. Others have recently shown that this region of the human collagenase gene has 75% sequence identity to polyoma virus enhancer alpha domain element 3 (PEA3) (21) and, indeed, it functions as a PEA3 element (22). The PEA3 element augments the response of the AP-1 sequence to phorbol or oncogenes in a fibroblast cell line when the PEA3/AP-1 unit is present as a tetramer in front of a heterologous promoter (22). However, as shown in Figure 1, the PEA3-like element of the stromelysin promoter is only 50% identical to the PEA 3 element originally identified in the polyoma virus enhancer (21) and only 63% identical to the human collagenase PEA 3-like element (22). We tested the putative role of this PEA3-like sequence in stromelysin gene expression by making a three base substitution in the

PEA3-like element in a region which others have shown to be required for PEA-3 activity (21). This mutation had a moderate effect on the phorbol or IL-1 response of the 0.05S fragment, decreasing the induction to approximately 70% of wt activity. Therefore, while a mutation in the AP-1-like region decreased the IL-1 induced activity to 35% of wt activity, the mutation in the PEA3-like element reduced IL-1 induction to 70% of wt activity. We conclude that this region of the stromelysin promoter, which has only limited sequence identity to the PEA3 element, does not play a major role in IL-1 induction. The differences between the phorbol induction of each of the mutants is not as great. The activity of the AP-1-like mutant is decreased to 55% of wt while that of the PEA3-like mutant is decreased to 70% of wt activity.

Immediately 5' of the PEA3-like sequence is a sequence with similarity to the nuclear inhibitor protein (NIP) binding sequence (23). In the human IL-3 promoter, a protein that is involved in negatively regulating the human IL-3 promoter binds to this sequence in activated T cells (23). The portion of the NIP



Figures 5a. and b. The sequences contained on the URS do not affect the affinity of the S AP-1 sequence for specific interactions with phorbol or IL-1 induced proteins. See the legend to Figure 3 for details. **c. and d.** The S AP-1 sequence does not affect the affinity of the URS for specific interactions with phorbol or IL-1 induced proteins. See the legend to Figure 3 for details.

sequence that is highly conserved between the stromelysin and the IL-3 promoters is also a region of the IL-3 promoter that is protected from chemical cleavage or DNase I digestion in the presence of nuclear proteins (23). We made a four base change in this region of the stromelysin promoter; the mutated bases included two of the protected G residues of the IL-3 promoter footprint (23). This mutation in the NIP-like region decreased the activity of the 0.05S fragment to 30 or 40% of wt phorbol or IL-1 induced activity. In contrast to its role as a negative regulatory element in the IL-3 promoter in activated T cells, this NIP-like sequence appears to function as a positive regulator in the stromelysin promoter in normal human fibroblasts. An alternative explanation is that the mutation made a negatively acting NIP-like sequence a stronger negatively acting element, thus preventing a strong positive response. However, based on the above data, the fact that mutating the NIP-like sequence has no detectable effect on the ability of the URS to compete for complex formation in untreated extracts (data not shown), and gel mobility shift data (see below) we favor the first explanation.

Therefore, 0.05S fragment of the stromelysin promoter requires at least two elements for the maximal response to phorbol or IL-1: the S AP-1 sequence and the NIP-like sequence of the URS.

Phorbol or IL-1 Induced Nuclear Proteins Interact with Specific Stromelysin Promoter Sequences

To further delineate the cooperative role of the S AP-1 sequence and the URS in producing the maximal induction of the 0.05S fragment in response to phorbol or IL-1, we assayed the ability of these fragments to bind IL-1 or phorbol induced nuclear proteins from normal human foreskin fibroblasts. We have observed that the basal level of DNA binding proteins varies from one batch of nuclear extracts to the next. However, the relative induction of binding activity with phorbol or IL-1 treatment is fairly constant. Figure 3 shows the nuclear protein complex formation when the S AP-1 oligo is used as a probe in gel mobility shift assays. Phorbol treatment leads to a greater amount of shifted probe compared to extracts from untreated cells. IL-1 treatment also increases the amount of shifted probe relative to extracts

from untreated cells, as well as resulting in the appearance of a slower migrating complex. Densitometric scanning of several different experiments indicates a 2 to 3 fold induction of DNA binding activity upon treatment with phorbol and a 2 to 4 fold induction with IL-1 treatment. Complex formation is specific and was competed in a dose dependent manner. A 50 fold molar excess of S AP-1 competitor abolished the phorbol or IL-1 induced shift while an AP-1 sequence with a mutation known to reduce the affinity for AP-1 protein (mtS AP-1) (17), no longer competed for binding to wild type S AP-1. Thus, our data show that a similar mutation in the AP-1-like sequence reduces activity of the 0.05S fragment in transfection assays and prevents binding of phorbol or IL-1 induced nuclear proteins in gel mobility shift assays.

To assay the ability of the URS to bind specific nuclear proteins, the experiments in Figure 4 were performed. Phorbol or IL-1 treatment leads to a change in the nuclear protein binding pattern on the URS probe compared to extracts from untreated cells. The top band is present in all extracts, the middle band is dominant in the untreated extracts, while the bottom band appears predominantly in phorbol or IL-1 extracts. Densitometric scanning of several different experiments indicates that the middle band is twice as intense in extracts from untreated cells compared to those from phorbol or IL-1 treated cells, while the bottom band is induced 2 fold in phorbol or IL-1 extracts compared to those from untreated cells. In extracts from either phorbol or IL-1 treated fibroblasts, all the bands were competed by a 50 fold molar excess of the URS oligo while an unrelated oligo, the S AP-1 sequence, did not compete at this concentration. (See Figures 4a and b). These findings indicate that the proteins are interacting with the URS in a sequence specific manner. Figures 4a and b also show that an oligo with a mutation in the PEA3-like sequence of the URS (mtURS Δ 3) had a reduced ability to compete for the formation of the top band in phorbol or IL-1 extracts, while it was still able to compete the bottom band. In fact, an oligo containing the PEA3 sequence from the polyoma virus enhancer (py PEA3) had a limited ability to compete the top band in IL-1 extracts while this effect was less pronounced in phorbol extracts. Since the top band is present in approximately the same amounts in extracts from untreated, phorbol, or IL-1 treated cells, and since the 0.05S fragment containing the PEA3-like mutation is still quite responsive to phorbol or IL-1, it is likely that the PEA3-like region of the URS has a limited role in phorbol or IL-1 regulation of the 0.05S fragment. However, we cannot rule out the possibility that even though the mutation in the PEA3-like sequence disrupts complex formation on the URS, it is not a functional mutation in a sequence required for induction.

Figure 4c shows that an oligo with a mutation in the NIP-like sequence (mtURS Δ 4) was still able to compete the top band in phorbol or IL-1 extracts. However, this mutant had a decreased ability to compete for bottom band formation compared to competitions with the wild type URS. The observed selective competition by the mutant oligos was confirmed by densitometric analysis. Therefore, there is a correlation between phorbol or IL-1 induced binding and phorbol or IL-1 induced activity of the 0.05S fragment involving the NIP-like sequence.

Characterization of the Protein-DNA Interactions on the Stromelysin Promoter Fragment

To begin to determine how the URS and S AP-1 sequence are cooperating, we tested whether the URS affects the affinity of the S AP-1 sequence. Figures 5a and b indicate that a 50 fold

molar excess of the 0.05S oligo abolished phorbol or IL-1 induced complex formation on the S AP-1 oligo and a mutation in the PEA3-like region (mt0.05S Δ 3) or the NIP-like region (mt0.05S Δ 4) had no detectable effect on the ability of the 0.05S fragment to compete. As expected, a mutation in the S AP-1 region (mt0.05S Δ 1) significantly diminished the ability of the 0.05S oligo to compete for complex formation on the S AP-1 oligo. Therefore, neither the PEA3-like region nor the NIP-like region have a detectable effect on the affinity of phorbol or IL-1 induced proteins for the S AP-1 sequence.

The experiments in Figures 5c and d were performed to find out if the S AP-1 sequence affects the affinity of the URS. Figure 5c and d show that a mutation in the S AP-1 (mt0.05S Δ 1) sequence had no effect on the ability of the 0.05S oligo to compete for binding to the URS probe in extracts from phorbol or IL-1 treated cells. Therefore, the S AP-1 sequence does not affect the affinity of phorbol or IL-1 induced nuclear proteins for the URS. As expected from the results in Figure 4, a mutation in the PEA3-like sequence (mt0.05S Δ 3) decreased the ability of the 0.05S oligo to compete for formation of the top complex from IL-1 extracts on the URS. We note that this mutation had a small effect in phorbol extracts, and allowed the 0.05S fragment to compete the bottom complex of IL-1 extracts better than the wt 0.05S oligo. Reasons for these potentially interesting observations are presently unclear. However, similar to Figure 4, a mutation in the NIP-like sequence (mt0.05S Δ 4) reduced competition for the bottom band in phorbol or IL-1 extracts.

CONCLUSIONS

Our data demonstrate that a 46 bp fragment of the stromelysin promoter (0.05S) is important for phorbol or IL-1 induced transcription. Further, we have defined elements within this fragment necessary for the response to these agents. The AP-1-like sequence is necessary but not sufficient for the maximal response and similarly, a sequence element contained on the URS, the NIP-like sequence of the stromelysin promoter, is also necessary but not sufficient for the maximal induction of the 0.05S fragment. Both the NIP-like region and the AP-1-like sequence are involved in binding phorbol or IL-1 induced nuclear proteins. The AP-1-like sequence and the NIP-like sequence cooperate, not through a detectable cooperative binding mechanism (see Figure 5), but either in an additive manner or through protein-protein interactions that increase the transactivating potential of the proteins bound to the 0.05S human stromelysin promoter fragment in response to phorbol or IL-1. In addition, a sequence that functions as a negative regulatory element in the human IL-3 promoter in activated T cells, seems to function as a positive regulator in the human stromelysin promoter when transfected into primary human fibroblasts.

AP-1 sequences are found in the promoters of many genes including human (4) and rabbit (20) collagenase and human IL-3 (23). In the human (22) and rabbit (Auble and Brinckerhoff, submitted) collagenase promoters, the PEA3 element and the AP-1 element functionally cooperate to provide the maximal response to phorbol. The human collagenase PEA3/AP-1 unit also drives transcription in response to the expression of oncogenes (22). In the IL-3 promoter, multiple elements, including an AP-1 sequence and the NIP element, cooperate to respond to phorbol in activated T cells (23). The human stromelysin promoter is also an example of a promoter that requires both an AP-1 sequence and another element(s) for the transcriptional response characteristic of endogenous gene

expression. It is possible that cooperation of the AP-1 element with other elements may provide a general mechanism whereby the AP-1 sequence is modulated to specifically and maximally respond to many different stimuli.

Interestingly, there is not a detectable significant difference between phorbol or IL-1 sequence requirements or in mobility shift banding patterns on the stromelysin promoter fragment. It is possible that different DNA binding proteins with the ability to give the same mobility shift pattern are involved. However, the similarities suggest that the signal transduction pathways of tumor promoting phorbol esters and inflammatory mediators such as IL-1 converge on the same transcriptional regulatory elements with the involvement of similar if not identical DNA binding proteins.

ACKNOWLEDGEMENTS

We thank Dr. Charles Cole, Dr. Paul Gardner, Dr. Nancy Speck, Steve Chamberlain, and Anne Delany for critical reading of the manuscript; Dr. David Auble for many helpful discussions; Immunex Corp. of Seattle, WA for the kind gift of human recombinant IL-1; and the Norris Cotton Cancer Center for defrayment of the cost of oligonucleotide synthesis. Karen Sirum-Connolly is a Norris Cotton Cancer Center Graduate Student Fellow. Supported by NIH grant #AR26599 and the RGK Foundation.

REFERENCES

- Mizel, S.B., Dayer, J.-M., Krane, S.M., and Mergenhagen, S.E. (1981)*Proc. Natl. Acad. Sci. U.S.A.* **78**, 2472.
- Harris, E.D., Jr. (1985) in *Textbook of Rheumatology*, Kelley, W., Harris, E.D., Jr., Ruddy, S., and Sledge, C., Eds., W.B. Saunders, Philadelphia, PA.
- Sirum, K.L., and Brinckerhoff, C.E. (1989)*Biochemistry* **28**, 8691.
- Angel, P., Baumann, I., Stein, B., Delius, H., Rahmsdorf, H.J., and Herrlich, P. (1987)*Mol. Cell. Biol.* **7**, 2256.
- Curran, T., and Franza, B.R., Jr. (1988)*Cell* **55**, 395.
- Muegge, K., Williams, T.M., Kant, J., Karin, M., Chiu, R., Schmidt, A., Siebenlist, U., Young, H.A., Durum, S.K. (1989)*Science* **246**, 249.
- Brenner, D.A., O'Hara, M., Angel, P., Chojkier, M., and Karin, M. (1989)*Nature* **337**, 661.
- Quinones, S., Saus, J., Otani, Y., Harris, E.D., Jr., and Kurkinen, M. (1989)*J. Biol. Chem.* **264**, 8339.
- Luckow, B., and Schutz, G. (1987)*Nucleic Acids Res.* **15**, 5490.
- Maniatis, T., Fritsch, E., and Sambrook, J. (1982)*Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Davis, C., Dignam, M., and Battey, J. (1973)*Basic Methods in Molecular Biology*, Elsevier, NY.
- Graham, F., and van der Eb, A. (1986)*Virology* **52**, 456.
- Gorman, C., Moffat, C., and Howard, B. (1982)*Mol. Cell. Biol.* **2**, 1044.
- Dignam, J.D., Lebowitz, R.M., and Roeder, R.G. (1983)*Nucleic Acids Res.* **11**, 1475.
- Ryan, W.A., Jr., Franza, B.R., Jr., and Gilman, M.Z. (1989)*EMBO J.* **8**, 1785.
- Gilman, M.Z., Wilson, R.N., and Weinberg, R.A. (1986)*Mol. Cell. Biol.* **6**, 4305.
- Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R.J., Rahmsdorf, H.J., Jonat, C., Herrlich, P., and Karin, M. (1987)*Cell* **49**, 729.
- Matrisian, L.M., Leroy, P., Ruhlmann, C., Gesnel, M., and Breathnach, R. (1986)*Mol. Cell. Biol.* **6**, 1679.
- Fritsch, S.M., and Ruley, H.E. (1987) *J. Biol. Chem.* **262**, 16300.
- Fini, M.E., Plucinska, I.M., Mayer, A.S., Gross, R.H., and Brinckerhoff, C.E. (1987) *Biochem.* **26**, 6156.
- Wasylyk, C., Flores, P., Gutman, A., and Wasylyk, B., (1989)*EMBO J.* **8**, 3371.
- Gutman, A., and Wasylyk, B. (1990)*EMBO J.* **9**, 2241.
- Mathey-Prevot, B., Andrews, N.C., Murphy, H.S., Kreissman, S.G., and Nathan, D.G. (1990)*Proc. Natl. Acad. Sci. U.S.A.* **87**, 5046.