Promoter elements in the transcriptional activation of the human stromelysin-1 gene by the inflammatory cytokine, interleukin 1

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Stromelysin-1, a tissue-remodelling metalloproteinase synthesized by fibroblasts, has proteolytic activity against a variety of extracellular matrix components. Stromelysin-1 gene transcription is induced by the inflammatory cytokine interleukin (IL)-1. In fibroblasts transiently transfected with constructs containing 5'-deletion mutants of the human stromelysin-1 gene promoter, IL-1-induced transcriptional activity was abolished with the removal of region -102 to -54. This region includes an AP-1 binding site at positions -70 to -64. The AP-1 site alone increased the basal activity of and conferred minimal IL-1 inducibility onto the heterologous gene promoter of thymidine kinase. Interestingly, although the removal of the AP-1 site from the native promoter (-1303 to +4) affected the absolute levels of IL-1-induced and basal promoter activity, it did not alter their ratio, indicating the involvement of regions outside the AP-1 site

in the IL-1 response. Of the stromelysin-1 5' flanking sequence examined, only the region -274 to -54 could confer IL-1 inducibility to a heterologous promoter independently of the AP-1 site. This region also bound specific nuclear factors. Further analysis revealed that the region composed of -86 to -71 and -63 to -54 could independently respond to IL-1 and bind protein of whole cell extracts. Protein binding to this region and to the AP-1 site was modestly induced by IL-1 treatment. From these results we conclude that, in fibroblasts, the AP-1 site (-70 to -64) is not necessary for the IL-1 response; however, it probably interacts through protein associations with the responsive region immediately surrounding it in the absolute transcriptional activation of the human stromelysin-1 gene by IL-1.

INTRODUCTION

Controlled deposition and turnover of extracellular matrix is critical to a number of basic biological processes which involve tissue remodelling, including the processes of embryogenesis, bone resorption and wound repair (for review, see [1]). Increasing evidence indicates that localized fluctuations in the activity of specific extracellular matrix-degrading metalloproteinases make significant contributions to this control [2,3]. So far, it has been possible to divide the human matrix-degrading metalloproteinases into three major subclasses (i.e. collagenases, type IV collagenases/gelatinases and stromelysins) on the basis of their substrate specificities (for review, see [4]). Stromelysin-1, the best characterized metalloproteinase of the stromelysin subclass, has a wide range of substrates, degrading collagen IV, fibronectin, laminin and cartilage proteoglycans [4,5].

The production of stromelysin-1 is under tight control. Enzyme synthesis and secretion are induced by a variety of treatments and extracellular signals such as cytokines, growth factors and oncogenic transformation [4,6,7]. Induction of human stromelysin-1 gene expression has been specifically linked to exposure of fibroblast cell cultures to the tumour-promoting phorbol ester phorbol 12-myristate 13-acetate (PMA) and the inflammatory cytokine interleukin (IL)-1 [8–11]. Conversely, stromelysin-1 gene expression is inhibited by retinoic acid, dexamethasone and transforming growth factor- β [9,10,12–15]. Sequence comparison of the rat, rabbit and human stromelysin-1 gene promoters reveals a high degree of similarity within the first 370 bp upstream

of the transcription start site, suggesting that this region may be critical to regulation [10]. At positions -70 to -64 in the human promoter is a region identical to the consensus binding site of the transcription factor AP-1 [8]; the sequence and position of this site are conserved in the rat and rabbit promoters. The AP-1 site is thought to contribute to the controlled expression of a number of genes. For example, the human collagenase AP-1 site confers inducibility to heterologous promoter constructs in response to tumour necrosis factor- α and PMA [16–18]. Interestingly, however, it has been demonstrated that mediation of native human collagenase gene transcription by PMA involves the co-operation of at least two protein-binding promoter domains, those of AP-1 and PEA3 [19].

We are particularly interested in the effects of IL-1 on human stromelysin-1 gene expression. IL-1 is secreted by activated monocytes and macrophages during the inflammatory response and is present in the synovial fluids of patients with rheumatoid arthritis [20]. In addition to promoting lymphocyte activation and adhesion to endothelial cells, IL-1 increases levels of stromelysin-1 and collagenase which, in turn, upset the extracellular matrix deposition/degradation equilibrium and mediate the invasive character of this disease [20,21]. By studying stromelysin-1 gene transcription in the context of the native promoter, we present here evidence that the AP-1 site and additional regions immediately surrounding it (-86 to -71 and -63 to -54) are involved in the response to IL-1 in human fibroblasts. The AP-1 site itself, however, is not necessary for activation of stromelysin-1 transcription by IL-1.

Abbreviations used: hGH, human growth hormone; IL, interleukin; NIP, nuclear inhibitor protein; PMA, phorbol 12-myristate 13-acetate; tk, thymidine kinase.

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EXPERIMENTAL

Cell culture

Primary human foreskin fibroblasts [10] and human hepatoma HepG2 cells (ATCC HB 8065) were maintained and subcultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum, penicillin (50 µg/ml), streptomycin (50 µg/ml) and neomycin (100 µg/ml). All experiments involving fibroblasts were performed between passages 4 and 10.

Plasmid construction

Human growth hormone (hGH) gene reporter constructs, containing the first 1.3 kb of the human stromelysin-1 gene promoter with [10] or without [8] the AP-1 site located between positions -70 and -64, were used to create *Bal31* nested deletion mutants essentially as described [22]. To generate mutants with varying 5' ends, the full-length 1.3 kb promoter constructs were linearized with *Xba*I, which cuts at -478, and then subjected to controlled *Bal31* digestion. The constructs were then cut with a second restriction endonuclease, *Hind*III, to remove all promoter sequence upstream of the *Xba*I site, followed by Klenow fill-in and religation. The exact deletion endpoints were determined by nucleotide sequence analysis [23].

To study the ability of specific stromelysin-1 5' flanking sequences to support transcription from a heterologous promoter, various fragments were attached upstream of the thymidine kinase (tk) gene promoter of BLGH [8]. The HXtkGH construct was obtained by cloning the corresponding *Hind*III/*Xba*I (-1303 to -479) fragment of the 1.3 kb wild-type stromelysin-1 promoter into *Hind*III/*Xba*I-digested BLGH. Similarly, XEtkGH was obtained by digesting the plasmid containing the 1.3 kb promoter with *Xba*I and *Eco*RI and cloning the fragment of interest (-478 to -275) into *Xba*I/*Bam*HI-digested BLGH after the addition of *Bam*HI linkers to the filled-in *Eco*RI site at position -275. To obtain EStkGH, the AP-1-lacking (-AP-1) 1.3 kb stromelysin-1 promoter [8] was first cut with *Eco*RI, the free ends were then filled-in with Klenow, and *Hind*III linkers were attached. The construct was then cut with *Sau*3A, which forms *Bam*HI-compatible ends, and the *Hind*III/*Sau*3A fragment (-274 to -54) was cloned into *Hind*III/*Bam*HI-digested BLGH. The construction of AP1-tkGH (p1XBLGH) and -53 stromelysin-1/hGH has been previously described [8,10].

Transient transfection and reporter gene assay

Transient gene expression assays using the hGH reporter constructs were performed on transfected HepG2 cells or primary human foreskin fibroblasts grown on 96- or 24-well microtitre plates respectively, as previously described [8]. Briefly, HepG2 cells were transfected overnight using the Cell Transfect Kit (Pharmacia); fibroblasts were transfected over 16 h using calcium phosphate. After rinsing, the media were then replaced. Human recombinant IL-1 β (5 units/ml) (Boehringer Mannheim) was added 24 h post-transfection and 48 h later the level of secreted hGH was measured by solid-phase radioimmunoassay (Nichols Institute, San Juan Capistrano, CA, U.S.A.). In HepG2 cells, the transfected DNA comprised 100% (w/w) reporter and was applied at a concentration of 50 ng/well; in fibroblasts, the amount transfected comprised 50% reporter/50% (w/w) carrier at a total concentration of 1.1 µg/well. Each experiment was performed in 3-8 wells for any given construct and treatment and was repeated at least three times.

Preparation of extracts

Nuclear extracts of foreskin fibroblasts were prepared according to the procedure of Dignam et al. [24]. Cells were scraped, collected by centrifugation and rinsed once in ice-cold PBS before the nuclei were isolated.

Whole cell extracts were prepared from fibroblasts grown in roller bottles (Bellco). The cells were removed by scraping the bottles with sterile glass beads (Fisher Scientific) and were collected by centrifugation. Total proteins were extracted essentially as described [25] with minor modifications. Phenylmethanesulphonyl fluoride (0.5 mM) and leupeptin (10 µM) were added to all buffers as additional proteinase inhibitors and total protein extract was desalted using a NAP-25 column (Pharmacia).

Protein concentrations of the extracts were determined using a bicinchoninic assay kit (Sigma Chemical Co.).

Gel shift

The *Eco*RI/*Ava*I (-274 to -11) fragment of the stromelysin-1 promoter was end-labelled using [α -³²P]dATP and the Klenow fragment of DNA polymerase I. Nuclear extracts (10 µg of protein) were incubated for 30 min at room temperature with 0.5 ng of probe (3000 c.p.m.) in a binding buffer (25 µl) containing 0.004 M Tris hydrochloride, pH 7.9, 0.012 M Hepes, pH 7.9, 0.06 M KCl, 0.001 M EDTA, 0.001 M dithiothreitol, 12% (v/v) glycerol, 100 µg/ml BSA and 1 µg of poly(dI-dC) (Pharmacia) [26]. In competing reactions, a 40-fold molar excess of the same unlabelled *Eco*RI/*Ava*I fragment and a 100-fold molar excess of unlabelled *Hae*III-digested ϕ X174 DNA were used as specific and non-specific competitors respectively. The DNA-protein complexes were separated in a non-denaturing 6% (w/v) polyacrylamide/Tris/glycine gel.

DNAase I footprinting

Whole cell extracts (100 µg of protein) were incubated with an *Ava*II/*Ava*I (-102 to -11) stromelysin-1 gene promoter fragment, labelled at position -11 on the coding strand using [α -³²P]dCTP and 1 ng of Klenow enzyme (20000 c.p.m.) in a total volume of 200 µl containing 0.4 µg of poly(dI-dC), 0.01 M Tris/HCl, pH 8, 0.005 M MgCl₂, 0.001 M CaCl₂, 0.1 M KCl, 0.002 M dithiothreitol, 50 µg/ml BSA and 2 µg/ml calf thymus DNA for 45 min at room temperature. DNAase I (0.32 units) was added for an additional period of 2 min at room temperature. Reactions were stopped, extracted and analysed in a denaturing 10% (w/v) polyacrylamide gel as described [27].

RESULTS

The region -102 to -54 of the human stromelysin-1 gene promoter is important to transcriptional activation by IL-1

In transient gene expression studies using 1.3 kb (-1303 to -11) of the human stromelysin-1 gene promoter and shorter fragments thereof we previously demonstrated that promoter activity is induced by IL-1 and that IL-1 response element(s) is(are) present within the region -478 to -11 [10]. In order to delineate more precisely the DNA elements contributing to the IL-1 response, we initially set out to locate those most proximal to the transcription start site by transient gene expression assay of *Bal31* deletion mutants. A group of mutants was constructed with progressive 5' promoter deletions, extending from -478 towards the transcription start site, and inserted into the hGH reporter vector. Table 1 shows that, as measured by the levels of

Table 1 Transient expression of 5'-deletion mutants of the stromelysin-1 gene promoter and contributions of the AP-1 binding site

For (a), 1.5 ng/ml secreted hGH in media corresponds to 6860 c.p.m. For (b), (c) and (d), 1.5 ng/ml secreted hGH in media corresponds to 3760, 400 and 1550 c.p.m. respectively. IL-1 response refers to the ratio of IL-1-induced activity to basal activity. For (c) and (d), growth hormone constructs containing 1.3 kb of stromelysin-1 gene 5' flanking sequence with (WT) or without (—AP1) the native AP-1 site located at positions —70 to —64 were used.

Expression system and construct transfected	Secreted hGH (c.p.m.)		IL-1 response
	— IL-1	+ IL-1	
(a) Primary human skin fibroblasts			
pΦGH	1531 ± 360	2302 ± 342	1.5
(—478 to —11) pΦGH	7148 ± 529	27785 ± 7340	3.9
(—201 to —11) pΦGH	5617 ± 1676	24448 ± 11147	4.4
(—102 to —11) pΦGH	2923 ± 602	12048 ± 1192	4.1
(—53 to —11) pΦGH	1938 ± 405	2192 ± 797	1.1
(b) Primary human skin fibroblasts			
BLGH (tkGH)	1209 ± 210	1420 ± 227	1.2
AP1-tkGH	2324 ± 1146	4103 ± 801	1.8
(c) Hep G2 cells			
WT	8440 ± 1750	22725 ± 7506	2.7
—AP1	1395 ± 155	1423 ± 361	1.0
(d) Primary human skin fibroblasts			
WT	4737 ± 1298	17216 ± 2520	3.6
—AP1	899 ± 374	4445 ± 1637	4.9

secreted hGH, stromelysin-1 promoter activity remains inducible by IL-1 treatment until the region —102 to —54 is deleted. Although both the absolute basal and induced promoter activities are reduced with the progressive shortening of the promoter fragment used to drive hGH transcription, IL-1 fold induction, i.e. the relative IL-1 response, expressed as the ratio between absolute induced and basal activity for a given construct, is not significantly affected by the deletion of —478 to —102. The basal and induced activities of the construct containing the shortest promoter fragment (—53 to —11) closely approximate those of the parent reporter vector, pΦGH, which contains no promoter sequence, and those of mock-transfected fibroblasts (results not shown), indicating that this construct has little if any promoter activity. From these results we conclude that the minimal region —102 to —54 of the human stromelysin-1 gene promoter is important to transcriptional activation by IL-1.

Involvement of the stromelysin-1 AP-1 site at —70 to —64 in IL-1-induced transcription

A sequence identical to the AP-1 binding site consensus, TGAGTCA, is located within the region —70 to —64 of the human stromelysin-1 promoter. This sequence has been found to bind the transcriptional activators c-Fos and c-Jun [8]. A number of reports have demonstrated that the AP-1 site alone attached to a heterologous promoter is responsive to a number of factors including PMA, retinoic acid and tumour necrosis factor-α [16–18]. We have also demonstrated that the human stromelysin-1 AP-1 site plays an important role in basal promoter activity [8]. Since the AP-1 site of the human stromelysin-1 gene falls within the minimal region shown to confer IL-1 inducibility, we were interested in determining whether the AP-1 site alone, removed from the context of the native promoter, could respond to IL-1 treatment. Therefore, a single copy of the AP-1 site attached upstream of the tk promoter in a hGH reporter vector was assayed for its response to IL-1 in transfected human fibroblasts. The results, presented in Table 1, demonstrate an increase in both the absolute basal and IL-1-induced promoter activity with

the addition of the AP-1 site to the tk promoter. The relative IL-1 response is only moderately increased when the AP-1 site is present in the construct, indicating that it is directly, but only weakly, responsive to IL-1 when isolated from its native promoter context.

Cell-type-specific contributions of the AP-1 site and additional regions of the stromelysin-1 gene promoter to IL-1 inducibility

To examine further the contributions of the AP-1 site and other regions of the stromelysin-1 gene promoter in the transcriptional activation conferred by IL-1, the activity of the native promoter, with the AP-1 site removed, was measured and compared with that of the completely intact promoter by transient gene expression assay. Results from a typical experiment are shown in Table 1. In HepG2 cells, removal of the AP-1 site from the stromelysin-1 promoter resulted in a decrease in absolute basal transcriptional activity as well as a complete loss of IL-1-induced activity. In primary fibroblasts, as in HepG2 cells, an absolute decrease in both basal and induced activity was observed using the —AP-1 promoter construct; however, the relative IL-1 response was not decreased compared with that of the intact promoter. Gene expression assays of promoter mutants containing single base changes within the AP-1 site and exhibiting decreased c-Fos and c-Jun binding activity [8] yielded similar results in fibroblasts; the absolute basal and induced activities were concomitantly reduced so that the relative IL-1 response remained unchanged in comparison to intact promoter activity (results not shown).

On the one hand, these data support the involvement of the AP-1 site in absolute basal and IL-1-induced stromelysin-1 promoter activity in fibroblasts. On the other hand, because the relative IL-1 response remained unchanged in fibroblasts even when the AP-1 site was removed, it is clear that the AP-1 site is not involved in the relative IL-1 response mechanism. Rather, it appears that other regions of the promoter act in conjunction in these cells to mediate the IL-1 response of the human stromelysin-1 gene.

Table 2 Enhancer activity of subregions of the 5' flanking region of the stromelysin-1 promoter

All constructs were transfected in primary human skin fibroblasts; 7500 c.p.m. corresponds to 1.5 ng/ml secreted hGH in media. IL-1 response refers to the ratio of IL-1-induced activity to basal activity.

Construct	Secreted hGH (c.p.m.)		IL-1 response
	– IL-1	+ IL-1	
BLGH	16916*	28696	1.7
(–1303 to –479) BLGH	7537	8891	1.2
(–478 to –275) BLGH	10565	12306	1.2
(–274 to –54)† BLGH	3032	15051	5.0

* Each value is the mean of 4–8 transfections.

† This promoter fragment does not contain the AP-1 site.

Figure 1 The human stromelysin-1 gene promoter (–274 to –11) binds soluble nuclear factors of control and IL-1-induced foreskin fibroblasts

An end-labelled double-stranded *EcoRI/AvaI* fragment was incubated with nuclear extract prepared from control fibroblasts (lanes 2–4) or fibroblasts treated for 5 h with 5 units/ml IL-1 (lanes 5–7). Specific (–274 to –11 fragment) and non-specific (*HaeIII*-digested ϕ X174) competitors were used in lanes 3 and 6 and lanes 4 and 7 respectively. Lane 1 contains probe electrophoresed without extract. Specific protein complexes (A–C) are indicated by arrows.

Identification of a region outside the AP-1 site that responds autonomously to IL-1 in primary fibroblasts

In order to identify other regions outside the AP-1 site that can independently respond to IL-1 treatment, different fragments of the first 1.3 kb of 5' flanking stromelysin-1 DNA were tested for their ability to enhance transcription from a heterologous promoter (tk in the BLGH reporter) in primary fibroblasts. Surprisingly, the results obtained, and presented in Table 2, indicate that all the stromelysin-1 promoter fragments tested could repress tk-directed basal transcription. For example, the fragment –274 to

Figure 2 Transient expression of 5' deletion mutants of the AP-1-less stromelysin-1 promoter

Ba31 deletion mutants of an AP-1-less stromelysin-1 promoter/hGH construct were assayed for their ability to direct hGH synthesis in control and IL-1-induced primary skin fibroblasts. The 5' endpoints of each promoter fragment tested is as indicated. The basal activity of the –53 construct was assigned a relative promoter activity of 1 and all other activities were expressed relative to this. The relative IL-1 response is indicated. The 3' endpoint of the constructs is +4, except that of the –53 construct which is –11. Results presented are the average of 8 transfections.

–54 repressed basal tk promoter activity 6-fold. However, the same fragment was the only one that could confer IL-1 inducibility to the tk promoter. In the typical experiment shown in Table 2 this enhancement was 5-fold. No contribution to the basal repression or the IL-1 response could be attributed to the AP-1 sequence located at positions –70 and –64 since it had been deleted from the –274 to –54 fragment before transfection. Because no regions tested upstream to the 1.3 kb of the transcription start site were able to independently confer IL-1 responsiveness to the tk promoter, their function must depend on the contributions of upstream or downstream sequences if they are involved in the IL-1 response *in vivo*.

The stromelysin-1 gene promoter (–274 to –11) binds the same nuclear factors in control and IL-1-induced fibroblasts

The ability of fibroblast nuclear extract components to bind specifically to the native stromelysin-1 gene promoter was examined by gel-shift assay (Figure 1). Using the fragment –274 to –11 as a probe and nuclear extract from control cells, six distinct complexes were observed. However, only three of these (A–C, Figure 1) appeared to be specific, as determined by separate competition experiments with an excess of the same fragment (specific competitor) and an excess of an unrelated DNA (non-specific competitor). Using nuclear extract from IL-1-induced cells, one of the non-specific complexes was lost but the number and migration rates of the specific complexes were the same as those obtained with control extract. Examination of the autoradiographic band intensities reveals that the amount of complex A is decreased, that of complex B is increased and that of complex C remains essentially unchanged in response to IL-1 treatment compared with controls. Gel-shift assays performed with nuclear extracts of fibroblasts at different passages gave identical results (results not shown). The results show that

Figure 3 Binding of proteins to specific sequences of the human stromelysin-1 gene promoter

DNAase I footprinting studies were performed using a fragment of the human stromelysin-1 promoter (–102 to –11) that was incubated either alone [lane 2; (–)], with whole cell extract of control fibroblasts (lane 3; –IL1) or with whole cell extract of fibroblasts induced with 5 units/ml IL-1 for 4 h (lane 4; +IL1). A G + A chemical sequence ladder (lane 1) serves as a marker. The lengths and positions of the protected regions (I, II and III) are stated in the text and depicted in Figure 4. These studies were performed in duplicate, resulting in the same protection pattern in each case.

although the region involved in independent IL-1 induction binds the same protein complexes whether or not IL-1 is present, IL-1 induction does cause some modulation in the amount of protein bound.

A minimal region surrounding the AP-1 site at –70 to –64 binds soluble factors and contributes to IL-1 inducibility

Once it was determined that a portion of the stromelysin-1 gene

promoter outside of the AP-1 site at positions –70 to –64 could autonomously respond to IL-1 treatment and that the same region interacted with soluble nuclear factors, we began to examine it in more detail in search of a defined IL-1 response sequence. By transient gene expression assay, we measured the IL-1 responsiveness of a series of deletion constructs of the stromelysin-1 gene promoter mutant containing no –70 to –64 AP-1 site (Figure 2). These experiments reveal that the deletion of the region –478 to –265 results in a significant and reproducible enhancement of the IL-1 response, possibly due to the presence of a negative regulatory sequence within the region. Furthermore, a large decrease in both absolute and relative IL-1 induction of transcriptional activity is observed when the promoter region –265 to –107 is deleted. There is again an increase in both absolute and relative induction in response to IL-1 following the further deletion of –107 to –86, possibly due to the contributions of another negative regulatory sequence. Inducibility is completely abolished with the removal of regions –86 to –71 and –63 to –54, indicating that this region contributes to the IL-1 response. No significant difference was detected in the basal transcriptional activity of any of the constructs tested.

The stromelysin-1 promoter protein-binding activity of whole cell extracts from control and IL-1-induced fibroblasts was investigated through DNAase I footprinting experiments. The results of such an experiment are shown in Figure 3. The footprint pattern reveals the presence of 3 areas of protection, I, II and III, in the promoter fragment –102 to –11 corresponding to the approximate positions of –85 to –76, –73 to –62 and –50 to –44 respectively (Figure 4). Area I falls within the minimal region shown to respond to IL-1 by transient gene expression assay of the –AP-1 deletion mutants (Figure 2). Area II includes the AP-1 site (–70 to –64) itself. IL-1 treatment results in a modest increase in the protection of all the regions.

DISCUSSION

The mechanisms by which the inflammatory cytokine IL-1 mediates activation of gene transcription have been the focus of extensive research in recent years. In this report we present evidence that, in the activation of human stromelysin-1 gene transcription in fibroblasts, IL-1 exerts its absolute effects through the co-operation of the AP-1 binding site at positions

Figure 4 IL-1-responsive region of the human stromelysin-1 gene promoter

Larger bold nucleotides denote IL-1 response region (–86 to –54). Approximate positions of nucleotides protected in DNAase I footprinting (I, II and III) are bracketed. Underlined region corresponds to region similar to that of rat haptoglobin A-element including 1 additional nucleotide in the centre of sequence. Sequences sharing similarity with the NIP [44] and the NF-IL6 consensus sequence are noted. A stromelysin-1 gene promoter sequence similar to positions 4 to 13 of the rat α_1 -acid glycoprotein DRE(AB) is on the complementary strand of the noted area and includes one additional nucleotide in the centre of the sequence.

–70 to –64 and the regions immediately surrounding it (–86 to –71 and –63 to –54), although the AP-1 site itself is not involved in the relative IL-1 induction mechanism. Several different promoter elements have been shown to respond to IL-1 depending on the gene. The simian virus 40 [28] and human IL-2 [29] gene promoters contain AP-1 sites that serve as IL-1 response elements. In addition to the AP-1 site, IL-1-induced simian virus 40 promoter activity involves a NF κ B-like binding site upstream of the AP-1 site, indicating the contribution of multiple *cis*-acting elements in the regulation of the cytokine response. Multiple elements have also been implicated in the IL-1 response patterns of a number of other genes, including human IL-6 [30–33], human IL-8 [34] and rat serum amyloid A1 [35]. IL-1 regulation of these genes involves both NF κ B and C/EBP-like binding sites. Neither of these sequences is similar to that of the IL-1 response region of the stromelysin-1 gene promoter surrounding the AP-1 site. However, as shown in Figure 4, within this additional response region lies a sequence, ATGAAAGCAAG, displaying similarity to the A-element of the rat haptoglobin promoter, ATGAAGCAAG [36]. The A-element was originally identified in the human haptoglobin promoter as one of three (A–C) separate but interacting regulatory regions [36]. Interestingly, the rat haptoglobin A-element has been identified as an IL-1 response sequence in hepatoma cells [37,38].

By gel shift and DNAase I footprinting analyses we have identified specific protein–DNA complexes and protein-protected regions within the stromelysin-1 promoter. We have not yet determined how the protein–DNA complexes and the precise protected regions are related. However, sequence comparison of the 3 areas resistant to DNAase I digestion reveals that areas I and II share the 5' nucleotides GGATGA (Figure 4). Whether this is any indication of common protein associations remains to be elucidated. In addition, it is noteworthy that area III lies outside of the promoter region found to be necessary for the IL-1 response. This suggests that this region is either not involved in IL-1-induced stromelysin-1 transcription or that its contribution to the IL-1 response is dependent upon the presence of additional upstream regions and the proteins that may come in contact with them. The fact that there is increased binding to this region in response to IL-1 favours the latter explanation.

In any event, IL-1 treatment caused only modest changes in the abundance of protein–DNA complexes as determined by gel shift and DNAase I footprinting experiments. No complexes or protected regions were lost and no additional complexes or protected regions arose. Other reports show that changes in DNA-binding activity do not always correlate with changes in transcriptional activity [28,34,39–43]. In addition to modulation in the amount of bound factors, IL-1-induced stromelysin-1 gene transcription may involve changes in factors that do not bind DNA directly, post-translational modifications and/or conformational changes in the DNA-binding proteins present in control extracts, and/or DNA modifications. These effects could then result in increased transcriptional activity without necessitating significant quantitative differences in DNA-binding factors.

Immediately upstream of the IL-1 response region described here is an 11 bp region identical to the nuclear inhibitor protein (NIP) binding site within the IL-3 promoter (Figure 4) [44]. Sirum-Connolly and Brinckerhoff [11] suggest that this region acts as a positive IL-1 and phorbol ester response element for human stromelysin-1 gene expression because a 4 nt mutation within this region diminished induced expression of a construct containing a –100 to –54 fragment of the stromelysin-1 promoter (including the AP-1 site) attached to the tk promoter.

However, we have found by transient gene expression analysis of *Bal31* deletion mutants that removal of the region –107 to –86, which contains the NIP-like sequence, actually augments the IL-1 response in the absence of the AP-1 site (Figure 2), suggesting that the deleted region contains a negative regulatory element. At present we do not know the basis of these differences in observations. Possibilities include the different IL-1 concentrations and media used for induction (5 units/ml in serum-containing medium versus 400 units/ml in serum-free medium), the different promoter context used in the assays, and the possibility that the mutant constructed by Sirum-Connolly and Brinckerhoff may actually be a stronger negative element than the wild-type. Similarly, it may be of significance to our apparently conflicting observations that NIP acts as a silencer of IL-3 transcription only in the absence of the adjacent AP-1 site. When the IL-3 AP-1 site is present, the negative effects of NIP seem to be overridden [44].

We have shown that the AP-1 site (–70 to –64) is required for basal expression of the human stromelysin-1 gene [8]. Here in fibroblasts we confirm those findings and also show that its contribution to the IL-1 response is dependent on the presence of additional responsive regions. Of the stromelysin-1 promoter fragments assayed, only the region –274 to –54 could respond to IL-1 independent of the AP-1 site. It is noteworthy that there is a decrease in absolute and relative induction by IL-1 with the deletion of region –265 to –107 in the –AP-1 promoter constructs that is only partially recovered with further deletion of the putative negative element. Within the –265 to –107 region are sequences displaying varying degrees of similarity to several *cis*-acting elements which include the NF-IL6 consensus sequence, TTNNGNAAT [32], and the complement of a portion of the IL-1-responsive DRE (AB) region of the rat α_1 -acid glycoprotein gene (Figure 4) [45,46]. At present we cannot yet rule out contributions of additional elements within 274 bp of the transcription start site or the contributions of regions even further upstream that may require interactions with other regions to be functional.

It is apparent, however, that precisely how stromelysin-1 transcription is regulated is at least in part a function of the cellular environment. Depending on the cell of study, different elements may play varying roles. In support of this, we have observed that the role of the stromelysin-1 AP-1 site (–70 to –64) in the IL-1 response mechanism is different in human HepG2 cells and in primary human fibroblasts. In HepG2 cells, there seems to be an absolute requirement for the AP-1 site in order to support the IL-1 response. In fibroblasts, however, the stromelysin-1 AP-1 site is not involved in the relative IL-1 response mechanism although it does contribute to basal activity and absolute levels of IL-1-induced activity. Other regions of the stromelysin-1 promoter mediate IL-1 induction of transcription in fibroblasts whether or not the AP-1 site is present. Since the same *cis*-acting DNA elements were assayed in each cell type, the regulatory differences presumably depend on cell-type-specific *trans*-acting elements or factors. IL-1 has been shown to induce AP-1 transcription factors in a cell-type-specific manner [29,47] and cell-type-specific differences in gene regulation have been observed for other IL-1-inducible genes [35,38,45,46]. Promoter environment also appears to play an important role in regulating gene transcription. We note here that when the AP-1 site was isolated from the native promoter environment, it conferred minimal IL-1 inducibility to a heterologous promoter (tk). Furthermore, the introduction of stromelysin-1 promoter sequence upstream of the tk promoter had a generalized effect of inhibiting the constitutive expression of the BLGH construct. It follows then that response element activity within a gene is

critically dependent on both the cellular background and the precise promoter structure [48–50]. It may prove to be more meaningful, therefore, to analyse transcriptional regulation from the standpoint of the native promoter context and the natural cellular background rather than from isolated DNA elements.

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REFERENCES

- Bernfield, M. (1989) *Curr. Opin. Cell Biol.* **1**, 953–1027
- Brenner, C. A., Adler, R. R., Rappolee, D. A., Pedersen, R. A. and Werb, Z. (1989) *Genes Dev.* **3**, 848–859
- Nakanishi, Y., Sugiura, F., Kishi, J.-I. and Hayakawa, T. (1986) *Dev. Biol.* **113**, 201–206
- Matrisian, L. M. (1990) *Trends Genet.* **6**, 121–125
- Okada, Y., Nagase, H. and Harris, E. D., Jr. (1986) *J. Biol. Chem.* **261**, 14245–14255
- Alexander, C. M. and Werb, Z. (1989) *Curr. Opin. Cell Biol.* **1**, 974–982
- Woessner, J. F., Jr. (1991) *FASEB J.* **5**, 2145–2154
- Buttice, G., Quinones, S. and Kurkinen, M. (1991) *Nucleic Acids Res.* **19**, 3723–3731
- Frisch, S. M. and Ruley, H. E. (1987) *J. Biol. Chem.* **262**, 16300–16304
- Quinones, S., Saus, J., Otani, Y., Harris, E. D., Jr. and Kurkinen, M. (1989) *J. Biol. Chem.* **264**, 8339–8344
- Sirum-Connolly, K. and Brinckerhoff, C. E. (1991) *Nucleic Acids Res.* **19**, 335–341
- Kerr, L. D., Miller, D. B. and Matrisian, L. M. (1990) *Cell* **61**, 267–278
- Kerr, L. D., Olashaw, N. E. and Matrisian, L. M. (1988) *J. Biol. Chem.* **263**, 16999–17005
- Nicholson, R. C., Mader, S., Nagpal, S., Leid, M., Rochette-Egly, C. and Chambon, P. (1990) *EMBO J.* **9**, 4443–4454
- Saus, J., Quinones, S., Otani, Y., Nagase, H., Harris, E. D., Jr. and Kurkinen, M. (1988) *J. Biol. Chem.* **263**, 6742–6745
- Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. and Karin, M. (1987) *Cell* **49**, 729–739
- Brenner, D. A., O'Hara, M., Angel, P., Chojkier, M. and Karin, M. (1989) *Nature (London)* **337**, 661–663
- Schonthal, A., Herrlich, P., Rahmsdorf, H. J. and Ponta, H. (1988) *Cell* **54**, 325–334
- Gutman, A. and Waslyk, B. (1990) *EMBO J.* **9**, 2241–2246
- Harris, E. D., Jr. (1988) in *Inflammation: Basic Principles and Clinical Correlates* (Gallin, J. I., Goldstein, I. M. and Snyderman, R., eds.), pp. 751–774, Raven Press, New York, NY
- Dinarello, C. A. (1988) in *Inflammation: Basic Principles and Clinical Correlates* (Gallin, J. I., Goldstein, I. M. and Snyderman, R., eds.), pp. 195–208, Raven Press, New York, NY
- Poncz, M., Solowiejczyk, D., Ballantine, M., Schwartz, E. and Surrey, S. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4298–4302
- Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463–5467
- Dignam, J. D., Martin, P. L., Shastry, B. S. and Roeder, R. G. (1983) *Methods Enzymol.* **101**, 582–598
- Manley, J. L., Fire, A., Samuels, M. and Sharp, P. A. (1983) *Methods Enzymol.* **101**, 569–583
- Chodosh, L. A. (1988) in *Current Protocols in Molecular Biology* (Ausubel, M. F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., eds.), John Wiley & Sons, New York, NY
- Brenowitz, M. (1988) in *Current Protocols in Molecular Biology* (Ausubel, M. F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., eds.), John Wiley & Sons, New York, NY
- Espel, E., Fromental, C., Reichenbach, P. and Nabholz, M. (1990) *EMBO J.* **9**, 929–937
- Muegge, K., Williams, T. M., Kant, J., Karin, M., Chiu, R., Schmidt, A., Siebenlist, U., Young, H. A. and Durum, S. K. (1989) *Science* **246**, 249–251
- Shimizu, H., Mitomo, K., Watanabe, T., Okamoto, S. and Yamamoto, K.-I. (1990) *Mol. Cell. Biol.* **10**, 561–568
- Zhang, Y., Lin, J.-X. and Vilcek, J. (1990) *Mol. Cell. Biol.* **10**, 3818–3823
- Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. and Kishimoto, T. (1990) *EMBO J.* **9**, 1897–1906
- Isshiki, H., Akira, S., Tanabe, O., Nakajima, T., Shimamoto, T., Hirano, T. and Kishimoto, T. (1990) *Mol. Cell. Biol.* **10**, 2757–2764
- Mukaida, N., Mahe, Y. and Matsushima, K. (1990) *J. Biol. Chem.* **265**, 21128–21133
- Li, X. and Liao, W. S. L. (1992) *Nucleic Acids Res.* **20**, 4765–4772
- Oliviero, S. and Cortese, R. (1989) *EMBO J.* **8**, 1145–1151
- Marinkovic, S. and Baumann, H. (1990) *Mol. Cell. Biol.* **10**, 1573–1583
- Baumann, H., Morella, K. K., Jahreis, G. P. and Marinkovic, S. (1990) *Mol. Cell. Biol.* **10**, 5967–5976
- Becker, P. B., Ruppert, S. and Schutz, G. (1987) *Cell* **51**, 435–443
- Devaux, B., Albrecht, G. and Keding, C. (1987) *Mol. Cell. Biol.* **7**, 4560–4563
- Herrera, R. E., Shaw, P. E. and Nordheim, A. (1989) *Nature (London)* **340**, 68–70
- Ron, D., Brasier, A. R., Wright, K. A., Tate, J. E. and Habener, J. F. (1990) *Mol. Cell. Biol.* **10**, 1023–1032
- Wilson, D. R., Juan, T. S.-C., Wilde, M. D., Fey, G. H. and Darlington, G. J. (1990) *Mol. Cell. Biol.* **10**, 6181–6191
- 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**, 5046–5050
- Won, K.-A. and Baumann, H. (1990) *Mol. Cell. Biol.* **10**, 3965–3978
- Won, K.-A. and Baumann, H. (1991) *Mol. Cell. Biol.* **11**, 3001–3008
- Yoza, B. K., Brooks, J. W. and Mizel, S. B. (1992) *Cell Growth Diff.* **3**, 677–684
- Ondek, B., Shepard, A. and Herr, W. (1987) *EMBO J.* **6**, 1017–1025
- van Dam, H., Offringa, R., Meijer, I., Stein, B., Smits, A. M., Herrlich, P., Bos, J. L. and van der Eb, A. J. (1990) *Mol. Cell. Biol.* **10**, 5857–5864
- Yamamoto, M., Ko, L. J., Leonard, M. W., Beug, H., Orkin, S. H. and Engel, J. D. (1990) *Genes Dev.* **4**, 1650–1662