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Cell-Type Specific Regulation of Human Interstitial Collagenase-1 Gene Expression by Interleukin-1β (IL-1β) in Human Fibroblasts and BC-8701 Breast Cancer Cells

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Abstract Interleukin-1β (IL-1β) is a potent cytokine that stimulates interstitial collagenase-1 (matrix metalloproteinase-1; MMP-1). In this study, we compared the mechanism(s) by which IL-1β induces collagenase gene expression in two very different cells, normal human foreskin fibroblasts (HFFs) and an aggressive breast cancer cell line, BC-8701 cells. Northern analysis showed that the time course of collagenase induction was distinct in the two cells: although both cells expressed low levels of MMP-1 constitutively, addition of IL-1β increased MMP-1 mRNA in HFFs by 1 h and levels remained high over a 24-h period. In contrast, MMP-1 levels in IL-1β-treated BC-8701 cells did not increase until 4 h, peaked by 12 h and then declined. To analyze the transcriptional response, we cloned and sequenced more than 4,300 bp of the human MMP-1 promoter, and from this promoter clone, we prepared a series of 5′-deletion constructs linked to the luciferase reporter and transiently transfected these constructs into both cell types to measure both basal and IL-1β induced transcription. When both cell types were uninduced, promoter fragments containing less than 2,900 bp gave only a minimal transcriptional response, while larger fragments showed increased transcriptional activity. With IL-1β treatment, significant responsiveness ($P < 0.001$) in HFFs was seen only with the larger fragments, while in the BC-8701 cells, all fragments were significantly induced with IL-1β. Finally, we found that IL-1β stabilized MMP-1 mRNA in normal fibroblasts, but not in BC-8701 breast cancer cells. We conclude that both the transcriptional and post-transcriptional regulation of MMP-1 gene expression by IL-1β is controlled by cell-type specific mechanisms, and we suggest that IL-1 induced MMP-1 expression in tumor cells and in neighboring stromal cells may amplify the invasive ability of tumor cells. J. Cell. Biochem. 66:322–336, 1977.

Key words: transcription; promoter; mRNA stability; nucleic acid sequence; matrix metalloproteinase

Collagenase-1 (matrix metalloproteinase-1; MMP-1) belongs to a family of at least 15 enzymes, each of which digests various components of the extracellular matrix [reviewed in Borden and Heller, 1997]. The collagenases degrade the interstitial collagens, types I, II and III, at neutral pH [Brinckerhoff, 1992; Arend and Dayer, 1993] and currently four of these enzymes have been identified. MMP-8 (collagenase-2) is a product of neutrophils [Harris, 1990, 1993; Brinckerhoff, 1992; Arend and Dayer, 1993] and chondrocytes [Cole et al., 1996]; MMP-13 (collagenase-3) was originally described in a breast carcinoma [Freije et al., 1994], but is also present in cartilage [Mitchell et al., 1996; Rebaul et al., 1996]; MT1-MMP contains a transmembrane segment [Sato et al., 1994] and has been demonstrated to digest the collagens types I, II and III [Ohuchi et al., 1997]; and MMP-1 (collagenase-1) is a major gene product of a variety of cell types that have been stimulated with phorbol esters, growth factors and cytokines [Harris, 1990; Brinckerhoff, 1992; Mackay et al., 1992; Borden and Heller, 1997]. Overexpression of all four collage-

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nases has been implicated in the pathophysiology of several diseases, including arthritis [Harris, 1990, 1993; Brinckerhoff, 1992; Arend and Dayer, 1993; Vincenti et al., 1996a] and tumor invasion/metastasis [MacDougall and Matrisian, 1995; Crawford and Matrisian, 1996]. In rheumatoid arthritis, the large quantity of MMP-1 produced by the proliferating fibroblasts that line the diseased joint is principally responsible for the irreversible destruction of collagens in cartilage, tendon and bone [Harris, 1990, 1993]. Similarly, destruction of the interstitial collagen is an integral part of tumor invasion and metastasis [MacDougall and Matrisian, 1995; Crawford and Matrisian, 1996; Barnes, 1988]. Along with the type IV collagenases (MMP-2 and MMP-9), which degrade basement membrane proteins, MMP-1 produced by tumor cells may contribute to this process by mediating degradation of collagen in vessel walls [Barnes, 1988], and facilitating the breakdown of connective tissue as the tumor cells metastasize to sites such as bone, lung and brain [MacDougall and Matrisian, 1995; Crawford and Matrisian, 1996].

Although some tumor cells produce high constitutive levels of MMP-1 [Templeton et al., 1990; Mackey et al., 1992; Allesandro et al., 1993; MacDougall and Matrisian, 1995; Crawford and Matrisian, 1996], in most cells, this production is low, and can be augmented by a variety of cytokines and growth factors. Among the most potent inducers are interleukin-1α and β. These are distinct proteins (17 kDa), synthesized mainly by monocytes and macrophages, that bind to a common transmembrane receptor and affect a variety of cellular functions, including proliferation, cell adhesion, immunomodulation, prostaglandin synthesis and induction of MMP-1 [Harris, 1990, 1993; Brinckerhoff, 1992; Arend and Dayer, 1993; Vincenti et al., 1996a; Dinarello, 1994; Karin, 1995; Deleuran et al., 1992]. Numerous in vivo and in vitro studies document that IL-1 is an important mediator in the pathogenesis of rheumatoid arthritis. In this disease, an autoimmune cascade recruits monocytes and macrophages to the inflamed joint, where these cells secrete IL-1 and induce MMP-1 gene expression in the synovial fibroblasts [reviewed in Arend and Dayer, 1993; Dinarello, 1994; and Deleuran et al., 1992; Kahle et al., 1992; Firestein et al., 1991]. Additionally, a role for IL-1 in tumor invasion is becoming apparent. IL-1 is found in several tumors [Tsuyuoka et al., 1994; Duncan et al., 1994; Reed et al., 1992; Suzuki et al., 1991; Wasserman et al., 1991], and may enhance tumor invasiveness [Mannel et al., 1994; Bani et al., 1991; Lauri et al., 1990; Giavazzi et al., 1990], perhaps by increasing adhesion to the vascular surface [Lauri et al., 1990; Giavazzi et al., 1990], stimulating tumor cell motility [Verhasselt et al., 1992] and inducing MMP-1 production. One tumor cell line with an aggressive collagenolytic phenotype is the BC-8701 breast carcinoma line. These cells were derived from a primary ductal infiltrating carcinoma, express MMP-1 constitutively, and respond to IL-1 [Allesandro et al., 1993].

Recent studies suggest that MMP gene expression may be regulated in a cell-type specific manner that may include both transcriptional and post-transcriptional mechanisms [reviewed by Benbow and Brinckerhoff, 1997]. Therefore, we compared the transcriptional and post-transcriptional regulation of collagenase-1 gene expression by IL-1β in normal skin fibroblasts and in the BC-8701 breast cancer cells. For transcriptional studies, we cloned and sequenced more than 4,300 bp of the human MMP-1 (collagenase-1) promoter and constructed a series of 5′-deletional fragments linked to the luciferase reporter. These constructs were transiently transfected into monolayer cultures of human foreskin fibroblasts (HFFs) and BC-8701 cells, and both basal and IL-1β stimulated transcription was assessed. For post-transcriptional analysis, we measured the stability of MMP-1 mRNA in both cell types, using a polymerase II inhibitor, 5,6-dichlorobenzimidizole riboside (DRB). We found that 1) the nucleic acid sequence of the human MMP-1 promoter contains a series of positive and negative cis-acting elements that may contribute to transcriptional regulation, 2) IL-1β induces transcription in fibroblasts and BC-8701 cells by different mechanisms and, 3) IL-1β substantially increases the half-life of collagenase mRNA in fibroblasts, but not in the BC-8701 breast cancer cells.

MATERIALS AND METHODS

Screening, Isolation, Sequencing and Characterization of a Genomic Clone Containing the Human Collagenase-1 Promoter

A 500 bp fragment derived from a BamH1/HindIII digest of a −517-+43 pCAT construct

(a generous gift from Dr. H.J. Ramsdorf) was used to screen a lambda phage EMBL3 human genomic library (Clontech). This library (peripheral blood leukocyte/Sau3AI partial digest) was plated at a density of approximately 1×10^5 plaques/150 mm plate and 10 plates were screened to represent a 2× coverage of the genome. Three positive clones were obtained and one clone remained after secondary and tertiary screening. Screening and preparation of bacteriophage DNA was performed according to standard methodology [Ausubel et al., 1987–1996; Sambrook et al., 1993]. The DNA isolated from the phage clone was digested with HindIII to take advantage of the HindIII site that had been reported previously at −4372 [Imai et al., 1994]. The HindIII fragment obtained was approximately 8 kb in length and was subcloned into pBSK− (Stratagene) and characterized by restriction digest analysis, which confirmed the presence and order of the restriction sites in the collagenase promoter as shown [Imai et al., 1994]. From the 8 kb fragment, 4.372 kb contained the human collagenase-1 promoter and was sequenced in its entirety. The top strand was sequenced using the ^{35}S-Sequenase™ (USB) method and the bottom strand was sequenced using the PRISM™ Ready Reaction Dyedeoxy™ Terminator Cycle Sequencing kit and the Model 373 Stretch/34 cm well system (Perkin-Elmer-Applied Biosystems, Inc.), following the standard protocol from the manufacturer. Computer analysis was done with the Transcription Factor Database (National Center for Biotechnology Information) and GeneInspector™ (Textco, Inc., Lebanon, NH) to determine the location of putative transcription factor binding site consensus sequences within the promoter.

Plasmid Constructs and 5′ Deletion Mutants

We used a human clone as a PCR template and primers at −517 bp and +63 bp to generate a fragment containing a HindIII site at the 5′ end and a BamHI site at the 3′ end. This product was sequenced and found to be identical to a previously published sequence [Angel et al., 1987a] except that our sequence contained a "T" where the published sequence contained an "A" at site −451 bp (see Fig. 2A). Since BamHI and BglII generate compatible overhanging ends, we ligated the PCR clone into the PXP2 luciferase vector using the BglII site in the polylinker [Brasier et al., 1989]. Through this process, the BamHI site is lost, so subsequent clones that were longer than −517 bp were cut with Kpn I at the 3′ end and ligated into this site at −517 bp ("PXP/517"). Some 5′-deletion promoter constructs were generated by using convenient restriction sites (HindIII at −4,372 bp; Pst1 at −3,300 bp; EcoR1 at −2,900 bp; EcoRV at −1,600 bp; and Bam H1 at −1,000 bp), blunting with Klenow and subcloning into PXP/517. In addition to the −512 bp construct, other deletional constructs (−125 bp, −315 bp, −776 bp, −1,948 bp, −2,240 bp, −3,156 bp and −4,008 bp) were constructed by PCR using primers that introduced a 5′ Hind III site with a −517 bp 3′ anchor, but (with the exception of −512 bp) are not presented because they did not provide any additional information (data not shown). All PCR constructs were sequenced to insure fidelity [Ausubel et al., 1987–1996; Sambrook et al., 1993].

Cell Culture

Human foreskins were obtained from the Birthing Pavilion at Mary Hitchcock Memorial Hospital (Lebanon, NH). Human foreskin fibroblasts (HFFs) were isolated as previously described [Delany and Brinckerhoff, 1992]. HFFs were grown and maintained in 150 mm diameter culture dishes (Sarstedt) in 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 µg/ml) in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) and used during passages 4–12 [Delany and Brinckerhoff, 1992]. BC-8701 breast carcinoma cells were obtained from Dr. David Woolley (University of Manchester, UK) [Allesandro et al., 1993], and were grown and maintained as described for the HFFs. For experiments, the cells were washed with Hank's balanced salt solution (HBSS; Gibco) and cultured in serum-free medium (DMEM plus 0.2% lactalbumin hydrolysate; DMEM/LH) supplemented with IL-1β (Promega) at a concentration of 10 ng/ml [Vincenti et al., 1994] and/or 5,6-dichlorobenzimidazole riboside at a concentration of 75 µM [Delany and Brinckerhoff, 1992] (DRB; Sigma).

Assay of Steady-State mRNA

Confluent cultures were washed and placed in 10 ml DMEM/LH and total cellular RNA was isolated using the TRIzol reagent (Gibco-BRL). Total RNA was quantitated by optical density, and 5–20 µg of RNA was subjected to Northern blot analysis [Ausubel et al., 1987–1996; Sambrook et al., 1993]. Blots were hybridized with

an [α^{32}P]-dCTP labeled probe (NEN; 12.5 µCi/reaction of 3,000 Ci/mmol) for 20 h at 56°C. Probes were labeled using the random prime labeling kit (Boehringer Mannheim). The blots were washed twice for 10 min at room temperature, followed by two 30-min washes at 56°C using 0.2× saline-sodium citrate, 0.5% sodium dodecyl sulfate. Hybridization probes used were cDNA clones of MMP-1 [Delany and Brinckerhoff, 1992] and c-jun (a gift from Dr. Inder Verma). Probe specific mRNA was detected by autoradiography. The signal obtained from the Northern blots was normalized to the signal for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to correct for slight differences in RNA loading.

Western Blot Analysis of Collagenase Protein

Culture medium (1 ml) was precipitated with 0.5 ml of cold 10% trichloroacetic acid (TCA) for 30 min on ice [Vincenti et al., 1994]. Protein was pelleted for 10 min at 13,000*g* at 4°C, briefly dried and resuspended in sample buffer. The samples were separated on 7.5% SDS-PAGE minigels, transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) using a Trans-Blot Cell (BioRad). Collagenase protein was detected as previously described [Vincenti et al., 1994]. Briefly, the membranes were blocked with 10% FBS for 30 min, then probed with human collagenase specific antiserum at a dilution of 1:10,000 overnight. The membranes were washed and specific antibody binding was detected using the Vectastain ABC kit (Vector Labs, Burlingame, CA).

Transfection and Luciferase Assay

The reporter plasmids cloned into the PXP2 luciferase vector were prepared using the Qiagen maxi prep kit (Qiagen). The plasmids were transiently transfected into HFFs or BC-8701 cells using the LipofectAMINE™ reagent (Gibco). Cells were plated at a density of 1.5×10^5/well in six-well cluster plates (Costar) in DMEM/10%FBS. The following day, the cells were washed, placed in serum free medium and transfected with 5 µg DNA and 7 µl LipofectAMINE reagent per well in HFFs, or 5 µg DNA and 2.5 µl LipofectAMINE reagent per well in the BC-8701 cells. After 5 h, 1 ml of DMEM/20%FBS was added. The next day, the cells were washed three times with HBSS and incubated in serum-free/LH medium alone or serum-free/LH medium containing 10 ng/ml IL-1β. Cell lysates were harvested 18–24 h after treatment and luciferase activity was determined on a ML 2250 Microtiter Plate Luminometer (Dynatech Laboratories) and reported as Relative Light Units (RLUs). The RLUs were normalized to the protein content of the lysate using a modification of the Lowry Assay (D_c Protein Assay, Biorad, Melville, NY). Each transfection was carried out in triplicate unless otherwise indicated; different preparations of DNA were used in at least four separate transfection experiments. Transfection efficiency was determined by Hirt's analysis as described previously [Ausubel et al., 1987–1996; Sambrook et al., 1993; Vincenti et al., 1994; White and Brinckerhoff, 1995].

mRNA Stability

5,6-Dichlorobenzimidazole riboside (DRB, Sigma) was dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 7.5 mM and diluted 1:100 for a final concentration of 75 µM [Ausubel et al., 1987–1996; Sambrook et al., 1993; Delany and Brinckerhoff, 1992]. Cells were cultured to confluency in 10% FBS, washed with HBSS and pre-treated with DMEM/LH alone, or with 10 ng/ml IL-1β for 8 h. Following pre-treatment, cells were washed with HBSS, replaced with fresh serum-free medium, and then treated with DRB. At various time points, cells were harvested for RNA using the TRIzol Reagent and assayed by Northern analysis as described above. Densitometry was performed on a Phosphorimager or Densitometer (Molecular Dynamics, PhosphorImager 445 SI) and analyzed with the IPLab Gel software (Signal Analytics Corporation).

RESULTS

Expression of the Endogenous Collagenase Gene in HFFs and BC-8701 Cells

As a first step in analyzing the induction of MMP-1 by these cells, we demonstrated that treatment with IL-1β increases collagenase mRNA and protein (Fig. 1). Cells were left untreated or were treated with IL-1β (10 ng/ml), and mRNA and culture medium were harvested at intervals. Northern blot analysis shows that untreated HFFs and BC-8701 cells (Fig. 1A and B, respectively) express low levels of collagenase mRNA, confirming previous studies [Vincenti et al., 1994; Delany and Brinckerhoff, 1992; Dalton et al., 1989; Mauviel et al.,

1988; Conca et al., 1991]. In HFFs, treatment with IL-1β increases collagenase mRNA within 1 h, and levels continue to increase substantially over the next 24 h (Fig. 1A). In BC-8701 cells, IL-1β-induced collagenase mRNA increases between 1 and 4 h, peaks by 12 h and then declines by 24 h (Fig. 1B). Thus, the time course of induction differs between the two cell types. We used Western blot analysis to confirm that levels of collagenase protein parallel levels of mRNA [Nagase et al., 1983]. Only low levels of collagenase protein are present in the culture medium of untreated cells, but in the presence of IL-1β, the levels increase (Fig. 1C and D). Since Western analysis measures an accumulation of protein in the culture medium over time, there is some increase in collagenase protein in the untreated cultures, as well as a more dramatic increase in the IL-1β treated cells.

Cloning and DNA Sequence Analysis of the Human MMP-1 Promoter

We cloned and sequenced both strands of a 4,372 bp clone of the human MMP-1 promoter

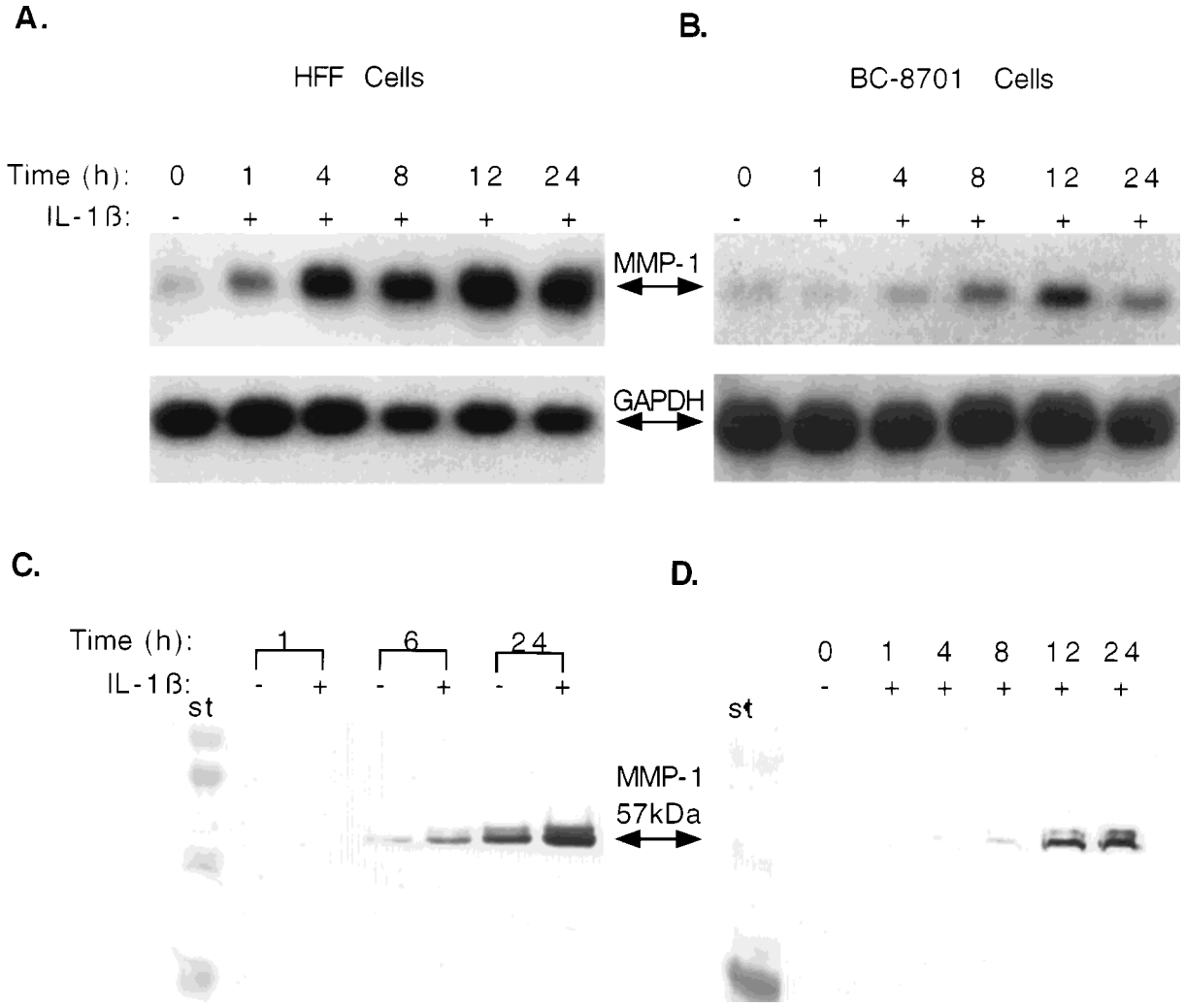

Fig. 1. Collagenase gene expression in human foreskin fibroblasts (HFFs) and BC-8701 cells. Confluent cultures were washed with HBSS and placed in DMEM/LH medium. Cells were left untreated or were treated with IL-1β (10 ng/ml), and mRNA and culture medium was harvested at intervals. **A and B:** RNA analysis. At the time points indicated, total RNA was isolated and 5 μg was subjected to Northern analysis. Collagenase mRNA was hybridized with a human MMP-1 cDNA probe and autoradiographed for 24 h. Control for loading is shown by using a probe specific for the housekeeping gene, GAPDH. **C and D:** Protein analysis. At the time intervals indicated, 1 ml of culture media was precipitated with 10% cold TCA, subjected to SDS-PAGE under reducing conditions, and transferred to a PVDF filter. Collagenase protein was visualized with a monospecific antibody and by using the Vectastain ABC kit. Low molecular weight standards (st) are shown in the first lane. The upper band is a glycosylated form of MMP-1 [Nagase et al., 1983]. −, no IL-1β added; +, IL-1β added. The arrows point to bands of interest.

(Fig. 2A). The proximal region of about 320 bp shares extensive homology with the rabbit MMP-1 promoter, including AP-1 sites at ~−70 and ~−186, a PEA3 element at ~−90, and a TTCA motif at ~−110 in both promoters [Angel et al., 1987a, 1987b; Imai et al., 1994; White and Brinckerhoff, 1995; Auble and Brinckerhoff, 1991; Chamberlain et al., 1993]. Each of these sequences is involved in transcriptional activation by phorbol myristate acetate, and their conservation between the two promoters confirms the experimental data, which shows that they are functionally important [White and Brinckerhoff, 1995; Auble and Brinckerhoff, 1991; Chamberlain et al., 1993].

Sequencing of the more distal regions reveals a number of AP-1 (consensus 5′-TGAGTCA-3′) and PEA3 (consensus 5′-AGGA-3′) sites. In a 700 bp region between −1,600 bp and −2,300 bp (Fig. 2A) there are several of these sites, and they have been implicated in basal transcription [Imai et al., 1994]. Additionally, PEA3 elements may contribute to MMP-1 gene expression, sometimes by cooperating with neighboring AP-1 sites [reviewed in Benbow and Brinckerhoff, 1997]. Of potential importance is the fact that our clone fails to contain the PEA3 site at −1,607 (5′-AAGA-3′), adjacent to the AP-1 site at −1,602 bp [Imai et al., 1994]. This may be due to an allelic variation, and may influence the transcriptional responsiveness of the promoter [Imai et al., 1994] (see Discussion). We also note the presence of the *Drosophila* homologue of an NFκB site (*dorsal;* 5′-ATGGAAAAA-3′) [Ghosh, 1991] at −2,886 bp. This site may participate in IL-1 inducibility, since NFκB sites are classic IL-1 response elements [Collins et al., 1995]. There are several putative silencer elements [Savagner et al., 1990; Cao et al., 1989; Moffat et al., 1996] located between −572 bp and −1,772 bp in the promoter that have the potential to repress transcription. In addition, there is an OCTA motif at −4,178 bp, which can also repress transcription [Zhen et al., 1996]. Two CREB sites at −1,540 and −3,187 bp are apparent, and it is possible that they, too, play a role in the down regulation of the collagenase gene by cAMP [Boyle et al., 1996]. Thus, the human collagenase gene may be regulated by a series of positive and negative cis-acting elements located throughout the promoter. Furthermore, these elements may function differentially in response to various stimuli and in a cell type-specific manner (see below).

Sequences in the Collagenase Promoter Control IL-1β Induced Transcription in a Cell Type-Specific Manner

We used our 4,372 bp clone of the human collagenase promoter to prepare a series of 5′-deletions, containing fragments of promoter DNA linked to the luciferase reporter (Fig. 2B). These fragments were transiently transfected into HFF and BC-8701 cells and both basal and IL-1β-induced transcriptional activity was measured. First, we examined basal transcription (solid bars, Fig. 3A and B), and found that in both cell types, fragments ranging from 512 bp to 1,600 bp gave only a minimal transcriptional response, while fragments greater than −1,600 bp showed increased transcriptional activity. This suggests that the basal activity is associated with distal regions of the promoter, and is similar in both cell types.

Next we tested the ability of each promoter construct to respond to IL-1β (hatched bars, Fig. 3A and B). In the HFFs, significant induction ($P < 0.001$) was seen only with fragments of the promoter containing at least 3,300 bp of

Fig. 2. The human collagenase-1 (MMP-1) promoter. **A:** Nucleic acid sequence analysis of 4,372 bp of the collagenase promoter. The MMP-1 promoter was cloned and sequenced as described in Materials and Methods. The underlined sequences from −2,265 bp through −1,549 bp and −517 bp through +63 bp have been previously reported [Imai et al., 1994, and Angel et al., 1987a, respectively]. The major consensus transcription factor elements are shown in boxes (including the TATA box). The putative silencers (P Sil) and anti-silencers (P Antisil) are either double underlined (forward direction) or single underlined (reverse direction) and the number of mismatches to previously described elements is shown in parentheses [Savagner et al., 1990; Cao et al., 1989; Moffat et al., 1996; Stover et al., 1994]. The single asterisks denote a discrepancy between our sequence and the previously reported sequence [Angel et al., 1987a; Imai et al., 1994]. The double asterisk indicates that our sequence does not contain a PEA3 element at −1,607 bp as previously described [Imai et al., 1994], since a "G" is absent. The arrow indicates the beginning of transcription. **B:** 5′-deletion constructs of the human MMP-1 promoter linked to the luciferase reporter. The full length (4,372 bp) construct shown indicates the various locations of the restriction sites which were used to generate the clones as indicated in the Materials and Methods. The asterisks show the two AP-1 sites that have been previously reported [Angel et al., 1987a, 1987b] and are included for orientation. The luciferase reporter is represented by the gray box.

A.

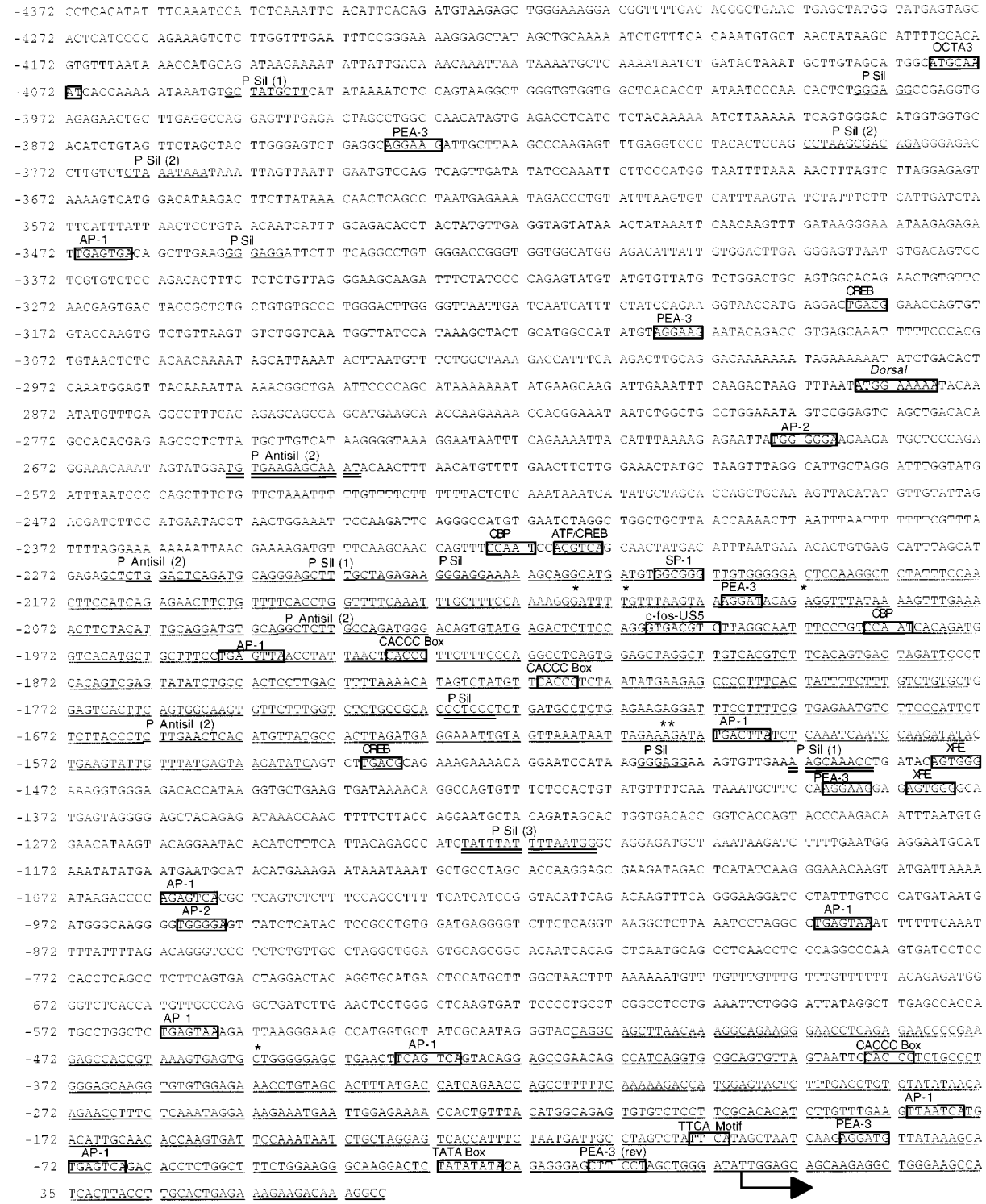
-4372 CCTCACATAT TTCAAATCCA TCTCAAATTC ACATTCACAG ATGTAAGAGC TGGGAAAGGA CGGTTTTGAC AGGGCTGAAC TGAGCTATGG TATGAGTAGC
-4272 ACTCATCCCC AGAAAGTCTC TTGGTTTGAA TTTCCGGGAA AAGGAGCTAT AGCTGCAAAA ATCTGTTTCA CAAATGTGCT AACTATAAGC ATTTTCCACA
-4172 GTGTTTAATA AACCATGCAG ATAAGAAAAT ATTATTGACA AACAAATTAA TAAAATGCTC AAAATAATCT GATACTAAAT GCTTGTAGCA TGGCATGCAT
-4072 ATCACCAAAA ATAAATGTGC TATGCTTCAT ATAAAATCTC CAGTAAGGCT GGGTGTGGTG GCTCACACCT ATAATCCCAA CACTCTGGGA GGCCGAGGTG
-3972 AGAGAACTGC TTGAGGCCAG GAGTTTGAGA CTAGCCTGGC CAACATAGTG AGACCTCATC TCTACAAAAA ATCTTAAAAA TCAGTGGGAC ATGGTGGTGC
-3872 ACATCTGTAG TTCTAGCTAC TTGGGAGTCT GAGGCAGGAA GATTGCTTAA GCCCAAGAGT TTGAGGTCCC TACACTCCAG CCTAAGCGAC AGAGGGAGAC
-3772 CTTGTCTCTA AATAAATAAA TTAGTTAATT GAATGTCCAG TCAGTTGATA TATCCAAATT CTTCCCATGG TAATTTTAAA AACTTTAGTC TTAGGAGAGT
-3672 AAAAGTCATG GACATAAGAC TTCTTATAAA CAACTCAGCC TAATGAGAAA TAGACCCTGT ATTTAAGTGT CATTTAAGTA TCTATTTCTT CATTGATCTA
-3572 TTCATTTATT AACTCCTGTA ACAATCATTT GCAGACACCT ACTATGTTGA GGTAGTATAA ACTATAAATT CAACAAGTTT GATAAGGGAA ATAAGAGAGA
-3472 TTGAGTGACA GCTTGAAGGG GAGGATTCTT TCAGGCCTGT GGGACCGGGT GGTGGCATGG AGACATTATT GTGGACTTGA GGGAGTTAAT GTGACAGTCC
-3372 TCGTGTCTCC AGACACTTTC TCTCTGTTAG GGAAGCAAGA TTTCTATCCC CAGAGTATGT ATGTGTTATG TCTGGACTGC AGTGGCACAG AACTGTGTTC
-3272 AACGAGTGAC TACCGCTCTG CTGTGTGCCC TGGGACTTGG GGTTAATTGA TCAATCATTT CTATCCAGAA GGTAACCATG AGGAGTGACG GAACCAGTGT
-3172 GTACCAAGTG TCTGTTAAGT GTCTGGTCAA TGGTTATCCA TAAAGCTACT GCATGGCCAT ATGTAGGAAG AATACAGACC GTGAGCAAAT TTTTCCCACG
-3072 TGTAACTCTC ACAACAAAAT AGCATTAAAT ACTTAATGTT TCTGGCTAAA GACCATTTCA AGACTTGCAG GACAAAAAAA TAGAAAAAAT ATCTGACACT
-2972 CAAATGGAGT TACAAAATTA AAACGGCTGA ATTCCCCAGC ATAAAAAAAT ATGAAGCAAG ATTGAAATTT CAAGACTAAG TTTAATATGG AAAAATACAA
-2872 ATATGTTTGA GGCCTTTCAC AGAGCAGCCA GCATGAAGCA ACCAAGAAAA CCACGGAAAT AATCTGGCTG CCTGGAAATA GTCCGGAGTC AGCTGACACA
-2772 GCCACACGAG AGCCCTCTTA TGCTTGTCAT AAGGGGTAAA GGAATAATTT CAGAAAATTA CATTTAAAAG AGAATTATGG GGGAAGAAGA TGCTCCCAGA
-2672 GGAAACAAAT AGTATGGATG TGAAGAGCAA ATACAACTTT AACATGTTTT GAACTTCTTG GAAACTATGC TAAGTTTAGG CATTGCTAGG ATTTGGTATG
-2572 ATTTAATCCC CAGCTTTCTG TTCTAAATTT TTGTTTTCTT TTTTACTCTC AAATAAATCA TATGCTAGCA CCAGCTGCAA AGTTACATAT GTTGTATTAG
-2472 ACGATCTTCC ATGAATACCT AACTGGAAAT TCCAAGATTC AGGGCCATGT GAATCTAGGC TGGCTGCTTA ACCAAAACTT AATTTAATTT TTTTCGTTTA
-2372 TTTTAGGAAA AAAAATTAAC GAAAAGATGT TTCAAGCAAC CAGTTTCCAA TCCACGTCAG CAACTATGAC ATTTAATGAA ACACTGTGAG CATTTAGCAT
-2272 GAGAGCTCTG GACTCAGATG CAGGGAGCTT TGCTAGAGAA GGGAGGAAAA AGCAGGCATG ATGTGGCGGC TTGTGGGGGA CTCCAAGGCT CTATTTCCAA
-2172 CTTCCATCAG AGAACTTCTG TTTTCACCTG GTTTTCAAAT TTGCTTTCCA AAAGGGATTT TGTTTAAGTA AAGGATACAG AGGTTTATAA AAGTTTGAAA
-2072 ACTTCTACAT TGCAGGATGT GCAGGCTCTT GCCAGATGGG ACAGTGTATG AGACTCTTCC AGGGTGACGT CTTAGGCAAT TTCCTGTCCA ATCACAGATG
-1972 GTCACATGCT GCTTTCCTGA GTTAACCTAT TAACTCACCC TTGTTTCCCA GGCCTCAGTG GAGCTAGGCT TGTCACGTCT TCACAGTGAC TAGATTCCCT
-1872 CACAGTCGAG TATATCTGCC ACTCCTTGAC TTTTAAAACA TAGTCTATGT TCACCCTCTA ATATGAAGAG CCCCTTTCAC TATTTTCTTT GTCTGTGCTG
-1772 GAGTCACTTC AGTGGCAAGT GTTCTTTGGT CTCTGCCGCA CCCTCCCTCT GATGCCTCTG AGAAGAGGAT TTCCTTTTCG TGAGAATGTC TTCCCATTCT
-1672 TCTTACCCTC TTGAACTCAC ATGTTATGCC ACTTAGATGA GGAAATTGTA GTTAAATAAT TAGAAAGATA TGACTTATCT CAAATCAATC CAAGATATAC
-1572 TGAAGTATTG TTTATGAGTA AGATATCAGT CTTGACCCAG AAAGAAAACA GGAATCCATA AGGGGAGGAA AGTGTTGAAA AGCAAACCTG ATACAGTGGG
-1472 AAAGGTGGGA GACACCATAA GGTGCTGAAG TGATAAAACA GGCCAGTGTT TCTCCACTGT ATGTTTTCAA TAAATGCTTC CAAGGAAGGA GAGTGGGGCA
-1372 TGAGTAGGGG AGCTACAGAG ATAAACCAAC TTTTCTTACC AGGAATGCTA CAGATAGCAC TGGTGACACC GGTCACCAGT ACCCAAGACA ATTTAATGTG
-1272 GAACATAAGT ACAGGAATAC ACATCTTTCA TTACAGAGCC ATGTATTTAT TTTAATGGGC AGGAGATGCT AAATAAGATC TTTTGAATGG AGGAATGCAT
-1172 AAATATATGA ATGAATGCAT ACATGAAAGA ATAAATAAAT GCTGCCTAGC ACCAAGGAGC GAAGATAGAC TCATATCAAG GGAAACAAGT ATGATTAAAA
-1072 ATAAGACCCC AGAGTCACGC TCAGTCTCTT TCCAGCCTTT TCATCATCCG GTACATTCAG ACAAGTTTCA GGGAAGGATC CTATTTGTCC CATGATAATG
-972 ATGGGCAAGG GGTGGGGAGT TATCTCATAC TCCGCCTGTG GATGAGGGGT CTTCTCAGGT AAGGCTCTTA AATCCTAGGC CTGAGTAAAT TTTTTCAAAT
-872 TTTATTTTAG ACAGGGTCCC TCTCTGTTGC CTAGGCTGGA GTGCAGCGGC ACAATCACAG CTCAATGCAG CCTCAACCTC CCAGGCCCAA GTGATCCTCC
-772 CACCTCAGCC TCTTCAGTGA CTAGGACTAC AGGTGCATGA CTCCATGCTT GGCTAACTTT AAAAAATGTT TGTTTGTTTG TTTGTTTTTT ACAGAGATGG
-672 GGTCTCACCA TGTTGCCCAG GCTGATCTTG AACTCCTGGG CTCAAGTGAT TCCCCTGCCT CGGCCTCCTG AAATTCTGGG ATTATAGGCT TGAGCCACCA
-572 TGCCTGGCTC TGAGTAAAGA TTAAGGGAAG CCATGGTGCT ATCGCAATAG GGTACCAGGC AGCTTAACAA AGGCAGAAGG GAACCTCAGA GAACCCCGAA
-472 GAGCCACCGT AAAGTGAGTG CTGGGGGAGC TGAACTTCAG TCAGTACAGG AGCCGAACAG CCATCAGGTG CGCAGTGTTA GTAATTGCAC CCTCTGCCCT
-372 GGGAGCAAGG TGTGTGGAGA AACCTGTAGC ACTTTATGAC CATCAGAACC AGCCTTTTTC AAAAAGACCA TGGAGTACTC TTTGACCTGT GTATATAACA
-272 AGAACCTTTC TCAAATAGGA AAGAAATGAA TTGGAGAAAA CCACTGTTTA CATGGCAGAG TGTGTCTCCT TCGCACACAT CTTGTTTGAA GTTAATCATG
-172 ACATTGCAAC ACCAAGTGAT TCCAAATAAT CTGCTAGGAG TCACCATTTC TAATGATTGC CTAGTCTATT CATAGCTAAT CAAGAGGATG TTATAAAGCA
-72 TGAGTCAGAC ACCTCTGGCT TTCTGGAAGG GCAAGGACTC TATATATACA GAGGGAGCTT CCTAGCTGGG ATATTGGAGC AGCAAGAGGC TGGGAAGCCA
35 TCACTTACCT TGCACTGAGA AAGAAGACAA AGGCC
OCTA3
P Sil
P Sil (1)
PEA-3
P Sil (2)
AP-1
P Sil
P Antisil (2)
OCTA3
CREB
PEA-3
Dorsal
AP-2
P Antisil (2)
OBP
ATF/CREB
P Antisil (2)
P Sil (1)
SP-1
PEA-3
c-fos-US5
OBP
CACCC Box
AP-1
CACCC Box
P Sil
P Antisil (2)
AP-1
CREB
P Sil
P Sil (1)
XRE
PEA-3
XRE
P Sil (3)
AP-1
AP-2
AP-1
AP-1
CACCC Box
AP-1
TTCA Motif
PEA-3
AP-1
TATA Box
PEA-3 (rev)

Figure 2

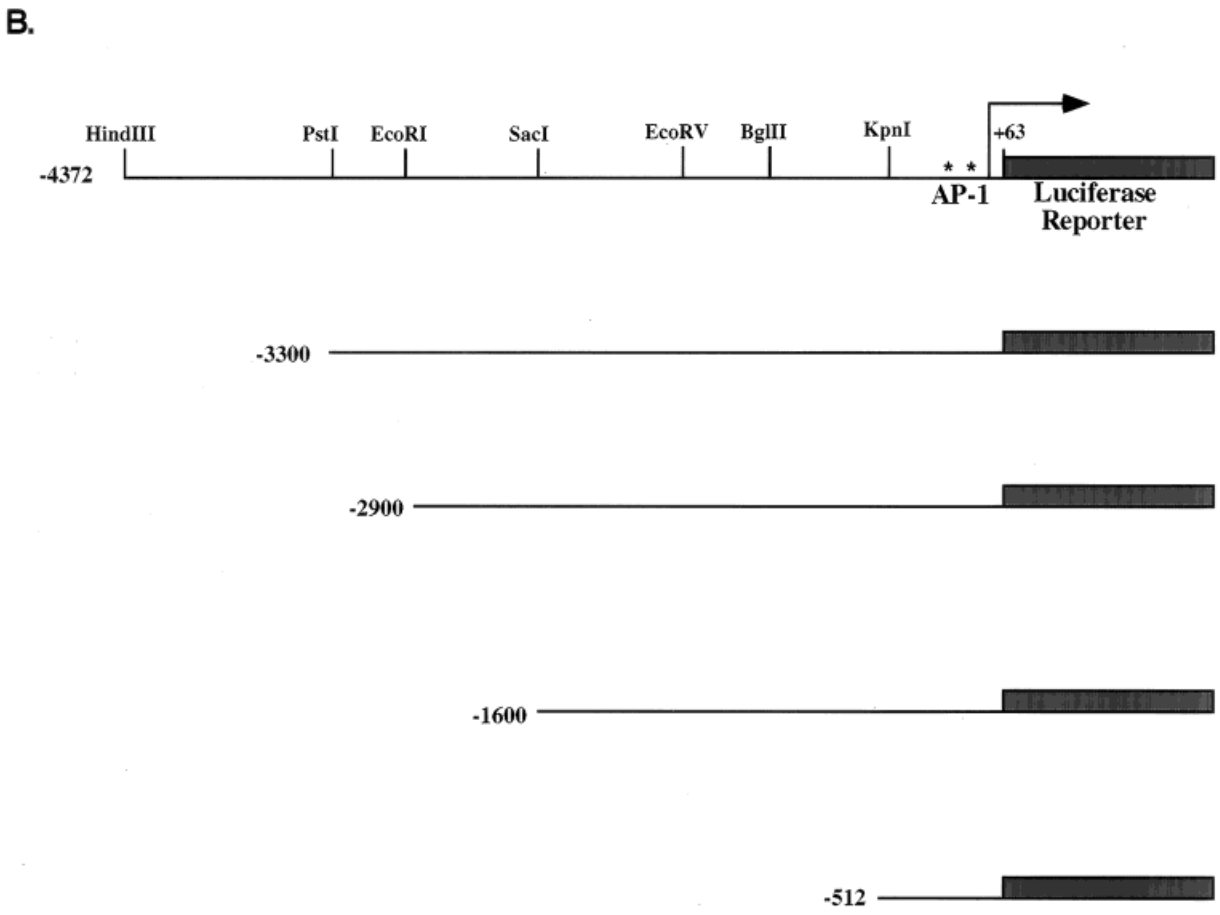

Figure 2 (continued)

DNA. In four separate experiments (Fig. 3A and data not shown), this induction was 1.5- to 3-fold, and is in keeping with the effect of several other cytokines and growth factors on MMP transcription [MacCachren et al., 1989; Delany and Brinckerhoff, 1992; Sudbeck et al., 1994; Sanz et al., 1995]. Moreover, the data suggest that, like basal transcription, the IL-1β-induced response is associated with the distal portions of the promoter. However, experiments with the IL-1 receptor antagonist indicate that this basal activity is not due to endogenous IL-1 production by these HFFs (data not shown).

Somewhat different results were seen when we examined IL-1β-induced transcription in the BC-8701 breast cancer cells (hatched bars, Fig. 3B). In contrast to the fibroblasts, all of the fragments tested in the BC-8701 cells responded significantly ($P < 0.001$) to IL-1β, including the smaller constructs. In all experiments, Hirt's assays [Vincenti et al., 1994; Ausubel et al., 1987–1996; Sambrook et al., 1993; White and Brinckerhoff, 1995] were performed to control for variations in the amount of transfected DNA taken up by the cells [Vincenti et al., 1994; White and Brinckerhoff, 1995], and demonstrate that the transfection efficiency of each construct was similar (data not shown). Thus, the results are due to the ability of the different promoter fragments to be transcriptionally activated. Our data indicate that proximal regions of the promoter can mediate IL-1β induction in the BC-8701 cells, but not in the HFFs, and suggest that the mechanisms governing IL-1β induction are different in the two cell types (see Discussion).

Expression of Endogenous Jun in HFFs and BC-8701 Cells

Induction of MMP-1 by phorbol esters involves an increase in c-JUN, which binds to AP-1 sites located at about −70 bp and −180 bp in the rabbit and human MMP-1 promoter [Angel et al., 1987a, 1987b; Auble and Brinckerhoff, 1991; White and Brinckerhoff, 1992; Chamberlain et al., 1993]. An increase in c-JUN has

also been implicated in the induction of MMP-1 by IL-1β [Conca et al., 1991; Lafyatis et al., 1990], and the data presented in Figure 3 suggest that these proximal AP-1 sites may participate in MMP-1 induction in BC-8701 cells, but not in HFFs. Therefore, we measured levels of c-jun mRNA in both cell types. Cells were left untreated or treated with IL-1β, and mRNA was harvested at various time intervals. Northern analysis of the HFFs (Fig. 4A) shows that jun is not detectable constitutively, but upon treatment with IL-1β, jun levels increase within 1 h, decrease by 6 h and return to low or undetectable levels by 12 h. Interestingly, the BC-8701 cells show a different pattern of jun expression (Fig. 4B). Low, but detectable levels of jun are seen constitutively (time "0"), and like the HFFs, increase by 1 h. However, unlike the HFFs, jun stays elevated. These results support the transcriptional data presented in Figure 3A and B, since the higher level of jun expression in BC-8701 cells may account for the significant induction with IL-1β in the smaller constructs.

IL-1β Increases the Stability of Collagenase mRNA in HFFs but Not in BC-8701 Cells

Previous studies of IL-1 induced collagenase gene expression in rabbit fibroblasts indicate that, along with transcription, mRNA stability contributes to the increase in steady-state mRNA [Vincenti et al., 1994]. The data presented in Figure 1 suggest there might be a post-transcriptional component to the regulation of MMP-1 in human fibroblasts, but not in the BC-8701 breast cancer cells. Treatment of HFFs with IL-1β results in a prolonged increase in collagenase mRNA and protein, while the increase in the BC-8701 cells is more transient. Since the transcriptional responses appear similar (Fig. 3), it is possible that post-transcriptional mechanisms account for this difference.

Therefore, we used the transcriptional inhibitor 5,6-dichlorobenzimidazole riboside (DRB) to measure the stability of MMP-1 transcripts [Delany and Brinckerhoff, 1992] in both cell types (Fig. 5). Cells were cultured to confluency and were pre-treated with serum-free medium alone, or with 10 ng/ml IL-1β [Dinarello, 1994] for 8 h. DRB was then added to selected cultures; RNA was harvested, assayed by Northern analysis and quantitated by phosphorimaging and densitometry. Treatment of cultures with DRB for more than 10 h resulted in cytotoxic effects, thus points beyond that time were not measured. Figure 5A and B shows the effects of IL-1β on mRNA stability in HFFs and BC-8701 cells. Several points are apparent. First, in untreated cultures of HFFs, there is a relatively rapid decay of the collagenase mRNA. Second, in HFFs pre-treated with IL-1β, this decay is abrogated, a finding that supports previous studies, which indicate a role for mRNA stability in regulating MMP-1 gene expression in rabbit fibroblasts [Vincenti et al., 1994]. Third, the stability of MMP-1 mRNA is greater in untreated cultures of BC-8701 cells than in the untreated HFFs, but IL-1β does not further stabilize the mRNA. Thus, our data demonstrate that IL-1β increases mRNA stability in human fibroblasts, but not in the BC-8701 cells, and suggest that the primary mechanism of MMP-1 gene regulation in the breast cancer cells is transcriptional.

DISCUSSION

In this study, we compared the mechanisms by which two different human cell types, normal skin fibroblasts and an aggressive breast cancer cell line, BC-8701 cells, respond to a potent MMP-1 inducer, IL-1β. Both cell types express low levels of this enzyme endogenously, and the time course of induction by IL-1β differed. To study the transcriptional response, we cloned and sequenced 4,372 bp of the human MMP-1 promoter, and from this clone, we prepared a series of 5′-deletion constructs linked to the luciferase reporter. These constructs were then transiently transfected into the fibroblasts and the breast cancer cells, so that we could compare basal and IL-1β induced transcription. In both cell types, basal transcription depends on distal sequences of the human MMP-1 promoter, as does IL-1β induced transcription in the fibroblasts. However, in the BC-8701 breast cancer cells, sequences in the proximal promoter can mediate IL-1β inducibility, while only sequences distal to −3,300 bp mediate IL-1β responsiveness in the HFFs (Fig. 3). Furthermore, our studies indicate that in fibroblasts, but not in the breast cancer cells, IL-1β substantially increases the stability of the collagenase mRNA. Thus, both the transcriptional and post-transcriptional mecha-

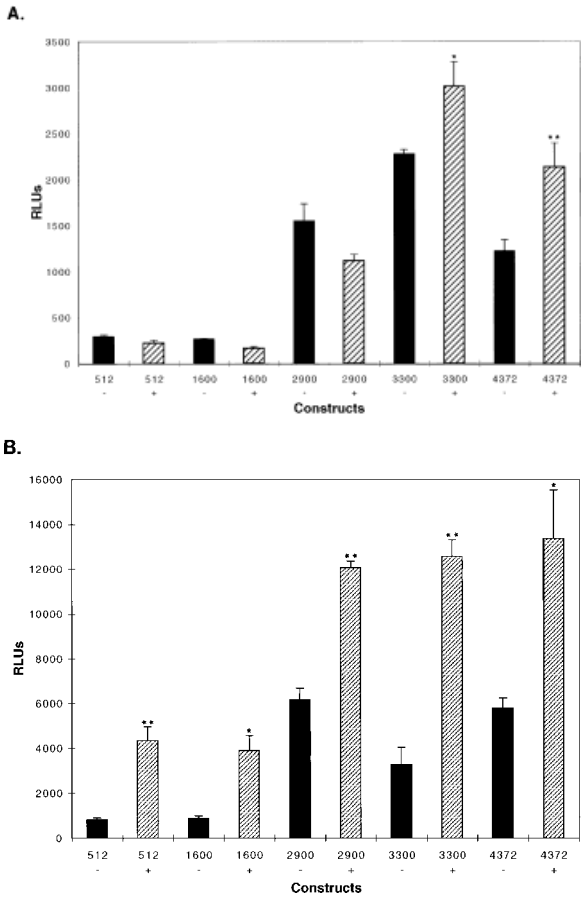

Fig. 3. Basal and IL-1β induced transcriptional activity of the human MMP-1 promoter and BC-8701 cells. **A:** Transient transfection in HFFs. For each transfection, 5 μg of construct DNA was combined with 7 μl LipofectAMINE (Gibco, BRL) and added to each well of 1.5×10^5 cells in DMEM/LH. Basal levels of transcription are indicated by the solid bars. Hatched bars represent IL-1β-induced transcription. The induction of IL-1β was either very significant (*$P < 0.002$) or extremely significant (** $P < 0.0009$). Each transfection was carried out in quadruplicate, and the error bars represent the standard error of the mean. **B:** Transcriptional activity in BC-8701 cells. Each construct was transfected in triplicate using 5 μg DNA with 2.5 μl LipofectAMINE, and the cells were either left untreated (solid bars) or treated with IL-1β (hatched bars) for 24 h. The induction by IL-1β was either very significant (*$P < 0.004$) or extremely significant (**$P < 0.0005$). For both A and B, the cells were harvested between 18 and 24 h, and cell lysates were read on a ML2250 Microtiter Plate Luminometer (Dynatech Laboratories). Luciferase readings are indicated as relative light units (RLUs).

nisms by which IL-1β regulates MMP-1 gene expression differ in these two cell types.

In analyzing the nucleic acid sequence of the human MMP-1 promoter, we note that the promoter contains a series of positive and negative regulatory sequences that may influence transcriptional activity (Fig. 2A). We also detected an allelic variation in our promoter clone which is potentially meaningful. A PEA3 site adjacent to the AP-1 site at −1,602 has been reported [Imai et al., 1994]. Our clone has the sequence 5′-AAGAT-3′ at this position, which fails to constitute a PEA3 site because a G at position −1,607 is missing. The presence of this G would create the sequence 5′-AAGGAT-3′, which contains a PEA3 consensus sequence [Ghosh, 1991]. Since cooperativity between adjacent AP-1 and PEA3 sites is well-documented in the induction of MMPs [reviewed in Benbow and Brinckerhoff, 1997], it is possible that this allelic difference could affect the transcriptional responsiveness of the promoter DNA. In addition, there is an NFκB-like site, 5′-ATGGAAAAA-3′ (*dorsal*) at −2,886 bp which may mediate IL-1β responsiveness [Collins et al., 1995]. *Dorsal* is a transcription factor which shows about 50% identity to the c-*rel* protein family [Steward, 1987]. However, since IL-1β inducibility is seen with smaller fragments of the promoter that do not contain this sequence, this site, alone, is unlikely to be responsible for IL-1β induction. Instead, it is likely to cooperate with other elements [Auble and Brinckerhoff, 1991; Chamberlain et al., 1993; Benbow and Brinckerhoff, 1997].

The lack of transcriptional activity seen in the proximal regions of the promoter and the decline in transcriptional activity in regions upstream of −4,000 bp are intriguing, and may reflect the negative regulatory elements scattered throughout the promoter. Analysis of the nucleic acid sequences (Fig. 2A) reveals the presence of several putative silencer elements located between −572 bp and −1,772 bp. These silencers have been described in several genes, including the collagen II [Savagner et al., 1990], human ϵ-globin [Cao et al., 1989] and the glutathione-S-transferase [Moffat et al., 1996] promoters. There are also several silencer/anti-silencer motifs [Stover et al., 1994] in the region between −1,653 bp and −2,672 bp. These types of anti-silencers require the presence of silencers in order to be effective, and have been described as important regulators of vimentin gene expression in MDA 231 breast cancer cells. The vimentin gene is regulated by a silencer element which binds a 95 kDa protein and by an over-riding anti-silencer which binds a 140 kDa protein [Stover et al., 1994]. Finally, there is an OCTA-binding site at −4,178 bp, which functions to repress transcription in neuronal cells [Zhen et al., 1996] and, therefore, may also

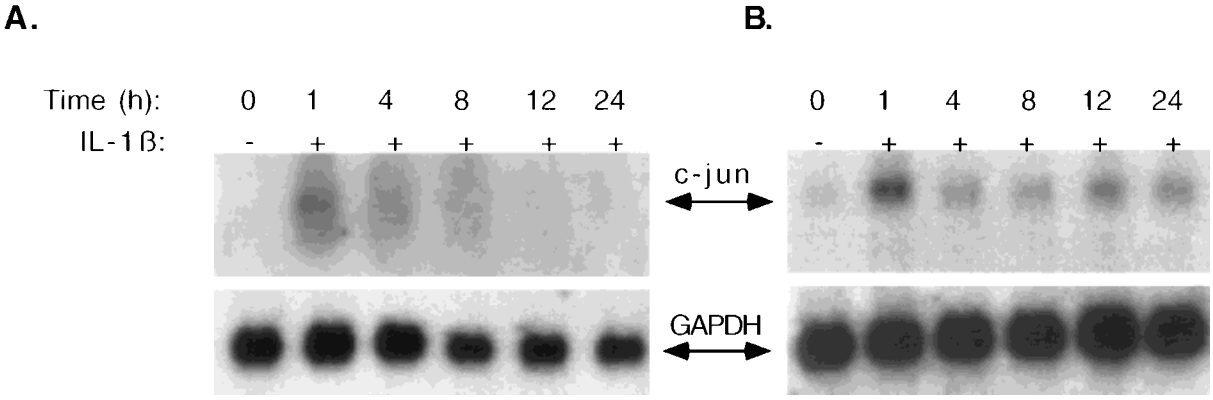

Fig. 4. Northern analysis of endogenous jun expression in HFFs and BC-8701 cells. Duplicate blots of those used in Figure 1A and B were probed with c-jun and autoradiographed for 48 h. Control for loading is shown by the GAPDH probe. To selected cultures 10 ng/ml IL-1β was added, as described in Figure 1. −, no IL-1β added; +, IL-1β added. **A:** HFFs. **B:** BC-8701 cells.

repress transcription in the MMP-1 promoter. It is also possible, as has been suggested for the vimentin gene [Stover et al., 1994], that one or more of the silencer elements may be active in repressing transcription of the human MMP-1 promoter. Some sequences are conserved between the rabbit and human MMP-1 promoters, particularly the AP-1 and PEA3 sites in the proximal region [Angel et al., 1987a, 1987b; Auble and Brinckerhoff, 1991; White and Brinckerhoff, 1995; Chamberlain et al., 1993]. However, the more distal regions are less conserved (Fig. 2A) [Imai et al., 1994; Vincenti et al., 1996b]. For example, the tandem array of AP-1 and PEA3 sites found in the human promoter are lacking in the rabbit gene [Vincenti et al., 1996b]. Furthermore, basal transcription of the rabbit gene depends primarily on the proximal promoter [Auble and Brinckerhoff, 1991; Chamberlain et al., 1993; White and Brinckerhoff, 1995], while the transcriptional activity of the human promoter depends on these upstream sequences (Fig. 3). Thus, there are substantial differences between the two promoters.

Previous studies have demonstrated that post-transcriptional mechanisms contribute to IL-1β induced gene expression. Indeed, IL-1 increases the stability of several mRNAs in various cell types [Michel and Quertermous, 1989; Elias and Lentz, 1990; Stoeckle, 1991; Guttridge et al., 1993; Ristimaki et al., 1994], and our data (Fig. 5) show that this mechanism also plays a major role in regulating the collagenase mRNA in human skin fibroblasts. There are three AUUUA motifs in the 3′ end of the collagenase mRNA [Vincenti et al., 1994], and these help regulate mRNA stability [Shaw and Kaman, 1986; Vincenti et al., 1994]. AU-rich regions were first documented in the 3′ end of several mRNAs that encode inflammatory cytokines with short half-lives, and it is thought that these sequences target the mRNAs for degradation [Shaw and Kaman, 1986]. Previously, using mutational analysis of the AUUUA sequences in the human collagenase mRNA, we demonstrated that their presence enhances mRNA degradation and that treating cells with IL-1β antagonizes this degradation [Vincenti et al., 1994], perhaps by inducing specific AU-binding proteins that complex with AU-rich regions and prevent mRNA decay [Shaw and Kaman, 1986; Stephen et al., 1992; Bohjanen et al., 1992; Whitelock et al., 1993]. Interestingly, the BC-8701 cells do not behave in a similar manner. Although the stability of the constitutively expressed collagenase mRNA may be greater in the breast cancer cells than in the fibroblasts, treatment of the tumor cells with IL-1β does not further stabilize the mRNA (Fig. 5).

It has been suggested [MacDougall and Matrisian, 1995] that collagenase-1 is most often seen in the stromal components of tumors, and that the lack of MMP-1 expression in tumor cells results from a tissue-restriction based on the epithelial lineage of many cancer cells. Therefore, it is noteworthy that the BC-8701 breast cancer cells, of epithelial origin, not only express MMP-1 constitutively, but also respond to IL-1β. However, the mechanisms controlling this expression are different from those seen in

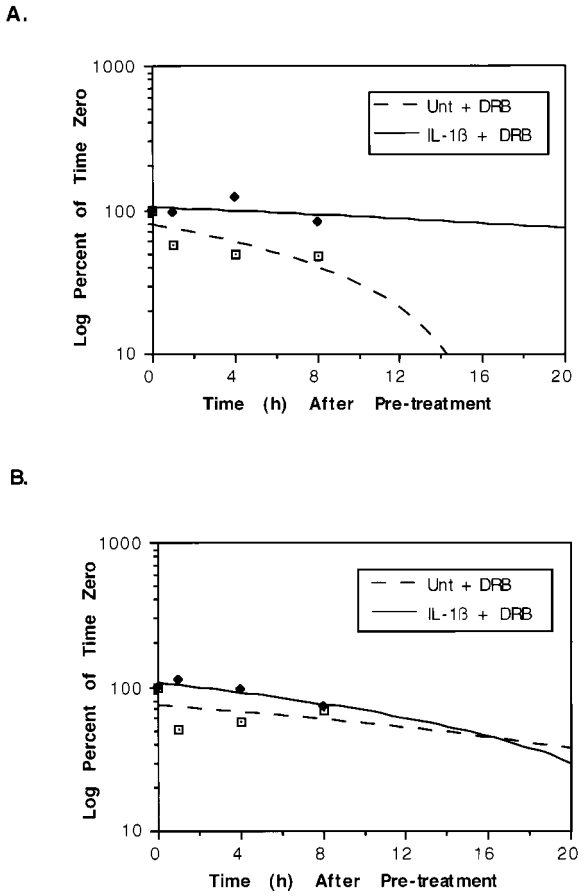

Fig. 5. Effect of IL-1β on the stability of MMP-1 mRNA. Cells were cultured to confluency and pre-treated with serum-free medium alone or with serum-free medium containing 10 ng/ml IL-1β for 8 h. Following the pre-treatment, IL-1β was removed and the transcriptional inhibitor 5,6-dichlorobenzimidazole riboside (DRB; indicated by a +) was added at a final concentration of 75 μM [Delany and Brinckerhoff, 1992; Brinckerhoff et al., 1986]. At selected time points, the cells were harvested for RNA and assayed by Northern analysis as described in Figure 1A. Phosphorimager analysis was performed (Molecular Dynamics, PhosphorImager 445 SI), along with the IPLab Gel software (Signal Analytics Corporation), to quantitate the density of the bands. **A:** HFFs. **B:** BC-8701 cells. Closed diamonds represent those cells treated with IL-1β and DRB; open squares represent those cells treated with DRB alone.

fibroblasts. Thus, distinct cell-type specific mechanisms regulate IL-1β-induced MMP-1 gene expression. The differences in transcriptional activation by IL-1β most likely reflect the complement of transcription factors present within the two cell types. The pattern of jun expression in the BC-8701 cells, for example, may account for the significant increase in transcription by IL-1β seen in the proximal regions of the promoter.

The ability of IL-1 to induce MMP-1 in stromal fibroblasts and chondrocytes has firmly established this cytokine as an important mediator of connective tissue invasion in arthritic disease [Harris, 1990, 1993; Brinckerhoff, 1992; Arend and Dayer, 1993]. Its role in mediating tumor invasiveness is less well documented, and may involve several mechanisms. IL-1 enhances tumor adhesion to the endothelial cell layer [Mannel et al., 1994], acts as a motility factor for some human breast cancer cells in vitro [Verhasselt et al., 1992], and stimulates MMP-1 gene expression by tumor cells. All of these processes increase the ability of tumor cells to degrade the extracellular matrix. Finally, by inducing a parallel increase in MMP-1 in neighboring stromal cells, IL-1 may further amplify the invasive potential of the tumor cells.

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