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Post-Transcriptional Regulation of Collagenase and Stromelysin Gene Expression by Epidermal Growth Factor and Dexamethasone in Cultured Human Fibroblasts

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Abstract Epidermal growth factor (EGF) is a ubiquitous fibroblast mitogen which also stimulates the synthesis of the extracellular matrix degrading metalloproteinases, collagenase, and stromelysin. Using primary cultures of human skin fibroblasts, we show that these metalloproteinase mRNAs are coordinately up-regulated by EGF; and that dexamethasone, a potent inhibitor of collagenase and stromelysin synthesis, coordinately down-regulates these EGF-induced mRNAs. Nuclear run-on assays showed that EGF increased transcription of collagenase and stromelysin ~2-fold over the untreated control, while repression by dexamethasone was difficult to detect. However, steady state mRNA levels were induced ~10-fold by EGF and co-treatment with dexamethasone decreased them to below control levels, suggesting modulation of mRNA stability. Thus, we measured the half-life of these mRNAs using "pulse-chase" methodology. Typically, the half-life of EGF-induced collagenase and stromelysin mRNAs was ~30 h, and co-treatment with dexamethasone decreased the half-life of these mRNAs by 30–50%. Additionally, we found that the transcription inhibitor DRB stabilized EGF-induced metalloproteinase mRNAs, suggesting an mRNA degradation pathway which requires transcription. Thus our data demonstrate that collagenase and stromelysin are coordinately regulated by EGF and by dexamethasone, primarily at the level of metalloproteinase mRNA stability.

Key words: RNA stability, metalloproteinases, half-life, DRB, glucocorticoid

EGF is a ubiquitous fibroblast mitogen [Carpenter and Cohen, 1979; Carpenter, 1981] which also stimulates production of the extracellular matrix degrading metalloproteinases collagenase and stromelysin [Edwards et al., 1987; Matrisian et al., 1985, 1986]. Collagenase (MMP1, matrix metalloproteinase 1) initiates the breakdown of collagen, while stromelysin (MMP3) degrades non-collagenous extracellular matrix components such as proteoglycans, laminin, elastin, and fibronectin [reviewed by Werb, 1989; Matrisian and Hogan, 1990]. Since these two fibroblast products can degrade almost all components of the extracellular matrix, the mechanisms regulating the expression of collagenase and stromelysin play an important role in controlling the connective tissue remodeling which occurs in normal processes, such as wound healing and uterine resorption, and in disease states such as rheumatoid arthritis and tumor invasion.

The expression of collagenase and stromelysin is often, but not always, coordinately regulated [Fini et al., 1987; Matrisian and Hogan, 1990; MacNaul et al., 1990; Otani et al., 1990; Circolo et al., 1991], and the transcriptional regulation of the collagenase and stromelysin genes by IL-1 or the tumor promoter phorbol myristate acetate (PMA) is the subject of intense study. This transcriptional regulation by IL-1 and PMA is mediated through the TRE (TPA-responsive element) [Angel et al., 1987; Schönthal et al., 1988; Brenner et al., 1989; Gutman and Wasylyk, 1990] as well as through other elements in the metalloproteinase promoters [Gutman and Wasylyk, 1990; Auble and Brinckerhoff, 1991; Sirum-Connolly and Brinckerhoff, 1991; Wasylyk et al., 1991]. The TRE is a binding site for Jun and Fos, two components of

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the AP-1 (Activator Protein 1) complex [reviewed by Curran and Franza, 1988; Distel and Speigelman, 1990; Vogt and Bos, 1990]; thus PMA, IL-1, and perhaps other cytokines may exert their effects on collagenase and stromelysin gene transcription by modulating the abundance and/or activity of Fos and Jun [Wasylyk et al., 1991; Kerr et al., 1988; Conca et al., 1989; McDonnell et al., 1990]. In contrast, dexamethasone is a potent inhibitor of metalloproteinase synthesis [Brinckerhoff, 1981; Brinckerhoff et al., 1986; Firestein et al., 1991]; and it acts by antagonizing the transcriptional induction of collagenase and stromelysin by IL-1 and PMA [Frisch and Ruley, 1987; Quinones et al., 1989; Schüle et al., 1990; Yang-Yen et al., 1990; Jonat et al., 1990; König et al., 1992].

We are interested in the mechanisms by which EGF stimulates metalloproteinase synthesis, and in how dexamethasone antagonizes this induction. Previous studies in rat fibroblasts showed that the induction of stromelysin by EGF requires c-*fos,* c-*jun,* and protein kinase C [McDonnell et al., 1990]; however, a *fos*-independent pathway has been reported in mouse NIH 3T3 cells [Kerr et al., 1988]. In this study, we used primary cultures of human skin fibroblasts and found that collagenase and stromelysin are coordinately up-regulated by EGF and down-regulated by dexamethasone. In addition, we found that although EGF induces collagenase and stromelysin expression by increasing gene transcription, its principle mode of action is to stabilize metalloproteinase mRNA, and this stabilization is antagonized by co-treatment with dexamethasone.

METHODS

Cell Culture

Human foreskin fibroblasts were obtained by routine circumcision from the Newborn Nursery at Mary Hitchcock Memorial Hospital (Lebanon, NH). Fibroblasts were isolated as we have previously described [Brinckerhoff et al., 1986; Sirum-Connolly and Brinckerhoff, 1991; Mitchell et al., 1991], and were grown in 10% fetal bovine serum (FBS) in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) and used for experiments during passages 3 to 11. For some experiments, the cells were cultured in serum-free medium (DMEM plus 0.2% lactalbumin hydrolysate, LH). The growth factors and drugs used in these experiments included EGF (Collaborative Research), phorbol myristate acetate (PMA; Sigma), 5,6-dichlorobenzimidazole riboside (DRB; Sigma), and dexamethasone (Sigma).

Nucleic Acid Hybridization

RNA was isolated by the method of Chirgwin et al. [1979]. RNA samples (5–20 μg) and standards (Bethesda Research Laboratory, BRL) were electrophoresed through agarose gels containing 0.66 M formaldehyde [Sambrook et al., 1989]. RNA was transferred to Gene Screen Plus (NEN) and hybridized with ^{32}P-labeled cDNAs under high stringency conditions, as described in Sirum and Brinckerhoff [1989]. cDNA inserts were ^{32}P-labeled using an oligo-labeling technique [Feinberg et al., 1983]. The cDNAs used were: the full length (1.9 kb) human stromelysin [Sirum and Brinckerhoff, 1989], the 1.7 kb EcoRI fragment of human collagenase [Brinckerhoff et al., 1987], and a 2 kb EcoRI/HindIII fragment of bovine pyruvate kinase (a generous gift of Neil Farber, Biogen Inc., Cambridge, MA). The resulting autoradiograms were quantitated densitometrically using an E-C Apparatus Corp. scanning densitometer with a Hewlett-Packard 3390A integrator. Unless otherwise noted, all blots were probed with cDNA for pyruvate kinase, a housekeeping gene. The signal obtained when the blots were probed with cDNAs for collagenase or stromelysin was normalized to the signal for pyruvate kinase, in order to correct for slight differences in RNA loading.

Nuclear Run-On Assay

The protocol used for the nuclear run-on assay was modified from that of Greenberg and Ziff [1984] and Marzluff and Huang [1985]. Confluent fibroblast cultures were treated with serum alone (DMEM-10% FBS) or with serum and EGF (10 ng/mL) or EGF plus dexamethasone (0.1 μM) for 24 or 48 h. Cells were harvested, and lysed by Dounce homogenization in buffer containing 0.3 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM Tris-HCl, pH 8.0, 0.5 mM DTT, and 0.2% Triton X-100. Nuclei were pelleted, washed, resuspended in freeze buffer (40% glycerol, 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.1 mM EDTA), and stored at −70°C. Cytoplasmic RNA was isolated from the supernatant of the detergent lysis by proteinase K treatment, followed by phenol/chloroform extraction and ethanol precipitation.

Isolated nuclei were transcribed in a 150 μL reaction volume in 16% glycerol, 25 mM Tris-

HCl, pH 7.5, 0.1 mM EDTA, 3 mM $MgCl_2$, 2 mM $MnCl_2$, 70 mM NaCl, 1 mg/mL BSA, 2.5 mM DTT, 1 mM each ATP, GTP, CTP, 0.5 μM UTP, 30 units Inhibit-ace (5 Prime-3 Prime), and 100 μCi ^{32}P-UTP (Amersham). Equal numbers of nuclei ($\sim 8 \times 10^6$) from each condition were used for the in vitro transcription reaction. The reactions were terminated and treated with DNase I (Worthington), and then proteinase K (BRL). The labeled RNA was extracted and unincorporated nucleotides were removed by sequential ethanol precipitations. Incorporation of ^{32}P into RNA was measured by TCA precipitation.

Gel-purified cDNAs (500 ng/slot) were immobilized onto Gene Screen Plus by slot blotting and equal numbers of counts ($\sim 7 \times 10^6$ cpm) were used for each hybridization. The membranes were hybridized and washed with high stringency, as described above. Specific hybridization was detected by autoradiography and quantitated by scanning densitometry. When α-amanitin (30 ng/mL; Sigma) was included in the transcription reactions, there was no detectable collagenase or stromelysin hybridization. The signal generated for collagenase and stromelysin mRNAs was normalized to that for pyruvate kinase as a final control for even loading of counts. Normally, the signal for pyruvate kinase varied little among the different samples.

mRNA Half-Life Determination for Collagenase and Stromelysin

The procedure used for measuring the half-life of metalloproteinase mRNAs was based on that of Brinckerhoff et al. [1986]. Confluent fibroblast cultures were washed with HBSS to remove traces of serum and were placed in DMEM-LH (serum-free medium) containing PMA (10 nM) or EGF (15 ng/mL) for 8 h, to induce metalloproteinase mRNA. RNA was labeled by the addition of 40 μCi/mL [5,6-^{3}H]uridine (25 μM; Amersham) for an additional 18 h. The cells were then subjected to an 8 h "pre-chase" with DMEM-LH containing 5 mM uridine and 2.5 mM cytidine (chase medium), which was necessary to deplete the intracellular pool of ^{3}H-uridine [Brinckerhoff et al., 1986]. If the pre-chase was omitted, the cells continued to incorporate ^{3}H-uridine into RNA even in the absence of exogenous ^{3}H-uridine (data not shown). The pre-chase medium was removed and the actual chase was begun by the addition of fresh chase medium. PMA or EGF was present during both the chase and the pre-chase, and in some experiments dexamethasone was added to the chase. At the appropriate times, cultures were terminated and the RNA was harvested.

To measure the decay of ^{3}H-collagenase and -stromelysin mRNAs, equal amounts (50–100 μg, depending on the experiment) of total ^{3}H-RNA from each sample were hybridized under high stringency conditions to immobilized gel-purified cDNAs (500 ng/slot) for collagenase and stromelysin, as described above. The membrane strips were washed with high stringency and radioactive RNA hybridizing to the immobilized DNA was quantitated by liquid scintillation counting: radioactivity was eluted from the membrane with Solvable (NEN) and counted in Atomlight (NEN) according to the manufacturer's instructions. Counts due to non-specific hybridization to linearized pUC19 (typically < 300 cpm) were subtracted from the signal for collagenase and stromelysin, and specific cpm hybridized to collagenase and stromelysin cDNAs was plotted vs. time; the half-life of collagenase and stromelysin mRNA was determined from the resulting graph, and by analyzing the data points using a computer program described in Brinckerhoff et al. [1986].

RESULTS

Collagenase and Stromelysin mRNAs Are Coordinately Regulated by EGF and Dexamethasone

Using Northern blot analysis, we first documented the coordinate induction of collagenase and stromelysin mRNAs by EGF and repression by dexamethasone (Fig. 1). RNA was isolated from untreated fibroblasts and from cells treated with EGF in the presence or absence of dexamethasone (0.1 μM) for 12, 24, or 48 h. Levels of collagenase and stromelysin mRNA were very low in untreated cells (Fig. 1, lanes 1, 4, and 7). However, EGF induced both mRNAs 5–10-fold over the untreated control by 12 h of treatment, and co-treatment with dexamethasone decreased these mRNA levels to below that of the untreated control (Fig. 1, lanes 1, 2, and 3). This coordinate regulation of collagenase and stromelysin mRNAs remained apparent throughout the 48 h time course. Levels of metalloproteinase induction were similar when the cells were treated in serum-free medium; and this EGF-induction and dexamethasone suppression of collagenase and stromelysin expression was also seen at the level of protein synthesis (data not shown).

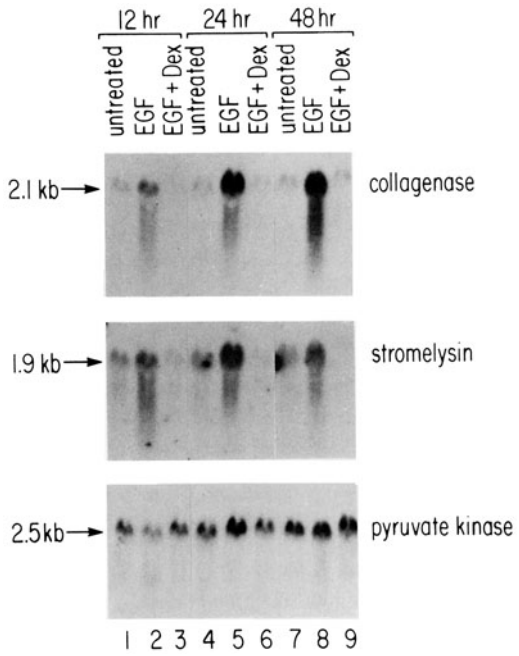

Fig. 1. Modulation of EGF-induced collagenase and stromelysin mRNA levels by dexamethasone. Human fibroblasts in DMEM-10% FBS were treated with 10 ng/mL EGF in the presence or absence of 0.1 μM dexamethasone for up to 48 h. Total RNA was isolated at appropriate time points and subjected to Northern blot analysis (20 μg RNA/lane), and probed with ^{32}P-labeled collagenase, stromelysin, and pyruvate kinase cDNAs.

EGF Increases Transcription of the Collagenase and Stromelysin Genes

We next used nuclear run-on transcription assays to determine the transcriptional component of the EGF and dexamethasone effects on metalloproteinase mRNA levels. Nuclei and the corresponding cytoplasmic RNA were isolated from fibroblasts treated for 24 or 48 h with EGF in the presence or absence of dexamethasone, and a representative of 4 experiments is shown in Figure 2. For both the 24 and 48 h time points, densitometric analysis showed that EGF increased collagenase and stromelysin gene transcription an average of ~2-fold over the untreated control (Fig. 2; data not shown). This modest increase in metalloproteinase transcription by EGF was similar to that seen when fibroblasts containing transiently transfected human or rat stromelysin promoter/reporter gene constructs were treated with EGF [Sirum and Brinckerhoff, 1989; McDonnell et al., 1990; Kerr et al., 1988]. However, significant down-regulation of transcription by dexamethasone was difficult to detect. As a positive control, we assessed

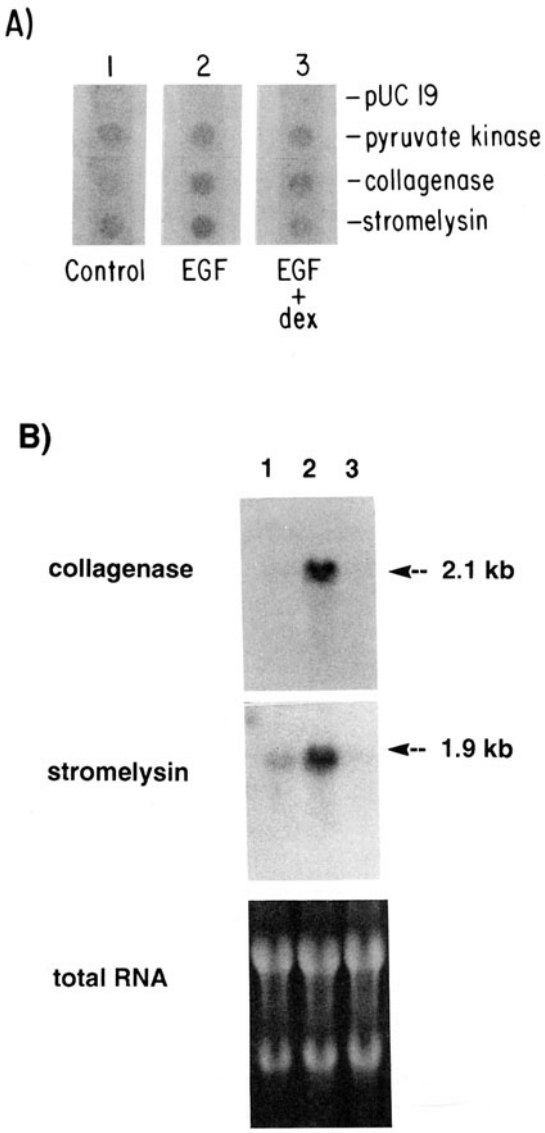

Fig. 2. Nuclear run-on analysis of metalloproteinase gene transcription in nuclei from fibroblasts treated with EGF and dexamethasone. Nuclei and cytoplasmic RNA were isolated from human fibroblasts treated for 24 h with DMEM-10% FBS alone (1, control), or in the presence of 10 ng/mL EGF (2), or EGF and 0.1 μM dexamethasone (3). **(A)** Nuclei were transcribed in vitro in the presence of ^{32}P-UTP, and labeled transcripts were hybridized to immobilized cDNAs for pyruvate kinase, collagenase, and stromelysin. Linearized pUC19 was used as a control for nonspecific hybridization. **(B)** Corresponding cytoplasmic RNA was subjected to Northern blot analysis and probed with ^{32}P-labeled cDNA for collagenase and stromelysin. Ethidium bromide staining of ribosomal RNA bands and hybridization to pyruvate kinase (data not shown) were used to document equal loading of RNA in each lane.

the effects of dexamethasone and EGF on cytoplasmic RNA isolated from the same cells as the nuclei. We found that both collagenase and stromelysin mRNAs were elevated 9- to 10-fold

over control in EGF treated cells (Fig. 2B), and co-treatment with dexamethasone repressed this induction to below control levels (Fig. 2B). Thus, it was clear that these small changes in gene transcription could not account for the changes in steady state levels of collagenase and stromelysin mRNA that were observed when fibroblasts were treated with EGF or EGF and dexamethasone (Fig. 2), suggesting a role for mRNA stabilization.

The Stability of EGF-Induced Collagenase and Stromelysin mRNAs Is Modulated by Dexamethasone

To test this hypothesis, we used "pulse-chase" methodology to measure the effects of EGF and dexamethasone on the half-life of collagenase and stromelysin mRNAs. Because untreated skin fibroblasts have such low constitutive levels of collagenase and stromelysin mRNA, we were unable to measure the half-life of the metalloproteinase mRNAs in untreated cells. Thus, we induced metalloproteinase mRNA by treating the fibroblasts with EGF and labeling the RNAs with ^{3}H-uridine [Brinckerhoff et al., 1986]. After 18 h of labeling, the medium was removed and replaced with cold "chase" medium containing EGF and excess uridine and cytidine, and dexamethasone was added to selected plates. Total RNA was prepared at appropriate times during the chase, and the turnover of ^{3}H-collagenase and -stromelysin mRNA was monitored by specific hybridization to immobilized cDNA and quantitated by scintillation counting. Table 1 shows the results of 2 experiments (exps. 1 and 2), where the half-life of collagenase mRNA ranged from 23 to 28 h, and that of stromelysin ranged from 16 to 29 h. This variability in the half-life of EGF-induced metalloproteinase mRNAs is similar to that seen by others [Brinckerhoff et al., 1986; Darnell, 1982], and could reflect the fact that the absolute level of induction varies from experiment to experiment, and, as has been demonstrated with PMA, this may impact on RNA stability [Brinckerhoff et al., 1986]. The stability of collagenase or stromelysin mRNA in fibroblasts treated with inducers such as IL-1, PMA, or cycloheximide has been measured [McCachren et al., 1989; Brinckerhoff et al., 1986; Otani et al., 1990], and the values we obtained are in the range of those observed by previous investigators.

Interestingly, we found that dexamethasone decreased the half-life of EGF-induced collagenase and stromelysin mRNA (Table I; exps. 1 and 2). This result is in keeping with those from our nuclear run-on experiments and our analysis of steady state mRNAs, which showed that dexamethasone had little or no effect on EGF-induced collagenase and stromelysin transcription, but greatly decreased steady state metalloproteinase mRNA levels. This suggests that dexamethasone modulates EGF-induced collagenase and stromelysin mRNAs at a post-transcriptional level.

TABLE I. Half-life of Collagenase and Stromelysin mRNA in Human Fibroblasts*

	COLLAGENASE			STROMELYSIN		
	PMA	EGF	EGF + Dex	PMA	EGF	EGF + Dex
Exp. 1	—	23 h	15 h	—	29 h	18 h
Exp. 2	—	28 h	13 h	—	16 h	11 h
Exp. 3[1]	7 h	25 h	—	7 h	44 h	—
Exp. 4	23 h	38 h	—	36 h	60 h	—

*The half-life of collagenase and stromelysin mRNAs was determined using pulse-chase methodology. Human fibroblasts in serum-free medium were induced with 10 nM PMA or 10 ng/mL EGF for 8 h and then pulse labeled with ^{3}H-uridine in the presence of inducer for 18 h. The cells were given a pre-chase and chase in the presence of PMA or EGF, and 0.1 μM dexamethasone was added to some of the cultures at the beginning of the chase. At appropriate times during the chase, total RNA was isolated and labeled RNAs were hybridized to immobilized cDNAs for collagenase and stromelysin. Specific hybridization was quantitated by liquid scintillation counting and the decay of ^{3}H-collagenase and -stromelysin mRNAs was plotted vs. hours of chase. The half-life of collagenase and stromelysin mRNAs was determined from the resulting graphs, and from a computer based program [Brinckerhoff et al., 1986].
[1]= experiment shown in Figure 3.

For comparison, we measured the stability of metalloproteinase mRNAs in fibroblasts treated with EGF or another inducer known to stabilize these mRNAs, PMA [Brinckerhoff et al., 1986; Otani et al., 1990]. We found that the half-life of the metalloproteinase mRNAs was longer in EGF-treated than in PMA-treated cells (Table I; exps. 3 and 4; Fig. 3). We found that in cells which received EGF, there was an initial lag in the decay curves, followed by a gradual decrease in the labeled mRNA. The shape of the metalloproteinase mRNA decay curves was different in PMA-treated cells (Fig. 3), suggesting that the stabilizing activity in these cells may be quantitatively or qualitatively distinct from that found in EGF-treated cells.

To determine the role of transcription in stabilizing metalloproteinase mRNAs in EGF-treated fibroblasts, we treated cells with the transcrip-

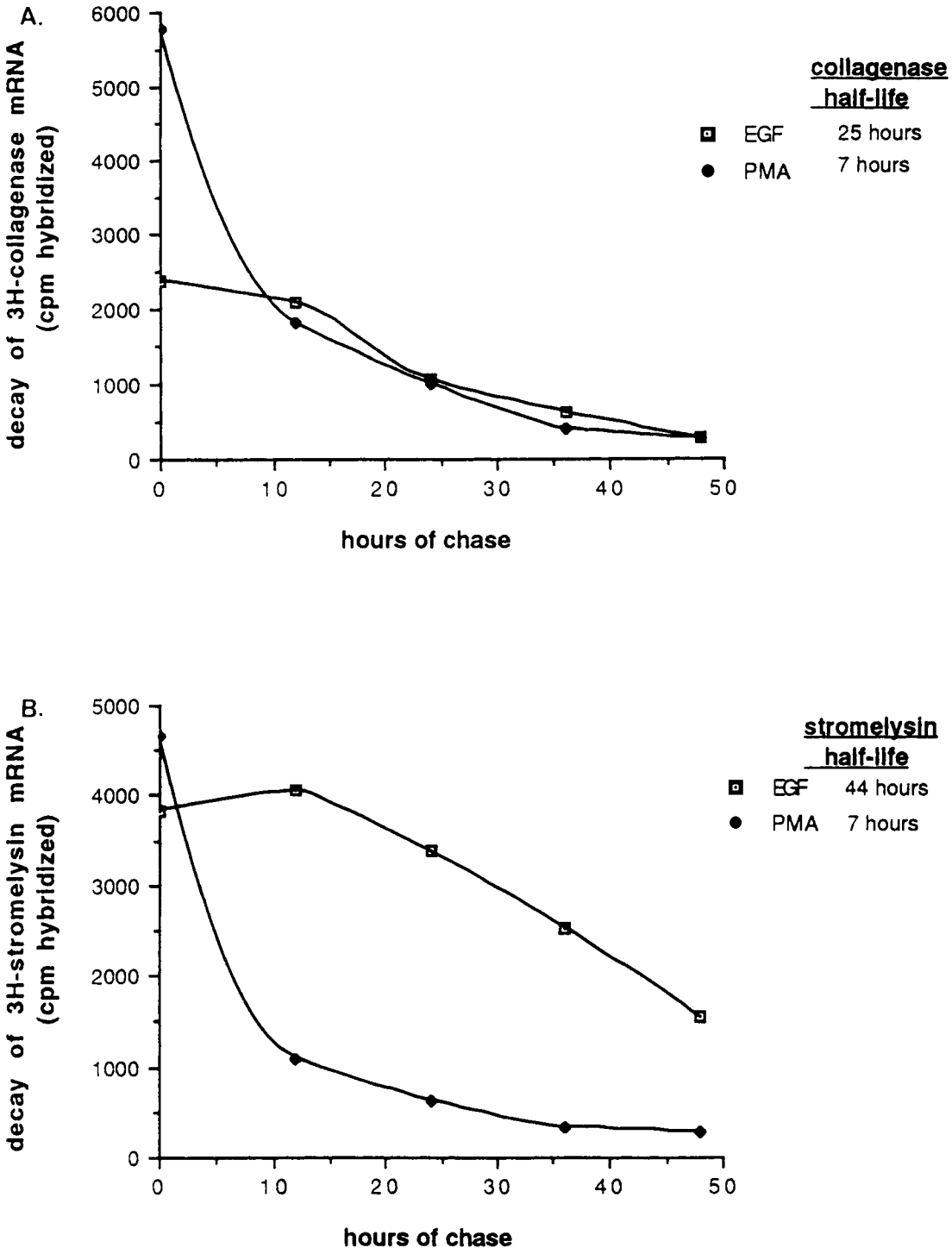

Fig. 3. Determination of collagenase and stromelysin mRNA half-life in PMA or EGF treated fibroblasts. Human fibroblasts in serum-free medium were treated with 10 nM PMA or 10 ng/mL EGF and pulse labeled with ^{3}H-uridine for 18 h. The cells were given a pre-chase and chase in the presence of PMA or EGF, and at the appropriate times during the chase total RNA was isolated. Labeled RNAs were hybridized to immobilized cDNAs for (A) collagenase and (B) stromelysin, and specific hybridization was quantitated by liquid scintillation counting.

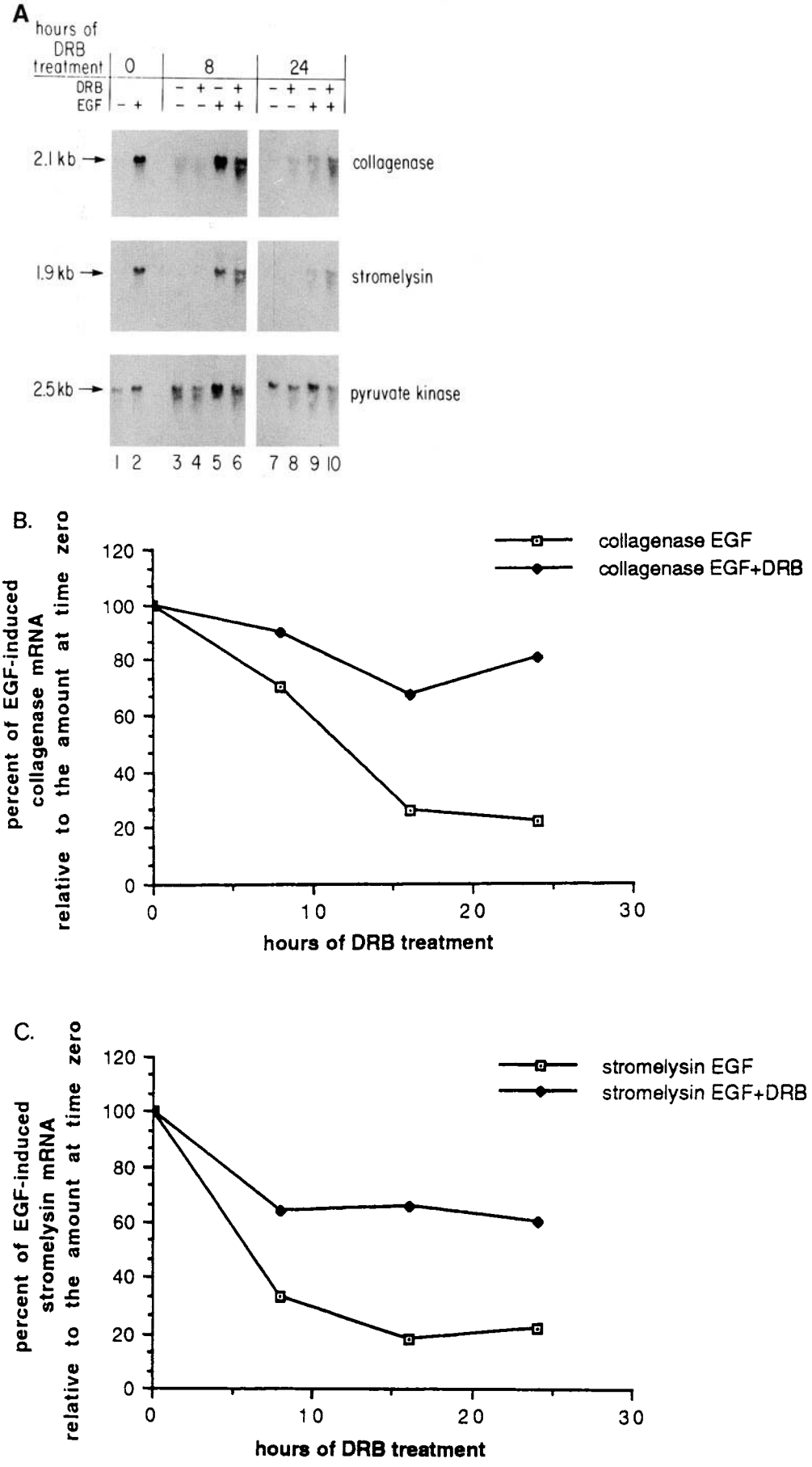

Fig. 4. (Legend appears on page 407.)

tion inhibitor DRB, in the presence or absence of EGF. Following an 8 h incubation with or without EGF, DRB (75 μM) was added to selected plates. This addition marked "time zero" and RNA isolated 8, 16, or 23 h later was subjected to Northern blot analysis (Fig. 4A) and densitometry (Fig. 4B,C). At "time zero," EGF increased metalloproteinase mRNAs 7–8-fold over the untreated controls. We found that the steady state mRNA levels for collagenase and stromelysin in cells treated with EGF alone decreased with time (Fig. 4; data not shown). This decrease contrasts with the data shown in Figure 1, where metalloproteinase mRNAs remained elevated. We attribute this discrepancy to the desensitization or down-regulation of the EGF receptor in serum-free medium [Gilligan et al., 1990; Honegger et al., 1990; Dong et al., 1991; Tartare et al., 1992]. However, in cells co-treated with DRB, this decrease in metalloproteinase mRNA was antagonized (Fig. 4), indicating that DRB stabilized collagenase and stromelysin mRNA in EGF-treated cells. DRB treatment did not appreciably affect metalloproteinase mRNA levels in untreated cells, nor did it influence the abundance of pyruvate kinase mRNA (Fig. 4; data not shown). Similar results were obtained from several different experiments and suggest that transcription is required for the degradation of EGF-induced collagenase and stromelysin mRNA. After 24 h of treatment with DRB, ^{3}H-leucine incorporation into the cell layer was ~50% of control (data not shown), and the cells remained adherent and appeared healthy, indicating that these changes in metalloproteinase mRNA levels are not due to overt DRB-mediated cell toxicity. Thus, we suggest that one component of the regulation of steady state collagenase and stromelysin mRNA levels results from a transcription-dependent degradation of metalloproteinase mRNAs, and that EGF treatment induces a metalloproteinase mRNA stabilizing activity which can be modulated by co-treatment with dexamethasone.

Fig. 4. DRB decreases the degradation of EGF-induced collagenase and stromelysin mRNAs. Human fibroblasts in serum-free medium were treated with or without 10 ng/mL EGF for 8 h. Following this period, at time zero, the transcription inhibitor DRB (5,6-dichlorobenzimidazole riboside; 75 μM) was added to some of the plates and total RNA was isolated 8, 16, and 24 h later. (**A**) Total RNA (10 μg RNA/lane) was subjected to Northern blot analysis, and probed with ^{32}P-labeled cDNA for collagenase, stromelysin, and pyruvate kinase. (**B** and **C**) The resulting autoradiograms were quantitated by scanning densitometry and normalized to hybridization to pyruvate kinase. The quantity of collagenase or stromelysin mRNA at the various time points is expressed relative to the amount found before the addition of DRB (time zero).

DISCUSSION

Using primary cultures of human fibroblasts, we have demonstrated the coordinate regulation of collagenase and stromelysin expression by EGF and by the glucocorticoid hormone dexamethasone. Our results show that while EGF modestly increases transcription of collagenase and stromelysin, its principle mode of action is to increase the stability of the metalloproteinase mRNAs, and that this increase in mRNA stability can be antagonized by co-treatment with dexamethasone. Additionally, we found that inhibiting transcription stabilized of collagenase and stromelysin mRNAs in EGF-treated cells, suggesting that these cells may contain a transcription-dependent factor which degrades metalloproteinase mRNAs.

Collagenase and stromelysin are often, but not always, coordinately expressed [Fini et al., 1987; Matrisian and Hogan, 1990; MacNaul et al., 1990; Otani et al., 1990; Circolo et al., 1991], and previous studies have ascribed transcriptional regulation as the major factor modulating metalloproteinase mRNA levels. Indeed the importance of the AP-1 element, as well as other additional sequences, in the positive and negative regulation of collagenase and stromelysin gene transcription has been demonstrated [Angel et al., 1987; Schönthal et al., 1988; Brenner et al., 1989; Gutman and Wasylyk, 1990; Auble and Brinckerhoff, 1991; Sirum-Connolly and Brinckerhoff, 1991; Wasylyk et al., 1991]. In contrast, we show that the principle mechanism by which EGF regulates collagenase and stromelysin mRNA expression is through changes in mRNA stability (Figs. 2 and 3; Table 1). Collagenase and stromelysin mRNAs were very stable in EGF-treated fibroblasts, and co-treatment with dexamethasone decreased this stability (Table 1). Glucocorticoid modulation of RNA stability has been demonstrated in other systems as well [Hamalainen et al., 1985; Peppel et al., 1991]. However, our results contrast with those of Brinckerhoff et al. [1986], who found that dexamethasone did not affect the half-life of collagenase mRNA in PMA-treated cells. This suggests that PMA and EGF may increase the stability of metalloproteinase mRNAs by distinct mechanisms. The destabilization of EGF-

induced metalloproteinase mRNAs by dexamethasone suggests that these cells may contain an RNA stabilizing factor that is inhibited by dexamethasone treatment. This metalloproteinase mRNA stabilizing factor could interfere with the activity of a specific ribonuclease, and, indeed, the presence of a metalloproteinase mRNA specific nuclease has been suggested [Brenner et al., 1989].

The selective degradation of collagenase and stromelysin mRNAs may involve specific conformational features and/or the primary sequence of the RNAs. Shaw and Kamen [1986] characterized an AU-rich destabilizing element (AUUUA) present in the 3′ terminus of numerous transiently expressed cytokine and proto-oncogene mRNAs. mRNAs containing this sequence are more stable in PMA-treated cells, and PMA may modulate a specific mRNA degradation pathway. AU-rich sequences, similar to those found by Shaw and Kamen, are repeated 3 times in the 3′ untranslated region of human collagenase and once in the 3′ untranslated region of human stromelysin mRNA [Sirum and Brinckerhoff, 1989; Whitham et al., 1986; Wilhelm et al., 1987]. This sequence is also found in the 3′ terminus of rabbit collagenase mRNA [Fini et al., 1987], which is stabilized by PMA in synovial fibroblasts [Brinckerhoff et al., 1986]. Metalloproteinase mRNAs are barely detectable in unstimulated fibroblast cultures; however, when the cells are stimulated with compounds such as PMA or EGF, collagenase and stromelysin become major products of these activated cells, and these metalloproteinase mRNAs are quite stable. Perhaps these agents modulate collagenase and stromelysin mRNA stability through a specific mRNA degradation pathway mediated by the AU-rich sequences.

A labile, cytosolic destabilizing factor that binds to the AU-rich region of the 3′ untranslated region of the c-*myc* mRNA has been described [Brewer and Ross, 1989; Brewer, 1991]. In addition, an adenosine-uridine binding factor (AUBF) which interacts with the AU-rich regions in the 3′ untranslated region of several oncogene and cytokine RNAs has been identified [Malter, 1989; Gillis and Malter, 1991], and the PMA-induced stabilization of AUUUA containing messages may be mediated by the binding of AUBF to these labile RNAs [Malter and Hong, 1991]. Our experiments utilizing the transcription inhibitor DRB indicate that transcription is necessary for the degradation of metalloproteinase mRNAs in the presence of EGF (Fig. 4). Perhaps fibroblasts contain a constitutive metalloproteinase-specific ribonuclease, and EGF treatment induces a labile metalloproteinase mRNA stabilizing factor which is degraded by a transcription-dependent pathway.

Regulation of collagenase and stromelysin expression by changes in mRNA stability is a level of gene regulation that has not been studied in great detail. In instances where fibroblasts may be exposed to cytokines such as EGF (e.g., wound healing, rheumatoid arthritis), it is possible that metalloproteinase mRNA stability plays an important role in determining the levels of collagenase and stromelysin mRNAs present in these cells.

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