Molecular Mechanisms Regulating the Production of Collagenase and TIMP in U937 Cells: Evidence for Involvement of Delayed Transcriptional Activation and Enhanced mRNA Stability†

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ABSTRACT: We have used the human promonocyte-like U937 cell line as a model to study the regulation of interstitial collagenase and tissue inhibitor of metalloproteinases (TIMP) during mononuclear phagocyte development. Our results show that differentiation of U937 cells with exposure to 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induces a temporally delayed (16–24 h) but marked increase in the biosynthesis and secretion of interstitial collagenase and TIMP. Similarly, steady-state mRNA levels for both proteins rose dramatically during the period of exposure, but again after considerable time delay (12–16 h). For interstitial collagenase, induction was transcriptionally regulated as demonstrated by nuclear run-on experiments, and required the synthesis of proteins as indicated by cycloheximide treatment. However, transcriptional activation of collagenase was never observed prior to 10–12 h; since c-fos is rapidly induced in U937 cells and largely disappears by 2 h (Mitchell et al., 1985), our data strongly suggest that collagenase induction in this system cannot be explained simply or entirely by an AP-1-dependent mechanism. Although TIMP steady-state mRNA levels also increased substantially with cellular differentiation, no transcription was detected by run-on experiments. However, TPA exposure markedly prolonged the half-life of TIMP mRNA from 4 h to >20 h. While cycloheximide treatment completely blocked TPA-mediated induction of collagenase mRNA, it only marginally interfered with simultaneously induced TIMP mRNA levels. Our results demonstrate that differentiation of U937 monocytic cells is accompanied by markedly enhanced production of both interstitial collagenase and TIMP. However, there are multiple, and perhaps differing, molecular mechanisms regulating these responses.

As monocytic cells differentiate in human tissues, they undergo characteristic morphologic, functional, and biochemical changes, including a marked transition in the profile of proteinases synthesized. Circulating peripheral blood monocytes contain the intracellular serine enzymes neutrophil elastase and cathepsin G, but they have little capacity for regulated production of metalloproteinases (Campbell et al., 1987, 1989). Upon migration into specific tissues and subsequent differentiation into macrophages, serine proteinase production rapidly ceases (Hanson et al., 1990). Simultaneously, developing tissue macrophages acquire the capacity for regulated synthesis and secretion of large amounts of metalloenzymes including interstitial collagenase, stromelysin, and 92-kDa type IV collagenase, as well as the tissue inhibitor of metalloproteinase (TIMP) (Welgus et al., 1985, 1990). The importance of macrophage production of metalloenzymes has recently been underscored by the demonstration of stromelysin gene expression from lipid-laden macrophages of atherosclerotic plaques (Henney et al., 1991) and of interstitial collagenase and stromelysin mRNAs in macrophage-like cells of the rheumatoid synovial pannus (McCachren et al., 1991).

While neutrophils and monocytes may rapidly deploy their complement of serine proteinases stored in granules, production of metalloenzymes and TIMP by mature mononuclear phagocytes involves the biosynthesis of new proteins which is subject to precise regulation by specific modulators (Welgus et al., 1990; Wahl & Winter, 1984; Werb, 1978). Lipopolysaccharide (LPS) potentiates the matrix-degradative phenotype of macrophages by coordinately stimulating the biosynthesis of all secreted metalloproteinases (Cury et al., 1988). Ingestion of particulate matter and exposure to collagenous substrates also stimulate enzyme production (Shapiro and Welgus, unpublished observations). In contrast, dexamethasone markedly suppresses the expression of all metalloenzymes and TIMP in human alveolar macrophages (Shapiro et al., 1991). Regulation of metalloenzyme synthesis by macrophages may also be enzyme-specific, varying in response to the same cytokine. For example, interferon-γ potently down-regulates the production of interstitial collagenase and stromelysin, but exhibits comparatively minor effects on the synthesis of 92-kDa type IV collagenase (Shapiro et al., 1990).

We (Senior et al., 1986), and others (Harris & Ralph, 1985), have used the human U937 cell line as a model to study processes involving the normal cellular differentiation of mononuclear phagocytes. Basal U937 cells divide rapidly, are nonadherent, and have a proteinase profile characteristic of promonocyte-like cells. In such cells, the serine proteinases elastase and cathepsin-G are stored intracellularly (Welgus et al., 1986). However, exposure to phorbol esters causes the cells to become adherent, stop division, and take on many of the properties of mature macrophages (Harris & Ralph, 1985). Of particular importance, this includes a shift from serine to metalloenzyme biosynthesis (Welgus et al., 1986). In the present report, we have studied TPA-induced differentiation

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of U937 cells as a model to gain insight into the gene regulation of interstitial collagenase and TIMP production that occurs during mononuclear phagocyte development. Our data indicate that TPA differentiation of U937 cells results in marked induction of the biosynthesis of both interstitial collagenase and TIMP, but only following a very prolonged temporal lag period. Furthermore, there are multiple and perhaps disparate molecular mechanisms controlling the induction of collagenase and TIMP biosynthesis. During U937 cell differentiation, interstitial collagenase undergoes transcriptional activation, but via a process that does not appear to be readily explainable by simple AP-1 activation by the c-fos/c-jun heterodimer. Enhanced production of TIMP involves a marked prolongation in its mRNA stability.

MATERIALS AND METHODS

Cell Culture. U937 cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (low endotoxin; <0.02 ng/mL; HyClone), 2 mM L-glutamine, 200 units/mL penicillin, and 200 μg/mL streptomycin. For induction of cell differentiation, U937 cells were plated at 1×10^6 cells/mL, and TPA (Sigma Chemicals, St. Louis, MO) was dissolved in DMSO at a stock concentration of 1×10^{-3} M and added at a final concentration of 1.5×10^{-8} M. This amount of DMSO (0.01 μL/mL of media) had no effect on collagenase or TIMP production in control cells.

Cultures of normal adult human skin fibroblasts were initiated from cells obtained from the American Type Culture Collection, Rockville, MD (CRL 1467). Fibroblasts were subcultured as monolayers in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Culture medium was obtained from the Tissue Culture Support Center, Washington University Medical Center. Cultures were maintained in humidified air with 5% CO_2 at 37 °C. All experiments involving either U937 cells or fibroblasts presented in this report were performed at least 4 separate times with essentially invariant results.

Reagents. Interstitial collagenase cDNA was kindly provided by G. I. Goldberg of Washington University School of Medicine, St. Louis, MO (Goldberg et al., 1986); TIMP cDNA was from D. F. Carmichael, Synergen Corp., Boulder, CO (Carmichael et al., 1986). Alu and pUC cDNA probes have been described previously (Ley et al., 1989). Cycloheximide (Sigma) was freshly diluted in culture medium to 1 mg/mL and used at a final concentration of 3 μg/mL. Actinomycin D (Sigma) was solubilized in 100% ethanol to 1 mg/mL and used at a final concentration of 5 μg/mL. This concentration of actinomycin D abolished further collagenase and TIMP secretion, and cells remained >85% viable for 12 h as verified by trypan blue staining.

Immunologic Assays. U937 cell cultures were exposed to nutrient medium alone or with added agents for the indicated times. Competitive binding ELISAs for human interstitial collagenase and TIMP were performed on U937 conditioned media samples as described previously (Cooper et al., 1982; Welgus & Stricklin, 1983). These assays have nanogram sensitivity, are specific for the respective proteins, and measure the total amount of each protein whether present in a free or a bound state (i.e., complexes of enzyme–inhibitor on enzyme–substrate). Due to minimal cross-reactivity between human and bovine TIMP, conditioned media samples containing 10% FBS were corrected for their small contribution of bovine TIMP (0.01 μg/mL immunoreactive material). This represented <2% of TIMP produced by differentiated U937 cells and resulted in a negligible background.

RNA Purification and Analysis. Total cellular RNA was isolated by the guanidinium/phenol extraction method (Chomczynski & Sacchi, 1987). Northern blot analysis of RNA was performed. Equal amounts (5 μg) of denatured RNA were fractionated on a 1.0% agarose gel containing 1.0 M formaldehyde and then transferred to nitrocellulose filters. The filters were dried in a vacuum at 80 °C for 2 h prior to prehybridization in 50% formamide, 5 × SSC, 50 mM $NaHPO_4$, pH 7.5, 5 × Denhardt's solution, 0.1% SDS, and 100 μg/mL denatured sonicated salmon sperm DNA. Hybridization was performed at 42 °C overnight with collagenase or TIMP cDNA probes labeled by nick-translation to a specific activity greater than 2×10^8 cpm/μg. The filters were washed 3 times for 5 min with 2 × SSC/0.1% SDA at room temperature, followed by two 15-min washes in 0.1 × SSC/0.1% SDS at 55 °C. The filters were dried and then subjected to autoradiography for 24–48 h at −70 °C.

Nuclear Run-on Analysis. The transcription rates of specific genes were measured by a nuclear run-on assay with isolated nuclei. A total of 2.5×10^7 cells were washed with 1 × HEBS (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM dextrose, and 20 mM Hepes, pH 7.05) and resuspended in hypotonic lysis buffer (10 mM Hepes, pH 8.0, 1.5 mM $MgCl_2$, and 10 mM KCl). After a 15-min incubation on ice, cell membranes were disrupted by five forceful ejections through a 22-gauge needle. Intact nuclei were pelleted by centrifugation at 2000*g* for 10 min, washed, and resuspended in 220 μL of transcription buffer (20 mM Tris, pH 8.0, 6 mM MgOAc, 84 mM KCl, 10 mM NH_4Cl, 300 mM EDTA, 1 mM dithiothreitol, and 10% glycerol). The final transcription reaction also contained 12 μg/mL creatine phosphokinase (Sigma), 250 μM unlabeled ATP, CTP, and UTP (Pharmacia LKB Biotechnology, Inc.), and 250 μCi of [α-^{32}P]GTP (ICN; >3000 Ci/mmol). The reaction mixture was incubated for 30 min at 30 °C, and RNA was purified by the guanidinium thiocyanate/phenol technique. A total of (5–15) × 10^6 cpm of labeled RNA were routinely obtained with this procedure. Nitrocellulose filters were slotted with NaOH and heat-denatured plasmid DNAs as previously described (Hanson et al., 1990). ^{32}P-Labeled RNA was resuspended in 500 μL of hybridization buffer (50% formamide, 4 × SSC, 2 × Denhardt's solution, 20 μg/mL *Escherichia coli* tRNA, 0.1% SDS, and 50 mM $NaHPO_4$, pH 7.5) and boiled for 10 min. Nascent RNA chains containing equal numbers of counts were used for each hybridization. Hybridizations were performed at 42 °C for 24 h in 10 mL of hybridization buffer. Filters were then washed twice for 15 min at room temperature with 2 × SSC/0.1% SDS, followed by two 15-min washes at 60 °C with 0.1 × SSC/0.1% SDS. Autoradiography was performed at −70 °C for 4–7 days.

RESULTS

Time Course of Interstitial Collagenase and TIMP Production following TPA-Induced Differentiation of U937 Cells. U937 cells were studied in the basal state, or they were induced to differentiate by exposure to phorbol ester. After various periods of incubation with TPA, U937 cell conditioned media were collected, and secreted levels of interstitial collagenase and TIMP were measured by ELISA. Collagenase secretion was not detected in untreated U937 cells (Figure 1). Following TPA-induced differentiation, a marked increase in production of interstitial collagenase was observed with levels >100 nM present in the media by 48 h (Figure 1). However, maximal collagenase release occurred only after 36 h, and followed a prolonged lag-period (at least 16-h post-TPA) during which time little enzyme was secreted.

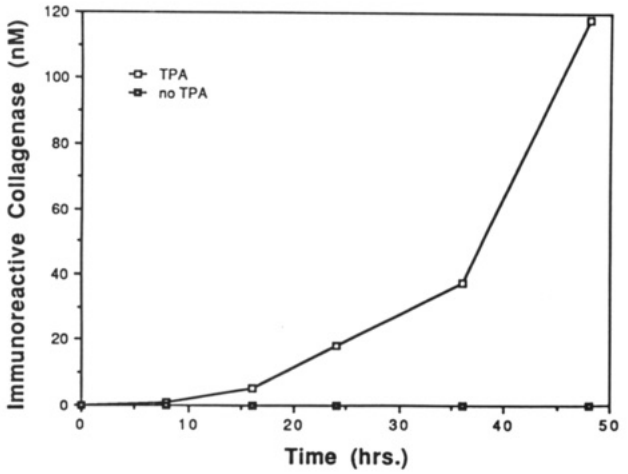

FIGURE 1: Time course of interstitial collagenase production during TPA-induced differentiation. U937 cells were cultured in the presence of TPA (1.5×10^{-8} M) (open squares) or media alone (shaded squares). Conditioned media were collected after various periods of incubation, and collagenase was determined by ELISA. Note that TPA differentiation markedly enhances collagenase release after 16 h of exposure. The maximal rate of collagenase secretion was observed between 36 and 48 h. This and all other experiments were repeated at least 4 times with essentially identical results. The data shown in Figures 1, 2, 3, and 4C were derived from the same experiment to simultaneously demonstrate changes in secreted protein, steady-state mRNA, and newly transcribed mRNA.

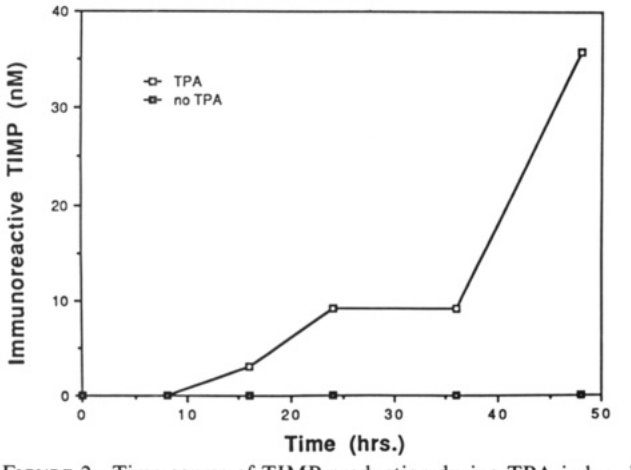

FIGURE 2: Time course of TIMP production during TPA-induced differentiation. U937 cells were cultured in the presence of TPA (1.5×10^{-8} M) (open squares) or media alone (shaded squares). Conditioned media were collected after various periods of incubation, and TIMP was determined by ELISA. Note that, similar to interstitial collagenase, TPA differentiation markedly enhances TIMP release after 16 h of exposure. Furthermore, the maximal rate of TIMP secretion was not achieved until 36–48 h of incubation.

Analysis of TIMP from identical conditioned media samples showed that inhibitor levels increased with a time course similar to that observed for interstitial collagenase induction. TPA-mediated differentiation of U937 cells resulted in markedly augmented TIMP release, but again after a lag-period of 16-h post-TPA exposure and with maximal secretion observed only after 36 h (Figure 2). Production of TIMP was greatly increased over amounts released by cells in the basal state, but absolute levels of secretion after 48 h of TPA exposure were approximately 30% those of collagenase on a molar basis. These features of collagenase and TIMP induction were consistently observed in several time course determinations. The results provided in Figures 1 and 2 are from the same experiment used to show steady-state mRNA levels (Figure 3) and transcriptional activity (Figure 4C).

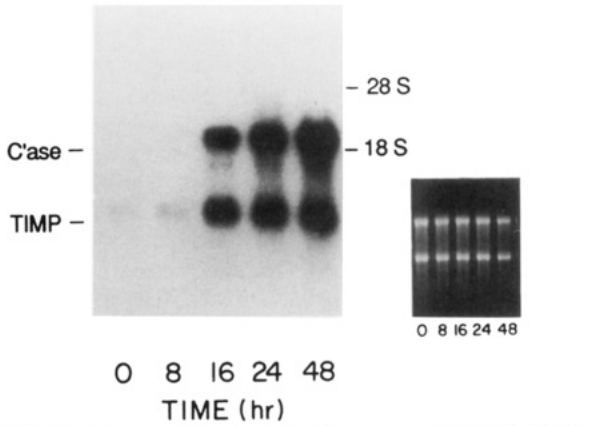

FIGURE 3: Time course of steady-state collagenase and TIMP mRNA levels during TPA-induced differentiation. U937 cells were cultured in the presence of TPA (1.5×10^{-8} M). Total cellular RNA was harvested after various periods of time by guanidinium/phenol extraction, and equal amounts of RNA (10 μg) were subjected to Northern hybridization sequentially with TIMP followed by interstitial collagenase cDNAs. Note that basal-state cells contained no detectable collagenase mRNA and little TIMP mRNA. By 16 h of exposure to TPA, steady-state mRNA levels increased markedly for both interstitial collagenase and TIMP. The inset demonstrates the quality and content of ribosomal RNA as shown by ethidium bromide staining.

Time Course of Steady-State mRNA Levels following TPA-Induced Differentiation of U937 Cells. Total cellular RNA was isolated from basal U937 cells and from differentiated cells at various times after the addition of TPA. Equal amounts of RNA were hybridized with a radiolabeled TIMP cDNA probe, and the Northern blot was then rehybridized with radiolabeled collagenase cDNA. The results shown in Figure 3 demonstrate no detectable collagenase and little detectable TIMP mRNA in basal U937 cells (time = 0 h). Following TPA exposure, steady-state mRNA levels for both genes increased markedly after a 16-h delay. In other experiments, little, if any, collagenase or TIMP mRNAs were detected at 12 h following phorbol induction (not shown). These data demonstrate that changes in interstitial collagenase and TIMP protein secretion are paralleled by increased steady-state levels of the respective mRNAs. Furthermore, these findings indicate that both products are regulated pretranslationally as U937 cells differentiate.

TPA-Induced Differentiation of U937 Cells Results in Delayed Transcription of the Interstitial Collagenase Gene. Nuclear run-on experiments were performed to directly assess rates of transcription. U937 cells were exposed to TPA for various periods of time, nuclei were isolated, and nascent RNA chains were allowed to transcribe in the presence of labeled nucleotides. RNA was isolated and hybridized to blotted, denatured cDNA. The slot blots in Figure 4A show that collagenase transcription was markedly induced at 24 h of TPA exposure. More complete time courses of collagenase transcriptional activity (Figure 4B,C) demonstrated no detectable transcription at 0 or 8 h, detectable transcription at 16 h and strong transcriptional activity at 24 h. In several other experiments, transcriptional activation of collagenase was never observed prior to 10 h following phorbol exposure (not shown). It should be noted that c-fos gene expression and c-jun gene expression are very early events in U937 cell differentiation, with transcripts accumulating within 15–30 min following phorbol exposure and dropping significantly by 2 h (Mitchell et al., 1985; Williams et al., 1990). Little or no c-fos protein is detectable after 2 h of exposure (Mitchell

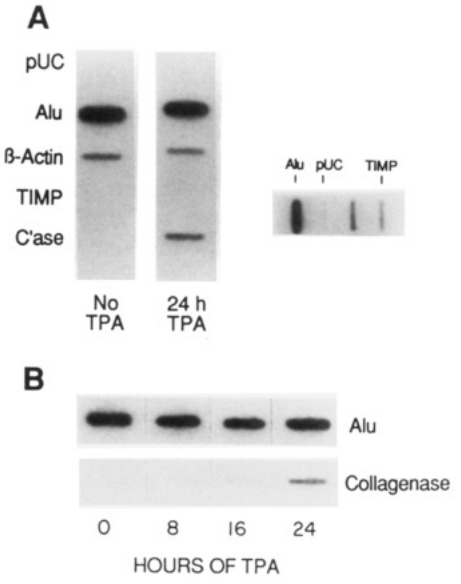

FIGURE 4: Run-on transcription analysis of U937 cells exposed to TPA. Nuclear run-on experiments were performed to directly assess transcriptional activity. (A) U937 cells were cultured in the presence of TPA (1.5×10^{-8} M) or media alone for 24 h. Nuclei prepared from these cells were allowed to transcribe in the presence of a radiolabeled nucleotide. Nascent RNA transcripts were isolated as described under Materials and Methods. A total of 2.0×10^6 cpm of each RNA sample was hybridized with a filter onto which target DNAs had been slotted. The control plasmids used in this study included pUC9, β-actin (1.9-kb β-actin cDNA in pUC9), and Alu (pUC9 containing a 3.6-kb *Eco*RI fragment that includes the genomic ϵ-globin gene and Alu sequences in the 5′ flank (Ley et al., 1989). The slot blots show that collagenase transcription is evident only following TPA exposure. TIMP transcription is not apparent either before or after TPA differentiation. (Inset) Nuclear run-on transcription was also performed on human dermal fibroblasts (CRL 1467). The blot demonstrates TIMP transcription in untreated fibroblasts. (B) U937 cells were cultured in the presence of TPA (1.5×10^{-8} M) for varying lengths of time, and then nuclear run-ons were performed as in (A). These same blots were used as controls in Ley et al. (1989). Note that collagenase transcription is not detected 8-h post-TPA exposure, becomes detectable at 16 h, and is prominent by 24 h. (C) U937 cells (from the same time course experiment shown in Figures 1, 2, and 3) were cultured in the presence of TPA (1.5×10^{-8} M) for varying lengths of time, and then nuclear run-ons were performed as described. Shown are densitometry values of collagenase and TIMP (relative to the control β-actin). Note that collagenase transcription is detectable only following an 8–16-h lag-time from TPA exposure and TIMP transcription is not detected at any time.

et al., 1985). We have shown that c-fos is transcriptionally induced within 15 min of TPA addition and that new

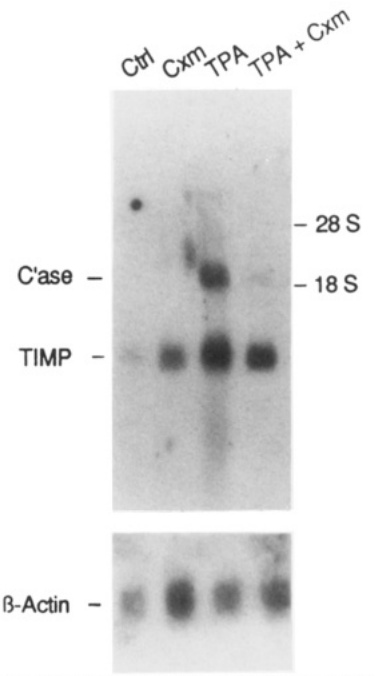

FIGURE 5: Effect of cycloheximide on steady-state mRNA levels of basal-state and TPA-differentiated U937 cells. U937 cells were exposed for 24 h to TPA (1.5×10^{-8} M), cycloheximide (3 μg/mL), neither agent (control) or both agents. Total cellular RNA was harvested, and equal amounts (5 μg) were used for Northern hybridization with cDNA probes for TIMP, interstitial collagenase, and β-actin. Note that TPA induction of collagenase mRNA is completely abolished in the presence of cycloheximide (TPA vs [TPA+Cxm]). Furthermore, cycloheximide itself causes some (1.5–2-fold when normalized for β-actin) increase in TIMP mRNA levels in basal-state cells (Cxm versus Ctrl), but only partially blocks TPA induction of TIMP mRNA (TPA vs [TPA+Cxm]).

transcription ceases at 2 h (T. J. Ley, unpublished observations).

In contrast to collagenase, newly synthesized TIMP transcripts were not detected at any time following TPA exposure of U937 cells (Figure 4A,C). This was the case in repeated experiments, even following prolonged (48 h) incubations in the presence of TPA, where collagenase transcription continued but TIMP was still not detectable (data not shown). To assess the sensitivity of the run-on assay for TIMP transcription, nuclei from human skin fibroblasts were also assayed. As shown in Figure 4A (inset), TIMP transcription was detectable in unstimulated cells. Furthermore, levels of TIMP protein secretion by unstimulated fibroblasts were approximately equivalent to those released by TPA-differentiated U937 cells.

Effect of Cycloheximide on the Induction of Collagenase and TIMP mRNA in Phorbol-Differentiated U937 Cells. To determine whether synthesis of proteins is required for collagenase and TIMP induction during cell differentiation, experiments using cycloheximide were performed. U937 cells were exposed for 24 h to TPA, cycloheximide, neither, or both agents (Figure 5). Total cellular RNA was harvested, and Northern hybridization was performed with cDNA probes for collagenase, TIMP, and β-actin. Interstitial collagenase mRNA was markedly induced by TPA, but this effect was completely inhibited by cycloheximide. In contrast to collagenase, cycloheximide only partially diminished TIMP mRNA induction following TPA exposure. Furthermore, cycloheximide alone reproducibly caused some increase in TIMP steady-state mRNA levels relative to β-actin. These data provide further evidence that the collagenase and TIMP genes may be disparately regulated by TPA exposure; enzyme

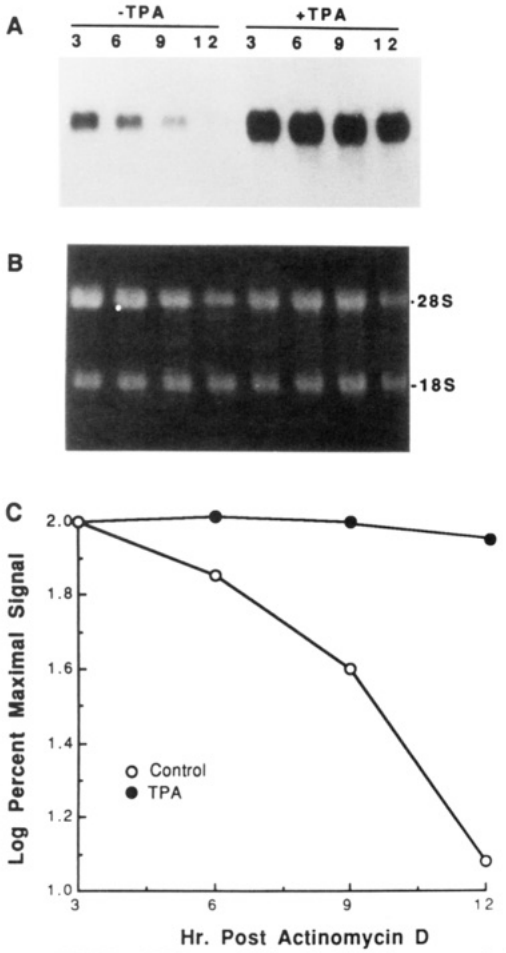

FIGURE 6: TIMP mRNA stability in basal-state and TPA-differentiated U937 cells. Basal and TPA-differentiated (24 h U937 cells were treated with actinomycin D (5 μg/mL) for various periods of time. (A) Total cellular RNA was harvested, and equal amounts of RNA were hybridized with a radiolabeled TIMP cDNA probe. (B) Ethidium bromide staining demonstrates quality and content of ribosomal RNA; 12 h of actinomycin D treatment did not significantly alter cell viability. (C) Densitometric scanning of (A) plotted in semilog form demonstrates near-linear decay of mRNAs. The half-life of TIMP mRNA for undifferentiated U937 cells is approximately 4 h (1 half-life between 3 and 7 h, for example). Following TPA-induced differentiation, TIMP mRNA was markedly stabilized with a substantially decreased slope of decay. Extrapolation of the data indicates a half-life in excess of 20 h (these calculations were confirmed by densitometry on less exposed autoradiographs as well as from independent experiments).

induction is absolutely dependent upon protein synthesis, while TIMP mRNA induction only partially requires such cellular activity.

TPA Causes a Pronounced Stabilization of TIMP mRNA. The effect of TPA exposure on transcript stability was assessed by actinomycin D experiments. Basal state and phorbol-differentiated U937 cells were treated with actinomycin D to inhibit further transcription. Total cellular RNA was harvested at various times after the addition of actinomycin D and hybridized with radiolabeled TIMP cDNA (Figure 6). Prior to TPA treatment, the decay of TIMP mRNA corresponded to a half-life of about 4 h. Following phorbol-induced differentiation, the mRNA half-life was increased markedly to >20 h. Determination of changes in collagenase mRNA half-life following U937 cell differentiation was not possible since steady-state mRNA levels were undetectable prior to differentiation using even more sensitive techniques [S1 nuclease protection assay and poly(A) RNA].

DISCUSSION

The present study provides insights into the mechanisms of interstitial collagenases and TIMP gene activation during mononuclear phagocyte differentiation. We have used TPA-induced differentiation of U937 cells as a model of monocyte/macrophage development, since we have previously shown that such cells mirror changes in the profile of serine and metalloproteinases exhibited by normal human promonocytes, monocytes, and macrophages undergoing cellular differentiation in vitro and in vivo (Welgus et al., 1985, 1986, 1990; Campbell et al., 1991). Basal U937 cells were induced to differentiate with TPA, and there was a marked but temporally delayed (>16 h) increase in the biosynthesis and release of both interstitial collagenase and TIMP. Steady-state mRNA levels for both proteins paralleled closely the secreted protein levels. For collagenase, we have shown that increased mRNA levels correspond to a temporally delayed onset of transcriptional activity. For TIMP, we were unable to demonstrate transcriptional changes; however, we did observe considerable (>5-fold) mRNA stabilization in response to TPA, which may account for a large proportion of the increased steady-state mRNA levels. It is possible that TIMP transcription is also affected, but this response was not detected by our nuclear run-on assay.

The temporal delay in the induction of collagenase transcription during phorbol differentiation of U937 cells stands in marked contrast to the stimulation of collagenase mediated by phorbol esters in fully differentiated cells, such as the human dermal fibroblast. In the latter cell type, increased steady-state levels of collagenase mRNA are detectable within the first hour of TPA exposure (Angel et al., 1987). Fibroblast collagenase stimulation requires activation of c-jun/c-fos, and this response can be duplicated with a minimal promoter containing only AP-1 recognition sites (Jonat et al., 1990; Yang-Yen et al., 1990; Schule et al., 1990). Although we have not identified the cis-acting elements responsible for collagenase induction in U937 cells, it seems possible that TPA eventually acts through the known AP-1 sites in the collagenase gene promoter. Our findings with U937 cells represent the first report to our knowledge of collagenase transcriptional activation by TPA occurring after a long lag phase. Significantly, the c-fos and c-jun genes are rapidly activated (within 15 min) in U937 cells following exposure to TPA (Mitchell et al., 1985; Williams et al., 1990). Transcription decreases for both genes within 1–2 h, and no c-fos protein is detectable by 2 h (Mitchell et al., 1985). While the precise molecular mechanisms responsible for collagenase induction in U937 cells will have to be addressed in our future studies, this process does not seem to be simply explained by a classic c-fos/c-jun heterodimer interacting with AP-1 sites in the collagenase promoter. Perhaps the delay in collagenase induction reflects the time necessary for the differentiating cell to acquire the appropriate machinery for signal transduction of an AP-1 response, or even for production of different transcription factors. In this regard, several recent reports provide other examples of collagenase gene activation that are not sufficiently explained by classic AP-1 responses (Auble & Brinckerhoff, 1991; Conca et al., 1991).

TIMP production is also regulated in various cell types by cytokines and other agents (Welgus et al., 1985; Werb, 1978; Shapiro et al., 1991; Clark et al., 1987). However, the mechanisms of TIMP gene regulation during cellular differentiation are poorly understood. The TIMP response to TPA has been studied in K562 chronic myeloid leukemia cells that undergo megakaryoblast-like differentiation in the pres-

ence of TPA (Ritta et al., 1990). In this cell line, TPA exposure results in an early (within 3 h) stimulation of TIMP biosynthesis with correspondingly rapid increases in steady-state mRNA. We have demonstrated a marked increase in TIMP protein secretion and steady-state mRNA levels in U937 cells exposed to TPA, but only after a prolonged time delay (16 h; Figures 2 and 3). However, we were unable to show increased transcriptional activity in U937 cells using nuclear run-on assays (Figure 4). We cannot explain our inability to detect TIMP transcription. Nuclear run-on assays are rather insensitive, and TIMP cDNA is <900 bp, providing a rather small target for labeled nascent RNA. Our data do not preclude the possibility that there is some increased transcriptional activity of TIMP induced by TPA, which when combined with a marked increase in mRNA stability results in the >10-fold increase in steady-state TIMP mRNA levels observed during U937 cell differentiation. Indeed, the contribution of enhanced mRNA stability may explain our failure to detect TIMP transcription in phorbol-differentiated U937 cells as contrasted with its ready demonstration in basal fibroblasts (Figure 4A) secreting comparable amounts of inhibitor protein.

The increased TIMP expression observed in TPA-differentiated U937 cells appears to be predominantly due to increased TIMP mRNA stability. We have demonstrated that basal U937 cells have a TIMP mRNA half-life of ~4 h, while differentiated U937 cell TIMP mRNA has a half-life in excess of 20 h (Figure 6). This finding suggests that U937 cells utilize multiple mechanisms to up-regulate expression of metalloproteinases and their counter-regulatory inhibitor. We do not yet know if 3′-untranslated or other specific mRNA sequences are important for mRNA stabilization, nor do we know the role of ribonucleases or translation in this process. Future studies will be directed to determine the mechanisms involved in TIMP mRNA stabilization.

The data in this report also suggest that molecular mechanisms controlling collagenase and TIMP induction in U937 cells are probably disparate. The contributions of transcriptional activation for collagenase and of mRNA stability for TIMP have already been discussed. Additional evidence for different mechanisms regulating induction of the two proteins is seen by response of the cells to cycloheximide. Cycloheximide has multiple effects on cells including inhibition of translation and in some instances mRNA stabilization (Shaw & Kamen, 1986; Brawerman, 1987). Cycloheximide completely ablated the TPA induction of interstitial collagenase mRNA, indicating that synthesis of new proteins is required for initiation of collagenase transcription. In contrast, cycloheximide only partially blocked TPA induction of TIMP mRNA. Furthermore, our data also show that TIMP mRNA levels were raised in basal-state cells by cycloheximide alone; no such response was obeserved for collagenase mRNA. Thus, the requirement of new protein synthesis differs for collagenase and TIMP gene activation in TPA-induced U937 cells.

The expression of metalloproteinases and TIMP by mononuclear phagocytes during growth, inflammation, and repair is under complex regulatory control. Our findings of coordinate up-regulation of these proteins in response to endotoxin (Cury et al., 1988) and their coordinate down-regulation with corticosteroids (Shapiro et al., 1991) initially suggested a simple paradigm whereby the cell either turned on or turned off all enzymes in response to various stimuli, and TIMP accompanied the enzymes to limit injury of normal tissue. As more potential mediators have been studied, it has become apparent that the control mechanisms are far more complex. For example, interferon-γ causes down-regulation of interstitial collagenase and stromelysin production, with little effect upon levels of secreted 92-kDa type IV collagenase or TIMP (Shapiro et al., 1990). Furthermore, these responses can be cell-type-specific. While dexamethasone suppresses TIMP production in mononuclear phagocytes, this agent fails to alter TIMP biosynthesis in human fibroblasts (Clark et al., 1987). The present study adds another level of complexity to the regulation of these genes by providing an example where coordinate up-regulation of interstitial collagenase and TIMP may be achieved by different molecular mechanisms. Further understanding of the regulation of the genes that encode these proteins may enhance our ability to intervene in pathologic processes where normal control mechanisms have become aberrant, resulting in tissue destruction or tumor invasion.

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REFERENCES

Angel, P., Baumann, I., & Stein, B. (1987) *Mol. Cell. Biol. 7*, 2256–2266.
Auble, D. T., & Brinckerhoff, C. E. (1991) *Biochemistry 30*, 4629–4635.
Brawerman, G. (1987) *Cell 48*, 5–6.
Campbell, E. J., Cury, J. D., & Lazarus, C. J. (1987) *J. Biol. Chem. 262*, 15862–15868.
Campbell, E. J., Silverman, E. K., & Campbell, M. A. (1989) *J. Immunol. 143*, 2961–2968.
Campbell, E. J., Cury, J. D., & Shapiro, S. D. (1991) *J. Immunol. 146*, 1286–1293.
Carmichael, D. F., Sommer, A., & Anderson, D. C. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2407–2411.
Chomczynski, P., & Sacchi, N. (1987) *Anal. Biochem. 162*, 156–159.
Clark, S. D., Kobayashi, D. K., & Welgus, H. G. (1987) *J. Clin. Invest. 80*, 1280–1288.
Conca, W., Auron, P. E., & Aounwath, M. (1991) *J. Biol. Chem. 266*, 6265–6268.
Cooper, T. W., Bauer, E. A., & Eisen, A. Z. (*Collagen Relat. Res. 3*, 205–216.
Cury, J. D., Campbell, E. J., & Lazarus, C. J. (1988) *J. Immunol. 141*, 4306–4312.
Goldberg, G. I., William, S. M., & Kroneberger, A. (1986) *J. Biol. Chem. 261*, 6600–6605.
Hanson, R. D., Connolly, N. L., & Burnett, D. E. (1990) *J. Biol. Chem. 265*, 1524–1530.
Harris, P., & Ralph, P. (1985) *J. Leukocyte Biol. 37*, 407–422.
Henney, A. M., Wakeley, P. R., & Davis, M. J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 8154–8158.
Jonat, G., Rahmsdorf, H. J., & Park, K.-K. (1990) *Cell 62*, 1189–1204.
Ley, T. J., Connolly, N. L., & Katamine, S. M. (1989) *Mol. Cell. Biol. 9*, 92–99.
McCachren, S. S. (1991) *Arthritis Rheum. 34*, 1085–1093.
Mitchell, R. L., Zokas, L., & Schreiber, R. D. (1985) *Cell 40*, 209–217.
Ritta, A., Partanen, H., & Pertovaara, L. (1990) *Blood 75*, 1974–1982.
Schule, R., Rangarajan, P. S., & Kliewer, S. (1990) *Cell 62*, 1217–1226.
Senior, R. M., Connolly, N. L., Campbell, E. J. & Welgus, H. G. (1986) in *Pulmonary Emphysema and Proteolysis 1986* (Taylor, J. C., & Mittman, C., Eds.) pp 219–226, Academic Press, Orlando.
Shapiro, S. D., Campbell, E. J., & Kobayashi, D. K. (1990) *J. Clin. Invest. 86*, 1204–1210.

Shapiro, S. D., Campbell, E. J., & Kobayashi, D. K. (1991) *J. Immunol. 146*, 2724–2729.
Shaw, G., & Kamen, R. (1986) *Cell 46*, 659–667.
Wahl, L. M., & Winter, C. C. (1984) *Arch. Biochem. Biophys. 230*, 661–667.
Welgus, H. G., & Stricklin, G. P. (1983) *J. Biol. Chem. 258*, 12259–12264.
Welgus, H. G., Campbell, E. J., & Bar-Shavit, Z. (1985) *J. Clin. Invest. 76*, 219–224.
Welgus, H. G., Connolly, N. L., & Senior, R. M. (1986) *J. Clin. Invest. 77*, 1675–1681.
Welgus, H. G., Campbell, E. J., & Cury, J. D. (1990) *J. Clin. Invest. 86*, 1496–1502.
Werb, Z. (1978) *J. Exp. Med. 147*, 1695–1712.
Williams, F., Wagner, F., & Karin, M. (1990) *J. Biol. Chem. 265*, 18166–18171.
Yang-Yen, H.-F., Chambard, J.-C., & Sun, Y.-L. (1990) *Cell 62*, 1205–1215.