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Authors
Aggeler, J
Frisch, SM
Werb, Z

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Collagenase Is a Major Gene Product of Induced Rabbit Synovial Fibroblasts

JUDITH AGGELER,* STEVEN M. FRISCH,*‡ and ZENA WERB*
*Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, California 94143; and ‡Department of Biochemistry, University of California, Berkeley, California 94720

ABSTRACT We have investigated the effects of the tumor-promoting phorbol diester, 12-O-tetradecanoylphorbol-13-acetate (TPA), on rabbit synovial fibroblasts, and found that this agent induced a major switch in gene expression in these cells that was marked by the specific induction of the neutral proteinase, collagenase, and was always accompanied by alterations in cell morphology. Procollagenase synthesis and secretion was first observed 6–12 h after the addition of TPA. The rate of collagenase production (1–5 U, or ~0.2–1 μg secreted procollagenase protein per 10^5 cells per 24 h) depended on the TPA concentration (1–400 ng/ml) and time of exposure (1–72 h). Procollagenase was the most prominent protein visible by direct silver staining or by autoradiography after SDS PAGE of [^{35}S]methionine-labeled proteins. The two procollagenase bands of M_r 53,000 and 57,000, which migrated as a family of spots on two-dimensional gels and were immunoprecipitated by antibodies to purified rabbit collagenase, accounted for 23% of the newly synthesized, secreted protein in TPA-treated cells. Cell-free translation of mRNA from TPA-treated cells in rabbit reticulocyte lysate produced a single band of immunoprecipitable preprocollagenase (M_r 55,000) as a major product (5% of total) that migrated as a single spot on two-dimensional gels. Secreted procollagenase, preprocollagenase, and active collagenase (purified to homogeneity; specific activity 1.2 × 10^4 U/mg protein) had related peptide maps. Two other major secreted proteins, a neutral metalloproteinase of M_r 51,000 and a polypeptide of M_r 47,000, were also induced by TPA. In contrast to the induction of these four polypeptides, TPA decreased synthesis and secretion of a number of proteins, including collagen and fibronectin. Thus, collagenase is a convenient marker for major alterations in the pattern of protein synthesis and secretion by rabbit synovial fibroblasts treated with TPA.

The extracellular scaffolding of loose connective tissue and basement membrane present in most tissues is composed of a complex matrix of proteins, many of which are relatively resistant to degradation by proteinases. This extracellular matrix is remodeled during development, growth, and healing, and matrix destruction is central to the pathology of many diseases. Therefore, it is important to identify and characterize those enzymes that can degrade specific matrix components, such as collagen, elastin, glycoproteins, and proteoglycans, and to understand how cells can control matrix structure by specific alterations in proteinase activity. For example, it has been suggested that increased secretion of both collagenase and plasminogen activator by tumor cells may play a central role in malignant tumor invasion and metastasis by allowing cells to migrate through complex connective tissue barriers and enter the blood circulation (22, 27).

We have been interested in the role of the specific neutral proteinase, collagenase, in both normal and pathologic processes. In previous studies we and others have demonstrated that a variety of agents can stimulate fibroblastic cells to produce collagenase, as determined by collagen-degrading activity (1, 3, 9, 14, 42). In these studies collagenase activities ranging over 5 orders of magnitude (0.001–10 U per 10^5 cells per 24 h) have been reported. In the present study we show that the phorbol diester tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA)[1], is one of the most potent

[1] *Abbreviations used in this paper*: DME, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; kd, kilodalton; preproCL, preprocollagenase; proCL, procollagenase; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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inducers of collagenase activity and that rabbit synovial fibroblasts treated with TPA are stimulated to produce collagenase as ≤5% of their total protein synthesis. We have correlated the synthesis and secretion of active collagenase and its inactive precursor procollagenase (proCL) by using enzymologic, immunochemical, and biosynthetic radiolabeling techniques and have shown that induction of collagenase marks a major switch in phenotypic expression in rabbit synovial fibroblasts.

MATERIALS AND METHODS

Cell Culture: Rabbit synovial fibroblasts were harvested from the knee joints of 4–6-wk-old New Zealand white rabbits and maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal bovine serum (FBS) (42). Cells were subcultured weekly (1:5) with 0.25% trypsin and used between passages 1 and 7. For most experiments cells were plated into flasks in DME-FBS 16–48 h before washing with Hanks' balanced salt solution and addition of serum-free medium supplemented with 0.2% lactalbumin hydrolysate. TPA (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol (1 mg/ml) and stored at −20°C.

Collagenase Assay: Enzymatic activity of collagenase was determined by means of a collagen fibril assay, using samples activated with L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-trypsin (10 μg/ml; Sigma Chemical Co.) for 30 min at 25°C (42, 43). Solubilized ^{14}C-labeled peptides were measured by liquid scintillation spectrometry. In all samples reported, the proportion of latent collagenase was always >98%, as determined by sham activation with trypsin premixed with soybean trypsin inhibitor. 1 U of collagenase activity hydrolyzes 1 μg of collagen per minute at 37°C.

Labeling of Cellular and Secreted Proteins: Cells plated into 12-well culture dishes (25-mm diam, Linbro Division, Flow Laboratories Inc., Hamden, CT) and exposed to TPA were pulse-labeled with 25 μCi/ml [^{35}S]-methionine (1,350 Ci/mmol, Amersham Corp., Arlington Heights, IL) in methionine-free DME. After 0.5–6 h, radiolabeled protein was precipitated from the medium by the addition of cold 45% trichloroacetic acid (44). To control for degradation of cellular and secreted proteins by endogenous proteinases, a cocktail of inhibitors of proteolytic enzymes (1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 10 mM *N*-ethylmaleimide, 1 μg/ml pepstatin) was added to the conditioned medium immediately after the labeling period in some experiments; no differences were observed in protein migration patterns with or without inhibitors. The washed protein pellets were resuspended in Laemmli sample buffer containing 0.5% β-mercaptoethanol, and boiled for 3 min before electrophoresis. Labeled cells were scraped into 0.5% Triton X-100, and diluted with 2 × modified Laemmli sample buffer.

SDS PAGE: SDS PAGE was carried out according to the method of Laemmli (19) on continuous gradient gels (usually 7–15%) with 3% stacking gels under reducing conditions (44). During the course of these experiments, we discovered that modifying the normal Laemmli sample buffer to reduce the concentration of β-mercaptoethanol to 0.5% improved the separation of proCL and the neutral metalloproteinase. Molecular weight markers used were cytochrome *c* (12 kilodaltons [kd]), lysozyme (14.3 kd), lactoglobin (18 kd), carbonic anhydrase (30 kd), immunoglobulins (25 and 50 kd), bovine serum albumin (68 kd), phosphorylase B (93 kd), macroglobulin (185 kd), myosin (200 kd), and filamin (240 kd). Dried gels were autoradiographed with Kodak X-Omat AR x-ray film, preflashed before exposure (20). Some autoradiographs were scanned with an integrating densitometer to determine the percentage of radioactivity incorporated into specific polypeptide bands. Two-dimensional PAGE using nonequilibrium pH gradient electrophoresis in the first dimension and 7–15% SDS PAGE slab gels in the second dimension was carried out as described by Jones (15).

The radiolabeled bands on the SDS gels that corresponded to procollagen(s) were identified by their sensitivity to purified clostridiopeptidase A (bacterial collagenase, Advanced Biofactures, Lynnbrook, NY). The procollagen bands contained hydroxyproline as determined by incorporation of [^{3}H]proline into hydroxyproline (16) and were susceptible to digestion with purified clostridiopeptidase A. Rabbit fibroblasts incorporated radioactivity into procollagen type I/procollagen type III/fibronectin in the ratio of approximately 1:2.5:2. Fibronectin was identified by its binding to *Staphylococcus aureus* (35).

Peptide mapping of [^{35}S]methionine-labeled collagenase, proCL, and preproCL was carried out according to the method of Cleveland et al. (11) by cutting labeled bands out of SDS PAGE gels and digesting them in situ with chymotrypsin (5–100 μg/lane) or with *S. aureus* V8 proteinase (5 μg/lane) at 22°C for 30 min. The peptide digests were analyzed by SDS PAGE, dried, and fluorographed as described. For comparison, collagenase secreted by rabbit synovial fibroblasts was purified essentially by the method of Cawston and Tyler (10) to a specific activity of 1.17 × 10^{4} U/mg protein (data not shown) and radiolabeled with ^{125}I (7) before peptide mapping. Active collagenase migrated as a doublet of 42 and 47 kd.

Immunochemical Procedures: Antisera against purified rabbit fibroblast collagenase were raised in sheep and characterized as immunologically monospecific (48, 49). For some experiments IgG fractions were prepared by affinity chromatography on protein A–Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). It should be noted that the binding of monomeric sheep IgG to staphylococcal protein A is quite poor (5–10% of total) (21); however, binding of sheep IgG immune complexes is quantitative, so that immunoprecipitation is easily accomplished (17). Collagenase was immunoprecipitated from medium by a modification of the method of Jones (15) using formalin-fixed *S. aureus* (Zysorbin, Zymed Labs, Burlingame, CA). Preabsorbed samples were incubated with 20–30 μg anticollagenase IgG for 30 min at room temperature and then precipitated by the addition of freshly prepared *S. aureus*. Washed *S. aureus* precipitates were resuspended in Laemmli sample buffer, boiled, and analyzed by SDS PAGE.

Cell-free Translation of Fibroblast mRNA: RNA was prepared from rabbit synovial fibroblasts according to the method of Monson (26). Cells were collected by scraping into 5 M guanidine thiocyanate (pH 8.0), 0.1% β-mercaptoethanol, 2% Sarkosyl (ICN Pharmaceuticals Inc., Plainview, NY), and 50 mM EDTA, and lysates were then overlaid onto a CsCl cushion and centrifuged for 22 h at 26,000 rpm in a Beckman Model L ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) with an SW27 rotor. The pellet was dissolved by heating in 1 mM EDTA, and RNA was precipitated with ethanol/lithium acetate. The final pellet was dissolved in H_2O at 1 mg/ml and stored at −80°C. The average yield was 40 μg total RNA/confluent T175 flask (~10^{7} cells). For isolation of polyA^{+}-containing RNA, samples of total rabbit RNA were passed over an oligo(dT)cellulose column (Collaborative Research, Boston, MA) according to the method of Aviv and Leder (5). Synovial fibroblast proteins were synthesized in vitro according to the method of Pelham and Jackson (32) using rabbit reticulocyte lysate (methionine-free, BRL, Bethesda, MD). A typical sample (30 μl) contained BRL reaction mixture, 0.08 M potassium acetate buffer (pH 7.2), 15 μCi [^{35}S]methionine, and 5 μg of total RNA or 0.5 μg of polyA^{+}-containing RNA. After 60 min at 30°C, the reaction was stopped by addition of 3 μg RNase A (Worthington Biochemical Corp., Freehold, NJ) followed by incubation at 30°C for 15 min, and addition of 2 × Laemmli sample buffer. In some cases, newly translated proteins were immunoprecipitated from these reaction mixtures before addition of sample buffer.

RESULTS

TPA Induces Procollagenase Production

TPA concentrations of 1–400 ng/ml (1.6 × 10^{-9} to 6.4 × 10^{-7} M) caused a dose-dependent increase in activatable proCL secreted by rabbit synovial fibroblasts (1–3). The range of TPA concentrations that induced proCL secretion is similar to that observed for other effects of TPA (31, 36), but the amount of collagenase activity produced was much greater than that previously reported for other cell types (9, 22, 27, 40, 41). When proCL secretion by TPA-treated cells was analyzed by SDS PAGE of [^{35}S]methionine-labeled proteins, dramatic differences were observed between the secreted proteins of control and TPA-stimulated cells (Fig. 1 *a*). Polypeptide bands of 47, 51, 53, and 57 kd were specifically increased, accounting for 40–50% of the secreted protein. The two polypeptides of 53 and 57 kd were identified as proCL by immunoprecipitation with specific IgG (Fig. 1 *b*); the 57 kd band is a more highly glycosylated form of proCL (28, 29). These proCL bands accounted for 23% of the labeled secreted protein in maximally stimulated cells, as judged by densitometry of one-dimensional gels. Based on the specific activity of purified active collagenase (1.17 ×10^{4} U/mg protein), this corresponds to secretion of ~0.4 μg of proCL per 10^{5} cells per 24 h, as confirmed by direct silver staining (25) of proCL in unconcentrated culture medium (not shown). [^{35}C]Methionine-labeled proCL activated by limited trypsin digestion was converted to active collagenase of 42 and 47 kd, identical to the purified enzyme (not shown). This is consistent with the concept that active collagenase is derived from its inactive

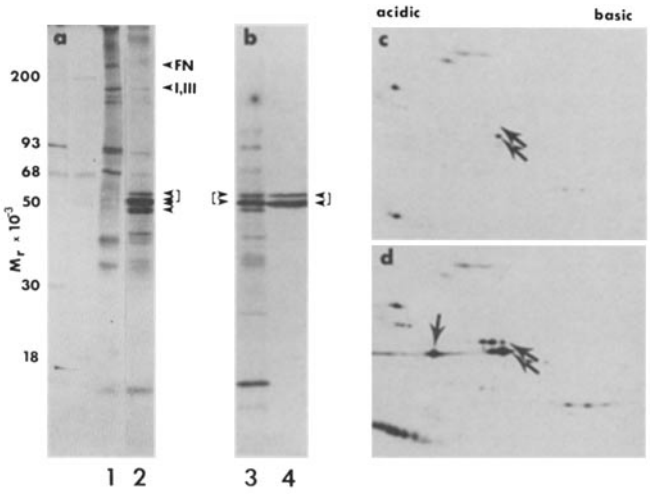

FIGURE 1 SDS PAGE demonstration of proCL secretion. Rabbit synovial fibroblasts cultured in the presence or absence of TPA were incubated with [^{35}S]methionine, and radiolabeled, secreted proteins were analyzed by SDS PAGE. (*a*) Secreted proteins from untreated control cells (lane *1*) or cells treated with 100 ng/ml TPA for 48 h before labeling (lane *2*). Migration of procollagen types I and III (*I*, *III*) and fibronectin (*FN*) are indicated by arrowheads. Migration of molecular weight markers is indicated at left. (*b*) Secreted proteins from TPA-treated cells before (lane *3*) and after (lane *4*) immunoprecipitation with specific antibodies to rabbit collagenase. Standard Laemmli sample buffer containing 5% β-mercaptoethanol was used in these experiments. In *a* and *b*, arrowheads indicate positions of the major polypeptides of 47, 51, 53, and 57 kd that are induced in TPA-treated cells, and brackets indicate the 53 and 57 kd proCL bands. (*c* and *d*) Part of the two-dimensional electropherograms of proteins secreted by control cells (*c*) or cells treated with 10 ng/ml TPA for 24 h (*d*). Arrows pointing to upper left indicate proCL spots and the arrow pointing downward indicates the migration of the 51-kd polypeptide.

precursor (proCL) by loss of a fragment of ~10 kd (10, 40). The 47 and 51 kd polypeptides were not immunoprecipitated by anticollagenase IgG, and two-dimensional gel electrophoresis showed that the 51 kd polypeptide was distinct from proCL (Fig. 1*d*). The 47 kd polypeptide appeared to be specific for TPA-treated cells (2) and was strongly induced only at very high concentrations of TPA (see Fig. 4). We have recently identified the 51 kd polypeptide as a neutral metalloproteinase (12).

The SDS PAGE patterns of proteins secreted by TPA-treated fibroblasts also indicate that the dramatic increase in proCL and 51 kd polypeptide secretion induced by TPA was accompanied by a decrease of a number of secreted proteins, including procollagen and fibronectin (Fig. 1*a*). The specific reduction of collagen synthesis in cells treated with TPA was confirmed by measuring incorporation of hydroxyproline into secreted proteins. Cells treated with TPA (20 ng/ml) for 23 h showed a 50% decrease in [^{3}H]hydroxyproline incorporation compared to untreated cells (0.75×10^4 dpm vs. 1.47×10^4 dpm). Because proCL is secreted as an inactive proenzyme, it is unlikely that this decrease in procollagen was due to extracellular degradation. When fibroblasts were plated directly onto [^{3}H]collagen layers (46) in the presence of TPA, no collagen degradation was detected even though proCL was present in the medium (data not shown).

Onset of ProCL Synthesis Occurs within 6–12 h of TPA Treatment

Increased production of collagenase was first observed in the medium of rabbit synovial fibroblasts between 6–12 h after the addition of TPA (Figs. 2 and 3). Although incubation with TPA for as little as 1 h was sufficient to induce subsequent collagenase secretion, a 6-h pulse of TPA produced a near-maximum effect when measured at 72 h (Fig. 2*a*). Accumulation of secreted collagenase in the medium often slowed 2–3 d after initial TPA treatment (Fig. 2*b*), probably owing to metabolic inactivation of the ester (30, 39). Cells treated continuously with TPA were maintained in a stimulated state for a longer time (Fig. 2*b*). The increase in collagenase activity, as measured enzymatically, was paralleled by increases in newly synthesized proCL protein analyzed by SDS PAGE (Fig. 3). Increased synthesis of proCL protein was observed as early as 6 h after addition of TPA (Fig. 3), and proCL represented a substantial proportion of the pulse-labeled, secreted protein even 72 h after induction, indicating that synthesis was sustained for several days after an initial 6-h treatment. As is the case for induction of collagenase by proteinases (42) and by cytochalasin B (14), distinct alterations in cell shape were always observed in TPA-stimulated cells during the initial 1–12 h induction period (2).

Collagenase Synthesis Rates Are Reflected by Translatable mRNA

The consistent delay in collagenase secretion after addition of TPA to cells suggested to us that specific changes in translatable collagenase mRNA might be taking place during the initial induction period (8). We therefore translated mRNA from control and TPA-treated cells in rabbit reticulocyte lysate and analyzed the polypeptides by one- and two-dimensional PAGE. In TPA-treated cells preprocollagenase (preproCL) (29) was identified as a major polypeptide of 55 kd that was immunoprecipitated by specific antibodies to rabbit collagenase and that constituted ~5% of the total in vitro synthesized protein (Fig. 4*a*). This was comparable to the percentage of proCL (53 kd) associated with cell layers after a short pulse-labeling period, which approximates the in vivo rate of protein synthesis. ProCL and preproCL were

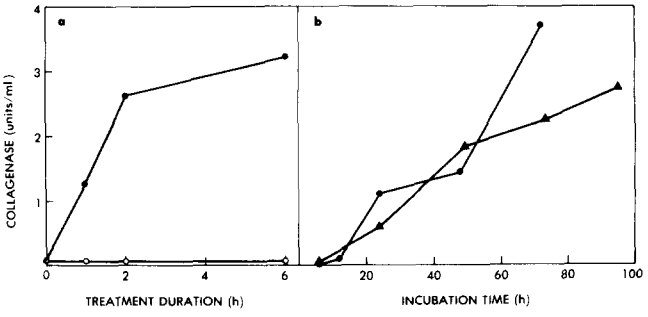

FIGURE 2 Effect of treatment duration on induction of collagenase by TPA. Rabbit synovial fibroblasts were incubated with 20 ng/ml TPA in serum-free medium. (*a*) Cells were incubated in the presence (●) or absence (○) of TPA for the times indicated and then washed and returned to serum-free medium for 72 h before assay. (*b*) Cells were incubated with TPA throughout the experiment (●) or for the first 6 h only (▲), and samples of medium were removed and assayed for collagenase activity at the times indicated.

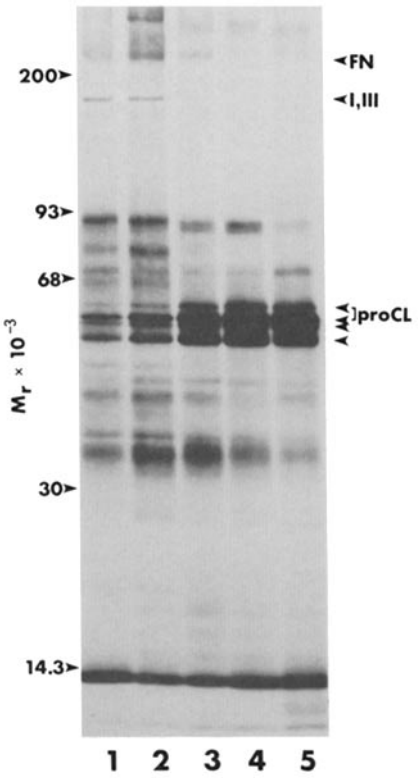

FIGURE 3 Time course of secretion of [^{35}S]methionine-labeled proCL. Rabbit synovial fibroblasts were preincubated with 100 ng/ml TPA for 6 h, washed, and returned to medium without TPA. At the times indicated, medium containing 25 μCi/ml [^{35}S]methionine was added. After 2 h the labeled, secreted proteins were precipitated with trichloroacetic acid and prepared for SDS PAGE, as described in Materials and Methods. Secreted polypeptides of TPA-treated cells after 6 h (lane *2*), 24 h (lane *3*), 48 h (lane *4*), or 72 h (lane *5*), or of untreated control cells (lane *1*). (In some experiments, control cells produced some proCL even in the absence of TPA.) Arrowheads indicate the positions of the four major polypeptides of 47, 51, 53, and 57 kd that are induced by TPA. *proCL*, procollagenase; *I,III*, procollagen types I and III; *FN*, fibronectin. Migration of molecular weight markers is indicated at left.

barely detectable among the polypeptides of control cells (Fig. 4, *a* and *b*). In contrast to secreted proCL, which migrated as a series of related species of 53 and 57 kd (Fig. 1 *d*), the 55 kd preproCL from cell-free translations migrated as a single spot on two-dimensional gels (Fig. 4 *c*). It is interesting to note that the 51 kd neutral metalloproteinase was detected only in TPA-treated cells. The precursor of this protein was not readily visible among the cell-free translation products of TPA-treated cells, but in preliminary experiments a polypeptide of ~53 kd was immunoprecipitated by specific antibodies against the proteinase (12, 13).

As a final confirmation of the identities of preproCL and proCL, the 53 and 55 kd bands were cut from one-dimensional SDS PAGE gels and partially digested with *S. aureus* V8 proteinase or with chymotrypsin. The resulting peptide maps showed that preproCL was highly related to secreted proCL and to purified active CL, except for two additional peptides in the V8 proteinase maps that may arise from the leader region (Fig. 5) (29).

DISCUSSION

We have described a cell culture system in which a major switch in protein secretion phenotype is induced in rabbit synovial fibroblasts treated with the tumor-promoting phorbol diester, TPA. As part of the altered gene expression, these cells are induced to synthesize and secrete the neutral proteinase, procollagenase. Because collagen is resistant to the action of most mammalian proteinases and is cleaved only by collagenase, detailed information about the control of collagenase secretion and activity is necessary to understand a wide variety of processes that involve either connective tissue destruction or remodeling. Collagenase accounted for up to 5% of the newly synthesized pulse-labeled protein and of the protein translated in vitro from TPA-induced cell RNA, and for ~23% of the secreted protein. Although the high specific activity of rabbit fibroblast collagenase (>10^4 U/mg) indicates that a large amount of degradation can be accomplished by a very small amount of collagenase protein, stimulated rabbit synovial fibroblasts secreted so much collagenase that it could easily be identified by autoradiography or by silver staining after SDS PAGE of unconcentrated medium. Other cell types typically secrete 1/10 to 1/1,000 of the collagenase activity of TPA-induced synovial fibroblasts, and all measurements of enzyme activity are complicated by balancing collagenase activity with inhibition by endogenous collagenase inhibitors (41).

In addition to the proCL doublet, TPA also induced synthesis and secretion of two major polypeptides of 47 and 51 kd. It has been reported that collagenase secretion is usually accompanied by the secretion of neutral metalloproteinase activities that degrade gelatin, casein, and proteoglycans (13, 45, 47), and these are separated from collagenase during purification (47). Recent experiments (12) have identified the 51-kd secreted protein as a metalloproteinase with multiple substrates, including proteoglycans, fibronectin, and laminin. The 47 kd polypeptide was induced only by very high concentrations of TPA (>50 ng/ml). TPA is a potent inducer of plasminogen activators (4, 23, 50), and it is possible that the 47 kd polypeptide is a plasminogen activator.

It is interesting to note that collagen, the specific substrate for collagenase, is synthesized and secreted by rabbit synovial fibroblasts. Our data indicate that procollagen synthesis was reduced in TPA-induced cells, a pattern opposite to that of collagenase. This negative control of procollagen synthesis in TPA-treated cells is one indication that stimulation of collagenase synthesis and secretion was a specific effect of TPA treatment, rather than a by-product of the generalized increase in protein secretion caused by this agent (2). This model system promises to be useful not only for elucidating the molecular mechanisms controlling the induction of a major gene product, but also for understanding the cellular control of connective tissue degradation and turnover (6).

In the present study we have used the tumor-promoting diester, TPA, to characterize the induction of collagenase production by rabbit synovial fibroblasts. This agent was chosen for these studies because of its potency as an inducer, but it has the drawbacks that its effects on cells are pleiomorphic, it persists in cells, and its mechanism of action is largely unknown. TPA has been reported to alter a variety of cellular functions, many of which appear to involve membranes, such as endocytosis and spreading (34), secretion (9,

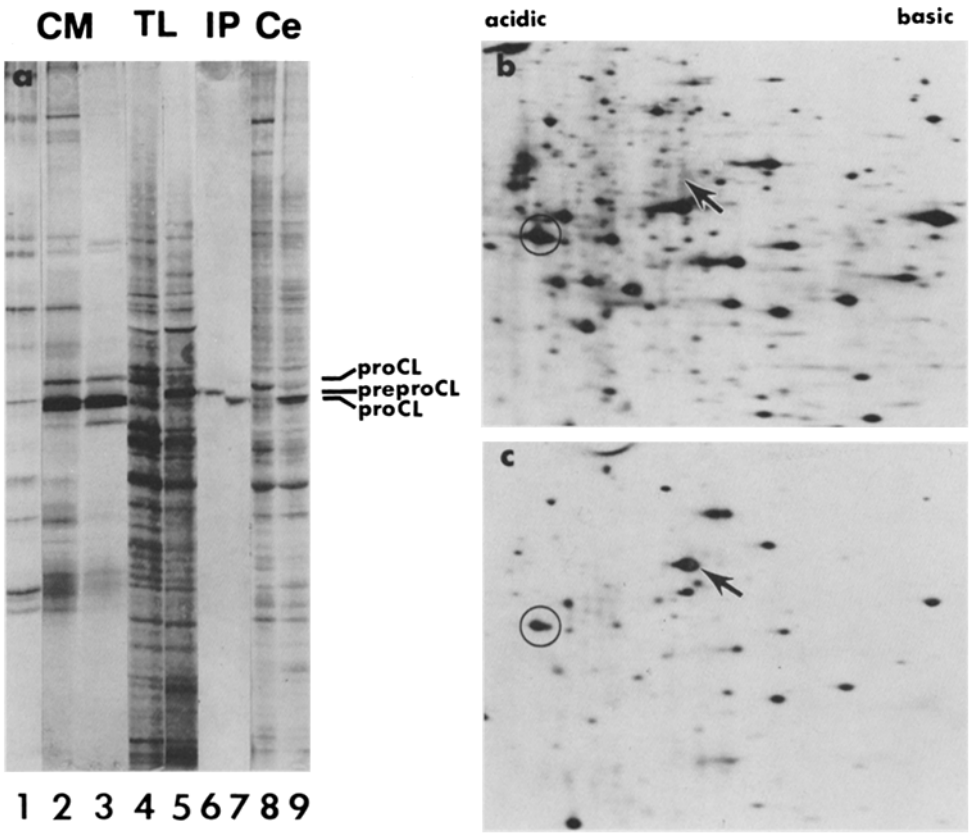

FIGURE 4 Induction of translatable mRNA for preproCL by TPA. (*a*) One-dimensional SDS PAGE of [^{35}S]-methionine-labeled proteins secreted (*CM*) by rabbit synovial fibroblasts after 24 h of treatment with 0 (lane *1*), 10 (lane *2*), or 50 ng/ml (lane *3*) TPA. [^{35}S]Methionine-labeled proteins from the cell-free translation (*TL*) of 5 μg total RNA isolated from control (lane *4*) or 400 ng/ml TPA-treated (lane *5*) cells. Immunoprecipitation (*IP*) of [^{35}S]methionine-labeled collagenase from cell-free translations of isolated RNA (lane *6*) or medium (lane *7*) from cells treated with 100 ng/ml TPA. [^{35}S]Methionine-labeled proteins of control (lane *8*) or 10 ng/ml TPA-treated (lane *9*) cells (Ce) pulse-labeled for 30 min. (*b* and *c*) Two-dimensional gel analyses of rabbit synovial fibroblast proteins synthesized by cell-free translation of equal amounts of mRNA derived from untreated control cells (*b*) or cells treated with 400 ng/ml TPA for 48 h (*c*). Arrow indicates preproCL (55 kd); the prominent actin spot is circled at the lower left in each panel.

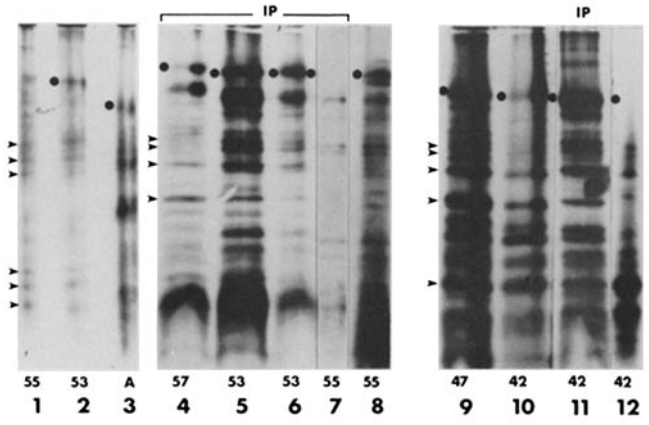

FIGURE 5 Peptide mapping of rabbit collagenase. To compare different forms of rabbit synovial fibroblast collagenase, radiolabeled bands were cut out of SDS PAGE gels, incubated in situ with *S. aureus* proteinase V8 (lanes *1–3*) or with chymotrypsin (lanes *4–12*), and reelectrophoresed. For lanes *1–11*, collagenase was labeled with [^{35}S]methionine; for lane *12*, purified collagenase was labeled in vitro with ^{125}I. Samples that were immunoprecipitated (*IP*) before digestion (lanes *4–7* and *11*) are indicated at top. Molecular weights of starting bands are given below each lane, and migration of the undigested proteins is indicated by a dot. Arrowheads indicate major common peptide fragments. Lane *1*, PreproCL from cell-free translation; lane *2*, secreted proCL; lane *3*, actin; lanes *4* and *5*, proCL secreted by TPA-treated cells; lane *6*, proCL immunoprecipitated from TPA-treated cell lysate; lanes *7* and *8*, preproCL from cell-free translations; lanes *9* and *10*, secreted proCL activated with trypsin; lane *11*, immunoprecipitated activated collagenase; lane *12*, purified active collagenase. Units of measure are kilodaltons.

27, 50), phagosome/lysosome fusion (18), and O_2 metabolism (39). In addition, TPA can affect the programmed differentiation of cells in culture (24, 38). One of the most interesting, but least understood, effects of TPA is its ability to cause morphologic changes in treated cells (36). These changes may be brought about by alterations in the complex association of cytoskeletal filaments (33, 37), especially in the binding of these filaments to the plasma membrane. We have found that alteration of cell morphology also accompanies proCL induction by TPA, and the relationship of collagenase induction to changes in cell shape is the subject of the accompanying paper (2).

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