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Increased Level of Translatable Collagenase Messenger Ribonucleic Acid in Rabbit Synovial Fibroblasts Treated with Phorbol Myristate Acetate or Crystals of Monosodium Urate Monohydrate†

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ABSTRACT: We studied mechanisms governing production of the neutral proteinase collagenase by synovial cells. We used a model system of monolayer cultures of rabbit synovial fibroblasts stimulated to produce collagenase by treatment with phorbol myristate acetate or crystals of monosodium urate monohydrate. mRNAs from these and untreated cells were translated in a wheat germ cell-free system. Collagenase was not present in the culture medium or in the in vitro translation products of mRNA from untreated cells but was present in both the medium and translation products of stimulated cells, as analyzed by gel electrophoresis and immunoprecipitation with monospecific antibody. Induction of collagenase was prevented by treatment of the cells with α-amanitin (2 μg/mL), an inhibitor of mRNA synthesis. We have concluded that the induction of collagenase synthesis by either phorbol myristate acetate or urate crystals is due to an increased level of translatable mRNA.

Synovial cells taken from patients with rheumatoid arthritis are known to synthesize and secrete large quantities of the neutral proteinase collagenase. The importance of this enzyme in mediating collagenolysis in rheumatoid disease is well established (Harris & Krane, 1974; Harris, 1978, 1981), but mechanisms controlling collagenase production are unclear.

We have developed a model system for rheumatoid synovium in which monolayer cultures of rabbit synovial fibroblasts can be stimulated experimentally to produce large amounts of collagenase. Untreated cultures secrete negligible amounts of this enzyme, but treatments with membrane active agents such as poly(ethylene glycol) (Brinckerhoff & Harris, 1978) or cytochalasin B (Harris et al., 1975), phagocytosis of insoluble debris (Werb & Reynolds, 1975), and a factor secreted by mononuclear cells (Dayer et al., 1976, 1978) are all capable of inducing collagenase synthesis. Two additional and potent inducers of collagenase are crystals of monosodium urate monohydrate (McMillan et al., 1981) and the tumor promoter phorbol myristate acetate (PMA) (Brinckerhoff et al., 1979).

No mechanism for collagenase induction by any of these stimuli has been postulated, although we previously measured a series of intracellular events occurring after addition of PMA (Brinckerhoff et al., 1979). In response to PMA, intracellular cyclic AMP levels increased temporarily at 10 min, followed by a transient decrease in DNA synthesis (maximum effect at 9–12 h), a rise in the PGE_2 level in culture medium (starting at 12–24 h), and a rise in collagenase activity that was detectable in the culture medium at about 24 h.

Since collagenase is not stored in cells after synthesis (Valle & Bauer, 1979), the 24-h lag period appeared to represent the time during which active cytoplasmic collagenase mRNA was produced. This could be the result of immediate synthesis of collagenase mRNA (in response to PMA or urate crystals) that remained in a translationally inactive form until 20–24 h, or it may represent the time needed for functionally active

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collagenase mRNA to be transcribed and/or processed.

Our experiments were directed at elucidating the mechanism(s) involved in the induction of collagenase synthesis. By utilizing the kinetics of induction, cell-free translation, and immunoprecipitation, we have been able to demonstrate that the major factor contributing to the appearance of collagenase is a large increase in the level of translatable collagenase mRNA.

Experimental Procedures

Cell Cultures. Monolayers of rabbit synovial fibroblasts were established from explants of synovium as previously described (Brinckerhoff & Harris, 1978; Brinckerhoff et al., 1979). Fibroblast-like cells growing to confluence were trypsinized (0.25% trypsin; Gibco, Grand Island, NY) and were passaged 1:2 in Dulbecco's modified Eagles medium (DMEM; Gibco) with 10% fetal calf serum (FCS; Gibco) with penicillin and streptomycin (50 μg/mL; Gibco) for up to five passages in 100-mm culture dishes (Falcon Plastics, Oxnard, CA).

All experiments were carried out with confluent cultures of cells. For some experiments, cultures were incubated in DMEM–10% FCS. For others, the cultures were washed 3 times in Hank's balanced salt solution (HBSS) to remove traces of serum, and the medium was replaced wth DMEM containing 0.2% lactalbumin hydrolysate (LH).

PMA (Consolidated Midland, Brewster, NY) was prepared in dimethyl sulfoxide at 5×10^{-4} M and stored at −20 °C. Just before use, it was diluted in HBSS and added at a final concentration of 10^{-8} M (0.01 μg/mL). Crystals of monosodium urate monohydrate (urate) were prepared by autoclaving a supersaturated (0.025 M) solution of crystals (pH 7.2) and incubating it without motion for 18 h at room temperature (Hasselbacher, 1979). Urate was added to the culture medium at a final concentration of 275 μg/mL. α-Amanitin (Calbiochem, LaJolla, CA) was stored at −20 °C at 1 mg/mL in phosphate-buffered saline and was added to cultures at a final concentration of 2 μg/mL.

Collagenase Assay. Latent collagenase was assayed in a standard assay with fibrils of reconstituted collagen (Harris et al., 1969) prepared from guinea pig skin as described by Glimcher et al. (1964). [^{3}H]Collagen was prepared by the method of Gisslow & McBride (1975). Latent collagenase in serum-free medium was activated with 10 μg/mL N^{α}-(*p*-tosyl)-L-phenylalanine chloromethyl ketone treated trypsin (Sigma, St. Louis, MO) or in serum-containing medium with 125 μg/mL trypsin for 30 min at room temperature, followed by addition of a 4-fold (weight) excess of soybean trypsin inhibitor (Sigma). Cells in culture dishes were washed 3 times in HBSS and 2 times in cold 5% trichloroacetic acid. The acid-insoluble fraction was solubilized in 1 mL of 0.2 N NaOH, and an aliquot portion was used for protein determination by the method of Lowry et al. (1951).

Extraction of RNA. Cells were removed from culture dishes with a rubber policeman by using calcium/magnesium-free phosphate-buffered saline and then pelleted. The pellet was lysed in 10 volumes of buffer [5% (w/v) $NaDodSO_4$,[1] 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 5 mM EDTA] and the lysate extracted 2 times with an equal volume of phenol–chloroform–isoamyl alcohol (50:50:1). The aqueous phase was collected and adjusted to 0.3 M NaCl, and the RNA was precipitated at −20 °C for 16 h with 2 volumes of absolute ethanol.

DNA was removed from the precipitate by treatment for 4–6 h with LiCl at a final concentration of 2.5 M on ice (Baltimore, 1966). The LiCl was removed by repeated ethanol precipitation. RNA was resuspended in H_2O and stored at −70 °C at A_{260}/mL = 80–100.

In Vitro Translation of mRNA and Analysis of Translation Products. The wheat germ translation system of Dobberstein & Blobel (1977) was used. Chemicals were purchased from Sigma, and [^{35}S]methionine (935 Ci/mmol) was from Amersham (Arlington Heights, IL). Wheat germ was a gift from General Mills (Minneapolis, MN).

The S-23 wheat germ was prepared according to Roman et al. (1976). Wheat germ was ground to a paste at 4 °C in buffer (90 mM KCl, 1.0 mM magnesium acetate, and 2 mM $CaCl_2$). The paste was centrifuged (23000*g*), and the supernatant was carefully removed and adjusted to 20 mM Tris–acetate, pH 7.6, and 2 mM magnesium acetate and then recentrifuged. The supernatant was passed through a Sephadex G-25 column equilibrated with 1 mM Tris–acetate, pH 7.6, 50 mM KCl, 4 mM dithiothreitol (DTT), and 1 mM magnesium acetate. The void volume was collected, clarified by centrifugation (23000*g*), and stored in aliquots at −70 °C.

A typical translation was carried out as follows. Mixture A was prepared containing 34 μL of distilled water, 27.5 μL of energy and amino acids [18.2 mM ATP, 1.82 mM GTP, 145.5 mM creatine phosphate, and 19 amino acids, minus methionine (each at 0.1 mM)], 35 μL of [^{35}S]methionine, and 50 μL of buffer (2 mL of 1 M Hepes–KOH, pH 7.5, 5.5 mL of 2 M potassium acetate, 0.25 mL of 2 M KCl, 0.34 mL of 0.5 M magnesium acetate, 0.8 mL of 10 mM spermine, 0.4 mL of 0.5 M DTT, and 0.71 mL of water). The pH was adjusted to 7.0 with 1.0 μL of 1 M KOH, and 2.5 μL of creatine phosphokinase (8 mg/mL) was added.

The translation mixture contained 7.5 μL of water, 7.5 μL of mixture A, 7.5 μL of S-23 wheat germ, 1.5 μL of ribonuclease inhibitor isolated from human placenta (Blackburn et al., 1977), and 1 μL of RNA in water (A_{260}/mL = 80–100). The reaction was run at 25 °C for 90 min. Five microliters was then removed to measure synthesis of Cl_3CCOOH-precipitable material and the remainder prepared for gel electrophoresis. The sample was precipitated with 10% trichloroacetic acid, and the pellet was resuspended in 20 μL of sample buffer [0.1 M Tris base, 0.02% of bromphenol blue, 50% (v/v) glycerol, 20 mM DTT, and 2% (w/v) $NaDodSO_4$], boiled for 2 min, and then loaded onto 10% acrylamide slab gels (Wyckoff et al., 1977). Gels were stained with 0.2% Coomassie Brilliant Blue R-250 (Bio-Rad, Richmond, CA) in methanol–water–acetic acid (5:5:1). Translation products were visualized by autoradiography using Kodak XRP-1 X-ray film.

Immunoprecipitation. Monospecific antibody was developed in sheep against rabbit synovial fibroblast collagenase (Vater et al., 1981). Synthesis of immunoreactive collagenase by cell-free translation was detected as described by Nagase et al. (1981) by incubating 5 μL of anti-(rabbit synovial collagenase) IgG (0.5 mg/mL) in a 100-μL translation reaction during the 90-min incubation. The following components were added, and the incubation was continued for 18 h at 23 °C: 4 μL of 10% $NaDodSO_4$, 20 μL of 20% Triton X-100, 10 μL of 1 M Tris-HCl, pH 8.6, 200 μL of 0.5 M arginine hydrochloride, pH 8.0, 13 μL of 100 mM methionine, 40 μL of 50 mM EDTA, 4 μL of 200 mM diisopropyl phosphorofluoridate and 4 μL of 2% NaN_3. The IgG-bound proteins were ex-

[1] Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IgG, immunoglobulin G.

Table I: Effect of Time of Exposure to PMA on Collagenase Production by Rabbit Synovial Fibroblasts[a]

hours exposure to PMA	units of collagenase [μg of collagen degraded h^{-1} (mg of cell protein)$^{-1}$]		
	expt I	expt II	expt III
0	2 ± 2	9 ± 1	3 ± 2
3	<1	5 ± 2	372 ± 31
6	<1	155 ± 21	320 ± 50
9	167 ± 50	91 ± 43	377 ± 55
12	309 ± 42	212 ± 64	426 ± 114
24	143 ± 23	318 ± 23	499 ± 81

[a] Cultures at approximately 8 × 10^5 cells/60-mm dish in DMEM–10% FCS were treated with PMA as indicated. The cultures were washed 3 times in Hank's balanced salt solution, and fresh DMEM–10% FCS without PMA was added for 48 h. The medium was then assayed for collagenolytic activity (±standard deviation) after activation of latent enzyme with 125 μg/mL trypsin, and the amount of protein/culture was determined.

tracted by adding 20 μL of protein A–Sepharose (Sigma) and incubating with continuous rotation for an additional 2 h at 23 °C. The protein A–Sepharose was pelleted and washed 5 times with the buffer described above and once with water, and the pellet was resuspended in 25 μL of $NaDodSO_4$ gel electrophoresis sample buffer and boiled for 2 min prior to application to $NaDodSO_4$–polyacrylamide gels.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. The medium from cultures of rabbit fibroblasts was run on $NaDodSO_4$–polyacrylamide gels with 10% total acrylamide concentration and 0.75 mm thick slab gels (Wyckoff et al., 1977). The culture medium (1 mL) was dialyzed against distilled water overnight, lyophilized, resuspended in 25 μL of sample buffer, and boiled for 2 min before application to the gel. Protein standards were purchased from Bio-Rad.

Results

PMA and Induction of Collagenase. Cultures were exposed to PMA for 3, 6, 9, 12, or 24 h to determine how long PMA must be present for induction of collagenase synthesis. PMA was washed away and the cells were incubated in fresh medium without PMA for an additional 48 h. This medium was then assayed for collagenase activity (Table I). In three experiments with cells cultured from three different rabbits the minimum exposure time required for appearance of collagenase in culture medium varied from as little as 3 h to as long as 9 h.

We next measured how long collagenase production could continue after removal of the PMA. Cells were treated with PMA for 24 h, washed, and cultured in fresh medium without PMA. The medium was replaced every 3 days for 12 days and subsequently assayed for enzyme activity. In Figure 1, it is shown that enzyme production by cells from which PMA had been removed continued for as long as 9 days. For the first 3 days the rate of collagenase production was nearly as great as that of cultures still treated with PMA, but the rate of production gradually decreased over the next 6 days. In cultures treated continuously with PMA, collagenase production was linear for 6 days. Longer treatments were usually toxic to the cells.

Effect of α-Amanitin on Collagenase Production by Synovial Fibroblasts Treated with PMA. We studied the effect of α-amanitin (an inhibitor of RNA polymerase II) on induction of collagenase synthesis. Cells were treated with PMA, in the presence or absence of 2 μg/mL α-amanitin, for 48 h, and the culture medium was assayed for collagenase activity. The results of three experiments are shown in Table II and indicate that treatment with α-amanitin decreased collagenase

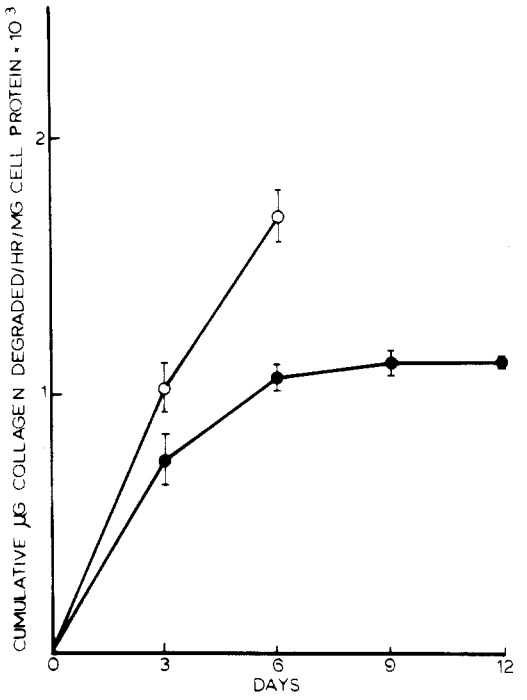

FIGURE 1: Continued production of collagenase by synovial fibroblasts after removal of PMA. Cells at approximately 8 × 10^5/60-mm culture dish in DMEM–10% FCS were treated with PMA (10^{-8} M) for 24 h. Selected cultures were washed 3 times in Hank's balanced salt solution and cultured in fresh medium without PMA; remaining cultures were continued in PMA. The medium was harvested and replaced every 3 days and was assayed for collagenolytic activity after activation with 125 μg/mL trypsin. (○) PMA continuously present; (●) PMA removed.

Table II: Effect of α-Amanitin on Collagenase Induction in Rabbit Synovial Fibroblasts Treated with PMA[a]

treatment of cells	units of collagenase [μg of collagen degraded h^{-1} (mg of cell protein)$^{-1}$]		
	expt I	expt II	expt III
untreated	<1	<1	<1
PMA	178 ± 65	295 ± 65	425 ± 68
PMA + α-amanitin	6 ± 3	46 ± 4	147 ± 64
α-amanitin	< 1	<1	<1

[a] Cells at approximately 8 × 10^5/60-mm culture dish in DMEM–10% FCS were treated with α-amanitin (2 μg/mL) for 2 h. PMA was then added, and the cultures were incubated for an additional 48 h. Culture medium was assayed for collagenolytic activity (±standard deviation) after activation with 125 μg/mL trypsin, and the protein content/culture was determined.

production by PMA-treated cells by 66%–97%. α-Amanitin had no effect on the activation of latent collagenase or on the activity of activated enzyme. Protein synthesis in cells treated with α-amanitin at 2 μg/mL, measured by incorporation of [^{3}H]leucine into trichloroacetic acid insoluble material during a 90-min pulse, was essentially the same as control values at 48 h: 1264 ± 15 cpm incorporated/mg of cell protein for the control compared with 1237 ± 106 for α-amanitin-treated cells. In contrast, cells treated with 4 μg/mL showed a 50% decrease in incorporation of [^{3}H]leucine. Similarly, we found that as little as 0.1 μg/mL actinomycin D inhibited collagenase induction; it also inhibited protein synthesis by 50%, but after only 6 h of the treatment.

In Vitro Translation of mRNA from Rabbit Synovial Fibroblasts. RNA extracted from untreated cells or from cells treated for 72 h with PMA or urate was examined for its ability to direct the incorporation of [^{35}S]methionine into protein in a wheat germ cell-free translation system. The translation products were analyzed by $NaDodSO_4$–poly-

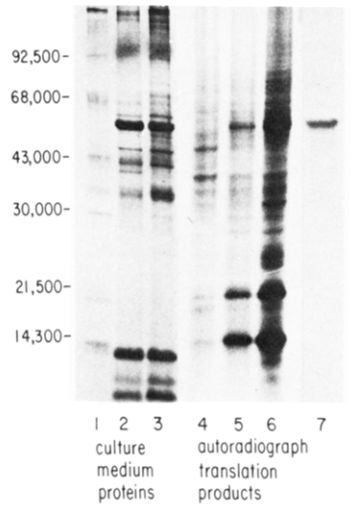

FIGURE 2: NaDodSO$_4$–polyacrylamide gel electrophoresis of culture medium and translation products from RNA from rabbit synovial fibroblast. Culture medium: (lane 1) untreated cells; (lane 2) PMA-treated cells (10^8 M); (lane 3) urate-treated cells (275 μg/mL). Autoradiograph of translation products: (lane 4) untreated cells; (lane 5) PMA-treated cells; (lane 6) urate-treated cells; (lane 7) immunoprecipitate, urate-treated cells.

acrylamide gel electrophoresis and autoradiography along with the proteins secreted into the culture medium by these same cells.

Figure 2 shows that medium from PMA- or urate-stimulated cells, but not from unstimulated cells, contained a protein band corresponding in electrophoretic mobility to purified rabbit synovial fibroblast collagenase (M_r 57 000) (Vater et al., 1981). However, it is yet to be identified whether all of the protein in the culture medium that corresponds to M_r 57 000 represents collagenase. A similar band was not present in the medium from control cells. Translation products of the RNA taken from stimulated cells also contained a protein at this molecular weight that was not found in the translation products of RNA from untreated cells.

For proof that the M_r 57 000 protein synthesized in vitro was indeed collagenase, the in vitro translation products were precipitated with sheep anti-(rabbit synovial collagenase) IgG as outlined under Experimental Procedures and then analyzed by gel electrophoresis. Figure 2 illustrates that the protein synthesized in vitro with M_r 57 000 was precipitated by the monospecific antibody to rabbit synovial fibroblast collagenase.

In addition to the collagenase protein, a number of other proteins were synthesized in vitro by stimulated cells and subsequently released into the culture medium. The most prominent of these had an approximate M_r of 14 000. A protein of slightly lower M_r was also present in culture medium from induced cells. The shift in M_r may reflect cleavage of the signal peptide as the protein was secreted in medium by cells (Blobel & Dobberstein, 1975). Any potential difference in M_r of the collagenase secreted from cells and that synthesized in the cell-free system could not be resolved with the 10% acrylamide gel used here.

Correlation of Collagenase Activity with the Appearance of Collagenase Protein. We tested whether the appearance of collagenase activity released by the cells into culture medium correlated with the appearance of mRNA for collagenase that could be translated by the wheat germ system. Synovial fibroblasts were stimulated with urate crystals. Culture medium was harvested and RNA was extracted from cells at days 1, 2, 3, and 4. The medium was assayed for collagenase activity, and the extracted RNA was assayed for its ability to direct

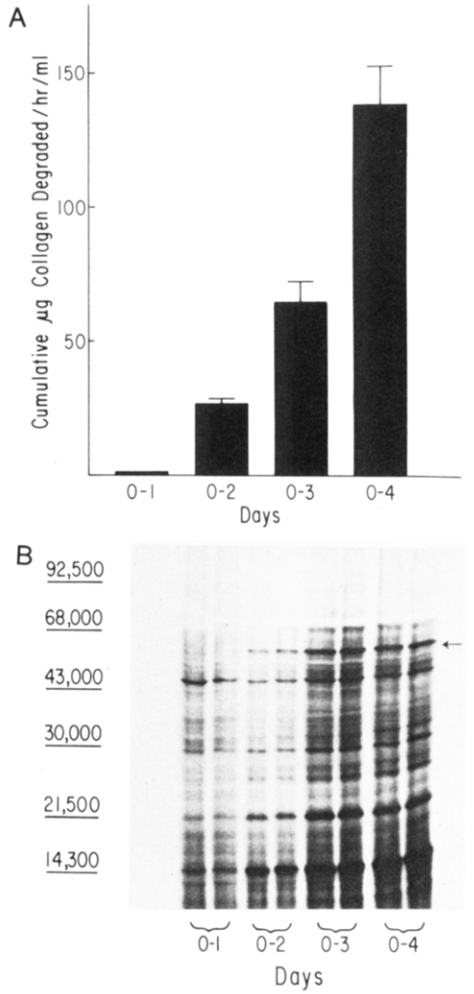

FIGURE 3: Correlation of collagenase activity with collagenase protein in translation products. Confluent monolayers of rabbit synovial fibroblasts were treated with urate (275 μg/mL) for 1, 2, 3, or 4 days. Each day, the culture medium was harvested and RNA extracted. The medium was assayed for collagenase activity (±standard deviation), and the mRNA was translated in the cell-free wheat germ system. (A) Collagenase activity in culture medium; (B) NaDodSO$_4$–polyacrylamide gel electrophoresis autoradiograph of translation products. The arrow indicates the position of collagenase.

the in vitro synthesis of collagenase. Results of the experiment are presented in Figure 3. No collagenase was detected in culture medium at day 1, but enzyme sufficient to degrade about 25 μg of collagen fibril h^{-1} mL^{-1} of culture medium at 37 °C was present by day 2 and increased in a linear manner over the next 2 days (Figure 3A).

Autoradiography of translation products of RNA from these cells revealed a similar pattern: mRNA coding for collagenase was not present at day 1 but was detectable by day 2 (Figure 3B). Note that as the cumulative amount of collagenase in the culture medium increased the level of collagenase from the in vitro translation remained relatively constant after day 2. This finding is consistent with continued synthesis of collagenase protein from a population of mRNA maintained at a constant level. Experiments correlating the appearance of collagenase protein and its mRNA within the cell following treatment with PMA gave similar results (data not shown).

Discussion

We have previously shown that treatment of monolayer cultures of rabbit synovial fibroblasts with PMA (Brinckerhoff et al., 1979) or urate crystals (McMillan et al., 1981) induced the synthesis of collagenase. We show here that this induction was related to an increase in the level of translatable messenger

RNA for this enzyme. Our evidence for this is the following: (1) Control cultures not treated with PMA or urate did not produce detectable amounts of collagenase as assayed by either $NaDodSO_4$–polyacrylamide gel electrophoresis or the enzyme activity of the culture medium. (2) It was not essential for PMA to be present continuously in culture medium for enzyme production to occur, but it must be present for at least 3 h. Once induced, enzyme production continued for several days after removal of PMA, although at decreasing levels. (3) $NaDodSO_4$–polyacrylamide gel electrophoresis and autoradiography of in vitro translation products of RNA from PMA- or urate-treated cells revealed the synthesis of a protein corresponding to latent collagenase (M_r 57 000). This protein was shown to be immunoreactive with monospecific antibody against purified rabbit synovial fibroblast. No immunoreactive protein was synthesized with RNA from control cells. (4) Induction of collagenase was inhibited by the presence of α-amanitin, an inhibitor of RNA polymerase II (Kedinger et al., 1970; Lindell et al., 1970; Hastie & Mahy, 1973).

PMA has been shown to induce the synthesis of at least two enzymes: ornithine decarboxylase in mouse skin (O'Brien, 1975; O'Brien et al., 1976) and in hamster and rat cells (O'Brien et al., 1979) and plasminogen activator in chick embryo fibroblasts (Wigler & Weinstein, 1976; Goldfarb & Quigley, 1978; Wilson & Reich, 1979) and in polymorphonuclear leucocytes (Granelli-Piperno et al., 1977). There is some preliminary evidence that induction of both these enzymes may be transcriptionally controlled and depend on the synthesis of new mRNAs, since inhibitors of RNA synthesis (actinomycin D and cordycepin) prevented their induction (O'Brien, 1975; Granelli-Piperno et al., 1977). The inhibitory effect was apparent within 6 h, presumably before translational efficiency had decreased.

Under our experimental conditions, we found that actinomycin D inhibited protein as well as RNA synthesis. We, therefore, used α-amanitin to determine whether RNA synthesis was required for induction of collagenase. The use of α-amanitin did not appear to inhibit protein synthesis, a finding consistent with other investigators, who reported that low concentrations (1–2 μg/mL) were not immediately lethal for cultured cells. Chan et al. (1972) reported a tripling of cell number when Chinese hamster ovary cells were exposed to 1 μg/mL α-amanitin. In addition, in our system α-amanitin itself did not interfere with either the activation or activity of collagenase.

Thus, our findings suggest, but do not prove, that new mRNA synthesis is necessary for induction of collagenase synthesis. They do not, however, tell us whether this is a direct activation of the collagenase gene(s) or an indirect effect, such as processing of hnRNA or other posttranscriptional events.

Data from a variety of systems have supported the concept that gene expression is controlled at a transcriptional or posttranscriptional level (Suzuki & Suzuki, 1974; Alton & Lodish, 1977; Palmiter et al., 1976; Roop et al., 1978; Lindquist, 1980; Thireos & Kafatos, 1980): generally, the appearance of mRNA in the cytoplasm can be closely correlated with the appearance of its protein product. In our system, as assayed by in vitro translation, the appearance of cytoplasmic mRNA was associated with the appearance of secreted collagenase (Figures 2 and 3), suggesting that the change in the level of collagenase secreted is the result of an increase in the level of mRNA.

Our results, therefore, tend to support transcriptional control as the major regulatory mechanism involved in collagenase induction. It is possible, however, that control of collagenase gene expression may also occur at posttranscriptional or translational levels, as has been described in other systems. The processing of hnRNA to mRNA has been shown to vary from one tissue to another. For example, in sea urchin, using unique-sequence hybridization probes, Wold (1978) showed that there were significant differences between the mRNA populations of blastula cells and adult intestine cells, while hnRNA populations remained essentially indistinguishable. Similar results have been observed in studies with mice (Davidson & Britten, 1979), guinea pigs (Craig et al., 1980), and tobacco (Kamalay & Goldberg, 1980).

At the translational level, the mRNA population of unfertilized eggs is translationally inactive, but upon fertilization, these mRNAs are "activated" and translated (Brachet et al., 1963). More specifically, the mRNA for myosin can exist in the cytoplasm in either an inactive or active form, depending on its interaction with a "translational-control" RNA (Heywood & Kennedy, 1976). When mRNAs are extracted from either of these systems, they are translated efficiently in vitro.

From our experiments, it seems unlikely that our results are due to an activation of inactive collagenase mRNA. More likely, they are due to de novo mRNA production. Distinguishing from among all the possibilities and quantitating levels of collagenase mRNA in synovial cells under different experimental conditions must await the availability of a cDNA probe.

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Dimerization of the Myosin Heads in Solution†

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ABSTRACT: It is shown, by means of analytical ultracentrifugation, that skeletal myosin S-1 exists in the form of a monomer–dimer mixture, in rapid reversible equilibrium, sensitive to the hydrostatic pressure, the temperature, and the composition of the buffer (at least, pH, ionic strength, presence or absence of a Mg–(phosphate compound), and presence or absence of Mg^{2+}). The dimer is predominant at high pH, at low ionic strength, in the presence of a Mg–(phosphate compound), at high pressure, and at low temperature. The monomer is predominant in the reverse conditions. At atmospheric pressure and at room temperature, in a buffer having a composition close to that of the physiological medium, but containing no Mg–(phosphate compound), the monomer is largely predominant (more than 90% at 1 mg/mL S-1). At atmospheric pressure and at room temperature, in a buffer containing a Mg–(phosphate compound) and having a composition close to that of the physiological medium, S-1 exists in the form of a monomer–dimer mixture, with a noticeable proportion of dimer (more than 25% at 1 mg/mL S-1 in the presence of 2 mM MgADP and 3 mM Mg^{2+}). In such buffers, the monomer:dimer ratio is extremely sensitive to both the pH and the ionic strength. The sedimentation coefficients of the monomer and the dimer are respectively 5.05 ± 0.05 S and 6.05 ± 0.05 S. The two protomers making up the dimer are stuck together in an end-to-end arrangement. Both the monomer and the dimer are highly hydrated (about 0.9 g of water/g of protein for the monomer and probably more for the dimer).

Actin and myosin are the two principal proteins found in the contractile apparatus of muscle and nonmuscle motile cells. The myosin molecule contains two globular heads at one end of a long tail. The heads are extremely important, since they each bear an actin-binding site, an enzymatic site, and maybe a regulatory site (Yee et al., 1980). Furthermore, they are involved in the generation of the contractile force, either directly (Huxley, 1969) or indirectly (Morel et al., 1976). Thus, it is of major importance to know all their properties. Here we show they can form dimers in solution, and this finding adds a new property, leading most likely to a yet countless number of consequences.

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