Aortic Smooth Muscle Cells in a Three-Dimensional Collagen Lattice Culture
Evidence for Posttranslational Regulation of Collagen Synthesis

Beatrix Redecker-Beuke, Michael Thie, Jürgen Rauterberg, Horst Robenek

Aortic smooth muscle cells were cultivated as monolayers on plastic or within collagen lattices with low- and high-serum supplementation, and the expression of mRNAs specific for pro alpha 1 (I) and pro alpha 1 (III) collagen were studied by slot blot hybridization. The steady-state levels of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNA of cells within collagen lattices were found to be higher than those grown on plastic, although the production of collagen was lower. The degradation of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs as revealed in the presence of actinomycin D was not affected by culturing the cells within a collagen lattice. In vitro translation assays of mRNAs of monolayer- and lattice-cultured cells showed no differences in translatability. These data suggest the involvement of posttranslational control of collagen production in collagen lattice-cultured smooth muscle cells. (Arterioscler Thromb. 1993;13:1572-1579.)

KEY WORDS • atherosclerosis • smooth muscle cells • collagen lattice culture • collagen • collagen synthesis • procollagen messenger RNA • in vitro translation

Overexpression of collagens is an important feature in the pathogenesis of atherosclerosis.1-2 The collagens are known to be synthesized by smooth muscle cells (SMCs) present within the atheromatous plaque. However, the regulatory mechanisms involved in the synthesis of collagens by ill-coordinated cells are unknown. An important question still to be answered in relation to the possible role of collagens in atherogenesis is, therefore, how collagen synthesis of SMCs is regulated under normal and pathophysiological conditions.

When healthy arterial SMCs are freed from their physiological matrix and are dispersed into monolayer culture on plastic, they begin to change their ultrastructural character. Cells show a modulation from the contractile state characteristic of the healthy vessel to the synthetic state typical of the atherosclerotic vessel, which is characterized by abundant rough endoplasmic reticulum and a large Golgi complex.3 Concomitantly, SMCs secrete large quantities of extracellular matrix proteins, ie, collagens, elastin, and proteoglycans.4,5 In vitro studies on the regulation of collagen synthesis suggest that cytokines and growth factors6 as well as cell-cell and cell-matrix7-10 interactions positively and negatively influence the collagen gene expression of cultured SMCs.

When monolayer-cultured SMCs are reintegrated into a tissue-like matrix, ie, a three-dimensional network of type I collagen, SMCs reduce their collagen synthesis and suppress their responsiveness to soluble growth mediators.11,12 To elucidate the mechanism responsible for the observed reduction of collagen production, we analyzed the molecular process involved in collagen biosynthesis of matrix-cultured cells. This article presents data concerning the level and the translational capacity of collagen mRNAs in SMCs grown within a type I collagen lattice with low- and high-serum supplementation. Our results showed that the steady-state levels of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNA of SMCs decrease with increases in serum concentration. At each concentration tested, steady-state levels of mRNA in lattice-cultured SMCs were higher than those in monolayer-cultured cells. The elevated collagen mRNAs of lattice-cultured cells, however, were not paralleled by elevated collagen levels. The stabilities of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs are not changed by culturing the SMCs within a collagen lattice. As the in vitro translation activity of mRNAs of lattice-cultured SMCs is not affected, it is concluded that control of collagen synthesis in SMCs is exerted at the posttranslational level, when the cells are in contact with a matrix of type I collagen.

Methods

Materials

Medium 199, fetal calf serum (FCS), penicillin, streptomycin, L-glutamine, trypsin-ethylenediamine-tetraacetate, and all kits were obtained from Boehringer, Mannheim, FRG. Collagenase CLS from
Clostridium histolyticum (Worthington, 204 U/mg) was purchased from Biochrom KG, Berlin, FRG. Elastase type III from porcine pancreas (62 U/mg), actinomyein D from Streptomyces species, and collagenase type III from Clostridium histolyticum were obtained from Sigma, Deisenhofen, FRG. L-[14C]Proline (9.69 GBq/mmol) was purchased from New England Nuclear, Dreieich, FRG. L-[35S]Methionine (37 TBq/mmol) and nylon membranes (Hybond-N+) were obtained from Amersham-Buchler, Braunschweig, FRG. Resin type M71 was purchased from Beckman, München, FRG.

The RNA labeling kit, the random priming DNA labeling kit, the digoxigenin (DIG) luminescent detection kit, and the translation kit were obtained from Boehringer.

Cell Culture

Thoracic aortas of 6- to 8-month-old female pigs were used for preparation of SMCs. Cells were released from the tunica media by enzymatic digestion according to the method of Chamley-Campbell et al13 with some modifications.14 Briefly, small pieces of the tunica media were first incubated with collagenase (600 U/mL) and afterward with elastase (30 U/mL) in serum-free medium 199 at 37°C for 30 minutes. Cells were counted using an electronic Coulter counter (Casy 1; Schärfe System, Reutlingen, FRG).

Preparation of Samples for Electron Microscopy

Cell cultures were fixed with Karnovsky's reagent, postfixed with OsO4, dehydrated in ethanol, cleared in propylene oxide, and embedded in Epon 812 as described previously.11 Ultrathin sections were double stained with uranyl acetate and lead citrate and were examined with a Philips EM 410 at 60 kV.

Collagen Synthesis

Collagen synthesis was determined as previously described.11 In brief, after incubation with radioactive proline, culture media were collected and collagen lattices and cell monolayers were washed three times with PBS at room temperature for 30 minutes each. The washing solutions were added to the collected culture media. Collagen lattices were frozen in liquid nitrogen, pulverized, lyophilized, and resuspended in 2 mL PBS. Cells grown in monolayers were removed by scraping. Cell suspensions were sonicated for 2 minutes. Samples were exhaustively dialyzed against 0.1 mol/L acetic acid, hydrolyzed for 24 hours at 110°C with 6N HCl, and subjected to ion exchange chromatography (resin type M71) for separation of hydroxyproline. Total amount of synthesized collagen, expressed as counts per minute/cells x 10^5, was determined by measuring the radioactivity of hydroxyproline in combined cell layer/substratum and liquid media.

RNA Extraction

Total cellular RNA was isolated from the cells with the guanidine isothiocyanate/phenol/chloroform extraction procedure of Chomczynski and Sacchi.14 The purity and the integrity of the RNA were checked by the quotient of A_{260 nm}/A_{280 nm} and by agarose gel electrophoresis. The RNA concentration was determined by its absorbance at 260 nm.

Hybridization of RNA

Hybridization of Northern blots and slot blots was performed with a 1.3-kb cDNA fragment in pGem3-plasmid (Hf 677) coding for human pro alpha 1 (I) collagen;15 a 1.8-kb cDNA fragment in pGem4-plasmid (pH III-1) coding for human pro alpha 1 (III) collagen;16 and a 3.6-kb HindIII fragment of pGem3zf(−) plasmid coding for human β-actin-related pseudogene HβAc-42.17

Before electrophoresis, RNA was denatured by heating at 65°C for 10 minutes in 50% formamide/2.2 mol/L formaldehyde, electrophoresed in a 1% agarose gel containing 2.2 mol/L formaldehyde–10 mmol/L sodium acetate–1 mmol/L EDTA–40 mmol/L sodium morphilinopropane sulfonate, pH 7.0,18 and transferred to nylon membranes by capillary blotting with 20×saline–sodium citrate (SSC) overnight (1×SSC=150 mmol/L NaCl and 15 mmol/L sodium citrate, pH 7.0). Slot blot analysis was performed to quantify the mRNA levels. For slot blot analysis RNA was denatured in a solution containing 6×SSC and 7% formaldehyde for 15 minutes at 60°C.19 Serial dilutions were made in 10×SSC, and aliquots were applied to a nylon membrane under gentle vacuum. Before hybridization the RNA was reversibly stained with methylene blue.18 The staining of the wet filters was documented by photocopying on...
overhead transparencies. Total RNA was determined by quantifying the transparencies by densitometric scanning. Thereafter, the blots were hybridized to DIG-UTP-labeled nucleic acids. DIG-UTP-labeled RNA probes were generated after linearization of the template DNA by in vitro transcription with an RNA labeling kit. DNA probes were DIG-UTP labeled after cutting of the insert by random priming.

The hybridizations were performed in 50% formamide/5x SSC/5% blocking reagent/0.1% N-lauroylsarcosine/0.1% sodium dodecyl sulfate (SDS) at 42°C for cDNA probes and at 68°C for RNA probes for 16 to 20 hours. The probes were used at a concentration of 25 ng/mL hybridization solution. The filters were washed two times for 5 minutes in 2x SSC and 0.1% SDS at room temperature and two times for 30 minutes in 0.1x SSC and 0.1% SDS at 68°C. The detection of the DIG-labeled probes was performed by enzyme immunoassay with luminescence. The luminescence was documented by exposure to x-ray films. RNA slot blots were quantified by densitometric analysis (Ultroscan XL, LKB, Freiburg, FRG). Determination of the relative amount of specific mRNA was done by normalizing the value of specific mRNA to the value of total RNA.

**Cell-Free Translation**

RNA from monolayer- and lattice-cultured SMCs was translated in a nuclease-treated reticulocyte lysate cell-free system. The radioactive tracer used was [35S]methionine. Before translation RNA (3 μg) was heated to 70°C for 30 seconds and quick cooled. Reaction mixtures contained 925 KBq [35S]methionine per 25 μL volume and were incubated for 60 minutes at 30°C. Preprocollagens were identified by their susceptibility to bacterial collagenase. Aliquots (12.5 μL) of cell-free translation reaction were incubated for 3 hours at 37°C with an equal volume of 20 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5, 5 mmol/L L-N-ethylmaleimide, and 10 mmol/L CaCl2 either with or without 10 U clostridial collagenase. The cell-free products were analyzed on 7% SDS-polyacrylamide slab gels with a 3% stacking region by using the gel system of Laemmli.21 Gels were subjected to fluorography. For comparison, [14C]formaldehyde-labeled type I collagen was also electrophoresed.

**Results**

**Morphology of Lattice-Cultured SMCs**

The ultrastructure of lattice-cultured SMCs (Fig 1A) was broadly comparable to that of the monolayer-cultured cells (Fig 1B), ie, cells exhibited the synthetic state characterized by a prominent Golgi apparatus, rough endoplasmic reticulum, and sparse intracytoplasmic filaments.3,12,23 An important characteristic of lattice cultures was the close contact between the SMCs and the exogenous collagen fibrils. Thus, collagen lattice culture did not alter the phenotype of SMCs, ie, cells remained in the synthetic state.

**Collagen Synthesis in Monolayer and Collagen Lattice Cultures**

The amount of collagen synthesized was estimated from the radioactivity associated with nondialyzable hydroxyproline in the cells, in the collagen lattices, and in the medium. Lattice-cultured SMCs and monolayer-cultured SMCs synthesized collagen under low and high serum concentrations. Monolayer cultures showed an increase in collagen synthesis with an increase in serum concentration (Table 1). SMCs within collagen lattices showed no differences in collagen synthesis when cultivated under either low or high serum concentrations (Table 1). In general, lattice-cultured SMCs showed lower collagen synthesis values than monolayer cultures. This behavior of lattice-cultured cells was comparable to that previously described.12

**Content of Collagen and Actin mRNAs in Monolayer and Collagen Lattice Cultures**

Porcine mRNAs hybridizing with probes directed against human pro alpha 1 (I) and pro alpha 1 (III) collagen mRNA and actin mRNA were characterized in Northern blots. Total RNA was isolated from SMCs that were grown as monolayers or within collagen lattices in medium supplemented with 10% FCS for 4 days. As shown in Fig 2, the probe for pro alpha 1 (I) collagen hybridized to two mRNA molecules, 6.4 and 4.9 kb in length, and the probe for pro alpha 1 (III) collagen hybridized to 5.8- and 4.9-kb mRNAs. The probe for actin hybridized to a single RNA with a length of 2.0 kb (not shown). No cross-hybridization was observed between these probes.

Total RNA was quantified from SMCs that were cultured on plastic and within collagen lattices according to the two different serum conditions described in “Methods.” After determining the integrity of the RNAs by gel electrophoresis, quantification was done by slot blot analyses. Triplicates of total RNA were immobilized on nylon membranes and hybridized with probes for pro alpha 1 (I) and pro alpha 1 (III) collagen mRNA and actin mRNA. The concentration of the mRNAs was determined by densitometry. The hybridization signals of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs and actin mRNAs referring to monolayer-cultured cells supplemented with 0.5% FCS were set at 100% (Table 2). In monolayer cultures and in collagen lattice cultures, the concentration of the mRNAs for pro alpha 1 (I) collagen and pro alpha 1 (III) collagen decreased when the cells were supplemented with 10% FCS in the place of 0.5% FCS. Compared with monolayer cultures, the steady-state levels of both collagen mRNA species were elevated in collagen lattice cultures. The concentration of actin mRNAs increased slightly in monolayer cultures when supplemented with 10% FCS compared with 0.5% FCS. In collagen lattice cultures the concentrations of actin mRNA were in general lower than in monolayer cultures. The concentration of mRNA decreased slightly in these cultures when supplemented with 10% FCS compared with 0.5% FCS.

**Stability of Collagen mRNAs in Monolayer and Collagen Lattice Cultures**

To examine the stability of collagen mRNAs, monolayer- and lattice-cultured SMCs were treated for 24 hours with 5 μg/mL actinomycin D in the presence of 0.5% FCS. The mRNAs specific for pro alpha 1 (I) and pro alpha 1 (III) collagen were quantified by slot blot hybridization of total RNA. The hybridization signals...
FIG 1. Photomicrographs showing ultrastructure of smooth muscle cells cultured within a type I collagen lattice (A) and as a monolayer on plastic (B) in medium supplemented with 10% fetal calf serum for 4 days. The cells exhibit the synthetic phenotype in both culture systems. In lattice culture (A) the cells are surrounded by collagen fibrils. n Indicates nucleus; cf, collagen fibrils; i, culture dish-medium interface; er, endoplasmic reticulum; m, mitochondria; and mf, myofilament. Bars=1 μm.

of the procollagen mRNAs of the untreated cells were set at 100%. After 24 hours of incubation of SMCs with actinomycin D the levels of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNA decreased. In lattice-cultured SMCs the reductions were comparable to those in monolayer-cultured cells (Table 3).
Table 1. Collagen Synthesis of Monolayer- and Collagen Lattice-Cultured SMCs

<table>
<thead>
<tr>
<th>Culture</th>
<th>Hydroxyproline, cpm/10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5% FCS</td>
</tr>
<tr>
<td>Monolayer</td>
<td>151 200±22 188</td>
</tr>
<tr>
<td>Collagen lattice</td>
<td>58 400±23 787</td>
</tr>
</tbody>
</table>

Smooth muscle cells (SMCs) were cultured as monolayers or within type I collagen lattices in medium supplemented with 10% fetal calf serum (FCS) for 2 days, and then cells were either switched to medium supplemented with 0.5% FCS or maintained in the high-serum medium (10% FCS) and incubated for 2 more days. Total amount of synthesized collagen, expressed as counts per minute hydroxyproline per 10⁶ cells, was determined by measuring the radioactivity of nondialyzable hydroxyproline in combined cell layer/substratum and liquid media. The values are mean±SEM of determinations from five separate cultures.

Fig 2. Northern blot analyses of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs extracted from lattice-cultured (L) or monolayer-cultured (M) smooth muscle cells. Both cell culture systems were cultured in medium supplemented with 10% fetal calf serum for 4 days. Total RNA (5 μg) was fractionated by 1% formaldehyde–agarose gel electrophoresis. Left, Staining of the 28S and 185 rRNA with methylene blue; middle, a subsequent hybridization with a probe for pro alpha 1 (I) collagen mRNA; and right, a hybridization with a probe for pro alpha 1 (II) collagen mRNA. The arrows indicate mRNA transcripts specific for pro alpha 1 (I) collagen (6.4, 4.9 kb) and pro alpha 1 (III) collagen (5.8, 4.9 kb).

Discussion

The results of this study showed that porcine aortic SMCs cultured within a three-dimensional matrix of type I collagen express pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs at higher levels than do control cells cultured on plastic. The increase in steady-state mRNA levels for the procollagens is not shared by RNA encoding actin, which is involved in cytoskeletal formation and the contractile apparatus of SMCs. However, SMCs cultured within collagen lattice in the presence of either 0.5% FCS or 10% FCS showed lower collagen production values than did cells cultured on plastic. Our data suggested, therefore, that the level of collagen mRNAs does not correlate with the level of collagen production in SMCs that have been transferred from a monolayer culture system to a collagen lattice culture system. In this respect, SMCs act quite differently from fibroblasts, in which the downregulation of collagen synthesis in lattice-cultured cells is reflected by a marked reduction of pro alpha 1 (I), pro alpha 2 (I), and pro alpha 1 (III) collagen mRNA.

We did not examine effects of the different heterogeneous cell-culture components used in our studies. Possible effects of species origin of collagen lattices have not yet been documented.

In many cases, differences in the amount of collagen synthesis appear to reflect differences in steady-state procollagen mRNA levels, implying primary control by transcription or RNA stabilization.

In recent years, however, several examples in which the procollagen mRNA levels do not coincide with the rate of collagen synthesis have been described. During sheep embryogenesis, type I collagen mRNA levels remain constant in the skin, although the rate of protein synthesis drops off sharply. High mRNA levels and low protein synthesis are also reported for laminin in normal fibroblasts/hepatoma cell hybrids and for fibronectin in malignant hepatoma cells. To elucidate the sites of regulation of collagen production in SMCs, we examined the translational capacity of the collagen mRNAs. When the mRNAs from lattice- and monolayer-cultured SMCs were translated in reticulocyte lysates, approximately equal amounts of preprocollagen chains were produced. Thus, we found that the translatability of the collagen mRNAs was not lower in lattice-cultured SMCs compared with monolayer-cultured SMCs. The degradation of pro alpha 1 (I) and pro alpha 1 (III) collagen mRNAs in the presence of actinomycin D was not affected by culturing the cells.
Thus, one might speculate that the concentration of procollagen mRNAs also contributes to the downregulation of collagen synthesis within a collagen lattice. These data suggested the involvement of posttranslational control of collagen production in collagen lattice-cultured SMCs. Further evidence for a posttranslational mechanism for the collagen regulation in lattice-cultured SMCs was apparent from our serum experiments. Although an increase in serum concentration resulted in a decrease of collagen mRNA content, the level of collagen production remained constant.

In the in vitro translation described here, the RNAs were highly purified and essentially free of protein, and translation was performed under controlled ionic conditions. Therefore, factors that could play a role in the regulation of collagen synthesis in collagen lattice culture were missing from our test. Potential candidates for feedback regulatory molecules might be propeptides of procollagens. As demonstrated by several groups,31–33 the N-terminal propeptide of type I procollagen, Col 1, is capable of inhibiting the synthesis of type I procollagen. Thus, one might speculate that the concentration and biological activity of these molecules would make them suitable candidates for the reduction of collagen synthesis in fibroblasts37 contract the collagen lattices and form a very dense matrix. The regulatory effects of collagen lattices can be reduced if contraction of the gels is inhibited. Paye et al38 demonstrated that transformed pulmonary epithelial rat cells only retract a collagen gel in the presence of fibronectin and factor XIII and that inhibition of collagen synthesis depends strongly on the degree of contraction. They also demonstrated that this effect is only partly due to the enhancement of collagen density. Gilly et al39 demonstrated that the retraction and the nature of the fibrillar protein that constitutes the lattices contribute to the lower level of procollagen mRNA in collagen lattice-cultured fibroblasts. Unemori and Werb40 report that the stimulation of collagenase synthesis in fibroblasts within a collagen gel does not occur if contraction of the gel is inhibited by mechanical means. The stimulation starts immediately if contraction of the gel is begun after the release of mechanical manipulation. These authors, however, find no connection between release and inhibition of collagen synthesis.

Elastic fibers adhere strongly to human skin fibroblasts and pig aortic SMCs.41 Hornebeck et al41 propose that the loss of the cell-elastin interaction leads to the modulation of the contractile to the synthetic phenotype of SMCs characteristic of the atherosclerotic vessel. To what degree this and other extracellular matrix components, such as proteoglycans organized in the type I collagen lattice,7,42 may regulate biosynthetic activity by direct interaction or by influ-

### Table 2. Levels of Specific mRNAs in Monolayer- and Collagen Lattice-Cultured SMCs

<table>
<thead>
<tr>
<th>Culture</th>
<th>Pro α 1 (I)</th>
<th>Pro α 1 (III)</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolayer 0.5% FCS</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Monolayer 10% FCS</td>
<td>62±9*</td>
<td>50±7*</td>
<td>111±6*</td>
</tr>
<tr>
<td>Collagen lattice 0.5% FCS</td>
<td>133±18*</td>
<td>172±54†</td>
<td>72±7*</td>
</tr>
<tr>
<td>Collagen lattice 10% FCS</td>
<td>117±17*</td>
<td>116±26†</td>
<td>51±6†</td>
</tr>
</tbody>
</table>

Smooth muscle cells (SMCs) were cultured as monolayers or within collagen lattices in medium supplemented with 10% fetal calf serum (FCS) for 2 days, and then cells were either switched to medium supplemented with 0.5% FCS or maintained in the high-serum medium (10% FCS) and incubated for 2 more days. Results were obtained from densitometric analyses of slot blot experiments. The mRNA levels were expressed as percentage of cells cultured as monolayers with medium containing 0.5% FCS, which was set at 100%. Data are mean±SEM of double determinations from four independent experiments.

*P<.01, †P<.05, mRNA levels of monolayer-cultured cells under high serum concentration and of lattice-cultured cells under low and high serum concentrations vs monolayer-cultured cells under low serum concentrations by Student's t test.

A few hours after lattice formation, SMCs as well as fibroblasts contract the collagen lattices and form a very dense matrix. The regulatory effects of collagen lattices can be reduced if contraction of the gels is inhibited. Paye et al demonstrated that transformed pulmonary epithelial rat cells only retracted a collagen gel in the presence of fibronectin and factor XIII and that inhibition of collagen synthesis depends strongly on the degree of contraction. They also demonstrated that this effect is only partly due to the enhancement of collagen density. Gilly et al demonstrated that the retraction and the nature of the fibrillar protein that constitutes the lattices contribute to the lower level of procollagen mRNA in collagen lattice-cultured fibroblasts. Unemori and Werb report that the stimulation of collagenase synthesis in fibroblasts within a collagen gel does not occur if contraction of the gel is inhibited by mechanical means. The stimulation starts immediately if contraction of the gel is begun after the release of mechanical manipulation. These authors, however, find no connection between release and inhibition of collagen synthesis.

Elastic fibers adhere strongly to human skin fibroblasts and pig aortic SMCs. Hornebeck et al propose that the loss of the cell-elastin interaction leads to the modulation of the contractile to the synthetic phenotype of SMCs characteristic of the atherosclerotic vessel. To what degree this and other extracellular matrix components, such as proteoglycans organized in the type I collagen lattice, may regulate biosynthetic activity by direct interaction or by influ-

### Table 3. Stabilities of Collagen mRNAs in Monolayer- and Lattice-Cultured SMCs

<table>
<thead>
<tr>
<th>Culture</th>
<th>Pro α 1 (I)</th>
<th>Pro α 1 (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolayer 0.5% FCS</td>
<td>100</td>
<td>42±6*</td>
</tr>
<tr>
<td>Collagen lattice 0.5% FCS</td>
<td>100</td>
<td>45±5*</td>
</tr>
</tbody>
</table>

Smooth muscle cells (SMCs) grew for 2 days in medium supplied with 10% fetal calf serum (FCS), and then cells were switched to medium supplemented with 0.5% FCS and were further incubated. On day 4 cells were treated with 5 μg/mL actinomycin D for 24 hours. RNAs were examined by slot blot analyses for mRNA levels of pro alpha 1 (I) and pro alpha 1 (III) collagen, and hybridization signals were quantified by densitometry. The hybridization signals of the untreated cells were set at 100%. The values are mean±SEM of double determinations from three independent experiments. + Indicates cells treated with actinomycin D; and −, untreated controls.

*P<.01 between treated cells and controls.
Fig 3. Autoradiographs showing translation products of mRNAs from smooth muscle cells cultured as a monolayer or within a collagen lattice. The cells were cultured for 2 days in medium supplemented with 10% fetal calf serum and then for 2 more days in medium supplemented with 0.5% fetal calf serum. Total RNA (3 μg) was translated in a reticulocyte lysate cell-free system with [14C]formaldehyde-labeled type I collagen is shown as a size marker.

Biocatalysis with or without collagenase before being fractionated on a 7% sodium dodecyl sulfate-polyacrylamide gel. [14C]Formaldehyde-labeled type I collagen is shown as a size marker.


collagenase

<table>
<thead>
<tr>
<th>type I collagen</th>
<th>monolayer</th>
<th>lattice</th>
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<tbody>
<tr>
<td>β₁₁</td>
<td></td>
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<td>β₁₂</td>
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In the pathogenesis of atherosclerosis a structural rearrangement of the collagen matrix, with a loss of interactions between cells and the extracellular matrix, takes place. The possibility exists that this process, in conjunction with growth factors, could cancel the feedback regulation of the collagen regulation.

Acknowledgments

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