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SPARC, a Secreted Protein Associated with Morphogenesis and Tissue Remodeling, Induces Expression of Metalloproteinases in Fibroblasts Through a Novel Extracellular Matrix-dependent Pathway

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Abstract. SPARC (osteonectin/BM40) is a secreted protein that modifies the interaction of cells with extracellular matrix (ECM). When we added SPARC to cultured rabbit synovial fibroblasts and analyzed the secreted proteins, we observed an increase in the expression of three metalloproteinases—collagenase, stromelysin, and the 92-kD gelatinase—that together can degrade both interstitial and basement membrane matrices. We further characterized the regulation of one of these metalloproteinases, collagenase, and showed that both collagenase mRNA and protein are upregulated in fibroblasts treated with SPARC. Experiments with synthetic SPARC peptides indicated that a region in the neutral α-helical domain III of the SPARC molecule, which previously had no described function, was involved in the regulation of collagenase expression by SPARC. A sequence in the carboxyl-terminal Ca^{2+}-binding domain IV exhibited similar activity, but to a lesser extent. SPARC induced collagenase expression in cells plated on collagen types I, II, III, and V, and on vitronectin, but not on collagen

type IV. SPARC also increased collagenase expression in fibroblasts plated on ECM produced by smooth muscle cells, but not in fibroblasts plated on a basement membrane-like ECM from Engelbreth-Holm-Swarm sarcoma. Collagenase was induced within 4 h in cells treated with phorbol diesters or plated on fibronectin fragments, but was induced after 8 h in cells treated with SPARC. A number of proteins were transiently secreted by SPARC-treated cells within 6 h of treatment. Conditioned medium that was harvested from cultures 7 h after the addition of SPARC, and depleted of residual SPARC, induced collagenase expression in untreated fibroblasts; thus, part of the regulation of collagenase expression by SPARC appears to be indirect and proceeds through a secreted intermediate. Because the interactions of cells with ECM play an important role in regulation of cell behavior and tissue morphogenesis, these results suggest that molecules like SPARC are important in modulating tissue remodeling and cell-ECM interactions.

THE interactions of a cell with its surrounding extracellular matrix (ECM)[1] can play an important role in the regulation of cell behavior and tissue architecture. While most cells in adult tissues remain anchored in place through specific interactions with tissue matrices, subsets of differentiated cells are specialized to move through the ECM. Biological cues can also induce normally stationary,

adherent cells to move over and through the ECM. In some cases, a specific interaction of a cell with certain matrices can help to stabilize or maintain a phenotype or particular tissue structure (Menko and Boettiger, 1987; Sorokin et al., 1990; Adams and Watt, 1990); in actively remodeling tissues, however, the cellular interaction with ECM is not static but is constantly changing (Mackie et al., 1988; ffrench-Constant et al., 1989; Gladson and Cheresh, 1991; Damsky et al., 1992).

Diversity in cell-ECM interactions is the consequence of several factors: the integration of the transcriptional regulation of ECM components and their receptors, the net accumulation of ECM constituents resulting from the balance of synthesis and degradation of ECM molecules, and the assembly of the ECM components into tissue-specific matrices (Cheresh et al., 1989; Dahl and Grabel, 1989; Dustin and Springer, 1991; for review see Hay, 1991; Damsky et al.,

1. *Abbreviations used in this paper:* CM, conditioned medium; DESPARC, a 90% pure fraction of SPARC; ECM, extracellular matrix; EHS, Engelbreth-Holm-Swarm; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin-1; LH, lactalbumin hydrolysate; RGD, Arg-Gly-Asp; RSF, rabbit synovial fibroblasts; RT, reverse transcription; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

1992). Cues provided by tissue-specific matrices are interpreted by cells in the decision to adhere, invade, migrate, secrete, or differentiate. There is strong correlative evidence that information transduced into the cell by adhesion of cells to specific matrices can lead to alterations in cell phenotype or to changes in gene expression (Menko and Boettiger, 1987; Werb et al., 1989; Sorokin et al., 1990; Adams and Watt, 1990; Talhouk et al., 1992; for review see Burridge et al., 1988; Hynes, 1992). Apart from establishing adhesive contacts with the ECM, a cell must somehow modify these contacts with the matrix, i.e., to de-adhere or break the interaction with the matrix. Controlled proteolysis might be one mechanism used by cells to diminish adhesive contacts with the ECM. Proteinases have been shown to be present at focal contacts in certain cells and can act to modulate the assembly of the actin cytoskeleton in these cells (Beckerle et al., 1987; Estreicher et al., 1990). ECM-degrading metalloproteinases are specifically enhanced in fibroblasts plated on fragments of fibronectin when compared with fibronectin or collagen type I (Werb et al., 1989), a response that could also diminish adhesion of cells to the ECM. Another way to effect changes in cell adhesion is to modify existing matrices by deposition or synthesis of additional matrix-associated molecules. Several ECM-associated molecules, including SPARC, thrombospondin, dermatan sulfate proteoglycans, and tenascin, have been shown to perturb the adhesion of cells to the matrix (Sage et al., 1989c; Lawler et al., 1988; Murphy-Ullrich and Hook, 1989; Chiquet-Ehrismann, 1991; Murphy-Ullrich et al., 1991). Thrombospondin and tenascin have both adhesive and anti-adhesive properties, and may interact with cells in a way that Arg-Gly-Asp (RGD) inhibits (Lawler et al., 1988; Bourdon and Ruoslahti, 1989), whereas SPARC acts in a manner insensitive to RGD (Sage et al., 1989c).

SPARC, tenascin, and thrombospondin are expressed transiently in tissues that are actively remodeling their matrix, where cells are dividing or migrating (for review see Chiquet-Ehrismann, 1991; Sage and Bornstein, 1991). Although SPARC is expressed transiently in a wide range of tissues during development, in adults SPARC is expressed chiefly in rapidly renewing populations of cells (Sage et al., 1989a,b). Metalloproteinase expression is also increased in regions of tissue where increased cell division, migration, or remodeling is observed (for review see Alexander and Werb, 1991). This is interesting because the expression of the ECM-degrading metalloproteinases collagenase and stromelysin correlates with perturbation of the actin cytoskeleton (Unemori and Werb, 1986), and tenascin, SPARC, and thrombospondin in culture have been shown to destabilize actin in focal contacts (Murphy-Ullrich et al., 1991).

The expression of collagenase and stromelysin is induced in cultured rabbit synovial fibroblasts (RSF) plated on fibronectin fragments or on anti-fibronectin receptor antibody but not on substrates of intact plasma fibronectin (Werb et al., 1989). This culture system might therefore be useful in the dissection of information transduced by cell-ECM interactions. Because SPARC diminishes adhesion of cultured cells by destabilizing focal contacts (Sage et al., 1989c; Murphy-Ullrich et al., 1991), we designed experiments to determine whether the addition of SPARC to cultured synovial fibroblasts alters the expression of metalloproteinases in these cells.

Materials and Methods

Cells and Cell Culture

RSF, isolated as described previously (Aggeler et al., 1984a,b) and used between passages 1 and 10, were cultured in DME (Cell Culture Facility, University of California, San Francisco), supplemented with 10% FBS (Hyclone Labs., Logan, UT). Cells (0.5–1 × 10⁵) were plated in uncoated 24 or 48-well tissue culture dishes (Costar Corp., Cambridge, MA), and cultured in DME containing 10% FBS for 3–4 h, after which the monolayers were washed and incubated further in DME supplemented with 0.2% lactalbumin hydrolysate (LH) (GIBCO BRL, Gaithersburg, MD). Cells were plated on ECM-coated dishes at a density of 10^5 and were cultured in one of two serum-free media, DME-LH or Fibroblast Growth Medium (Clonetics Corp., San Diego, CA).

Preparation of ECM Ligand Substrates

Fibronectin was purchased from Collaborative Research (Bedford, MA) and Boehringer Mannheim Corp. (Indianapolis, IN); fibronectin fragments and vitronectin were purchased from Telios Pharmaceuticals (San Diego, CA). Bovine collagen type I (Vitrogen) was purchased in a solution of 0.1 N HCl from the Collagen Corp. (Palo Alto, CA); collagen types I, II, III, IV, V were purchased from Collaborative Research or from Eureka Laboratories (Sacramento, CA) and reconstituted as directed by the manufacturer. Culture dishes were coated with fibronectin or vitronectin at 10–20 μg/ml by incubating them overnight (9–15 h) in PBS, pH 7.4, at 4°C. Human collagen types I, II, III, IV, V, or bovine collagen type I were diluted from acidic solutions into distilled water to a concentration of 20 μg/ml and culture dishes were further incubated in this solution overnight (9–15 h) at 4°C. Unoccupied binding sites were blocked by incubation with 0.2% BSA (Sigma Chem. Co., St. Louis, MO) at ambient temperature for 2 h; wells were then washed three times with PBS and used immediately. Culture dishes coated with a basement membrane matrix from Engelbreth-Holm-Swarm (EHS) sarcoma (Alexander and Werb, 1992) or the smooth muscle matrix from R22 rat smooth muscle cells (Werb et al., 1980) were prepared as described previously.

Addition of SPARC or SPARC Peptides to Cell Cultures

SPARC was prepared from mouse parietal yolk sac (PYS-2) cells as described previously (Sage et al., 1989c). Purified SPARC was solubilized in PBS at a concentration of 250 μg/ml and used at a final concentration of 10–40 μg/ml; SPARC was used as a soluble ligand except where noted otherwise. In many experiments we used a slightly less pure (90%) fraction of SPARC (DESPARC), isolated by anion-exchange chromatography, which contains a carrier protein for SPARC; this protein was more stable and available in larger quantities than pure SPARC. The synthetic peptides used in this study were synthesized, characterized, and used as described previously (Lane and Sage, 1990).

Antibodies

The mouse anti–rabbit collagenase mAbs used for immunoprecipitations and analysis of secreted collagenase in this study were described by Werb et al. (1989). The mouse anti–human stromelysin antibody, SL188.2 (Wilhelm et al., 1992) used in the immunoblots was a generous gift of Dr. Scott Wilhelm (Miles Research, West Haven, CT). The horseradish peroxidase-conjugated and biotinylated secondary antibodies were purchased from Sigma Chem. Co. Texas red–streptavidin conjugate was purchased from Amersham Corp. (Arlington Heights, IL). Goat anti-SPARC antibody was prepared with purified SPARC as antigen, and its specificity characterized by immunoprecipitation and immunoblotting; the titer was measured by ELISA, and was maximal at 1:500 and half-maximal at 1:250.

To deplete SPARC from the medium, we incubated 500 μl of antiserum or normal goat serum with 100 μl packed protein G-Sepharose beads (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) for 1 h and washed them extensively with PBS, and then incubated the beads with 200 μl conditioned medium (CM) for 1 h.

Biosynthetic Labeling of Secreted Proteins and Analysis of Metalloproteinases

Proteins were biosynthetically labeled by incubating cultures with 50–100

μCi/ml of [^{35}S]methionine (Expresslabel; New England Nuclear, Boston, MA) for 2–4 h in methionine-free DME at 37°C. Secreted proteins were precipitated from the CM with quinine sulfate and SDS as described (Unemori and Werb, 1986). Samples were then analyzed by SDS-PAGE using the Laemmli buffering system followed by fluorography (En^{3}Hance, New England Nuclear), as previously described (Unemori and Werb, 1986). Alternatively, collagenase in the radiolabeled CM was immunoprecipitated with a mixture of anti-collagenase mAbs (Werb et al., 1989). Zymography was used to analyze the gelatinases in the CM (Werb et al., 1989). The stromelysin and collagenase protein in the enriched culture supernatants was also identified by immunoblotting procedures (Harlow and Lane, 1988) with the use of anti-human stromelysin mAb (Wilhelm et al., 1992) and anti-rabbit collagenase mAbs (Werb et al., 1989). Briefly the CM was resolved by SDS-PAGE and the proteins were transferred to Immobilon P membranes (New England Nuclear). Unoccupied binding sites on the membrane were blocked by incubation with 3% BSA in TBS (150 mM NaCl, 20 mM Tris-HCl, pH 7.5), washed once with TBS, and incubated with the primary antibody for 1–2 h. The membrane was washed briefly with TBS, and then washed three times for 20 min with a solution of TBS containing 0.5% Tween-20 (Sigma Chem. Co.). After incubation with a horseradish peroxidase–conjugated secondary anti-mouse antibody, the membrane was washed as described above, and specific bands were visualized by enhanced chemiluminescence (Amersham Corp.) as described by the manufacturer. To quantify the amount of collagenase in the CM, we applied dilutions of the CM to nitrocellulose membranes (Schleicher and Schuell, Inc., Keene, NH) using a slot-blot filtration manifold (Schleicher and Schuell, Inc.). The filters were blocked and incubated with anti-collagenase antibodies as described above. The resulting films were scanned by laser densitometry and the amounts of collagenase in the CM were compared.

RNA Isolation, Polymerase Chain Reaction, Hybridization Conditions, and cDNA Probes

Total cellular RNA was isolated from cultured cells and 1-μg samples were analyzed by reverse transcription (RT) and amplification of specific sequences by the PCR (Rappolee et al., 1989). Synthetic primers used to amplify collagenase cDNA sequences were selected from regions of identity in the rabbit and human cDNA sequences. The collagenase primer pair (nucleotides 1154–1174 and 1433–1453 in the rabbit collagenase gene [Brenner et al., 1989]) amplified a 300-bp fragment of the transcribed cDNA. Synthetic primers used to amplify cDNA sequences coding for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) spanned the sequence 3308–3337 and 3649–3672 in the rat gene, and produced a 241-bp fragment (Rappolee et al., 1989). Amplifications with both primer pairs were performed with 4 mM MgCl$_2$ at an annealing temperature of 60°C.

PCR products were analyzed by agarose gel electrophoresis followed by staining with ethidium bromide. For quantification of the amplified product, negatives of the gels were analyzed by scanning densitometry, and the area under the peak corresponding to specific bands was plotted against the dilution of the RT mixture used in that amplification. The amounts of GAPDH and collagenase mRNA in the RT mixture were compared in the linear portion of the curve. To analyze mRNA by blotting, we applied a series of linear dilutions of total RNA to nylon membranes using a slot-blot filtration manifold (Sambrook et al., 1989). The filter was probed with ^{32}P-labeled cDNA inserts from pCL1, a clone of rabbit collagenase (Frisch et al., 1987), and human γ-actin (Engel et al., 1981; a gift of L. Kedes, Stanford University, Palo Alto, CA) as described (Werb et al., 1989).

Results

The Expression of Metalloproteinases Is Upregulated in Synovial Fibroblasts Treated with SPARC

To determine if SPARC alters expression of proteins synthesized by RSF, we added purified SPARC or DESPARC to adherent RSF in culture. We compared the expression of proteins secreted by SPARC-treated cells with that of proteins secreted by untreated cells. We also incubated cells with the phorbol diester 12-O-tetradecanoylphorbol-13-acetate (TPA), which rapidly induces the expression of metalloproteinases in many cell types (Frisch and Werb,
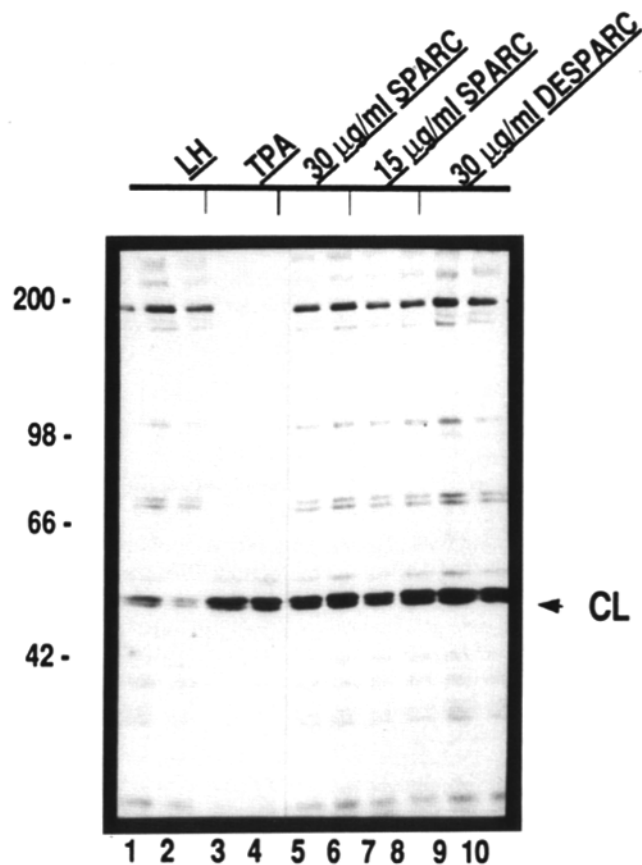

Figure 1. SPARC induces the synthesis and secretion of proteins in synovial fibroblasts. Freshly trypsinized RSF were cultured in DME-10% FBS in 48-well plates for 3 h. The cells were incubated further in DME-LH (*LH*, lanes *1* and *2*, in duplicate) or DME-LH supplemented with either 100 ng/ml TPA (lanes *3* and *4*, in duplicate), 30 μg/ml pure SPARC (lanes *5* and *6*, in duplicate), 15 μg/ml pure SPARC (lanes *7* and *8*, in duplicate), or 30 μg/ml DESPARC (lanes *9* and *10*, in duplicate) for 30 h. The CM was removed, and the proteins were biosynthetically labeled by incubation of cells with [^{35}S]methionine in methionine-free DME for 2 h. The radiolabeled secreted proteins were concentrated with quinine sulfate-SDS and analyzed by SDS-PAGE and autoradiography. Procollagenase (*CL*), migrating at 53 and 57 kD, is indicated on the right. Molecular weight ($\times 10^{-3}$) markers are indicated on the left.

1989). SPARC induced the synthesis of biosynthetically labeled secreted proteins migrating between 50 and 60 kD that are characteristic of the proenzyme forms of collagenase and stromelysin (Fig. 1). We detected no difference in the pattern of metalloproteinase expression in RSF induced by pure SPARC and DESPARC, and, unless otherwise stated, the two were used interchangeably in later experiments.

To identify and quantify the metalloproteinases in the CM harvested from SPARC-treated RSF, we used mAbs against collagenase and stromelysin. Immunoprecipitable collagenase represented >30% of the radiolabeled protein secreted by RSF after treatment with SPARC for 40 h (Fig. 2 A). Increases in total collagenase and stromelysin were also detected by immunoblotting CM harvested from SPARC-treated RSF (Fig. 2 B). Changes in proteolytic activity were evident on zymograms that used a gelatin substrate incorporated into the resolving gel. Increases in the gelatinolytic doublet of collagenase, migrating at 57 and 53 kD, and of the
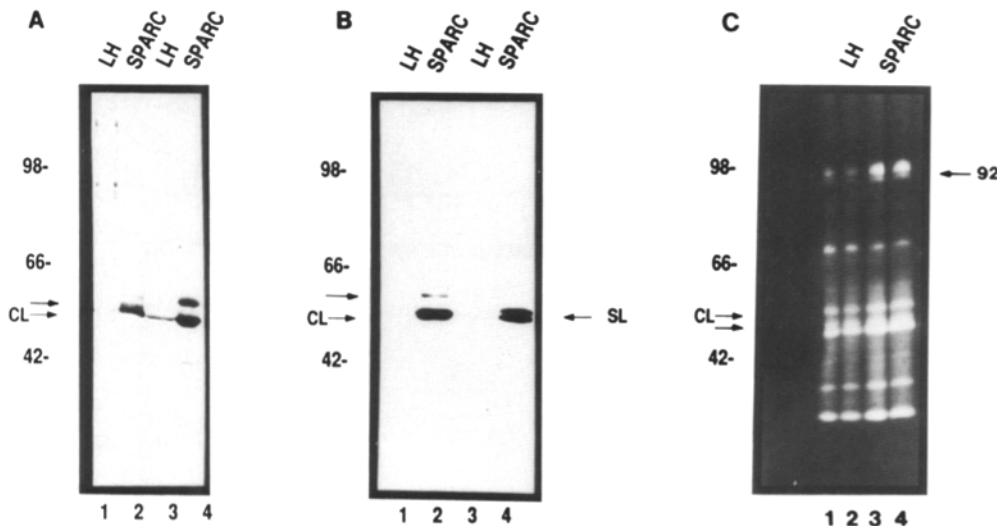
A LH SPARC LH SPARC

98-

66-

CL→
42-

1 2 3 4

B LH SPARC LH SPARC

98-

66-

CL→   — SL
42-

1 2 3 4

C LH SPARC

98-   ← 92

66-

CL→
42-

1 2 3 4

Figure 2. SPARC induces the expression of collagenase protein in synovial fibroblasts. (*A*) Freshly trypsinized RSF plated in 48-well plates were allowed to attach and spread in DME-10% FBS for 3 h. The medium was removed, the monolayers were washed and cells were incubated further in DME-LH or in DME-LH supplemented with 30 μg/ml DESPARC for 40 h. The CM was removed and saved for later analysis. The proteins were biosynthetically labeled for 3 h by incubation of cells with 50 μCi/ml of [^{35}S]methionine in methionine-free DME. Samples of CM containing radiolabeled secreted proteins from untreated RSF (*LH*, lanes *1* and *3*) or RSF treated with DESPARC (*SPARC*, lanes *2* and *4*) were concentrated with quinine sulfate-SDS (lanes *1* and *2*) to show total proteins secreted into the medium, or proteins secreted into the medium were immunoprecipitated with anti-collagenase mAbs (lanes *3* and *4*) and the precipitates were separated on a 10% SDS-polyacrylamide gel and analyzed by autoradiography. The doublet of procollagenase (*CL*) is indicated by arrows. (*B*) CM harvested from untreated RSF (*LH*, lanes *1* and *3*) or SPARC-treated RSF (*SPARC*, lanes *2* and *4*) was separated on a 10% SDS-polyacrylamide gel under denaturing conditions, and the proteins were transferred to membranes and analyzed by immunoblotting with anti-collagenase mAbs (lanes *1* and *2*) or an anti-stromelysin mAb (lanes *3* and *4*). Procollagenase (*CL*) and prostromelysin (*SL*) are indicated by arrows. (*C*) The proteinase content of the 40-h CM harvested from untreated RSF or from RSF treated with SPARC was analyzed by zymography. The proteins were separated on a 10% polyacrylamide gel that contained 0.1% gelatin under nondenaturing conditions. The zymograms were stained with Coomassie blue R250 after development overnight (14 h). Proteolytic degradation of gelatin appears as a clear band on a dark background. Note the induction of gelatinases migrating at 92, 57, and 53 kD corresponding to the proenzyme forms of the 92-kD gelatinase (*92*) and collagenase (*CL*). Molecular weight ($\times 10^{-3}$) markers are indicated on the left.

92-kD gelatinase show that these metalloproteinases are induced by SPARC (Fig. 2 *C*). In the rest of the experiments described in this report, we concentrated on the effects of SPARC on the regulation of collagenase gene expression.

Regardless of the basal level of collagenase secretion, SPARC reproducibly upregulated the expression of collagenase in RSF. The induction of collagenase in RSF treated with SPARC, measured as radiolabeled secreted collagenase protein, was quantified by scanning densitometry of autoradiographs (Table I). There was a mean 5.3-fold increase ($\pm$ 2.7 SD) in the expression of collagenase in cultures treated with SPARC, compared with untreated cultures ($n = 8$ experiments, 2–3 replicates per treatment, $p < 0.01$; Student's *t* test). By comparison TPA induced an 8.6-fold increase ($\pm$ 1.8 SD) in collagenase expression. Induction of collagenase expression in RSF was found using three preparations of SPARC and in RSF strains derived from six rabbits.

In the preceding experiments we examined the effect of SPARC on the amounts of collagenase protein secreted by RSF. We used RT-PCR to analyze the effect of SPARC on mRNA for collagenase. The accumulation of collagenase mRNA increased fivefold in RSF treated with SPARC (Table I), and when normalized for mRNA for the housekeeping gene GAPDH (Fig. 3), this increase correlated with the expression of collagenase protein by RSF treated with SPARC. The data analyzed in Fig. 3 *B* are an average of three experiments. In a separate blotting analysis of RNA from one experiment, a series of sequential dilutions of RNA was applied to nylon membranes with a filtration manifold, and the membrane was probed with ^{32}P-labeled cDNA probes for collagenase and γ-actin. The autoradiographs were scanned

and, normalizing to the level of γ-actin expression, we saw a threefold increase in collagenase mRNA in cultures treated with SPARC compared with untreated control cultures; this increase was comparable to the levels obtained with RT-PCR (data not shown).

Previous studies have indicated that induction of collagenase and stromelysin gene expression strongly correlates with cell rounding, although treatment of RSF with a func-

Table I. Collagenase Expression in RSF Treated with SPARC and SPARC Peptides

Peptide*	Collagenase induction	G₀/S delay‡	Shape change§
	(*-fold*)		
SPARC	5.3 ± 2.7‖	Yes	Yes
1.1	1.2 ± 0.4	No	Yes
2.1	1.1 ± 0.	Yes	No
3.2	10.0 ± 1.7‖	No	No
3.4	1.1 ± 0.5‖	No	No
4.2	4.3 ± 0.5‖	No	Yes
TPA	8.6 ± 1.8‖	ND	Yes

To facilitate comparison of results from five experiments, we expressed data as "-fold induction" by normalizing data from treated to untreated cultures in individual experiments. For SPARC, eight different experiments were averaged. Data are shown as mean ± SD.
* See Fig. 6 A for location of peptides in the SPARC molecule. Peptides had the same structure as that described by Lane and Sage (1990) and were added to RSF at 0.8 mM for 30 h. SPARC was added at 30 μg/ml.
‡ From Funk and Sage (1991).
§ From Lane and Sage (1990).
‖ Significantly different from untreated controls ($p < 0.05$, Kruskall Wallis test).
ND, Not done.

A

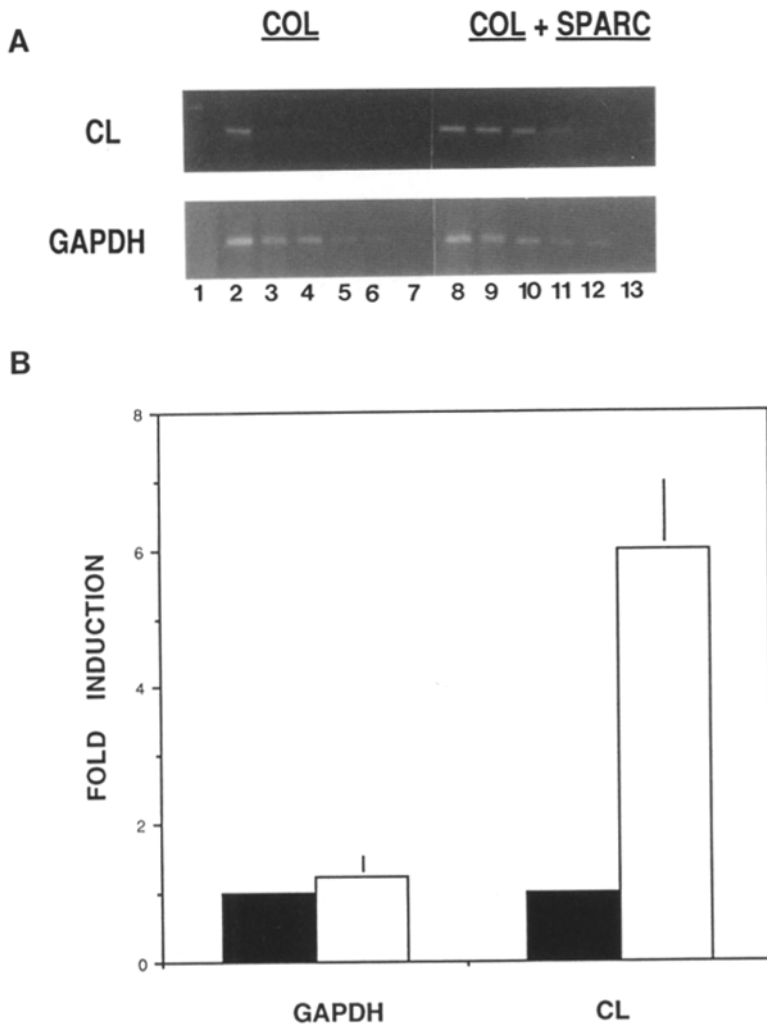COL   COL + SPARC

B

Figure 3. The expression of collagenase mRNA is up-regulated by SPARC. Freshly trypsinized RSF were cultured on type I collagen-coated wells in DME-LH or in DME-LH containing 30 μg/ml SPARC for 30 h. The total cellular RNA was isolated and analyzed by RT-PCR. (*A*) Sequences in cDNA were amplified with the use of specific primers for collagenase cDNA and GAPDH cDNA to yield 300- and 241-bp fragments, respectively. The products were separated on agarose gels and stained with ethidium bromide. For amplification of the collagenase (*CL*) mRNA, the cDNA derived by RT of 2 μg RNA from control (*COL*) and SPARC-treated RSF (*COL + SPARC*) was diluted to 10^{-2} (lanes *2* and *8*), 5×10^{-2} (lanes *3* and *9*), 10^{-3} (lanes *4* and *10*), 5×10^{-3} (lanes *5* and *11*), 10^{-4} (lanes *6* and *12*), or 5×10^{-4} (lanes *7* and *13*). For amplification of GAPDH mRNA, the cDNA derived by RT of 2 μg RNA from untreated RSF and RSF plated on collagen and treated with SPARC was diluted to 10^{-1} (lanes *2* and *8*), 5×10^{-1} (lanes *3* and *9*), 10^{-2} (lanes *4* and *10*), 5×10^{-2} (lanes *5* and *11*), 10^{-3} (lanes *6* and *12*), and 5×10^{-3} (lanes *7* and *13*); lane *1* contained no template. (*B*) The data from three independent experiments were quantified by densitometry and are expressed as -fold induction (the ratio of values from SPARC-treated to untreated RSF values). Shaded bars represent mean values from untreated cells plated on collagen, and open bars represent values from cells plated on collagen and treated with SPARC. Lines at the end of bars indicate SD.

tion-perturbing anti-fibronectin receptor antibody can induce collagenase without significant change in cell shape (Aggeler et al., 1984*b*; Werb et al., 1989). As in the regulation of collagenase in cells plated on fibronectin fragments or treated with anti-fibronectin receptor antibody, SPARC induced collagenase expression without a major morphological change (Fig. 4). This contrasts with the marked rounding of endothelial cells and nuchal fibroblasts treated with SPARC (Sage et al., 1989*c*).

The Induction of Collagenase by SPARC Is ECM-Specific

Because SPARC expression is tightly regulated during development and tissue remodeling, we wished to determine if there are particular ECM contexts in which SPARC initiates a remodeling cascade involving metalloproteinase expression. Accordingly, we plated fibroblasts in serum-free medium in wells that had been coated with collagen types I, II, III, IV, or V, with vitronectin, or with the more complex matrices synthesized by EHS tumor cells and R22 smooth muscle cells.

SPARC increased collagenase synthesis 2–3-fold in RSF that were plated on vitronectin or collagens of types I, II, III, and V, but not in cells plated on collagen type IV (Fig. 5). SPARC was an inductive ligand for cells plated on R22 ma-
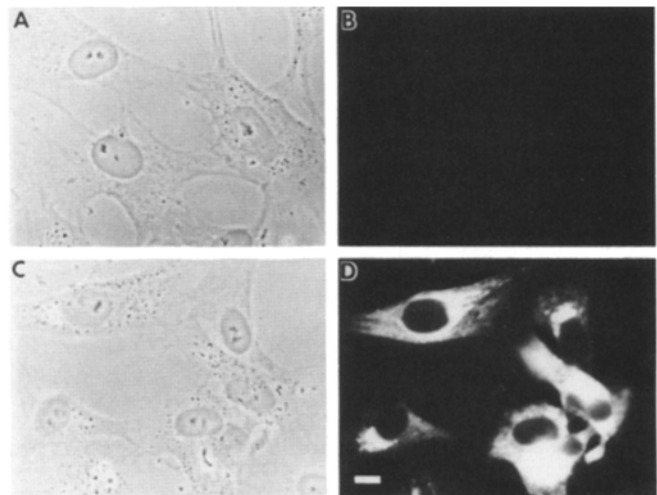

Figure 4. SPARC induces collagenase without inducing cell rounding in RSF. Freshly trypsinized RSF were cultured on glass coverslips in DME-10% FBS for 3 h. The cells were incubated in DME-LH (*A* and *B*) or in DME-LH supplemented with 30 μg/ml DESPARC (*C* and *D*) for 30 h, then fixed, permeabilized, and stained with anti-collagenase mAbs. Paired phase-contrast (*A* and *C*) and immunofluorescent micrographs (*B* and *D*) are shown. Bar, 10 μm.
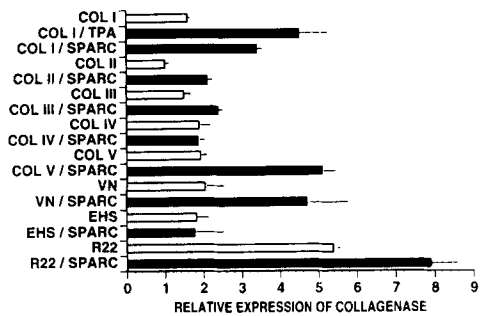
Figure 5. SPARC induces collagenase expression in matrix-specific fashion. RSF were plated in 48-well plates that were coated with ECM proteins—collagens (*COL*) of types I, II, III, IV, or V; vitronectin (*VN*), EHS matrix, R22 matrix—in Fibroblast Growth Medium alone or with 30 µg/ml DESPARC (*SPARC*) and incubated for 30 h. The proteins were biosynthetically labeled by incubating cells with [^{35}S]methionine, and the labeled secreted collagenase was quantified by scanning densitometry after SDS-PAGE and autoradiography. Open bars indicate collagenase synthesized by untreated RSF plated on the indicated matrix molecule. Shaded bars indicate total collagenase secreted into the culture medium from RSF plated on the indicated matrix molecule and treated with 30 µg/ml SPARC. Means of duplicate samples are shown; lines at end of bars indicate range.

trix (which consists predominantly of elastin and collagen types I and III), but not for cells plated on EHS matrix (predominantly laminin, collagen type IV, and heparan sulfate proteoglycan) (Fig. 5). Because the basal secretion of collagenase on EHS matrix was no lower than it was on other matrices in this experiment, and because cells plated on another basement membrane component (pure collagen type IV) did not regulate collagenase in response to SPARC, it is unlikely that TGF-β, which is known to be associated with some preparations of EHS matrix, abrogated the effect of SPARC in these experiments. These results indicate that cells treated with SPARC synthesize more collagenase than do untreated fibroblasts plated on the matrices.

SPARC Peptides Also Induce Collagenase Expression

Sequence analysis has indicated that there are four unique structural domains in the SPARC molecule (Mason et al., 1986; Engel et al., 1987): I, an acidic Ca^{2+}-binding domain; II, a cysteine-rich domain; III, a neutral α-helical sequence containing a serine proteinase-sensitive site; and IV, a high-affinity Ca^{2+}-binding EF handlike structure (Fig. 6). One approach that has been used successfully to define the structural motifs of the SPARC molecule that affect cell
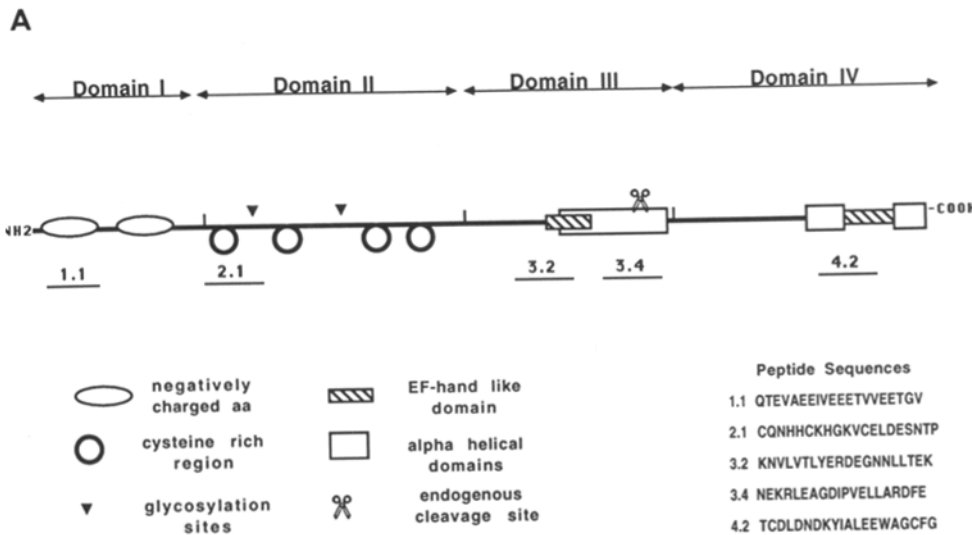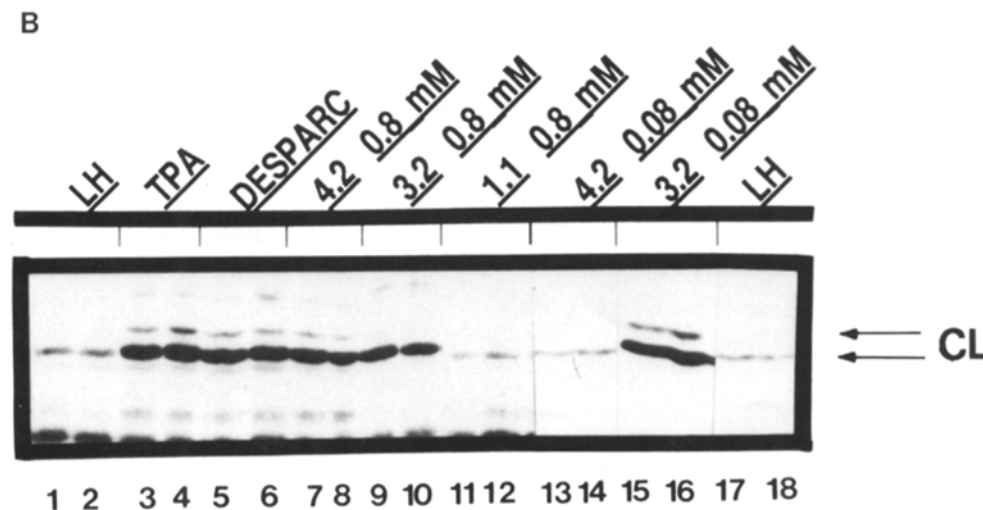
A

B

Figure 6. SPARC peptides from domains III and IV regulate collagenase expression. (*A*) Schematic diagram of the SPARC molecule showing the location of specific domains and the synthetic peptides (1.1, 2.1, 3.2, 3.4, and 4.2) used in this study. (*B*) The synthetic peptides (0.8 mM or 0.08 mM) or 30 µg/ml DESPARC were added to RSF plated in 48-well plates. Cultures were incubated for 24 h, and then radiolabeled with [^{35}S]methionine for 2 h. Biosynthetically labeled secreted proteins were analyzed by SDS-PAGE and autoradiography. This panel shows the induction of collagenase in RSF incubated with DME-LH alone (*LH*, lanes *1*, *2*, *17*, and *18*), with 100 ng/ml TPA (lanes *3* and *4*), with DESPARC (lanes *5* and *6*), or with synthetic peptide 4.2 (0.8 mM) (lanes *7* and *8*), peptide 3.2 (0.8 mM) (lanes *9* and *10*), peptide 1.1 (0.8 mM) (lanes *11* and *12*), peptide 4.2 (0.08 mM) (lanes *13* and *14*), or peptide 3.2 (0.08 mM) (lanes *15* and *16*).

Peptide Sequences

1.1 QTEVAEEIVEEETVVEETGV

2.1 CQNHHCKHGKVCELDESNTP

3.2 KNVLVTLYERDEGNNLLTEK

3.4 NEKRLEAGDIPVELLARDFE

4.2 TCDLDNDKYIALEEWAGCFG

A

COL COL COL
    SPARC TPA

2-4 HR -

4-6 HR -

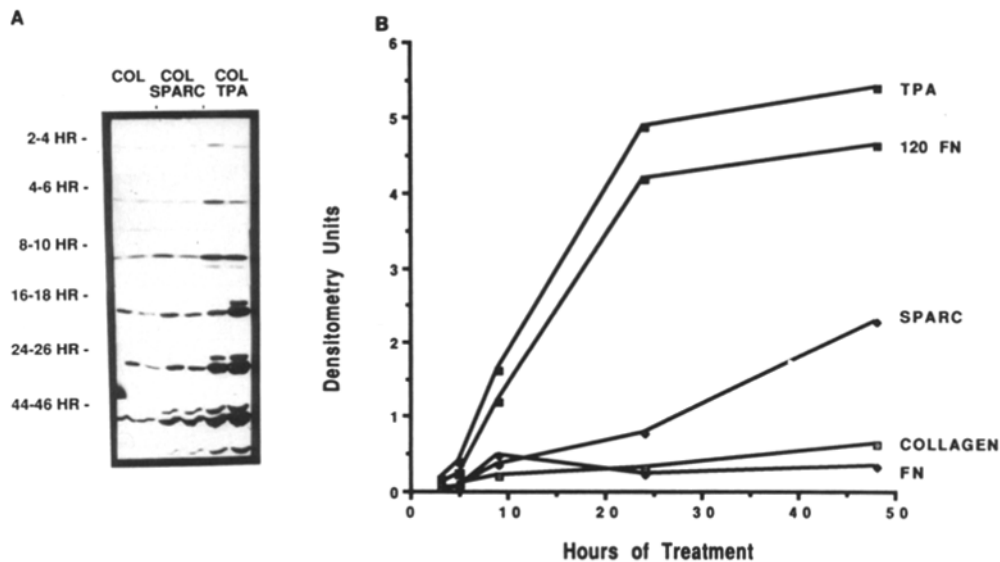8-10 HR -

16-18 HR -

24-26 HR -

44-46 HR -

Figure 7. SPARC induces collagenase expression in RSF with slower kinetics than is seen for TPA or fibronectin fragments. Freshly trypsinized RSF were plated in wells coated with collagen type I in DME-LH alone (*COL*), or in DME-LH supplemented with 30 μg/ml SPARC (*COL SPARC*), or with 100 ng/ml TPA (*COL TPA*), or in wells coated with fibronectin (*FN*), or with the 120-kD chymotrypsin fragment of fibronectin (*120 FN*). The cultures were incubated for 2-44 h, and proteins were biosynthetically labeled by incubation with 100 μCi/ml of [³⁵S]methionine for 2 h. The radiolabeled secreted collagenase was analyzed by immunoprecipitation with anti-collagenase mAbs, and the precipitates were resolved by electrophoresis on a 10% SDS-polyacrylamide gel. (*A*) Autoradiogram of the immunoprecipitated radiolabeled collagenase protein. (*B*) Quantification of secreted collagenase by densitometry of autoradiographs.

adhesion (Lane and Sage, 1990) or the cell cycle (Funk and Sage, 1991) is the synthesis of peptides corresponding to regions in domains I-IV and the monitoring of their efficacy in mimicking SPARC function. We tested a panel of synthetic peptides spanning portions of each of the four domains of the SPARC molecule (Lane and Sage, 1990) to define which domains in the SPARC molecule confer regulation of metalloproteinases in RSF.

Peptides in domain I (1.1) and domain IV (4.2) destabilize adhesion of cells to the ECM and thus mimic the antiadhesive properties of SPARC. Addition of peptide 4.2, at 0.8 mM, to cultures of adherent RSF induced collagenase expression (Fig. 6, Table I). We detected no significant induction of collagenase in response to peptide 1.1 (Fig. 6). Peptide 2.1, like intact SPARC, delays the G₀/S transition when added to endothelial cells in culture (Funk and Sage, 1991); however, it did not induce collagenase expression in RSF (Fig. 6, Table I). Surprisingly, we saw an induction of collagenase in cells treated with peptide 3.2, which is in the neutral α-helical domain III; peptide 3.2 was inductive at 0.08 mM, which is a tenfold lower concentration than was inductive with peptide 4.2. Similar results were obtained with two different preparations of peptide 3.2. Peptides representing nearby sequences, both amino-terminal (peptide 2.1) and carboxyl-terminal (peptide 3.4), did not induce collagenase expression (Table I). These results suggest that regions in domain III and IV in the SPARC molecule induce metalloproteinase expression in fibroblasts.

The Regulation of Collagenase Expression by SPARC Is Temporally Distinct from Induction by TPA or Fibronectin Fragments

Previous experiments have shown that fibronectin fragments and TPA induce collagenase expression within 4 h (Werb et al., 1989). To characterize the timing of the induction of collagenase by SPARC, we compared the time course of collagenase induction by SPARC, TPA, and fragments of

fibronectin. RSF were plated in serum-free medium in collagen-coated wells, some of which were supplemented with 30 μg/ml DESPARC or 100 ng/ml TPA, or in wells that had been coated with fibronectin or the 120-kD chymotrypsin fragment of fibronectin. At various times (2-44 h) after plating we incubated the cultures with [³⁵S]methionine for 2 h, immunoprecipitated the radiolabeled secreted collagenase from the CM, and analyzed the immunoprecipitates by SDS-PAGE and autoradiography. Collagenase synthesis was detected in SPARC-treated cultures after 8 h but was apparent within 4 h in cultures plated on fibronectin fragments or treated with TPA (Fig. 7). When we analyzed the total proteins secreted by RSF after a 2-6-h incubation with SPARC, we noted the transient induction of several proteins that were not secreted by RSF treated with TPA (Fig. 8).

SPARC Induces Collagenase Expression by an Indirect Mechanism

The data presented in Figs. 6, 7, and 8 suggest at least two potential mechanisms by which SPARC may regulate collagenase expression in fibroblasts. The slow induction of collagenase (>10 h) in RSF treated with SPARC is reminiscent of the time course of collagenase induction in RSF elicited by agents that act by shape-dependent mechanisms, such as cytochalasins or culture in retracted collagen gels (Unemori and Werb, 1986). In our experiments, however, SPARC did not appear to alter the morphology of RSF. Alternatively, the induction of collagenase in cells treated with SPARC may be mediated by one of the molecules induced in SPARC-treated cultures before collagenase expression is induced. Although the experiment with SPARC peptides also suggests that two domains in the SPARC molecule regulate the expression of collagenase, possibly by distinct mechanisms, for simplicity we decided to clarify the mechanism by which native, intact SPARC affects the expression of collagenase in fibroblasts.

To determine if the increase in collagenase expression was a direct response to treatment of cells with SPARC, or was
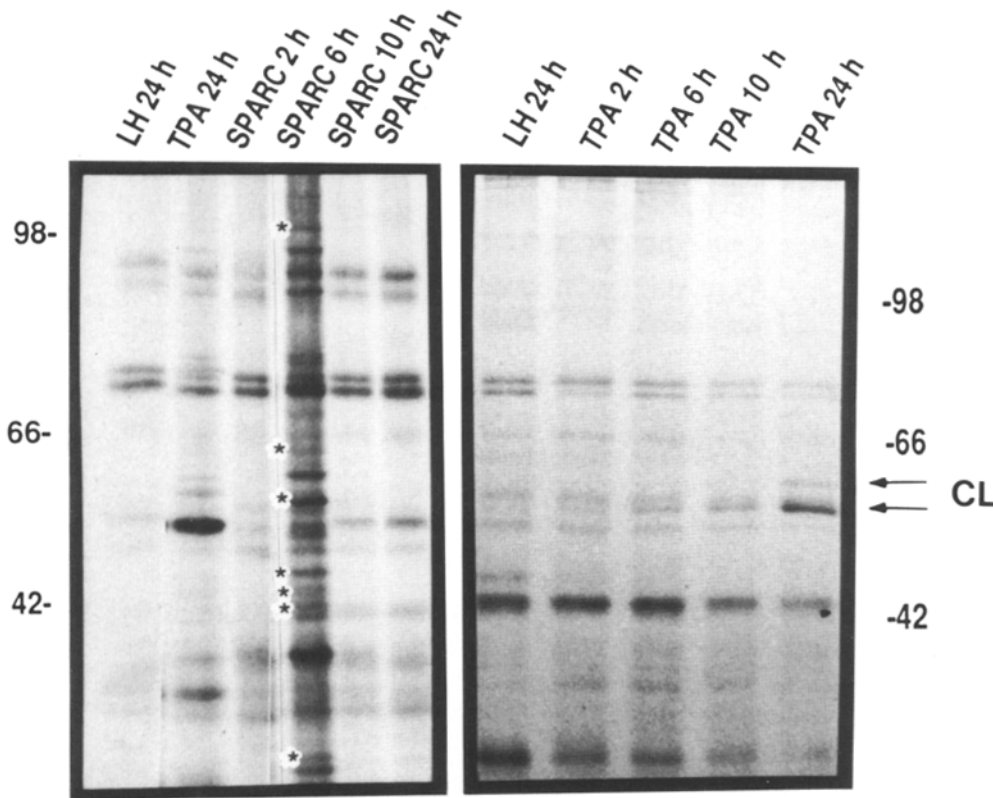
Figure 8. SPARC treatment transiently induces the expression of several novel secreted proteins. RSF were plated in 48-well plates in DME-10% FBS for 3 h. The medium was then replaced with DME-LH alone (*LH*) or with DME-LH supplemented with 100 ng/ml TPA (*TPA*), or 30 μg/ml DESPARC (*SPARC*). After incubation of cells for 2, 6, 10, or 24 h, the proteins were biosynthetically labeled by incubating cultures with [35S]methionine, and the total secreted proteins were concentrated and analyzed by SDS-PAGE and autoradiography. The bands representing procollagenase are indicated at the right. The novel SPARC-induced proteins are indicated by asterisks. Molecular weight ($\times 10^{-3}$) markers are indicated. Data shown are from one experiment; however, the same results were obtained in a duplicate experiment.

secondary to the action of a SPARC-induced molecule, we plated three sets of parallel cultures of RSF in wells coated with collagen type I, with or without 30 μg/ml pure SPARC or DESPARC (Fig. 9 *A*). One set of cultures was incubated with pure SPARC or DESPARC for 24 h. Another set of cultures was incubated with or without pure SPARC or DESPARC for 7 h, the medium was removed and saved, and the cultures were incubated further in DME-LH for 17 h. From the medium that was removed and saved, we depleted the residual SPARC by incubation of the CM with goat anti-SPARC IgG bound to protein G beads; we incubated a third set of cultures, plated on collagen type I, with this SPARC-depleted CM for 15 h. To confirm that SPARC, and not a contaminating molecule in the preparation of DESPARC, induced the expression of collagenase, we removed SPARC from a duplicate sample of SPARC-supplemented culture medium by preadsorption with goat anti-SPARC IgG-loaded protein G beads before the addition of this medium to cultures. To ensure that SPARC was depleted from the samples incubated with anti-SPARC IgG beads, we subjected duplicate samples of SPARC, or CM harvested from SPARC-treated or control cultures, before and after incubation with anti-SPARC IgG, to analysis by SDS-PAGE under non-denaturing conditions. No residual SPARC was detected in the SPARC-depleted CM by immunoblotting (data not shown). To eliminate the possibility that serum-derived factors associated with the washed IgG-loaded protein G beads may induce collagenase expression, we also incubated cultures for 24 h with medium that had been preabsorbed with protein G beads loaded with IgG from normal goat serum. All cultures were incubated with [35S]methionine 24 h after initial plating, and the biosynthetically labeled secreted collagenase was analyzed by immunoprecipitation. These ex-

periments were performed with both DESPARC and pure SPARC. The data represent an average of three experiments.

We saw a 3.5-fold increase in collagenase expression in cultures incubated with SPARC for 24 h (Fig. 9 *B*). In cultures incubated with SPARC for only 7 h, collagenase expression remained at basal levels, similar to that of untreated controls. Interestingly, we saw a 2.5-fold increase in collagenase expression in cells incubated with the SPARC-depleted CM harvested from cultures treated with SPARC for 7 h, whereas cells cultured in SPARC-depleted CM harvested from untreated cells synthesized basal levels of collagenase. Cells that were incubated with medium from which SPARC was preadsorbed before incubation, or cells incubated with medium that was preincubated with protein G beads containing normal goat serum IgG, also synthesized basal levels of collagenase. These results indicate that the mechanism of regulation of collagenase by SPARC is different from that of TPA or fibronectin fragments. The increased expression of collagenase associated with SPARC may, therefore, be mediated in part through a secreted factor that is produced during the first 7 h of treatment with SPARC.

The upregulation of collagenase expression in response to peptide growth factors and cytokines is rapid and is initiated within 2–6 h of exposure to the inducing agent (Frisch and Werb, 1989). If the regulation of collagenase by SPARC is mediated by the production of a secreted intermediate such as a cytokine, one prediction would be that the induction of collagenase expression in cells incubated with SPARC-depleted CM harvested from cells treated with SPARC for 7 h would be faster than that seen in cultures incubated with SPARC. To analyze the kinetics of induction, we compared the number of cells staining positive by immunofluorescence for cell-associated collagenase from cultures treated with
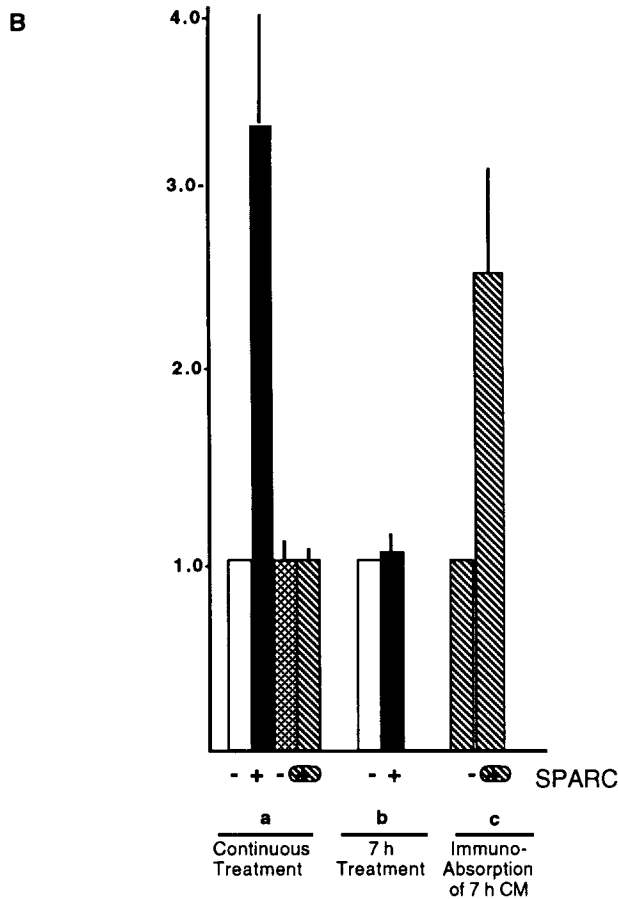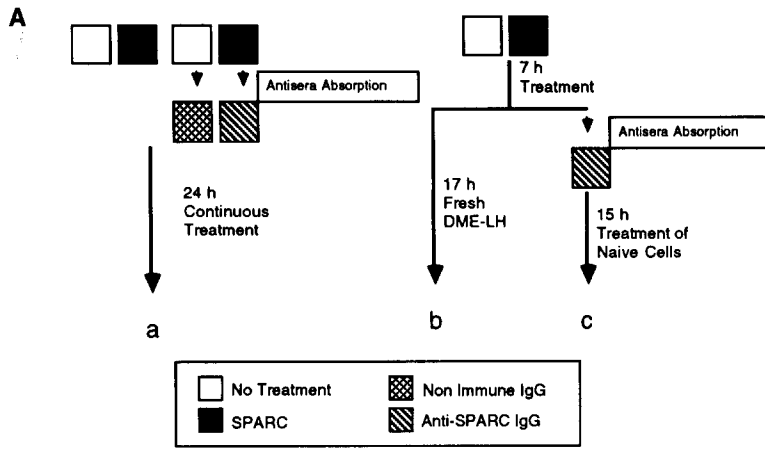
A

| | No Treatment | | Non Immune IgG |
| SPARC | | Anti-SPARC IgG |

B

- + -⊗ - + -⊗ **SPARC**

| a | b | c |
| Continuous Treatment | 7 h Treatment | Immuno-Absorption of 7 h CM |

Figure 9. SPARC indirectly induces collagenase expression through a secreted intermediate. (*A*) RSF were plated on wells coated with collagen type I and incubated in Fibroblast Growth Medium, with or without 30 µg/ml DESPARC. One set of cultures (*a*) was incubated for 24 h. Experimental controls included RSF plated on type I collagen–coated wells in Fibroblast Growth Medium supplemented with DESPARC that had been preincubated with protein G beads loaded with goat anti-SPARC IgG, or in Fibroblast Growth Medium containing protein G beads loaded with IgG from normal goat serum. In a second set of cultures (*b*), the CM was removed 7 h after plating, and the cultures were incubated further in unsupplemented Fibroblast Growth Medium for 17 h. The CM that was harvested from cells incubated for 7 h in Fibroblast Growth Medium with or without SPARC was depleted of residual SPARC by immunoprecipitation with protein G beads loaded with goat anti-SPARC IgG; this SPARC-depleted medium was added to a third set of cultures (*c*) that had been plated on wells coated with collagen type I, and the cultures were incubated for an additional 15 h. At 24 h after the initiation of the experiment, proteins were biosynthetically labeled by incubation of cells with [³⁵S]methionine. The radiolabeled secreted collagenase was immunoprecipitated with anti-collagenase mAbs, and analyzed by SDS-PAGE followed by autoradiography. (*B*) The autoradiograms of the RSF treated as described in *A* from three independent experiments were quantified by densitometry. The data are expressed as -fold induction. Plus and minus signs indicate treatment with or without SPARC.

SPARC for 35 h, or with SPARC-depleted CM harvested from SPARC-treated cultures for 10 or 35 h (counting about 500 cells in 4–5 microscopic fields). We then studied RSF plated on collagen-coated coverslips and treated with SPARC-depleted CM from cultures incubated in serum-free medium with or without 30 µg/ml pure SPARC for 6.5 h at 10 and 35 h of treatment. RSF treated with SPARC for 10 h showed little induction of collagenase (see Fig. 7). However, at 10 h we saw a twofold increase in the total secreted collagenase and a 3.5-fold increase in the collagenase-positive cells in the cultures treated with CM from SPARC-treated cultures, whereas there was no change in secreted col-

lagenase from cells treated with control CM. The increase in collagenase-positive cells compared with the increase in secreted collagenase reflects the short time elapsed to accumulate induced collagenase during the 10 h of culture time. By 35 h, the cells incubated with SPARC continuously showed a 4.3-fold increase in secreted collagenase and a 3.0-fold increase in collagenase-positive cells, which was similar to the fourfold increase in secreted collagenase and 2.7-fold increase in collagenase-positive cells seen in cultures treated with 6.5 h CM from SPARC-treated cells, compared with untreated controls or cells treated with CM from control cultures. The increase in collagenase-positive cells in cultures

incubated with SPARC for 35 h, or with SPARC-depleted, SPARC-conditioned medium for 10 and 35 h was significantly different from control cultures at all time points ($p < 0.05$, Student-Newman-Kuels test). The increase in the collagenase-positive cells in cultures treated for 10 h with SPARC-depleted CM from SPARC-treated cultures was not significantly different from cultures treated with pure SPARC for 35 h. Taken together, these data support the concept that a SPARC-induced secreted intermediate is at least a part of the cascade by which SPARC induces the expression of collagenase in RSF.

Discussion

The decision not to adhere to a substrate has developmental and biological consequences. This concept has been derived from studies on neurite outgrowth and patterning of the nervous system (Tomaselli et al., 1987; Dodd et al., 1988; Klambt et al., 1991; Bovolenta and Dodd, 1991), from studies of the complex adhesive interactions of cells in the immune system (Kieffer and Phillips, 1990; Butcher, 1991) and from characterization of the migration of embryonic cell populations (Riou et al., 1990; ffrench-Constant et al., 1991; Hynes and Lander, 1992). One proposed mechanism for diminishing the adhesion of cells to the ECM is through ECM-associated molecules with anti-adhesive properties (for review see Chiquet-Ehrismann, 1991; Sage and Bornstein, 1991). A number of structurally dissimilar molecules with anti-adhesive properties that modulate cell-ECM interactions have been described: SPARC, tenascin, thrombospondin, dermatan sulfate proteoglycans, and scatter factor have defined anti-adhesive effects on cells in culture (for review see Chiquet-Ehrismann, 1991; Sage and Bornstein, 1991). The expression of these proteins is tightly regulated to produce developmental patterns and to respond to tissue injury; moreover, they localize to regions of tissue where cell division, migration, and ECM remodeling are prevalent (for review see Chiquet-Ehrismann, 1991; Sage and Bornstein, 1991).

In this report we have presented evidence that one of these anti-adhesive, ECM-associated molecules, SPARC, at a concentration of ~ 0.5 nM, which is within physiological levels (Maillard et al., 1992), initiates a cascade of events that result in the increased expression of the ECM-remodeling metalloproteinases. SPARC induced the expression of collagenase, stomelysin, and 92-kD gelatinase, metalloproteinases capable of degrading both basement membranes and interstitial connective tissue matrices. Collagenase specifically cleaves native, fibrillar collagens, whereas the 92-kD gelatinase degrades type IV collagen and denatured collagen. Stromelysin can degrade many molecules in the ECM, including proteoglycans and fibronectin (for review see Alexander and Werb, 1991). These results suggest that SPARC, in addition to its characterized effects on cell shape, may alter the nature of the ECM presented to the cell.

Cellular responses to growth factors have traditionally been divided by temporal parameters into immediate, intermediate, and delayed effects. Likewise, cells can respond to SPARC within minutes of treatment, but there are also downstream effects that can be measured hours to days after exposure. Immediate responses include actin disassembly and accumulation of mRNA coding for plasminogen activator

inhibitor-1 in bovine aortic endothelial cells (Hasselaar et al., 1991). Intermediate responses include a delay in the G_o/S transition in cycling bovine aortic endothelial cells (Funk and Sage, 1991). The generation of a SPARC-induced secreted molecule that regulates collagenase expression in synovial fibroblasts is another intermediate response to SPARC, whereas a delayed response, the induction of metalloproteinases in cells treated with SPARC, occurs over a period of days.

When we further characterized the regulation of collagenase in RSF, we noted that the regulation of collagenase by SPARC was distinct from the regulation of collagenase conferred by TPA or ligands acting through the fibronectin receptor. Collagenase expression induced by SPARC proceeded with kinetics similar to that conferred by treatments that act by a shape-dependent mechanism, such as culture on polyhydroxyethyl methacrylate, in retracted collagen gels, or with cytochalasins. However, there are several reasons why it is unlikely that SPARC acts solely through a shape-dependent mechanism: (a) In contrast to the marked changes in the morphology of bovine nuchal fibroblasts or aortic endothelial cells mediated by SPARC (Sage et al., 1989c), RSF in culture remained well spread, although we may not have detected the subtle, transient alterations in the cytoskeleton that were described by Murphy-Ullrich et al. (1991); (b) we detected the synthesis of several novel SPARC-induced proteins that were secreted transiently before collagenase expression; (c) supernatants that were removed from SPARC-treated cultures and depleted of SPARC induced collagenase expression in untreated fibroblasts, whereas cultures from which SPARC was removed after 7 h of incubation did not, after further incubation, increase their expression of collagenase; furthermore, (d) the increase in cells staining for collagenase in cultures incubated with this SPARC-depleted, SPARC-conditioned medium for only 10 h was the same as that seen in cultures incubated continuously with pure SPARC for 35 h.

Thus, one important feature of this induction is the synthesis or secretion of an intermediary molecule. The nature of this secreted intermediate is not known at present. In preliminary experiments in which we used RT-PCR to measure cytokines expressed by RSF after incubation with SPARC for 7 h, we have observed increases in mRNA for basic FGF and tumor necrosis factor-α and a decrease in mRNA for TGF-β (unpublished observations). It is known that tumor necrosis factor-α and basic FGF upregulate the expression of collagenase in cultured fibroblasts (for review see Frisch and Werb, 1989). Because TGF-β both inhibits the expression of metalloproteinases and induces the expression of the tissue inhibitor of metalloproteinases, further studies to characterize this secreted intermediate will prove interesting.

There are other examples of autocrine regulation of collagenase expression in fibroblasts. The induction of collagenase by ultraviolet irradiation of fibroblasts also proceeds through an uncharacterized, secreted intermediate (for review see Herrlich et al., 1992). In synovial cells both serum amyloid alpha and β_2-microglobulin, which are induced in cells treated with TPA or interleukin-1 (IL-1), have been shown to induce the expression of collagenase in untreated fibroblasts (Brinckerhoff et al., 1989). In other culture systems, changes in adhesion correlate with the synthesis or secretion of growth factors (Hedin et al., 1989; Shaw

et al., 1990). Collagenase expression in fibroblasts is increased in response to a number of growth factors and cytokines that can be synthesized or stored in fibroblasts, including PDGF, basic FGF, tumor necrosis factor-α, and IL-1 (for review see Frisch and Werb, 1989). It is possible that any of these factors may be the secreted intermediate.

Collagenase expression is regulated in synovial fibroblasts by cytochalasins, by culture on a substrate with altered adhesive properties (polyhydroxyethyl methacrylate), or in retracted collagen gels through mechanisms that involve a change in the actin cytoskeleton (Aggeler et al., 1984b; Unemori and Werb, 1986), in which cell shape is altered from an extended, well-spread morphology to a rounded, cuboidal morphology. There may also be a cytoskeletal component to the induction of collagenase in RSF treated with SPARC. Although these cells remained spread and adherent, nuchal fibroblasts and aortic endothelial cells rapidly reorganize their actin cytoskeleton and lose their focal contacts after exposure to SPARC (Lane and Sage, 1990). Although a SPARC receptor has not yet been fully characterized, we cannot exclude the possibility that SPARC, acting through a specific cell surface receptor for SPARC, induces collagenase expression in fibroblasts in vivo.

That SPARC induced collagenase expression in cells plated on a smooth muscle matrix is consistent with the observations of Raines et al. (1992), who noted that SPARC and PDGF are increased in the neointima of atherosclerotic plaque. SPARC has been shown to delay the G_o/S transition of cycling cells (Funk and Sage, 1991), to play a role in calcifying tissue (Termine et al., 1981), and to have defined effects on adhesion of cells to ECM (Lane and Sage, 1990). SPARC binds to PDGF in such a way as to block the interaction of PDGF (AB and BB) with its receptor (Raines et al., 1992). Although both PDGF and IL-1 induce collagenase expression in synovial cells, the addition of PDGF to IL-1–stimulated cells diminishes the proliferation and metalloproteinase expression induced by IL-1 (Kumkumian et al., 1989). Therefore, the sequestration of PDGF by SPARC may have several distinct consequences, such as modifying the cellular response to specific cues and/or initiating a mitogenic or remodeling cascade in the atherosclerotic plaque.

We have used the induction of metalloproteinase expression in cultured cells as an assay to expand the structure-function analysis of the SPARC protein. Domain IV contains a high-affinity Ca^{2+}-binding site that diminishes the adhesion of cells in culture; this domain also mediates the interaction of SPARC with immobilized collagen type I. Our work supports the existing evidence that this carboxyl-terminal Ca^{2+} binding domain is important for SPARC function. We anticipated that peptide 4.2, derived from SPARC sequences representing the carboxyl-terminal Ca^{2+}-binding domain, would induce collagenase expression in RSF because this peptide causes cultured fibroblasts and aortic endothelial cells to assume a rounder morphology (Lane and Sage, 1990). In addition, antibodies raised against peptide 4.2 react against native SPARC. Peptide 4.2 binds to collagen type I and blocks the interaction of native SPARC with collagen type I (Lane and Sage, 1990). In contrast, peptide 1.1, which spans sequences in the amino-terminal Ca^+-binding domain I of the molecule, also induces a cell shape change; however, peptide 1.1 did not cause significant changes in collagenase expression in RSF. Peptide 1.1 does not inhibit SPARC bind-

ing to immobilized collagen I (Lane and Sage, 1990). Our data also suggest that the presumptive extended α-helical domain is important for some functions of SPARC. We did not anticipate that peptide 3.2, located in a stable helical structure of domain III in the SPARC molecule (Engel et al., 1987; Bolander et al., 1988; Lane and Sage, 1990), would regulate collagenase expression in RSF to a significant degree. It is possible that proteolytic cleavage changes the conformational constraints in the SPARC molecule and that these changes in turn alter the accessibility and/or function of the α-helical domain. Comparison of SPARC cDNA sequences from *Xenopus* and several mammalian species shows that there is a high degree of sequence homology in the carboxyl-terminal domain IV and in domain III, but not in the negatively charged domain I (Damjanovski et al., 1992). Taken together, these data indicate that domains III and IV are functionally important for the interaction of SPARC with ECM or cells and for functioning of SPARC in vivo.

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