SPARC Regulates Processing of Procollagen I and Collagen Fibrillogenesis in Dermal Fibroblasts*

Received for publication, January 8, 2007, and in revised form, April 13, 2007 Published, JBC Papers in Press, May 23, 2007, DOI 10.1074/jbc.M700167200

Tyler J. Rentz†, Felicita Poobalarahi‡, Paul Bornstein§, E. Helene Sage¶, and Amy D. Bradshaw¶††

From the †Department of Medicine, Medical University of South Carolina, Charleston, South Carolina 29412, the ‡Department of Biochemistry, University of Washington, Seattle, Washington 98195, and the ¶Hope Heart Program, Benaroya Research Institute at Virginia Mason, Seattle, Washington 98101

A characterization of the factors that control collagen fibril formation is critical for an understanding of tissue organization and the mechanisms that lead to fibrosis. SPARC (secreted protein acidic and rich in cysteine) is a counter-adhesive protein that binds collagens. Herein we show that collagen fibrils in SPARC-null skin from mice 1 month of age were inefficient in fibril aggregation and accumulated in the diameter range of 60–70 nm, a proposed intermediate in collagen fibril growth. In vitro, procollagen I produced by SPARC-null dermal fibroblasts demonstrated an initial preferential association with cell layers, in comparison to that produced by wild-type fibroblasts. However, the collagen I produced by SPARC-null cells was not efficiently incorporated into detergent-insoluble fractions. Coincident with an initial increase in cell association, greater amounts of total collagen I were present as processed forms in SPARC-null versus wild-type cells. Addition of recombinant SPARC reversed collagen I association with cell layers and decreased the processing of procollagen I in SPARC-null cells. Although collagen fibers formed on the surface of SPARC-null fibroblasts earlier than those on wild-type cells, fibers on SPARC-null fibroblasts did not persist. We conclude that SPARC mediates the association of procollagen I with cells, as well as its processing and incorporation into the extracellular matrix.

Matricellular proteins are defined as proteins that are associated with the extracellular matrix (ECM) but are not considered structural components of the ECM, in contrast to classical ECM proteins such as laminin and collagen I (1,2). SPARC is a prototypic matricellular protein that exhibits counter-adhesive and anti-proliferative activity when added to cultured cells (3). SPARC has been shown to bind to a number of ECM proteins including collagens I, III, and IV (3).

Expression of SPARC is elevated during development and decreases upon differentiation in a majority of tissues (4). However, expression of SPARC persists in tissues in which ECM remodeling is ongoing, such as bone and gut epithelia (4). Increased levels of SPARC are detected in response to injury where ECM remodeling is initiated, with fibroses in liver, lungs, and kidney, and in the skin of individuals with scleroderma (5–8). Hence, SPARC expression patterns implicate this protein as an important mediator of collagen I deposition and/or remodeling.

The α1(I) and α2(I) subunits of procollagen I are synthesized with N- and C-propeptides that are enzymatically released by specific proteases to yield processed collagen I (9). Processing of procollagen I to collagen I is essential for correct assembly of collagen fibrils. Spatial and temporal regulation of procollagen processing has been proposed as a potential regulatory event in collagen fibril assembly (9). For example, antibodies against the N-propeptide of collagen I were immunolocalized exclusively to the surface of collagen fibrils that were 20–40 nm in diameter (10). Retention of the propeptides of procollagen I following incorporation into fibrils might present a steric hindrance to fibril fusion and thus limit fibril expansion. Alternatively, Watson et al. (11) reported that pN collagen I (procollagen I with N-propeptides retained) could be incorporated into collagen fibrils with the N-propeptide accommodated within the fibril. The authors suggested that the N-propeptide acts to enhance collagen fibril aggregation (11). In this case, premature processing of the N-propeptide would limit fibril expansion. The factors that influence the spatial and temporal regulation of procollagen I conversion to collagen I are predicted to have a profound influence on collagen deposition (9).

SPARC-null mice display a number of phenotypic abnormalities, the majority of which are manifested in aberrant ECM structure and assembly, consistent with a function of SPARC as a modulator of cell-ECM interactions (4). Adult SPARC-null mice exhibit decreased amounts of collagen in skin and bone (12, 13). In addition, fibrotic deposition of collagen is diminished in the absence of SPARC. For example, streptozotocin-induced renal fibrosis in SPARC-null mice was reduced in comparison to that of wild-type (14). Increased expression of SPARC is associated with collagen production, and the lack of SPARC results in decreased collagen accumulation. SPARC is therefore implicated as a key regulator of collagen incorporation in tissues.

To investigate further the mechanisms by which SPARC influences collagen deposition, we cultured SPARC-null and wild-type (WT) primary dermal fibroblasts and characterized collagen I maturation and fiber formation in vitro. Our studies...
indicate that, in the absence of SPARC, an initial increase in the levels of procollagen I in cell layers was coupled with an increase in the conversion of procollagen I to collagen I. The addition of recombinant SPARC (rSPARC) reduced the levels of procollagen I associated with cell layers and was accompanied by a decrease in conversion of procollagen I. Enhanced association of procollagen I with SPARC-null cells did not lead to a greater incorporation of collagen into detergent-insoluble fractions. In fact, SPARC-null fibroblasts demonstrated substantially less incorporation of collagen into detergent-insoluble fractions after extended culture. Likewise, collagen fibers formed sooner on the surface of SPARC-null cells but did not persist, in comparison to fibers on WT cells.

EXPERIMENTAL PROCEDURES

Reagents—Cell culture reagents were from Invitrogen. rSPARC was generated by baculovirus infection of insect cells and purified as described in Ref. 15. Anti-murine collagen I antibodies were from MD Biosciences (Zurich, Switzerland). Antibodies generated against the C-propeptide of collagen α1(I) (LF41) were provided by Dr. L. Fisher (National Institutes of Health) (16). Anti-actin antibodies were from Sigma.

Electron Microscopy—The SPARC-null mouse colony used in these studies has been described previously (17). Sections of skin were generated from 3 WT and 5 SPARC-null mice at 1 month of age and were prepared for electron microscopy (EM) as described in Ref. 12. Collagen fibril diameters were measured in scanned images generated from electron micrographs with NIH Image software. Collagen fibrils in at least 3 fields derived from sections of skin from each mouse were quantified; 2699 WT fibrils and 2658 SPARC-null fibrils were measured.

Primary Cell Culture—Primary dermal fibroblasts were isolated from age-matched WT and SPARC-null animals based upon the protocol described in Ref. 18 with the following exceptions: 1) the collagenase solution used in these experiments was 1/10 Blendzyme 3 (Roche Applied Science) in Dulbecco’s modified Eagle’s medium (Invitrogen), 2) cell preparations were rinsed 3 times in growth media (Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and antibiotic/antimycotic solution) prior to final plating, and 3) cells were filtered through a 100-μm cell strainer prior to final plating. The majority of animals used in these experiments were 2–3 months of age. Dermal fibroblasts were cultured in growth media supplemented with 50 μg/ml sodium ascorbate to induce collagen production and secretion as indicated. All experiments were performed with dermal fibroblasts between passages 1 and 4.

Analysis of ECM Production and Deposition—Primary dermal fibroblasts plated at equal concentrations were labeled with 25 μCi/ml [2,3,4,5-3H]proline (PerkinElmer Life Sciences) in growth media for 18–24 h to assess collagen production in media and cell layers from WT and SPARC-null cells. Cell layers were collected by scraping in hot SDS-PAGE buffer with dithiothreitol, separated by SDS-PAGE, and proteins were resolved on 7% SDS-polyacrylamide gels. Bands representing procollagen and collagen I were detected by fluorography on X-Omat film. Immunoblot analysis was performed by transfer of separated proteins to nitrocellulose and detection with anti-murine collagen I antibodies. Chemiluminescence was used to detect secondary antibodies conjugated to horseradish peroxidase. Quantification of protein bands was performed with NIH Image software.

Metabolically labeled conditioned media generated by equal amounts of WT and SPARC-null cells grown in ascorbate for 18–24 h were incubated with an anti-N-propeptide rabbit polyclonal antibody against recombinant mouse N-propeptide that was produced in insect cells, or polyclonal anti-murine SPARC antibodies (R&D Systems, Minneapolis, MN). Primary antibodies were precipitated with protein A/G-Sepharose beads (GE Healthcare). Immune complexes were boiled in SDS-PAGE buffer with dithiothreitol, separated by SDS-PAGE, and exposed to film.

Pulse-chase experiments were performed by labeling equal amounts of WT and SPARC-null fibroblasts in growth media. Ascorbate was added ~2 h prior to pulse labeling with 25 μCi/ml [3H]proline for 12 min (pulse label). The pulse labeling media were removed, and the cell layer was rinsed in phosphate-buffered saline followed by incubation in chase media (growth media with ascorbate and 1 mg/ml unlabelled proline) for the indicated time periods. Cell layers were collected in SDS-PAGE buffer with dithiothreitol, and proteins were resolved on 7% SDS-polyacrylamide gels. Bands representing procollagen and collagen I were detected by fluorography and were quantified by the use of NIH Image software.

Immunohistochemistry—Age-matched WT and SPARC-null cells were plated in equal numbers on glass coverslips in growth media. Cells were treated with 50 μg/ml ascorbate and were grown for the indicated number of days. Cell layers were fixed in 4% paraformaldehyde in phosphate-buffered saline (pH 7.5) for 30–45 min. Coverslips were blocked in 1% normal goat serum in Tris-buffered saline (blocking solution) prior to addi-
tion of anti-murine collagen I antibodies (1:200 dilution in blocking solution) or anti-cellular fibronectin (1:200 in blocking solution, Sigma). Primary antibodies were detected with goat anti-mouse fluorescein-conjugated secondary antibodies (Jackson Laboratories). Coverslips were mounted in Anti-Fade reagent (Molecular Probes, Eugene, OR) and were viewed on a Leica microscope equipped for epifluorescence.

RESULTS

EM of Collagen Fibrils from 1-Month-old Mice—Thin collagen fibrils characteristic of young dermis undergo lateral fusion to generate collagen fibrils of increasing diameter. We have reported that collagen fibrils in adult SPARC-null skin were smaller and more uniform in diameter than those of WT mice (12). We sought to determine whether the absence of SPARC was associated with phenotypic differences in collagen fibril morphology in the dermis during times characterized by active collagen fibril aggregation. As shown in Fig. 1, a more limited range of fibril diameters was observed in SPARC-null skin in comparison to WT at 1 month of age. Although the average diameter of WT fibrils was slightly larger than SPARC-null fibrils (60.2 versus 53.8 nm), the standard deviation of the WT fibril population was 24, whereas that of SPARC-null fibrils was 14.6, which is indicative of a more restricted range of fibril diameters in null dermis. Whereas collagen fibrils as large as 220 nm were observed in WT dermis, SPARC-null fibrils larger than 110 nm were not found. The frequency of SPARC-null collagen fibrils with diameters of 60–70 nm was disproportionately represented in SPARC-null skin. Thirty-seven percent of measured SPARC-null collagen fibrils were 60–70 nm in diameter, whereas 20% of WT fibrils were in this range. We conclude that in the absence of SPARC, collagen fibril accretion is compromised, as early as 1 month after birth.

Production and Deposition of Collagen I by SPARC-null Dermal Fibroblasts—Primary dermal fibroblasts from age-matched WT and SPARC-null mice were cultured to examine differences in collagen deposition that might implicate SPARC in collagen fibril formation. We have found that collagen secretion by primary murine dermal fibroblasts is greatly enhanced by the addition of ascorbate. As shown in Fig. 2, collagen produced by SPARC-null fibroblasts displayed an increased interaction with detergent-soluble cell layers in comparison to WT (+/+) cells, whereas an increase in collagen I was observed in SPARC-null versus WT cell layer fractions. Quantification of collagen α1(I) protein bands generated by fluorography from 7 separate cell isolations. The ratio of null α1(I)/WT α1(I) was ≤1 in conditioned media, but ratios >1 for null α1(I)/WT α1(I) were observed in cell layers.
Contrast to the distribution of collagen observed in WT fibroblasts (Fig. 2B). As detergent-insoluble fractions did not completely dissolve in boiling Laemmli buffer, we relied on hydroxyproline analysis (see below) for quantification of collagen in detergent-insoluble cell extracts.

To characterize further the association of collagen I with separate cellular fractions, we performed hydroxyproline analysis of detergent-extracted WT and SPARC-null cells. As shown in Fig. 3A, the percent of hydroxyproline normalized to total protein (Hyp/total protein) associated with detergent-soluble layers was higher in the absence of SPARC after 4 h of collagen secretion. In contrast, the percent of Hyp/total protein in detergent-insoluble extracts was reduced in SPARC-null versus WT cells.

Deposition and accumulation of ECM occur over several days in cultured cells. We sought to determine whether initial increases in cell-associated collagen influenced deposition of collagen by SPARC-null cells over a 5-day time period. Hydroxyproline analysis carried out on cell layers from WT and SPARC-null fibroblasts cultured for 1, 2, and 5 days after ascorbate addition indicated that initial increases in cell-associated collagen did not result in increased collagen deposition. A significant decrease in collagen accumulation in detergent-insoluble cell layers in null versus WT cells was observed (Fig. 3B). The amounts of collagen were decreased in conditioned media from SPARC-null fibroblasts in comparison to WT levels at each day (data not shown).

We conclude from these results that in the absence of SPARC, procollagen I initially associated with cell surfaces to a greater extent than procollagen I produced concomitantly with SPARC. However, SPARC-null fibroblasts did not incorporate collagen as efficiently into an insoluble matrix.

Enhanced Procollagen Processing in the Absence of SPARC—

As procollagen processing is a critical component of collagen I ECM assembly, we asked whether SPARC influenced procollagen I processing in vitro. Fig. 4 is a representative immunoblot from conditioned media and detergent-soluble cell layers col...
SPARC Regulates Collagen Deposition

A  
Day:  
1  2  1  2  

|   | Media | Detergent-Soluble |
|---|-------|-------------------|
|+/+| pro pC | pro pC α1(l) |
|-/-| pro pC | pro pC α1(l) |

B  
Day:  
1  2  1  2  

|   | Detergent-Soluble |
|---|-------------------|
|+/+| Actin |
|-/-| Actin |

C  
Day 1  Day 2  Pro pC  Pro pC  WT Null  WT Null

D  
Day 1  Day 2  Pro pC α1(I)  Pro pC α1(I)  WT Null  WT Null

FIGURE 4. Increases in processed collagen α1(I) were detected in SPARC-null cells and conditioned media. A, immunoblot analysis using anti-collagen I antibodies showed elevated levels of processed collagen intermediate pC over procollagen I (pro; arrows) in media conditioned by SPARC-null versus WT cells. Detergent-soluble SPARC-null (-/-) cell layers exhibited increased amounts of fully processed α1(I) (α1(I); arrows) in comparison to WT (+/+ ) cells that contained higher levels of procollagen I (pro; arrows). Parallel cultures of null and WT cells from days 1 and 2 following addition of ascorbate are shown. B, actin levels in detergent-soluble fractions confirm equal cellular contribution from each genotype. C, quantification of band intensities from A exhibited a higher proportion of collagen I present as pC in SPARC-null media fractions (white bars) than in WT media (black bars). D, quantification of band intensities from A demonstrated that SPARC-null detergent-soluble fractions (white bars) contained higher amounts of processed collagen α1(I) at days 1 and 2 in comparison to WT fractions (black bars). Pro, procollagen α1(I); pC, pC-collagen α1(I); α1(I), mature collagen α1(I).

An enhancement of procollagen I processing is expected to result in the production of greater amounts of cleaved propeptides in conditioned media. Immunoprecipitation was carried out with antibodies against the N-propeptide of collagen I to determine whether increased amounts of cleaved N-propeptide were observed in media conditioned by SPARC-null fibroblasts. A representative experiment is shown in Fig. 5A. Although a slight decrease in amounts of collagen I was observed in the starting material of SPARC-null versus WT conditioned media, increased amounts of N-propeptide were consistently found associated with SPARC-null conditioned media. As shown in Fig. 5B, quantification of three separate immunoprecipitation experiments with different primary cell isolates confirmed a reproducible increase in processed N-propeptide in media conditioned by SPARC-null versus WT fibroblasts.

We performed pulse-chase experiments to determine whether the absence of SPARC influenced procollagen I processing and/or whether longer periods of time were required to detect differences in procollagen I conversion in SPARC-null fibroblasts. A representative pulse-chase experiment is shown in Fig. 6A. Whereas mature collagen I was detected in SPARC-null cell layers at 36 min of chase, mature collagen I in WT cells was not detectable until 60 min of chase. An immunoblot analysis with anti-collagen I antibodies is shown in Fig. 6A, lower panel, to demonstrate that approximately equal amounts of total collagen I were present in SPARC-null and WT cells. The immunoblot also demonstrated the increase in processed collagen I associated with SPARC-null cells. Fig. 6B shows quantification from three separate experiments demonstrating that less time following pulse labeling was required to detect processed collagen I in SPARC-null versus WT cells. We conclude that SPARC diminishes procollagen I binding to cell surfaces and functions as a regulatory component in the conversion of procollagen I to collagen I.
rSPARC Reverses Increases in Cell-associated Collagen I and in Collagen I Processing in SPARC-null Cells—We asked whether restoration of SPARC activity to SPARC-null fibroblasts reversed the differences in procollagen conversion in SPARC-null cells. Addition of recombinant SPARC (rSPARC) to SPARC-null fibroblasts led to an appreciable decrease in procollagen I associated with null cell layers (Fig. 7A, compare lane 3 (−rSPARC) to lane 4 (+rSPARC)). Coincident with a decrease in overall amounts of cell-associated collagen I, a significant inhibition of procollagen processing was evident upon addition of rSPARC.

Quantification from three separate experiments is shown in Fig. 7, B and C. We consistently recorded a decrease in collagen I in detergent-soluble SPARC-null cell layers treated with rSPARC, in comparison to that of control null cells treated with equal amounts of nonspecific protein (ovalbumin, Fig. 7B). In addition, the proportion of collagen present as processed collagen α1(I) in detergent-soluble fractions was significantly decreased by addition of rSPARC (Fig. 7C). The decrease in amounts of procollagen I converted into collagen I was accompanied by a decrease in the levels of collagen I associated with SPARC-null cell layers.

Fiber Formation on SPARC-null Versus WT Fibroblasts—Immunofluorescence studies to detect collagen fibers on the surface of WT and SPARC-null dermal fibroblasts were carried out to discern whether the differences seen in collagen I processing influenced collagen fiber formation in vitro. At 1 (Fig. 8, C and D) and 2 h (E and F) after addition of ascorbate, more collagen immunoreactivity was associated with SPARC-null cells (D and F) in comparison to WT cells (C and E). These results are consistent with the initial increases in cell-associated collagen as described in Fig. 2.

After addition of ascorbate for 1 day, collagen fibers were apparent on WT and SPARC-null fibroblasts (Fig. 9, A and B). However, 3 days after treatment with ascorbate, collagen fibers on the surface of SPARC-null fibroblasts (Fig. 9D) were diminished, whereas those associated with WT cells persisted (Fig. 9C). Collagen fibers on WT fibroblasts at day 3 appeared to have increased staining over those present at day 1. We conclude that collagen fibers on WT cells continued to develop with time as expected, whereas collagen fibers formed in the absence of SPARC did not persist.

DISCUSSION

In this study, we have found that collagen fibrils generated in the
absence of SPARC in mice at 1 month of age appeared to be stalled at a stage of fibril aggregation and accumulated as proposed intermediates in fibril fusion (22). Previous studies demonstrated a substantial decrease in amounts of collagen in SPARC-null dermis (12). This observation suggested to us that SPARC was essential for collagen fibril growth and deposition in skin. To elucidate the cellular basis of the SPARC-null collagen phenotype, we investigated collagen I production in SPARC-null dermal fibroblasts. We have shown, by a number of different techniques, that SPARC-null dermal fibroblasts exhibited an increased association of collagen I with cell layers and enhanced processing of procollagen α1(Ⅰ) to collagen α1(Ⅰ). The processed collagen I was not efficiently incorporated into SPARC-null detergent-insoluble cell layers, and collagen fibers that formed on SPARC-null cells did not persist to the same extent as those on WT cells. These results indicate that regulation of collagen association with cell surfaces by SPARC is crit-
We do not observe significant differences in procollagen processing in conditioned media generated from metabolic labeling experiments, a finding that we attribute to the timing of secretion of the labeled procollagens. Upon addition of ascorbate, accumulated procollagen within intracellular organelles will be secreted prior to newly synthesized, labeled procollagen. We postulate that cell binding sites for procollagen will bind unlabeled procollagen released prior to labeled procollagen. Hence, we predict that differences in procollagen conversion are more readily detected by immunoblot analysis, which represents the entire pool of secreted procollagen, rather than by fluorography of labeled procollagen, which detects only a portion of the secreted pool.

The C-propeptide of collagen I directs in intracellular trimer formation in the endoplasmic reticulum (9). Removal of the C-propeptide by bone morphogenic protein-1 (or related tolloid family members) is thought to be a critical step in collagen I deposition, as its removal decreases the solubility of procollagen I to a level similar to that of processed collagen I (21, 29). Although association of pC collagen I with cell layers is not observed in some cell types, Lamande and Bateman (30) reported that mutations that nullified glycosylation of the C-propeptide in primary murine fibroblasts resulted in significant increases in pC collagen I in the media of cells expressing the mutant form of collagen I versus WT collagen I with fully glycosylated C-propeptide. Hence, glycosylation of the C-propeptide of collagen I increased interactions with cell surfaces. We found higher levels of pC relative to pN collagen I in detergent-soluble cell layers produced by primary dermal fibroblasts. We predict that the majority of pN collagen I resides in the detergent-insoluble fraction.

Whereas pC collagen I is not thought to be included in collagen fibrils, pN collagen I incorporation in growing fibrils has been observed in developing skin and tendon by EM immunolocalization (10, 31). Incorporation of pN collagen I might limit collagen fibril diameter, such that regulation of N-propeptide removal by ADAMTS-2 (or related family members ADAMTS-3 and -13) might control further fibril aggregation and growth (32, 33). In the event that N-propeptide functions as a facilitator of collagen fibril accretion, removal of N-propeptide prior to fibril incorporation would be expected to result in smaller collagen fibrils (11). Interestingly, SPARC-null dermis exhibited small collagen fibril diameters at both 1 month and in adults. Hence premature cleavage of N-propeptide resulting in inefficient collagen fibril fusion would be consistent with a function of SPARC in modulation of procollagen I processing at the cell surface. We consider the most likely explanation for increased processing in SPARC-null cells to be an increase in receptor engagement of procollagen I that retains procollagen within extracellular matrix deposition, in decreased fibronectin assembly and a reduction in fibronectin-induced stress fiber formation. As Barker et al. (34) did not
SPARC Regulates Collagen Deposition

induce collagen production by addition of ascorbate, the effects of SPARC on collagen production in these cells were not assessed. We have also observed a slight reduction in fibronectin fibrils on the surface of SPARC-null dermal fibroblasts in the absence of ascorbate (data not shown). However, differences in fibronectin fibrils were not detectable between SPARC-null and WT fibroblasts in the presence of ascorbate (data not shown). As collagen and fibronectin fibril assembly have been shown to be coincident in vitro, collagen I production by SPARC-null fibroblasts at early times after ascorbate addition might mask alterations in fibronectin assembly (35, 36). Possibly, a diminished capacity to assemble fibronectin fibrils over time could contribute to the decreased stability of collagen fibers on SPARC-null cell surfaces observed at times >3 days (Fig. 9).

SPARC-null mice develop cataracts that appear to be based, at least in part, on aberrant ECM assembly by lens epithelial cells (37). SPARC might function to reduce lens epithelial cell binding to collagen IV, another SPARC-binding collagen. Immunohistochemistry of lens basement membrane from SPARC-null mice demonstrated disorganized collagen IV associated with β1 integrin-positive cellular protrusions into the matrix (38). Failure of lens epithelial cells to release collagen IV might mask alterations in fibronectin assembly (35, 36). Possibly, a diminished capacity to assemble fibronectin fibrils over time could contribute to the decreased stability of collagen fibers on SPARC-null lens. Recently, Canty and Kadler (39) reported that some procollagen I conversion to collagen I takes place in intracellular compartments within embryonic tendon. We cannot rule out that some processing of procollagen I in our dermal fibroblast cultures might take place within cells. As SPARC shares characteristics with heat shock proteins, the possibility that SPARC might function in the capacity of a chaperone is conceivable. In this case, procollagen I produced in the absence of SPARC might exhibit properties distinct from those of WT procollagen I that influence cell surface association. Notably, in assays of protein folding, addition of SPARC resulted in disulfide rearrangement of a model substrate that was consistent with chaperone-like activity (40). However, SPARC has a secretory signal peptide, contains no recognizable endoplasmic reticulum or Golgi apparatus retention sequences, and is readily detected in conditioned media from a number of different cell types (3). In the event that SPARC does perform a chaperone-like function for procollagen I (and/or other ECM proteins), SPARC is likely to be secreted with procollagen I.

A growing number of extracellular proteins are known to participate in collagen fibril formation. Decorin, lumican, fibromodulin, biglycan, and mimecan regulate collagen fibril diameters, presumably through binding of these proteoglycans to collagen fibrils in the extracellular space, where they serve either to inhibit or promote fibril aggregation (41–44). In addition, disruption of the genes encoding the matricellular proteins tenascin X, thrombospondin 2, osteopontin, dermatopontin, and hevin have revealed a function for these proteins in collagen fibril formation (45–49). In the majority of cases, decreased expression of the aforementioned collagen fibril-effector proteins resulted in increased collagen fibril diameters with irregular profiles, in contrast to SPARC-null collagen fibrils that are small and regular in diameter. SPARC is the first reported collagen fibril effector protein to influence procollagen I processing events.

SPARC expression is often associated with fibrosis. Recently, Zhou et al. (50) reported that reduction of SPARC expression in scleroderma fibroblasts by RNA interference was sufficient to reduce collagen expression by these cells that otherwise exhibit increased expression of SPARC and collagen I, in comparison to normal fibroblasts. SPARC therefore is an attractive target for the development of strategies to counteract fibrotic deposition of collagen. We postulate that disruptions in procollagen I processing influenced by SPARC result in decreased stability of collagen I fibrils. SPARC is therefore identified as an essential modulator of collagen I-cell interaction that regulates the deposition of stable collagen I fibrils in the extracellular space.

Acknowledgments—We express appreciation to Juliet Carbon, Carrie Murri, and Gail Workman for expert technical assistance.
26. Gardner, H., Broberg, A., Pozzi, A., Laato, M., and Heino, J. (1999) J. Cell Sci. 112, 263–272
27. Ravanti, L., Heino, J., Lopez-Otin, C., and Kahari, V. M. (1999) J. Biol. Chem. 274, 2446–2455
28. Lee, W., Sodek, J., and McCulloch, C. A. (1996) J. Cell Physiol. 168, 695–704
29. Kessler, E., Takahara, K., Biniaminov, L., Brusel, M., and Greenspan, D. S. (1996) Science 271, 360–362
30. Lamande, S. R., and Bateman, J. F. (1995) J. Biol. Chem. 270, 17858–17865
31. Fleischmajer, R., Perlish, J. S., Timpl, R., and Olsen, B. R. (1988) J. Histochem. Cytochem. 36, 1425–1432
32. Colige, A., Sieron, A. L., Li, S. W., Schwarze, U., Wertelecki, W., Wilcox, W., Krakow, D., Cohn, D. H., Reardon, W., Byers, P. H., Lapiere, C. M., Prockop, D. J., and Nusgens, B. V. (1999) Am. J. Hum. Genet. 65, 308–317
33. Colige, A., Vandenbergh, L., Thiry, M., Lambert, C. A., Van Beeumen, J., Li, S. W., Prockop, D. J., Lapiere, C. M., and Nusgens, B. V. (2002) J. Biol. Chem. 277, 5756–5766
34. Barker, T. H., Baneyx, G., Cardo-Vila, M., Workman, G. A., Weaver, M., Menon, P. M., Dedhar, S., Rempel, S. A., Arap, W., Pasqualini, R., Vogel, V., and Sage, E. H. (2005) J. Biol. Chem. 280, 36483–36493
35. Li, S., Van Den Diepstraten, C., D’Souza, S. J., Chan, B. M., and Pickering, J. G. (2003) Am. J. Pathol. 163, 1045–1056
36. Velling, T., Risteli, J., Wennerberg, K., Mosher, D. F., and Johansson, S. (2002) J. Biol. Chem. 277, 37377–37381
37. Yan, Q., Clark, J. I., Wight, T. N., and Sage, E. H. (2002) J. Cell Sci. 115, 2747–2756
38. Yan, Q., Blake, D., Clark, J. I., and Sage, E. H. (2003) J. Histochem. Cytochem. 51, 503–511
39. Canty, E. G., and Kadler, K. E. (2005) J. Cell Sci. 118, 1341–1353
40. Emerson, R. O., Sage, E. H., Ghosh, J. G., and Clark, J. I. (2006) J. Cell Biochem. 98, 701–705
41. Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V. (1997) J. Cell Biol. 136, 729–743
42. Ezura, Y., Chakravarti, S., Oldberg, A., Chervoneva, I., and Birk, D. E. (2000) J. Cell Biol. 151, 779–788
43. Corsi, A., Xu, T., Chen, X. D., Boyde, A., Liang, J., Mankani, M., Sommer, B., Iozzo, R. V., Eichstetter, I., Robey, P. G., Bianco, P., and Young, M. F. (2002) J. Bone Miner. Res. 17, 1180–1189
44. Tasheva, E. S., Koester, A., Paulsen, A. Q., Garrett, A. S., Boyle, D. L., Davidson, H. J., Song, M., Fox, N., and Conrad, G. W. (2002) Mol. Vis. 8, 407–415
45. Mao, J. R., Taylor, G., Dean, W. B., Wagner, D. R., Afzal, V., Lotz, J. C., Rubin, E. M., and Bristow, J. (2002) Nat. Genet. 30, 421–425
46. Kyriakides, T. R., Zhu, Y. H., Smith, L. T., Bain, S. D., Yang, Z., Lin, M. T., Danielson, K. G., Iozzo, R. V., LaMarca, M., McKinney, C. E., Ginn, E. L., and Bornstein, P. (1998) J. Cell Biol. 140, 419–430
47. Liaw, L., Birk, D. E., Ballas, C. B., Whitsitt, J. S., Davidson, J. M., and Hogan, B. L. (1998) J. Clin. Invest. 101, 1468–1478
48. Takeda, U., Utani, A., Wu, J., Adachi, E., Koseki, H., Taniguchi, M., Matsuzato, T., Ohashi, T., Sato, M., and Shinkai, H. (2002) J. Investig. Dermatol. 119, 678–683
49. Sullivan, M. M., Barker, T. H., Funk, S. E., Karchin, A., Seo, N. S., Hook, M., Sanders, J., Starcher, B., Wight, T. N., Puolakkainen, P., and Sage, E. H. (2006) J. Biol. Chem. 281, 27621–27632
50. Zhou, X., Tan, F. K., Guo, X., and Arnett, F. C. (2006) Arthritis. Rheum. 54, 2626–2631