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Cell Biology

The Ca^{2+}-binding glycoprotein SPARC modulates cell cycle progression in bovine aortic endothelial cells

(extracellular matrix)

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ABSTRACT **SPARC (secreted protein, acidic and rich in cysteine) is an extracellular, Ca^{2+}-binding protein associated with cellular populations undergoing migration, proliferation, and/or differentiation. Active preparations of SPARC bind to specific components of the extracellular matrix and cause mesenchymal cells to assume a rounded phenotype. In this study we show that SPARC modulates the progression of bovine aortic endothelial cells through the cell cycle. At a concentration of 20 μg/ml, SPARC inhibited the incorporation of [^{3}H]thymidine into newly synthesized DNA by ≈ 70%, as compared to control cultures within 24 hr after the release from G_0 phase. The effect was dose-dependent and reached >90% inhibition at 30 μg of SPARC per ml after 24 hr. A 20-residue synthetic peptide (termed 2.1) from a non-Ca^{2+}-binding, disulfide-rich domain of SPARC also exhibited a dose-dependent inhibition of [^{3}H]thymidine uptake in endothelial cells within 24 hr after release from G_0 phase. An inhibition of 50% was seen with peptide 2.1 at a 0.4 mM concentration. Peptides from other regions of the SPARC protein did not produce this effect. Maximum inhibition of [^{3}H]thymidine uptake by SPARC and peptide 2.1 occurred during the early-to-middle G_1 phase of the endothelial-cell cycle. From 0–12 hr after release from G_0 phase, cells exhibited delayed entry into S phase, which normally occurred at 24 ± 2 hr. These results were further corroborated by flow cytometry. In the presence of SPARC at 20 μg/ml, 72% fewer cells were in S phase after a 24-hr period; a similar, but less marked, reduction was seen with peptide 2.1. Peptide 2.1 did not cause cell rounding, whereas peptide 1.1, a highly efficient inhibitor of endothelial-cell spreading, exhibited essentially no activity with respect to cell-cycle progression. It therefore appears that the transient, inhibitory effect of SPARC on the entry of endothelial cells into S phase does not depend on the overt changes in cell shape mediated through cytoskeletal rearrangement.**

Regulatory signals affecting cellular growth and DNA synthesis operate on both positive and negative levels (1). Examples of the latter are the "tumor-suppressor" or "growth-suppressor" gene products, and the former includes polypeptide mitogens specific for different types of cells. In addition to the mitogenic growth factors, other proteins have been shown to play a role in stimulating cellular proliferation, such as the extracellular proteases thrombin (for review, see ref. 2) and urokinase (3), the extracellular matrix (ECM) component fibronectin (4), and intracellular calmodulin (5). Negative control of mesenchymal-cell proliferation has also been described: a cell-surface fraction from confluent endothelial cells has been shown to inhibit endothelial-cell growth (6), and heparin prevents the progression of vascular smooth muscle cells through the G_1 phase of the cell cycle (7, 8). Mechanisms accounting for inhibitory effects on cell growth are largely unknown, although two pathways involving actin reorganization (9) and protein kinase C (7) have been experimentally confirmed.

Studies in our laboratory have focused on the characterization of ECM proteins and their modulation of cellular behavior and phenotype. We initially identified a glycoprotein secreted by endothelial cells that were cultured at subconfluent density and exhibited high levels of proliferation and migration (10, 11). This "culture shock" protein was subsequently termed SPARC (secreted protein, acidic and rich in cysteine) by Mason *et al.* (12), who isolated the corresponding cDNA from parietal endoderm, a differentiated cellular population that is highly migratory but nonproliferating. SPARC is also identical to the proteins osteonectin (13, 14) and BM-40 (15) and is highly conserved among several mammalian species.

In the developing mouse, SPARC mRNA and protein have been localized to areas of active tissue morphogenesis (16, 17). Expression of SPARC in the adult mouse appears confined to cellular populations that exhibit high rates of turnover (e.g., epithelium of the intestinal crypts), secretion (steroidogenic cells), and remodeling (e.g., lactating mammary gland) (16, 17). Recent experiments have shown that SPARC effectively inhibits cell spreading (particularly that of endothelial cells and fibroblasts) and binds to specific components of the connective tissue ECM in a Ca^{2+}-dependent manner (18). Although the mechanism promoting changes in cell shape is not presently understood, we have proposed that interaction of SPARC with the cell surface results in the transcriptional activation or repression of specific gene products that, in turn, regulate association of the cell with its ECM (T. F. Lane, L. Iruela-Arispe, and H.S., unpublished data; P. Hasselaar, D. Loskutoff, M. Sawdey, and H.S., unpublished data).

Experiments designed to test directly the effect of SPARC on cell-cycle progression have not been performed. In the present study we addressed this question by analysis of the $G_0 \rightarrow$ S-phase transition in bovine aortic endothelial cells (BAEC) cultured in the presence of purified preparations of SPARC and synthetic SPARC peptides. Our results show that SPARC and a 20-residue peptide from a disulfide-bonded domain of SPARC both repress DNA synthesis in BAEC, as measured by [^{3}H]thymidine incorporation and delay the entry of cells into S phase. An inhibitory effect of secreted proteins such as SPARC on cell-cycle progression might facilitate the temporary withdrawal from the cell cycle that often occurs after cellular responses to injury or developmental signals.

MATERIALS AND METHODS

Protein and Peptide Reagents. SPARC was purified from murine PYS-2 cell culture medium as described (18). Biolog-

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Abbreviations: BAEC, bovine aortic endothelial cell(s); DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; FBS, fetal bovine serum; PBS, phosphate-buffered saline.

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ical activity of individual preparations was determined quantitatively by a cell-rounding assay (18). Protein was solubilized in phosphate-buffered saline (PBS) at ≈300 μg/ml and used on cells at a concentration of 20 μg/ml of culture medium. Concentration was determined by measurement of absorbance at 280 nm ($\in {}^{1mM,\ 280}_{1cm} = 0.838$) recalculated from the amino acid composition of murine SPARC according to Gill and von Hippel (19, 20). Synthetic peptides representing discrete domains of SPARC (20) were solubilized in PBS and used at a concentration of 0.9 mg/ml of culture medium (0.4 mM). Location and sequence of these peptides are shown diagrammatically in Fig. 1.

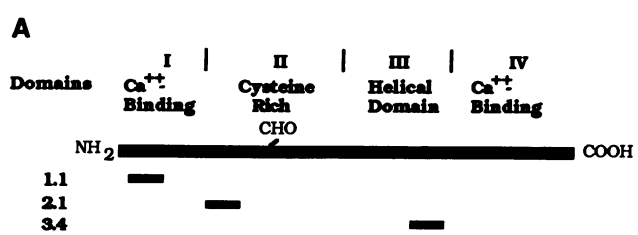
Endothelial-Cell Culture. Adult BAEC were isolated as described (18). Cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma) buffered with 27 mM sodium bicarbonate and 25 mM Hepes and supplemented with 10% fetal bovine serum (FBS) (Flow Laboratories). For experiments on cell-cycle kinetics, contact-inhibited BAEC were synchronized by incubation in serum-free DMEM for 72 hr. Of these cells, 95–97% were shown by flow cytometry to be arrested in $G_0(G_1)$. Quiescent cultures were dissociated by brief digestion with trypsin, washed with 10% FBS/DMEM, and replated in 2.5% FBS/DMEM at ≈10,000 cells per cm^2.

Flow Cytometric Analysis. Synchronized cells were plated in 24-well plates (Corning) in the presence of 0.4 mM SPARC peptides, SPARC at 20 μg/ml, or PBS. At various time points, duplicate wells were washed with 0.2% EDTA in buffered saline, exposed to trypsin, and washed with 10% FBS/DMEM; the nuclei were then isolated and stained with 4,6-diamidino-2-phenylindole (Accurate Chemicals, Westbury, NY) by a modification of the method of Reid *et al.* (22). The cells were resuspended in a buffer containing 0.85% NaCl, 0.1% Tris·HCl (pH 7.4), 0.1% Nonidet P40, 10% (vol/vol) dimethyl sulfoxide, 2 mM Ca^{2+}, 2 mM Mg^{2+}, and 4,6-diamidino-2-phenylindole at 10 μg/ml. Samples were frozen and stored at −70°C until all wells had been processed. Flow cytometry was done by P. Rabinovitch (University of Washington) on an ICP-22 (Ortho Diagnostics), and data were analyzed on an IBM AT computer with MULTICYCLE (Phoenix Flow Systems, San Diego) as described (23). The use of RNase to extinguish non-DNA-related fluorescence was found not necessary for subcultured BAEC (24).

Incorporation of [^{3}H]Thymidine. Quiescent cultures were treated with trypsin and plated as described above in the presence of 5 μCi/ml [*methyl*-^{3}H]thymidine (NEN; 1 Ci = 37 GBq) and 0.4 mM SPARC peptides, SPARC at 20 μg/ml, or PBS. At 2-hr intervals, starting at 10 hr, duplicate wells were washed twice with cold PBS, fixed with ice-cold 10% trichloroacetic acid for 25 min, washed with cold ethanol:ether (2:1), and allowed to air-dry. Trichloroacetic acid-insoluble material was hydrolyzed with 200 μl of 0.4 M NaOH at 60°C for 25 min, neutralized with an equal volume of glacial acetic acid, and dissolved in 4 ml Ecolume (ICN) for scintillation counting.

To ascertain that there was no significant cell loss due to rounding, duplicate wells were washed twice with 0.2% EDTA, released by brief exposure to trypsin, washed in 10% FBS/DMEM, and counted in a hemocytometer before fixation in trichloroacetic acid. Fixed cell suspensions were solubilized for 25 min in 10% trichloroacetic acid at 90°C according to a modification of a standard technique (25). The effects of added SPARC and peptides on cell spreading were assessed quantitatively as described by Lane and Sage (20). By this method, a "rounding index" is calculated for each culture according to a scoring system for cell morphology as determined by light microscopy.

Post-Release Incorporation of [^{3}H]Thymidine. To determine the period in the cell cycle during which cells are sensitive to SPARC, quiescent cells were plated as described above in 24-well plates in medium containing [^{3}H]thymidine. At various times SPARC at 20 μg/ml, 0.4 mM peptides, or PBS was added to duplicate wells. Thirty hours from time of plating, all wells were washed twice in cold PBS, fixed in ice-cold 10% trichloroacetic acid, and solubilized as described above. To count attached cells, before solubilization two fields from the center of each fixed well were photographed on a Zeiss inverted microscope with phase-contract optics.



B

Peptide	Sequence	Location
1.1	QTEV AEEIVE EETVVEETGV	5- 23
2.1	CQNHHCKHG KVCELDESNTP	54- 73
3.4	NEKRLEAGDI PVELLARDFE	184- 203

FIG. 1. Diagrammatical representation of the SPARC protein with the location and sequence of synthetic peptides. (*A*) Peptides are named with reference to the predicted domain structure of murine SPARC (21). ■, 20 amino acids. (*B*) Sequences (in single-letter code) of synthetic peptides reported in this study. Sequences were derived from the amino acid sequence predicted from murine SPARC cDNA (12). Cysteine residues are underlined.

RESULTS

Early studies in which preparations of SPARC were tested for mitogenic activity on cultures of BAEC showed no significant increase in cell number when growth in 8–16% FBS was monitored over 4 days (11). In some experiments, a reduction in cell number was seen at earlier time points; these negative results were difficult to interpret because high levels of SPARC *in vitro* were generally associated with proliferating cells (11). In the present experiments we have examined the effect of highly purified preparations of SPARC and of synthetic SPARC peptides on the cell cycle of cultured BAEC. The following specific questions were addressed: (*i*) Is SPARC stimulatory, inhibitory, or noneffective for growth of BAEC, as measured by the incorporation of [^{3}H]thymidine? (*ii*) Can a specific peptide sequence be identified that mimics the observed effect of the intact SPARC protein? (*iii*) Is there a correlation between the ability of SPARC to inhibit cell spreading and to perturb the cell cycle? (*iv*) At what point(s) in the cell cycle are BAEC sensitive to SPARC and SPARC peptides, and (*v*) What is the distribution of BAEC in the cell cycle after exposure to SPARC and SPARC peptides as measured by flow cytometry?

For the experiments described in this study we used low-passage, homogeneous cultures of BAEC that were growth-arrested at confluence by culture for several days in the absence of FBS. This treatment was not deleterious to the cells as judged by morphology, response to serum, and plating efficiency; by flow cytometry, the number of cells arrested in $G_0(G_1)$ phase was >95% (data not shown). In previous experiments we showed that native SPARC, at concentrations between 2 and 50 μg/ml, inhibited the spreading of newly plated BAEC and caused rounding of confluent monolayers (18). Fig. 2 shows the morphology of representative cultures of BAEC at 24 hr after exposure to SPARC or to SPARC peptides 2.1 and 3.4. Although other 20-residue SPARC peptides, at concentrations between 0.1 and 0.8 mM, caused a rounded morphology when added to BAEC (20), peptides 2.1 and 3.4 clearly did not (Fig. 2; see Fig. 1 for sequences and locations of these peptides in the SPARC

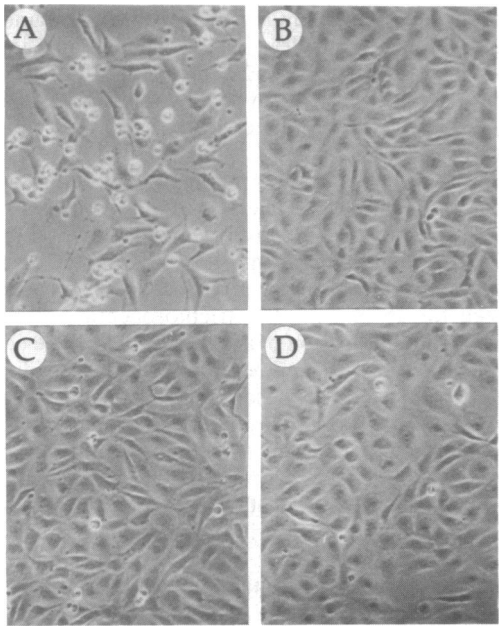


FIG. 2. Morphology of BAEC treated with SPARC and SPARC peptides. Cell monolayers are shown 24 hr after being plated in DMEM/2.5% FBS containing SPARC at 20 μg/ml (*A*), 0.4 mM peptide 2.1 (*B*), 0.4 mM peptide 3.4 (*C*), or PBS (*D*). (×100.)

protein). Rounding indices for the cultures shown in Fig. 2 were: *A*, 2.14; *B*, 1.07; *C*, 1.16; *D*, 1.17, where an index of 1 indicates a culture with only spread cells, and an index of 3 indicates a culture consisting of entirely rounded cells. We have used a concentration of 20 μg of SPARC per ml for the experiments described herein; however, we note that similar effects on the $G_0 \rightarrow$ S transition of the BAEC cycle were seen with lower concentrations of SPARC that did not promote overt cell rounding.

Fig. 3*A* shows a standard, [^{3}H]thymidine incorporation assay in BAEC after release from a quiescent, growth-arrested state. With no additions other than PBS, cells exhibited maximum incorporation of isotope at 26–28 hr after replating at subconfluent density in the presence of 2.5% FBS and [^{3}H]thymidine. In contrast, cells replated under the same conditions, but with SPARC, exhibited a time lag of 8–10 hr in apparent DNA synthesis, in comparison to controls (Fig. 3*A*). After ≈36 hr, levels of isotope incorporation in SPARC-treated cells approached but did not reach control levels. These altered kinetics could reflect differences in the rate at which BAEC traverse S phase in the presence of SPARC, or that fewer cells were actually making DNA. This point is addressed quantitatively in subsequent experiments.

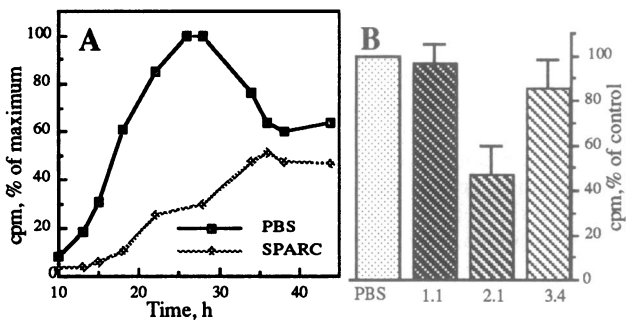


FIG. 3. Effects of SPARC on DNA synthesis in BAEC. (*A*) Kinetics of DNA synthesis in BAEC. To determine when cells enter S phase, contact-inhibited, serum-deprived cells were released from growth-arrest by replating at subconfluent density in growth medium containing 2.5% FBS, [^{3}H]thymidine, and SPARC at 20 μg/ml or PBS in an equivalent volume. At the times indicated, duplicate cultures were washed with cold PBS, fixed in 10% trichloroacetic acid and solubilized in 0.4 M NaOH. Points represent the average of two independent experiments. No cpm above background were detected before 10 hr. (*B*) SPARC peptides affect cell growth. Growth-arrested BAEC were replated as described in *A* in the presence of 0.4 mM SPARC peptides and [^{3}H]thymidine. After 24 hr duplicate monolayers were washed and fixed in 10% trichloroacetic acid. Bars (± SD) represent incorporated radioactivity as percentage of the value seen with the PBS control and were normalized for cell number. Values shown are the means of four independent experiments.

In Fig. 3*B* are shown the levels of [^{3}H]thymidine incorporated into BAEC DNA 24 hr after addition of SPARC peptides 1.1, 2.1, and 3.4. To adjust for differences in initial plating density or cell losses during the experiments, incorporated cpm were normalized with respect to cell number. In the presence of 0.4 mM peptide 1.1, the level of [^{3}H]thymidine incorporation was nearly identical to that of control cultures that received only PBS; 0.4 mM peptide 3.4 caused a slight reduction (≈15%). In contrast, a mean inhibition of 50% was seen when cells were incubated 24 hr in the presence of 0.4 mM peptide 2.1 (Fig. 3*B*).

Both SPARC and peptide 2.1 reduced the incorporation of [^{3}H]thymidine into BAEC DNA in a dose-dependent manner. As shown in Fig. 4*A*, an inhibition of 90% was achieved at a concentration of 30 μg of SPARC per ml, whereas an inhibition of 50% was seen with 8 μg of SPARC per ml. The latter concentration of SPARC is equivalent to 0.24 μM and is within the range reported effective for the inhibition of cell spreading (18). Likewise, peptide 2.1 exhibited an inhibitory effect on [^{3}H]thymidine incorporation of 50% at a concentration of 0.4 mM (Fig. 4*B*). Although this is the first biological effect seen with peptide 2.1, other SPARC peptides have demonstrated optimal activities between 0.2 and 0.8 mM (e.g., peptides 1.1 and 4.2 inhibited cell spreading) (20). Because peptide 2.1 had no obvious effect on cell shape (Fig. 2*B*), the anti-spreading activity of SPARC appears to be separate from the ability of the protein to depress DNA synthesis in BAEC undergoing a synchronized transition from G_0 to S phase.

The experiments summarized in Fig. 5 were designed to identify an interval of time or specific time points at which BAEC were responsive to SPARC and SPARC peptides. Growth-arrested cells were plated at subconfluent density in growth medium containing [^{3}H]thymidine. Labeling was continued for 30 hr, during which time, at prescribed intervals, SPARC or SPARC peptides were added to the cells. After release from quiescence, the addition of PBS or peptide 3.4 resulted in essentially no change in incorporation of [^{3}H]thymidine over the 30-hr period (Fig. 5). In contrast, maximum

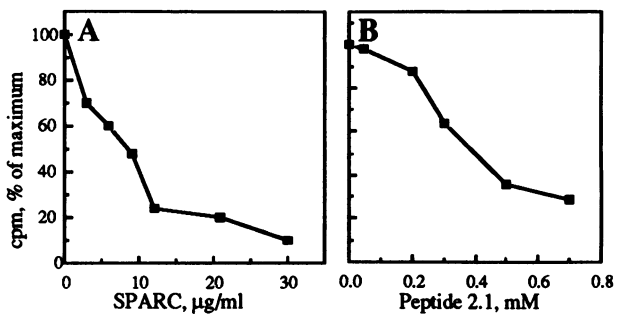


FIG. 4. Inhibition of DNA synthesis is dose-dependent. Growth-arrested BAEC were released by plating at subconfluent density in growth medium with increased amounts of SPARC (*A*) or peptide 2.1 (*B*) and [^{3}H]thymidine at 5 μCi/ml. After 24 hr incubation, cells were counted and fixed as described in Fig. 3 (*B*). Points represent the average of duplicate wells.

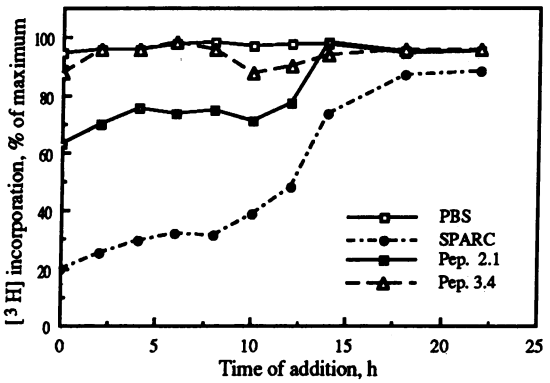


FIG. 5. BAEC are sensitive to SPARC before the onset of S phase. Quiescent BAEC were plated at subconfluent density in growth medium containing [^{3}H]thymidine at 5 μCi/ml. At the indicated times after release from growth-arrest, SPARC at 20 μg/ml, 0.4 mM peptides, or PBS was added to duplicate cultures. Incorporated isotope was measured by liquid-scintillation counting of trichloroacetic acid-insoluble material in the cell layer at 30 hr after plating. Values are plotted as percent of the maximum level of incorporation observed in the wells to which PBS was added at 8 hr. Curves shown are from one experiment and are representative of data from three independent experiments.

inhibition of incorporation occurred when SPARC or peptide 2.1 was added at plating, and an inhibition of 70% occurred within the first 8 hr of the G_1 phase of the cell cycle. After 12 hr, SPARC was considerably less effective in reducing DNA synthesis in these cultures. Similar kinetics were observed with peptide 2.1, although, as shown also in Fig. 3, the peptide inhibited the incorporation of isotope to a lesser extent.

We used flow cytometry to assess the position of BAEC in the cell cycle after exposure to SPARC and SPARC peptides. Cells released from G_0 phase and plated in growth medium containing the reagents were processed for flow cytometry from 0–50 hr. Time points representing 2-hr intervals, as well as overlapping time points, were covered within the four experiments that were performed. The data shown in Fig. 6*A* represent the number of cells in S phase plotted as percent of the total population, from 10 to 45 hr. At 22–24 hr after release from G_0 phase, cultures treated with both PBS and peptide 3.4 reached a maximum level with respect to the number of cells in S phase. A delay of 2–4 hr accompanied by a 20% decrease in S-phase cells, was seen in cultures treated with peptide 2.1 (Fig. 6*A*). In the presence of 20 μg of SPARC per ml, there was a 72% decrease at 22 hr, compared to controls. These results were reproduced in four experiments. Some variation in the number of cells undergoing DNA synthesis was observed among experiments due to differences in growth rates of clonal isolates. Studies from other laboratories have indicated a considerable range of variability in cell-cycle parameters of BAEC as a function of propagation *in vitro* (24). For this reason it was important to perform internal controls (e.g., addition of null peptide or buffer only) within the same strain and subculture of BAEC. As illustrated in Fig. 6*B*, the effect of SPARC was dose-dependent. After 24 hr, 20 μg of SPARC per ml reduced the number of S-phase cells by $\approx$70%, and further reductions were apparent at higher concentrations.

DISCUSSION

The inhibitory effect of SPARC on the progression of the BAEC cycle that we have shown in this study allows a further refinement of the purported role of this secreted, Ca^{2+}-sensitive glycoprotein in cellular proliferation. A significant proportion of published studies on SPARC (osteonectin, BM-40, 43-kDa protein) have noted an apparent association of this protein with tissues or cells undergoing morphogen-

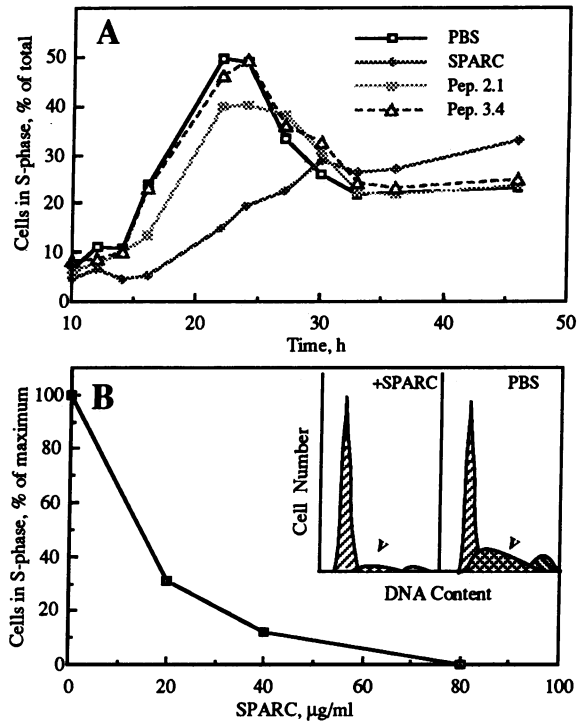


FIG. 6. Effects of SPARC and SPARC peptides on DNA synthesis: flow cytometric analysis. (*A*) SPARC delays entry into S phase. Growth-arrested BAEC were plated at subconfluent density in growth medium containing SPARC at 20 μg/ml, 0.4 mM SPARC peptides, or PBS. At the times indicated, duplicate cultures were processed for flow cytometry. Cells were photographed at 24 hr, as shown in Fig. 2. Each point represents the average of two samples. (*B*) The inhibition is dose-dependent. Growth-arrested cells were plated as described in *A* with various concentrations of SPARC and processed for flow cytometry after 24 hr. (*Inset*) Flow cytometry patterns of SPARC-treated (*Left*) and control cultures (*Right*) at 26 hr. Arrowheads indicate locations of S-phase peaks.

esis, repair, remodeling, culture shock, or metastatic invasion (11, 12, 16–18, 26, 27). Because high levels of SPARC protein and mRNA were often seen in nonproliferating, but actively secreting, cells (e.g., Leydig and Sertoli cells) and migrating cells (e.g., trophoblastic giant and parietal endoderm cells) (16, 28, 29), it appeared that this extracellular protein might direct a metabolic pathway ancillary to the cell cycle. Although no study has claimed SPARC to be stimulatory to cellular growth, this communication demonstrates that SPARC might inhibit proliferation. We further speculate that SPARC is activated after cells have initially proliferated and might function to withdraw cells temporarily from the cell cycle in preparation for other events, such as migration.

The identification of SPARC as an anti-spreading factor for certain cells, coupled with its ability to effect marked changes in cell shape, suggested to us that the apparent proliferation in the presence of SPARC was probably a consequence of the changes in cell shape (18). However, potentially common events mediated by SPARC between, for example, mitosis and cellular migration, both of which require detachment from an ECM, have not been identified experimentally. Whether cell rounding facilitates progression through the cell cycle or, alternatively, is permissive for cell migration due to an inhibition of S phase, had therefore not been determined for SPARC.

A study by Ingber (4) demonstrated conclusively that fibronectin, an abundant ECM component and a product of capillary endothelial cells, stimulated DNA synthesis when presented to these cells on a substrate but not in solution; cell spreading was, therefore, directly correlated with progres-

sion from G_0 to S phase. The assembly of stress fibers and focal contacts, a consequence of cell spreading, may also facilitate entry into S phase in the presence of a growth-promoting stimulus. It has, in fact, been shown that disruption of actin filaments and promotion of cell rounding by dihydrocytochalasin inhibited DNA synthesis in cultured fibroblasts (9). The proposed function of SPARC as an anti-spreading factor that retards cell-cycle progression would be compatible with these studies.

The ability of a cell to switch between the G_0 and G_1 phases of the cell cycle is a fundamental property that allows normal, postembryonic cells to proliferate at optimal rates (30). It is felt that extracellular factors are responsible for determining the rate at which a quiescent cell enters G_1 and, conversely, whether cells in G_1 will progress through the cell cycle or revert to G_0. In contrast, intracellular regulatory factors, possibly including proteins such as calmodulin (5), predominate after entry of cells into S phase (30). Although few studies address matrix components as extracellular factors that regulate progression of cells between G_0 and S phases, density-arrested smooth muscle cells were shown to upregulate mRNA levels specifically for type III collagen (31). This observation is potentially interesting to us because SPARC binds to type III collagen (18). If the increased levels of this collagen produced by growth-arrested cells sequestered more SPARC than the ECM assembled by proliferating cells, SPARC could function to maintain a block in the progression from G_0 to S phase.

Specific gene products have been identified that inhibit progression of the cell cycle. For example, expression of growth arrest-specific (*gas*) mRNAs is suppressed when mammalian cells are stimulated to transit from G_0 to G_1 phase (32). Exogenous Myo D1, a nuclear protein that regulates muscle-specific gene expression, inhibited the transition of nonmyogenic cells from G_0 to S phase (33). Moreover, inhibition of S phase, which is necessary for differentiation in this system, occurred via a domain separate from that shown to regulate myogenesis. We have demonstrated in this study that SPARC peptide 2.1, but not peptides tested from other regions of SPARC, has an inhibitory effect on the incorporation of [^{3}H]thymidine into BAEC DNA. On a molar basis, however, this peptide was consistently less effective than intact SPARC. The significance of specialized domains within the SPARC protein that perform apparently disparate functions is presently not understood.

Inhibitors of proliferation have also been described for cells of the vascular wall. Analogs of cAMP and compounds that increase intracellular cAMP were inhibitory for BAEC, although the phase within the cell cycle at which these agents were functional was not addressed (34). Endogenous secreted inhibitors of BAEC proliferation have been found by Sorgente *et al.* (35), who showed a dose-dependent effect on the incorporation of [^{3}H]thymidine by synchronized cultures. In smooth muscle cells, the secreted glycosaminoglycan heparin specifically inhibited progression into the S phase of the cell cycle during the last 4 hr of G_1 phase (7). Pretreatment of synchronized cells with heparin blocked induction of S-phase-specific histone H3 mRNA, but *c-myc* and *c-fos* mRNAs, which are expressed during the transition from G_0 to G_1 phase, were unaltered (8). As we have found for SPARC, heparin not only depressed entry of cells into S phase but also modulated protein synthesis, motility, and cellular interaction with the ECM (18, 20, 36–38). Clearly a matrix-associated group of extracellular regulators that includes fibronectin, collagen, heparin, and now SPARC must be evaluated in studies addressing the responses of cells of the vessel wall to injury as well as to developmental cues.

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