# A Permanently Proliferating Rat Vascular Smooth Muscle Cell with Maintained Expression of Smooth Muscle Characteristics, Including Actin of the Vascular Smooth Muscle Type

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ABSTRACT Cells of an established clonal line (RVF-SMC) derived from rat vena cava are described by light and electron microscope methods and biochemical analysis of the major proteins. The cells are flat, and they moderately elongate and form monolayers. They are characterized by prominent cables of microfilament bundles decoratable with antibodies to actin and $\alpha$-actinin. These bundles contain numerous densely stained bodies and are often flanked by typical rows of surface caveolae and vesicles. The cells are rich in intermediate-sized filaments of the vimentin type but do not show detectable amounts of desmin and cytokeratin filaments. Isoelectric focusing and protein chemical studies have revealed actin heterogeneity. In addition to the two cytoplasmic actins, $\beta$ and $\gamma$, common to proliferating cells, two smooth muscle-type actins (an acidic $\alpha$-like and a $\gamma$-like) are found. The major ($\alpha$-type) vascular smooth muscle actin accounts for 28% of the total cellular actin. No skeletal muscle or cardiac muscle actin has been detected. The synthesis of large amounts of actin and vimentin and the presence of at least three actins, including $\alpha$-like actin, have also been demonstrated by in vitro translation of isolated poly(A)$^+$ mRNAs. This is, to our knowledge, the first case of expression of smooth muscle-type actin in a permanently growing cell. We conclude that permanent cell growth and proliferation is compatible with the maintained expression of several characteristic cell features of the differentiated vascular smooth muscle cell, including the formation of smooth muscle-type actin.

Studies of biological processes in cells cultured in vitro are often limited by the fact that pronounced changes of cell character occur during culturing. One prominent line of changes observed during culturing involves the loss of certain structures, functions, and chemical compounds that are typical for the differentiated state of the original cell. Another frequently found phenomenon is the expression of structures and chemical compounds in the cultured cell that are not found in the cell *in situ*. Typical examples include the formation of collagen type I in chondrocytes (24) and of intermediate-sized filaments of the vimentin type in cultured cells from epithelial, neuronal, and musole origin (e.g., see references 2, 11, and 12).

Drastic changes of cell character and composition have also been described in cultures of muscle cells, smooth muscle cells of vascular origin included (e.g., see references 6–8 and 21; for an excellent review, see reference 7). For example, in vertebrate muscle cells grown in culture, usually the muscle-specific actins and myosins appear to be greatly reduced in amount or even completely lost, concomitant with the expression and increase of the nonmuscle types of actin and myosin; in established cell lines, including those derived from myogenic embryonal tissue, only nonmuscle actins (i.e., $\beta$- and $\gamma$-actin) have been found (for myosin, see references 4, 6, and 7; for actin, see references 7, 14, 16, 18, 19, 28–30, 31, 33, and 34). Similar observations



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have been made in cell cultures of an insect, *Drosophila melanogaster*, in which two muscle-specific forms of actin (actin I and "stable actin III") have been found in differentiating and mature "super-contractile" muscle and in mature thorax muscle, which are not present in cells permanently growing in culture (e.g., see references 13 and 17). Rubenstein and Spudich (28) have mentioned that in their primary and secondary cultures of "chick embryo fibroblasts" some muscle actin is produced that has not been further characterized.

Here we describe a smooth muscle cell line derived from the wall of a rat vein, which has maintained certain smooth muscle cell features and continuously produces, in addition to non-muscle $\beta$- and $\gamma$-actins, relatively high amounts of smooth muscle actin typical of mammalian vascular smooth muscle cells *in situ*. This allows us to identify the cell line (RVF-SMC, for rat vein fibroblastoidal–smooth muscle cell; cf. references 10 and 12) as a vascular smooth muscle–derived line and represents, to our knowledge, the first case of a cultured cell producing vascular smooth muscle–type actin.

## MATERIALS AND METHODS

### Cells and Tissue

The cell culture was originally obtained from the vena cava inferior of an adult rat (~250 g body weight). The preparation and maintenance of the cell culture have been described (10). Cloned cell cultures were grown in "Dulbecco's Minimal Essential Medium" (DMEM) supplemented with 20% fetal calf serum and weekly transferred and seeded (1:10 or 1:20) after trypsination (0.14% trypsin; 1:250; Difco Laboratories, Detroit, Mich.; in phosphate-buffered saline (PBS) containing 0.04% EDTA). Cells were usually grown in monolayer culture in plastic dishes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) or in roller bottles. At the time of submission of this manuscript, the cells were in passage 198. In some experiments, cells were treated with $10^{-6}$ M Colcemid for 12 or 24 h. For radioactive labeling of proteins, cells were allowed to incorporate [$^{35}$S]methionine (50 $\mu$Ci/ml; The Radiochemical Centre, Amersham, England) for 20 h.

For comparison, dissected portions (~1 cm long) of rat vena cava inferior were directly frozen and used for analysis of proteins.

### Electron Microscopy

Cells grown on glass cover slips or on the plastic dish were fixed and processed for electron microscopy of thin sections essentially as described (cf. reference 10).

### Immunofluorescence Microscopy

Cells grown on cover slips were processed for indirect immunofluorescence microscopy, with and without initial fixation in 3.7% formaldehyde, as described (9–12). Antibodies to murine vimentin (9–12), avian desmin (9), bovine prekeratin (9, 12), and chick gizzard actin (cf. reference 9) have been described. Rabbit antibodies to the carboxyterminal propeptide of murine procollagen T were kindly provided by Dr. B. R. Olsen (Department of Biochemistry, Rutgers Medical School, Piscataway, N. J.).

### Gel Electrophoresis

Whole-cell proteins from PBS-rinsed monolayers and cytoskeletal preparations extracted with high salt and the nonionic detergent Triton X-100 were obtained as described (11, 12). One-dimensional gel electrophoresis, using an SDS-polyacrylamide system, was as described (cf. references 9, 11, and 12). Isoelectric focusing in 9 M urea and two-dimensional gel electrophoresis was essentially according to O'Farrell (25), sometimes using the modifications of Kelly and Cotman (20). Gels were stained with Coomassie Blue or processed for autoradiofluorography.

### Isolation of mRNA and In Vitro Translation

Cells grown in roller cultures were briefly rinsed with cold (4°C) PBS, scraped off, and lysed in 140 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, containing 0.5% Nonidet P-40 (NP-40), under gentle homogenization with a loosely fitting Dounce homogenizer. Nuclei and large particles were removed by centrifugation at 800 *g* for 10 min, and the supernate obtained was adjusted to 0.25 M sucrose and centrifuged at 12,000 *g* for 15 min. RNA was directly extracted from the "postmitochondrial" supernate by use of the phenol-chloroform procedure. Poly(A)-containing RNA was isolated by chromatography on oligo(dT)-cellulose (1) by use of 0.4 M NaCl, 10 mM triethanolamine-HCl (pH 7.5), 1 mM EDTA, 0.5% SDS for application and the same buffer without NaCl for elution. Sizes and purity of RNA preparations were routinely controlled by gel electrophoresis on 1.5% agarose gels in borate buffer (50 mM boric acid, 5 mM sodium tetraborate, 10 mM $Na_2SO_4$, 1 mM EDTA, pH 8.0). In vitro translation assays were performed essentially according to Pelham and Jackson (26). Translation products were characterized by one- and two-dimensional gel electrophoresis.

### Characterization of Actins by Their Amino-terminal Tryptic Peptides

The method used has been described (31–33). Briefly, the procedure is based on the finding that the six currently known mammalian actins differ by amino acid exchanges in their amino-terminal tryptic peptide (residues 1–18 or 2–18), which allows a clear separation of five of the six peptides by a two-dimensional paper electrophoretic system. The only peptides not resolved are the ones from skeletal muscle and cardiac muscle, which require a further cleavage to yield separatable peptides. Substitution of the cystein residues by $^{14}$C-carboxymethylation increases the sensitivity of the technique and facilitates direct quantitation of the analysis. The procedure is not limited to purified actin but can be used directly on total cellular protein extracted by 8 M urea because of the unique electrophoretic properties of the actin N-terminal peptides.

## RESULTS

### Light Microscopy

Cultures were examined between passage 8 and 180 by light and electron microscopy, immunofluorescence microscopy, and the gel electrophoretic characterization of the major proteins. The typical morphology of the cell line at relatively low density is presented in Fig. 1 *a*. The cells exhibited a tendency to grow in groups of closely attached cells. At confluency they appeared in a monolayer of slightly elongated cells arranged in a typical "shingle" pattern. When cells were allowed to grow to higher densities, groups or foci of multilayered cells were often seen, in association with deposits of extracellular matrix material similar to the extracellular material produced by short-term cultures of smooth muscle cells (cf. reference 15; see below). During mitosis the cells rounded off (Fig. 1 *a*) but still seemed to remain attached to the substratum. The cell doubling time was ~22 h.

The cells showed pronounced "cables", i.e., bundles of microfilaments, intensely decoratable with antibodies to actin (Fig. 1 *b*) and, in an interrupted pattern, $\alpha$-actinin (not shown here; cf. reference 7). Especially conspicuous were the bundles of intermediate-sized filaments of the vimentin type, which displayed typical "wavy" arrays (Fig. 1 *c*) and were translocated into the characteristic perinuclear aggregates upon treatment with Colcemid. The cells were not significantly stained by antibodies to prekeratin, indicating their nonepithelial character (9–11), and to desmin (not shown here). An endothelial origin for these cells seemed to be excluded by their morphology, growth rate, and growth behavior, and the absence of detectable factor VIII-like proteins (not shown here). At all cell densities the cells produced procollagen type I, which by immunofluorescence microscopy appeared to be contained in elements of the endoplasmic reticulum (ER) and in granular structures most probably representing secretory vesicles (not shown here; for collagen synthesis in cultured smooth muscle cells, see references 3 and 7).

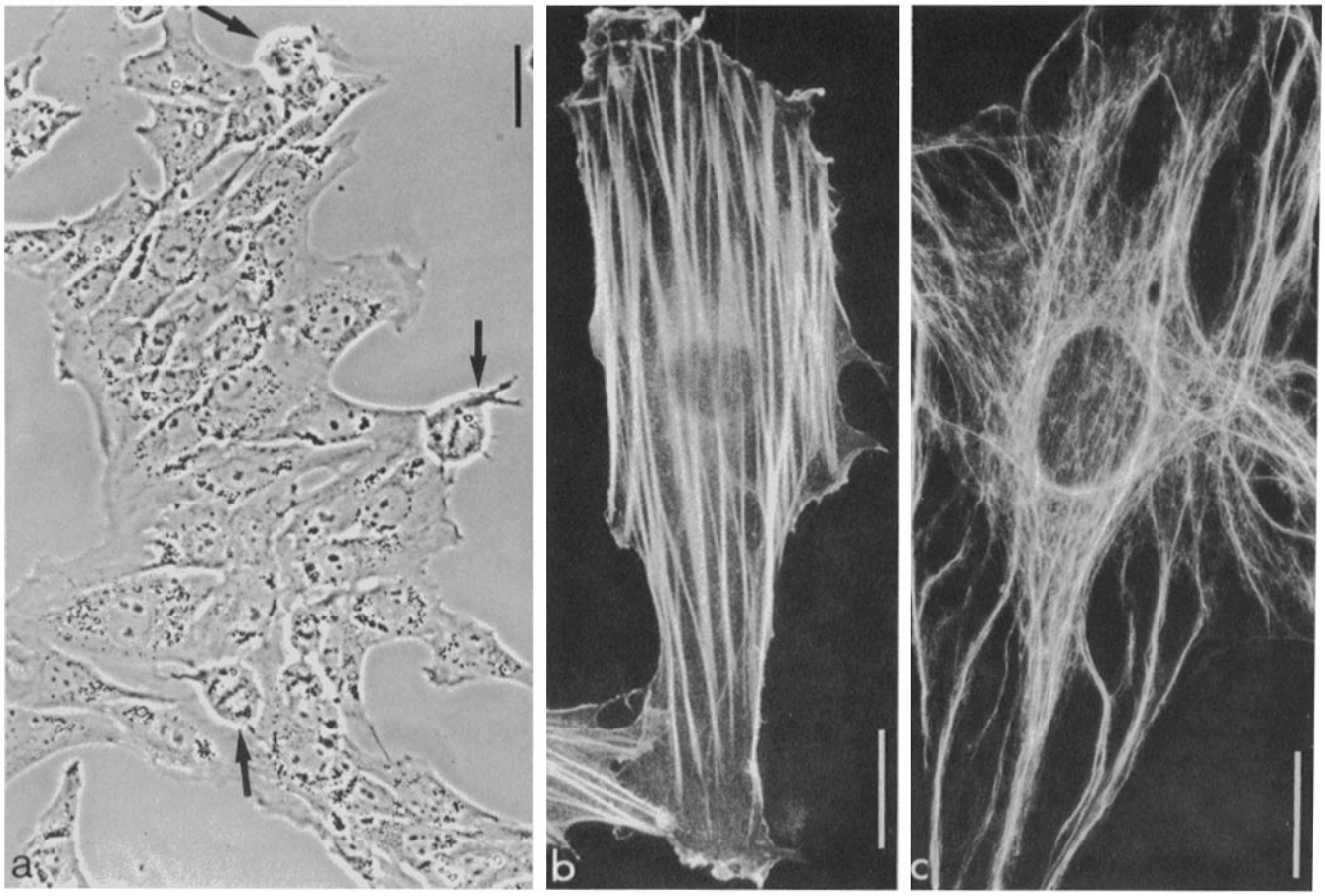


FIGURE 1 Morphology of vascular smooth muscle cells, line RVF-SMC, derived from rat vena cava by phase-contrast microscopy of the typical growth pattern (*a*) and by immunofluorescence microscopy, using antibodies to actin (*b*) and vimentin (*c*). Note the tendency of the flat cells to form coherent groups (*a*) and well-developed bundles of actin-containing microfilaments ("cables", *b*), and intermediate-sized filaments of the vimentin type (*c*). Note also positive staining with actin antibodies in ruffling zones (*b*). Arrows in *a* denote mitoses. Bars: *a*, 40 μm; *b* and *c*, 20 μm. *a*, × 270; *b* and *c*, × 800.

## *Electron Microscopy*

The cells contained conspicuous microfilament bundles of various dimensions, including exceedingly thick ones (Fig. 2 *a–c*), most of which were located toward the bottom side of the cell, i.e., parallel to the substratum. These microfilament bundles, which corresponded to the cables decorated by actin antibodies (see above), were characterized by many streaklike or ellipsoidal densely stained "dark bodies" (Fig. 2 *b* and *c*) similar to those described in smooth muscle cells, including secondary cultures (cf. references 5, 7, 8, 15, 21, and 27). Besides microfilaments, intermediate-sized filaments were seen to be associated with these dense bodies (Fig. 2 *b* and *c*).

Extensive arrays of variable numbers of dictyosomes often in perinuclear locations were observed (not shown here; for similar observations see, e.g., reference 8). Rough endoplasmic reticulum usually appeared as dilated cisternal sacs containing fuzzy, fibrillar material (not shown here; for similar observations, see references 5, 7, 8, and 21). Smooth-surfaced membrane elements included a special vesicle type containing heavily stained aggregates of probably secretory material and various forms of lysosomelike vesicles, many of which contained membranelike whorls or myelin figures (not shown here) of the type described by Fowler et al. (8) for cultured smooth muscle cells from bovine aorta. Characteristic membrane structures were also arrays of peripheral smooth-surfaced vesicles or surface membrane invaginations with diameters of 40–80 nm, often arranged in rows located between adjacent microfilament bundles (Fig. 2 *a*). Such conspicuous arrays of vesicles and/or surface cavernae are generally found in smooth muscle cells, both *in situ* and in culture (e.g., references 7, 8, 21, and 27). The cells were connected by extensive junctions of the intermediate type and gap junctions. Typical desmosomes were not found.

The cells produced and accumulated, especially at high cell densities, masses of extracellular fibrillar material (Fig. 2 *d* and *e*) that was similar to the extracellular fibril matrix described in short-term cultures of smooth muscle cells by various authors (27; see also references 7 and 15). At least two different filament types could be distinguished in these extracellular deposits. One type consisted of long, unbranched filaments 8–11 nm in diameter with an unstained, apparently hollow core (Fig. 2 *e*) identical in morphology to the "microfibrils" of elastic material known to be produced by smooth muscle cells (27; for review, see reference 7). The other component comprised thin, densely stained and fasciated 5- to 7-nm filaments without a special substructure.

## *Major Cytoskeletal Proteins*

The major polypeptides of RVF-SM cells separated by one-dimensional SDS-polyacrylamide gel electrophoresis are

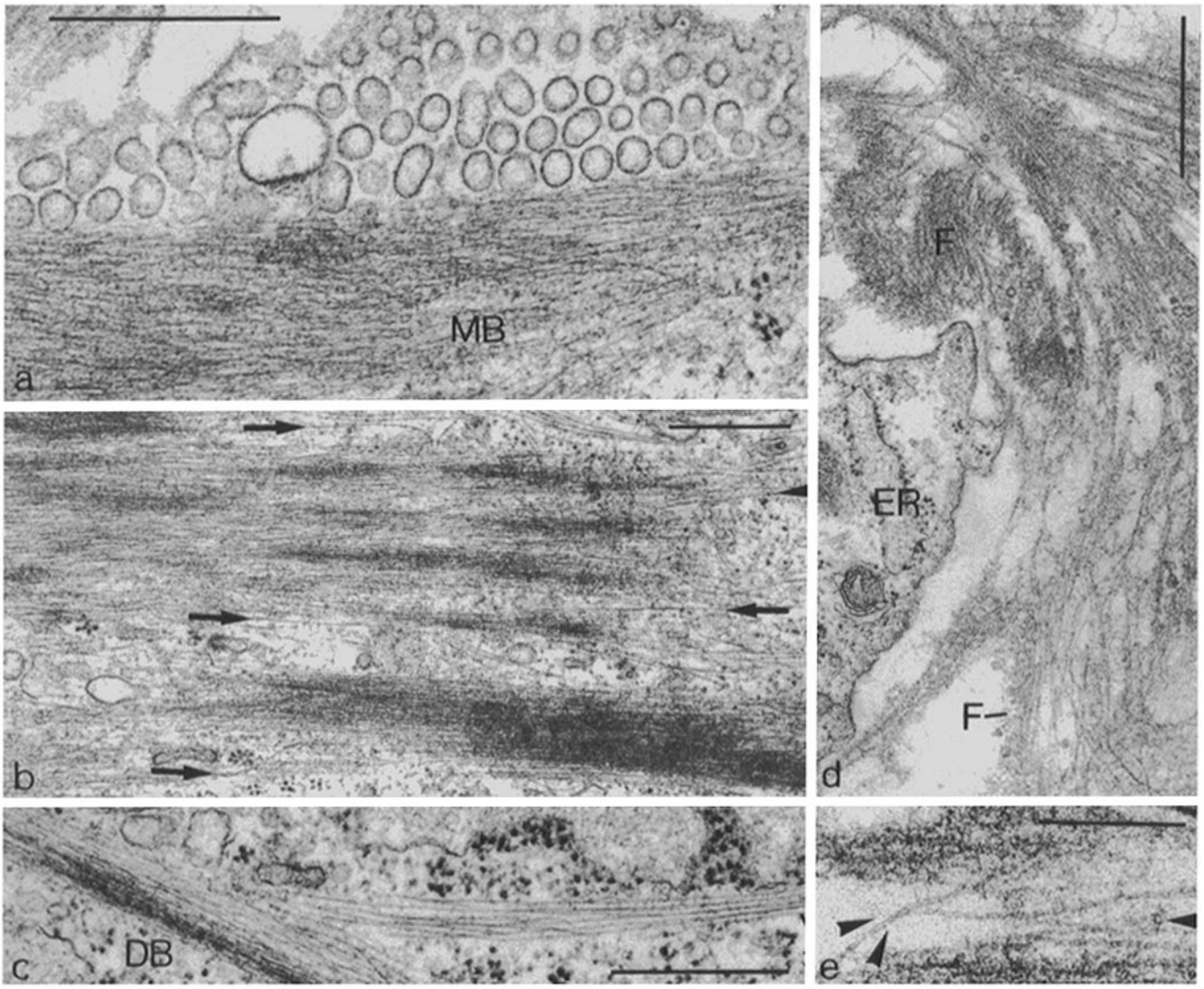


FIGURE 2 Electron microscopy of rat vascular smooth muscle cells (RVF-SMC line) of microfilament bundles (*MB*; cables) that contain numerous densely stained bodies (*DB*; *b* and *c*). Note that intermediate-sized filaments (some are denoted by arrows in *b*) are often aligned and/or intimately interwoven with these microfilament bundles and seem also to be associated with dense bodies (*c*). Note typical arrays of peripheral vesicles and/or surface invaginations that often form rows or other regular arrays (*a*). Dense cultures of these cells show typical masses of densely stained bundles of extracellular fibrillar material (*F* in *d*) in which two components are distinguished: thin filaments, and thicker (8- to 11-nm) filaments with an electron-translucent, apparently hollow core (some are denoted by arrowheads in *e*). *ER*, endoplasmic reticulum. Bars: *a–c*, 0.5 μm; *d*, 1 μm; and *e*, 0.2 μm. *a*, × 67,000; *b*, × 35,000; *c*, × 53,000; *d*, × 24,000; and *e*, × 110,000.

shown in Fig. 3 (slot *2*). The predominant protein was actin, which from densitometer tracings and radioactivity determinations accounted for ~11% of total protein. The second most frequent protein in total cellular extracts was vimentin. When cytoskeletons were prepared by a combined treatment with nonionic detergent and high salt buffer, actin and vimentin again constituted the majority of the proteins present (Fig. 3, slot *4*). A significant polypeptide band corresponding to desmin was not found in these cytoskeletons (cf. slots *3* and *4* in Fig. 3). A high molecular weight protein (~230,000), probably fibronectin, was also prominent in cytoskeleton preparations (Fig. 3, slots *2* and *4*). When in vitro translation products of poly(A)-containing messenger RNA were examined in this one-dimensional system, actin was again the most abundant protein (Fig. 3, slot *6*) and vimentin, together with an as yet unidentified component of ~51,000 mol wt, was among the next frequent polypeptides synthesized.

Several different actin types were present in RVF-SM cells. Fig. 4 shows the isoelectric focusing pattern of actins extracted from an acetone powder of the cells and purified by DNase I affinity chromatography (23). Comparison is made with nonmuscle β- and γ-actin from bovine brain and the actins from bovine aorta tissue in which the α-like vascular smooth muscle type is the major actin species (33). These reference actins have previously been characterized by complete amino acid sequence analysis (31–33). Three different actin species could be distinguished in RVF-SM cells (Fig. 4), two of which had the same isoelectric point as nonmuscle β- and γ-actin. The third actin species had a slightly more acidic isoelectric point and comigrated with the α-like vascular smooth muscle actin type of bovine aorta (for isoelectric focusing of vertebrate actins, see references 14, 18, 19, and 28–34). The ratio of the three actin

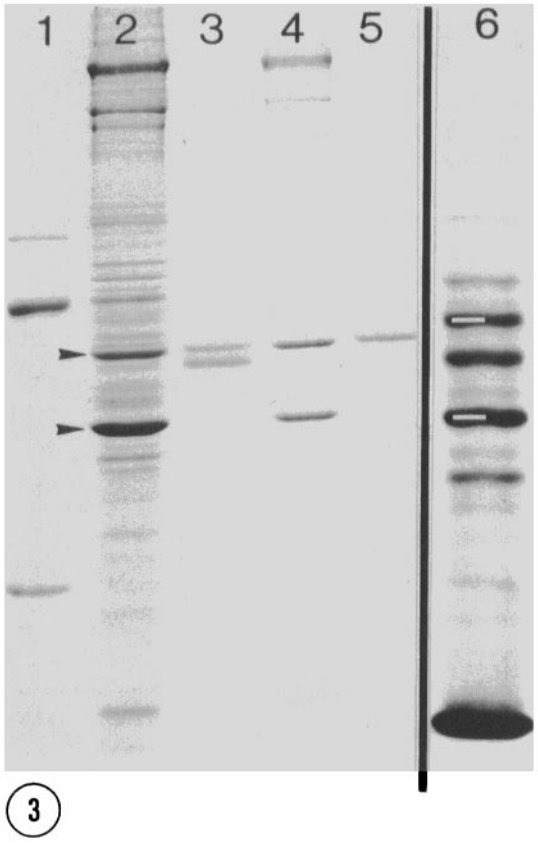


FIGURE 3 One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of total Coomassie Blue-stained cell proteins of cultured rat vascular smooth muscle cells (RVF-SMC line, slot *2*) and cytoskeletons prepared therefrom (slot *4*), in comparison with reference proteins: slot *1* presents, from top to bottom, phosphorylase *a*, bovine serum albumin, and chymotrypsinogen; slot *3* contains purified murine vimentin and porcine desmin (cf. references 9, 11, and 12; slot *5* presents vimentin. The gel shown in slot *6* presents an autoradiofluorogram of [$^{35}$S]methionine-labeled total proteins synthesized in vitro, using total poly(A)$^+$ mRNA isolated from RVF-SM cells. The two major cytoplasmic protein components, vimentin and actin (indicated by arrowheads and white bars, respectively), have been identified by Coomassie Blue staining of co-electrophoresed authentic actin and vimentin.

species detected ($\alpha$-like:$\beta$:$\gamma$) was ~3:5:1 as determined by densitometric scanning of the Coomassie Blue-stained gels.

The different actin types present in RVF-SM cells were further identified by their characteristic N-terminal tryptic peptides. The differences between different actins are particularly striking in the number and positioning of aspartic acid, glutamic acid, and cystein residues in the N-terminal tryptic peptides (residue nos. 1–18 or 2–18; cf. references 31–33). Electrophoresis at pH 3.3 and at pH 6.5 enabled the separation of the different peptides according to their specific aspartic and glutamic acid content. Fig. 5*A* shows as a reference a reconstituted mixture of the $^{14}$C-carboxymethylated N-terminal tryptic peptides of five of the six actins commonly found in higher vertebrates ($\alpha$-like smooth muscle actin, $\gamma$-like smooth muscle actin, $\alpha$–skeletal muscle actin, and the two nonmuscle actins, $\beta$ and $\gamma$). When this procedure was used for RVF-SMC actin, four different actin species were resolved (Fig. 5*B*): the $\alpha$-like and the $\gamma$-like smooth muscle actins (spots *1* and *2*, respectively) and the nonmuscle $\beta$- and $\gamma$-actins (spots *3* and *4*). The different N-terminal tryptic peptides were further identified by the digestion products obtained with two different proteolytic enzymes (thermolysin and the protease of *Staphylococcus aureus V 8*). The typical pattern of radioactively labeled secondary peptides obtained in parallel with the reference peptides (data not shown here) confirmed the assignment of the four actin species.

Table I summarizes the relative amounts of the four different actin N-terminal peptides. The ratios were calculated from the radioactivity incorporated by the carboxymethylation at the cysteine residues, taking into account the presence of one cysteine residue in the nonmuscle actins and two cysteine residues in the two smooth muscle actins (for sequence data, see reference 31). The (nonmuscle) $\beta$-actin was by far the most abundant species (51%), whereas the nonmuscle $\gamma$-actin was present in unusually small amounts (~13%). The $\alpha$-like vascular smooth muscle actin was present at ~29%, whereas the second ($\gamma$-like) smooth muscle actin accounted for only a small proportion of the total actin (~7%). Similar results were obtained when the procedure was performed on total extracted cellular actin, using urea extraction (Table I). All four actins found in RVF-SM cells were also observed in vena cava tissue, although at somewhat different ratios (Fig. 5*C*). A similar complexity of actin expression in RVF-SM cells was observed when the in vitro translation products obtained from total poly(A)$^+$-containing messenger RNA were analyzed by two-dimensional electrophoresis (Fig. 6*c*; note that in this system nonmuscle $\gamma$-actin and smooth muscle $\gamma$-actin are not resolved as separate species).

In cytoskeletal preparations, vimentin (apparent $M_r$, 57,000; apparent pI, 5.30) was the only identifiable intermediate-filament protein present (Fig. 6*a* and *b*). A series of characteristic proteolytic breakdown products of vimentin were noticed in two-dimensional gel electrophoresis (Fig. 6*b*) that could be significantly reduced when the samples were first denatured by heating with SDS (20), before the isoelectric focusing in urea. We did not observe significant amounts of cytoskeletal proteins that by size or pI resembled mammalian desmin (Fig. 6*a* and *b*), although this protein is frequent in various smooth muscle-rich organs (uterus, taenia coli, and stomach; 19, 22; cf. reference 9). Similarly, vimentin was the only intermediate filament protein detected on one- and two-dimensional gel electrophoresis of proteins synthesized in vitro from poly(A)$^+$-mRNA

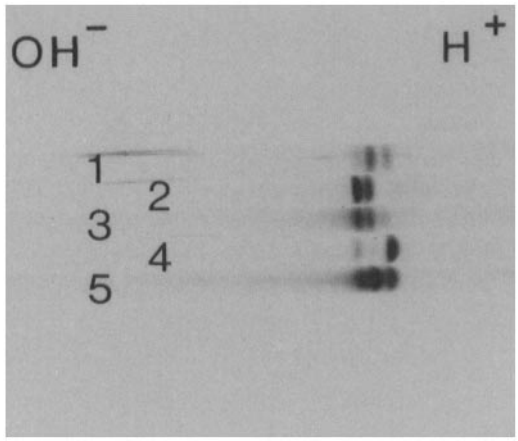


FIGURE 4 Isoelectric focusing (IEF) analysis of DNase I-purified actins from RVF-SM cells (gel *1*). This RVF-SMC actin was mixed with actins from bovine brain and aorta (in gels *3* and *5*, respectively). Gels *2* and *4* contain bovine brain actin (gel *2*) and bovine aorta actin (gel *4*).

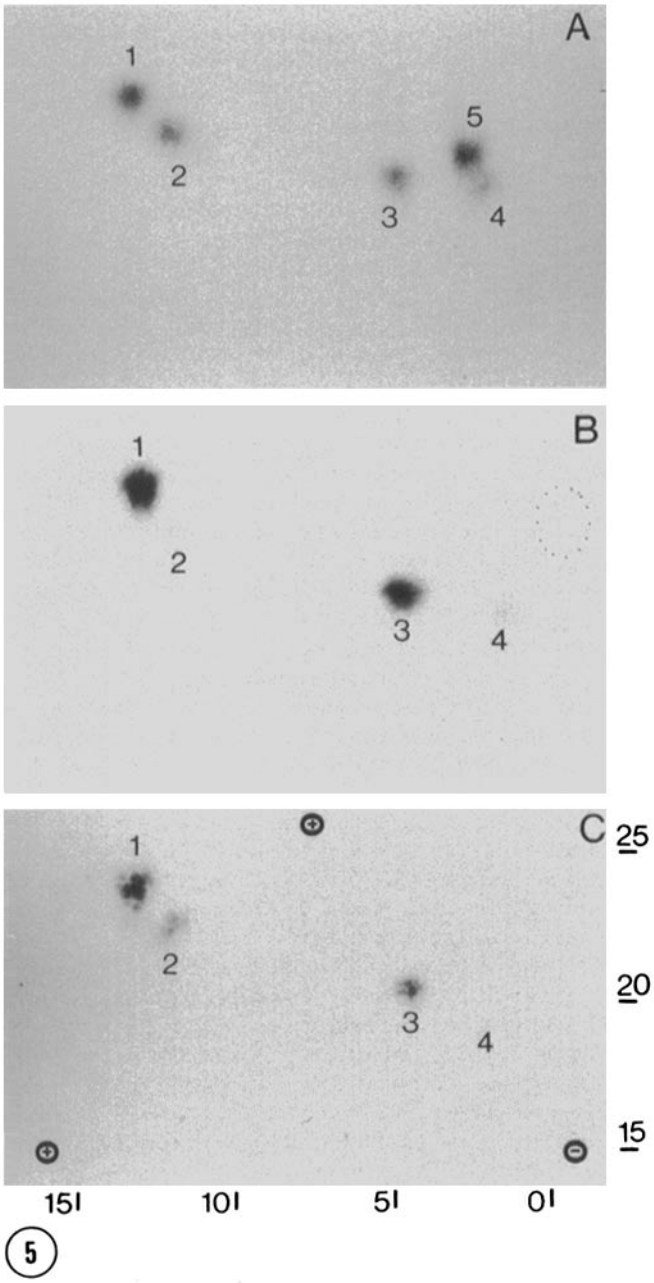


FIGURE 5 Two-dimensional paper electrophoretic separation of actin N-terminal tryptic peptides radioactively labeled with [$^{14}$C]iodoacetate. *A* shows a reconstituted peptide mixture of aorta smooth muscle actin (*1*), stomach smooth muscle actin (*2*), β-nonmuscle actin (*3*), γ-nonmuscle actin (*4*), and skeletal muscle actin (*5*). *B* shows the peptide separation pattern obtained from total cell extracts of RVF-SM cells, and *C* shows the pattern of actin from rat vena cava tissue. Numbers in *B* and *C* refer to the reference peptides shown in *A*. Horizontal separation is by electrophoresis at pH 3.3, and vertical separation is by electrophoresis at pH 6.5 (distances in centimeters on both axes have been measured from the origin). The dotted spot in *B* indicates the position of Orange G used as colored marker to follow pH 6.5 electrophoresis.

(Fig. 6*c*). Protein comigrating with mammalian desmin was not detected in translation assays.

## DISCUSSION

The present study describes a clonal cell line, derived from the walls of the vena cava of rat, that has retained several characteristics of its origin, i.e. vascular smooth muscle cells. This cell line (RVF-SMC) grows and proliferates with an efficiency comparable to that of other cultured cells. Its morphology exhibits a series of features that have been repeatedly described in short-term cultures of smooth muscle cells and are commonly considered to be typical of smooth muscle cells in culture (7). Such features include abundance of dilated RER containing fibrillar material, extended Golgi apparatus often in perinuclear arrangements, abundance of characteristic lysosomelike vesicles containing myelinlike figures, typical surface invaginations or peripheral vesicles, high density of microfilament bundles, and numerous "dark bodies", and formation of extracellular fibrillar masses that include typical "hollow" filaments of the type described in microfibrils of elastic material (3, 5–8, 15, 21, 27). The cells produce type I procollagen, and probably secrete collagen I.

The most remarkable property of the cell line appears to be the continual synthesis of the vascular smooth muscle type of actin. This actin species has been characterized by isoelectric focusing analysis and identification of the N-terminal tryptic peptide. To our knowledge, this is the first demonstration of the expression of vascular smooth muscle actin in a permanently proliferating cell line. In addition, the cells express, like all proliferating cells, the usual cytoplasmic actins β and γ, although with an unusually high emphasis on β (β:γ ratio, 5:1). Furthermore, a small amount of the second smooth muscle actin (γ-like) has been noticed. Examination of the total actin of rat vena cava tissue from which RVF-SM cells are derived shows also the presence of the same four different actin species, although the two smooth muscle types are present in higher relative amounts than in the cultured cells. Rat vena cava contains several different cell types in addition to vascular smooth muscle cells. Therefore, we cannot decide at the moment whether the vein smooth muscle cells *in situ* express only the smooth muscle actin types and acquire the nonmuscle actins only upon culturing or whether they also contain at least some non-muscle actin *in situ*. In addition, it remains unclear whether the two smooth muscle actins (γ-like and α-like) are expressed *in situ* in the same or in different cells. A further interesting question currently unresolved is the distribution of the four actins in the cultured cells: are they present in the same microfilament structures or are some of them differently organized and serving different functions?

RVF-SM cells are rich in intermediate-sized filaments of the

TABLE I

*Ratios of the Different Actins Present in RVF-SM Cells and in Rat Vena Cava Tissue*

| | RVF-SM cells | | |
|---|---|---|---|
| Actin types | DNase I-purified actin | Total cell extract actin | Rat vena cava |
| α vascular type | 28.3 | 29.8 | 37.4 |
| γ-like smooth muscle type | 6.9 | 6.6 | 14.3 |
| β nonmuscle | 51.0 | 51.5 | 36.5 |
| γ nonmuscle | 13.6 | 12.1 | 11.8 |
| α skeletal muscle | — | — | — |

Amounts of actin (% of total actin) in RVF-SM cells (collected from passages nos. 96–102) and rat vena cava were calculated from the amounts of radioactivity incorporated in the separated actin N-terminal tryptic peptides. The separation pattern of the peptides from total RVF-SM cell extracts and from rat vena cava is shown in Fig. 5 *B* and *C*.

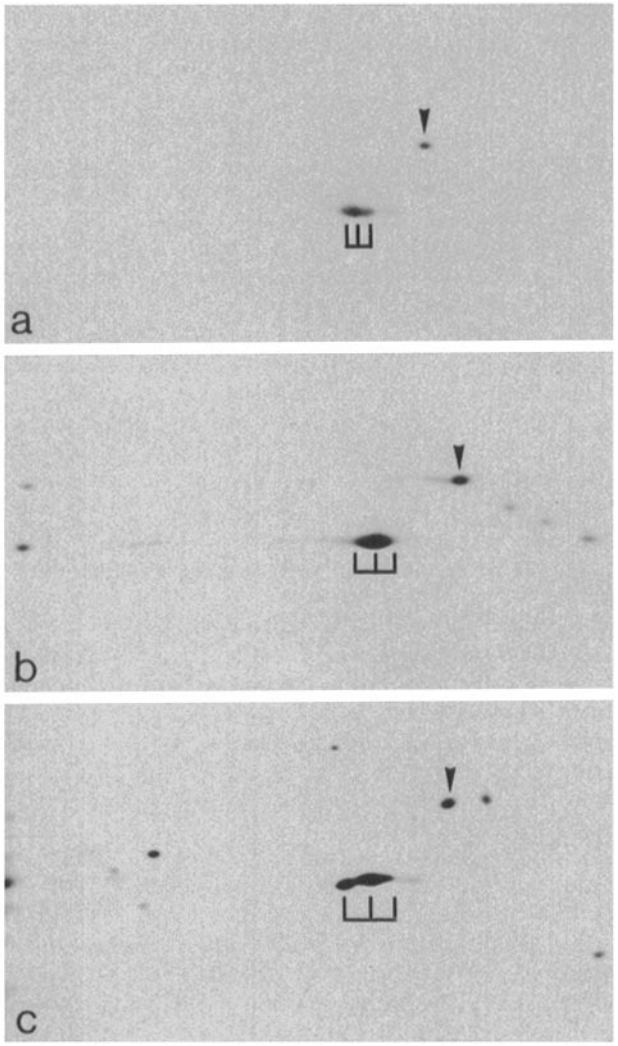


FIGURE 6 Two-dimensional gel electrophoresis (IEF from left to right, SDS-PAGE from top to bottom) showing the major proteins of whole RVF-SM cells (*a*) and cytoskeletons prepared therefrom (*b*), using Coomassie Blue staining. Fig. 6 *c* presents a fluorogram after [$^{35}$S]methionine labeling of proteins synthesized in vitro from total poly(A)-containing mRNA. Brackets denote the actins present; arrowheads denote vimentin. Note breakdown products of vimentin (at right margin of *b*) and absence of a component comigrating with mammalian desmin.

vimentin type. Desmin, a major cytoskeletal protein of various muscle-derived or myogenic cells (e.g., see references 18, 19, and 22), has not been found in these cells. This finding could indicate a "takeover" by vimentin filaments in culture—as experienced previously in many cultured cells (12)—concomitant with a loss of desmin filaments. On the other hand, in view of our finding that smooth muscle cells present in walls of veins and arteries, notably the tunica media of the aorta, contain large amounts of vimentin but very little, if any, desmin (these authors, unpublished observation), it is also possible that vascular smooth muscle cells differ from other smooth muscle tissues and contain vimentin rather than desmin filaments. Hence, the absence of desmin in RVF-SM cells may simply reflect the origin of these cells from vimentin-containing vascular smooth muscle cells.

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