Proc. Natl. Acad. Sci. USA
Vol. 78, No. 1, pp. 298–302, January 1981
Cell Biology

Vascular smooth muscle cells differ from other smooth muscle cells: Predominance of vimentin filaments and a specific α-type actin

(intermediate filaments/desmin/muscle differentiation/blood vessels/atherosclerosis)

GIULIO GABBIANI*, ERIKA SCHMID†, STEFANIE WINTER†, CHRISTINE CHAPONNIER*, CATHERINE DE CHASTONAY*, JOEL VANDEKERCKHOVE‡, KLAUS WEBER‡, AND WERNER W. FRANKE†

*Department of Pathology, University of Geneva, 1211 Geneva 4, Switzerland; † Department of Membrane Biology and Biochemistry, Institute for Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany; and ‡Max Planck Institute for Biophysical Chemistry, D-3400 Goettingen, Federal Republic of Germany

Communicated by Earl P. Benditt, September 2, 1980

ABSTRACT **Smooth muscle cells of the digestive, respiratory, and urogenital tracts contain desmin as their major, if not exclusive, intermediate-sized filament constituent and also show a predominance of γ-type smooth muscle actin. We have now examined smooth muscle tissue of different blood vessels (e.g., aorta, small arteries, arterioles, venules, and vena cava) from various mammals (man, cow, pig, rabbit, rat) by one- and two-dimensional gel electrophoresis of cell proteins and by immunofluorescence microscopy using antibodies to different intermediate-sized filament proteins. Intermediate-sized filaments of vascular smooth muscle cells contain abundant amounts of vimentin and little, if any, desmin. On gel electrophoresis, vascular smooth muscle vimentin appears as two isoelectric variants of apparent pI values of 5.30 and 5.29, shows the characteristic series of proteolytic fragments, and is one of the major cell proteins. Thus vimentin has been demonstrated in a smooth muscle cell present in the body. Vascular smooth muscle cells are also distinguished by the predominance of a smooth muscle-specific α-type actin, whereas γ-type smooth muscle actin is present only as a minor component. It is proposed that the intermediate filament and actin composition of vascular smooth muscle cells reflects a differentiation pathway separate from that of other smooth muscle cells and may be related to special functions and pathological disorders of blood vessels.**

Different cell types contain distinct species of contractile and cytoskeletal proteins. This is especially evident in the class of the intermediate-sized (7- to 11-nm) filaments (refs. 1–4, for review see ref. 5). Thus, epithelial cells contain intermediate filament proteins related to epidermal prekeratin (2, 6–9) and mesenchymal cells contain filaments of the vimentin type (1, 2, 4). Vimentin has also been reported to exist in various cultured cells, nonmesenchymal ones included (1, 2, 10, 11), and in association with Z-disks of striated muscle (ref. 12; see, however, ref. 13). The major protein constituent of intermediate filaments of chicken gizzard smooth muscle ["desmin" (14)], which is similar, if not identical, to the intermediate filament protein predominant in smooth muscle of mammalian colon ["skeletin" (15, 16)], has been shown to occur in differentiated smooth muscle cells of the digestive and urogenital tracts of chicken (1, 4, 14, 17–20) and mammals (20–22), in cultured cardiac myocytes (18, 23), in filaments of skeletal myotubes (13, 18, 24), in Z-lines of mature skeletal and cardiac muscle of birds and mammals (3, 13, 14, 17–19, 22, 24), and in intercalated disks of heart muscle (14, 22).

To date, vimentin has not been carefully demonstrated in smooth muscle (refs. 14–17, 19, 20, and 25; for cultured smooth muscle cells see, however, ref. 18), though antibodies to vimentin have been shown to react strongly with the wall of small arteries (2, 26).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

It has recently been noted that the actin composition of smooth muscle tissue of bovine aorta is unique in that it shows a predominance of a smooth muscle α-type actin with the amino-terminal sequence Glu-Glu-Glu-Asp-Ser, which is different in two amino acid positions from the γ-type smooth muscle actin predominant in bovine rumen and uterus and in chicken gizzard (27, 28).

Here we report that vascular smooth muscle cells of various species, humans included, profoundly differ from smooth muscle cells of digestive, respiratory, and urogenital tract by the presence of large amounts of vimentin filaments and of α-actin.

MATERIALS AND METHODS

Segments of the aorta were removed from male or female adult rats, cows, pigs, and rabbits, and were directly frozen either at −150°C (in isopentane) for immunofluorescence microscopy (8, 29) or at −20°C for protein analysis. In some experiments, lyophilized tissue was used. Alternatively, aortic tissue was directly extracted in the sample buffer used for polyacrylamide gel electrophoresis (see below). Cytoskeletons were prepared by extraction with high-salt buffers and Triton X-100 as described for chicken gizzard (2, 22, 30). Pieces of aorta were dissected and stripped pure media was prepared. Parts of the inferior vena cava were removed from rats and processed as described for aortic tissue. Heart muscle tissue and smooth muscle-rich tissues of uterus, trachea, stomach, small intestine, and colon were removed and processed as described for aorta.

Human tissues were collected as follows: aorta was obtained at autopsy from 4 males and 4 females with a mean age of 57 years (47 to 73). The cross section of the whole wall was used for immunofluorescent staining. For protein analysis intima and adventitia were stripped off with a fine pair of forceps and the integrity of the media was verified by light microscopy. Uterus was obtained from a 40-year-old female hysterectomized for a uterine myoma. The endometrium was carefully eliminated and normal myometrium was used for immunofluorescence and protein analysis. Human trachea and cardiac tissue were obtained at autopsy from two females aged 76 and 50, respectively, without macroscopically and microscopically visible respiratory or cardiac pathology, with the exception of thickening of coronary intima.

The preparation of guinea pig antisera and monospecific antibodies against murine and human vimentin (2, 11), desmin from chicken gizzard (22, 30), and bovine prekeratin (2, 6, 22) as well as procedures used for immunofluorescence microscopy on frozen sections (22, 29) were as previously described.

One-dimensional gel electrophoresis was essentially according to Laemmli (31). In two-dimensional gel electrophoresis (32) the modification of Kelly and Cotman (33) was used in some ex-

periments in order to minimize proteolytic degradation of vimentin in the 9.5 M urea buffer used for isoelectric focusing (12, 34).

Actins were purified and used for isoelectric focusing analysis and characterization of their amino-terminal tryptic peptides as described (27, 28).

RESULTS

Gel Electrophoresis. Smooth muscle-rich tissue and cytoskeletal fractions from stomach, small intestine, colon, and uterus all showed a prominent desmin band and comparatively little protein in the position of vimentin (e.g., Fig. 1, lane a) as shown by one-dimensional gel electrophoresis of total tissue proteins and cytoskeletal fractions. At the same time, in all species examined, aortic and venous tissues showed a prominent polypeptide band comigrating with purified mammalian vimentin (2, 11). In order to minimize the problem of contamination with vimentin-containing cells of non-smooth-muscle origin, we also analyzed stripped aortic media, because this tissue contains only smooth muscle cells (35). Fig. 1, lane b shows an example of protein from stripped aortic media. A faint band of electrophoretic mobility slightly higher than that of vimentin was noted only in some preparations of aortic tissue; comparison and coelectrophoresis with desmin showed that this polypeptide band had significantly higher mobility than authentic mammalian desmin (2, 22).

Two-dimensional gel electrophoresis of aortic tissue or cytoskeletons showed the presence of relatively large proportions of

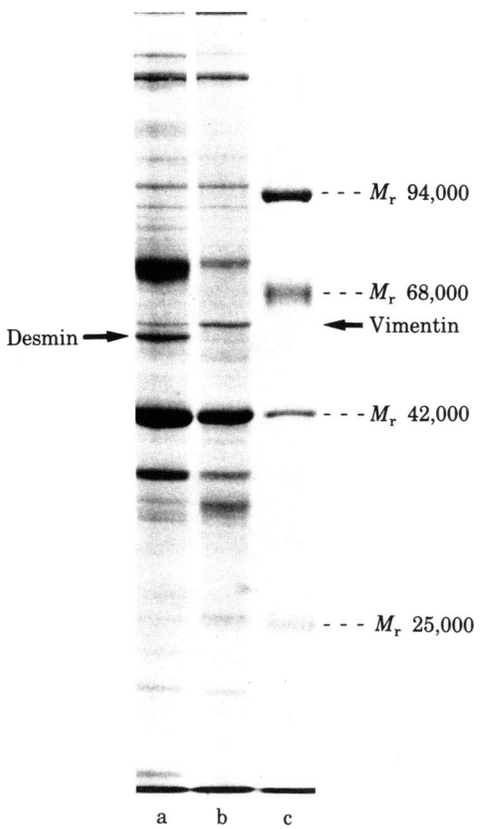

FIG. 1. One-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis of proteins of human uterus smooth muscle (lane a), stripped pure media of human aorta (lane b), and reference proteins (lane c; from top to bottom: phosphorylase, bovine serum albumin, skeletal actin, and chymotrypsinogen A). Arrows indicate the positions of vimentin and desmin.

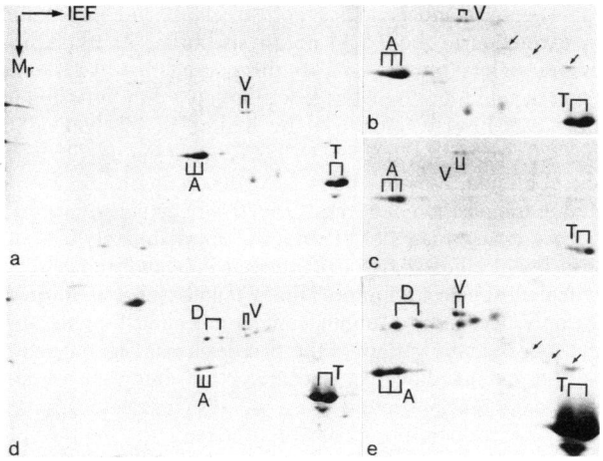

FIG. 2. Two-dimensional gel electrophoresis (orientation is indicated in *a*: IEF, isoelectric focusing; M_r, second electrophoresis in the presence of $NaDodSO_4$ for estimation of M_r values) of proteins of stripped media of human aorta (*a–c*) and human uterus tissue (*d* and *e*). Brackets indicate the positions of actin (A; α-, β-, and γ-type actins are separated in *c*; see also Fig. 3*b*), tropomyosin (T), vimentin (V), and desmin (D in *d* and *e*). Proteolytic fragments of vimentin (some are denoted by arrows in *b* and *e*) are conspicuous in samples directly lysed in 9.5 M urea lysis buffer (32) but are reduced in samples denatured prior to isoelectric focusing (*c*; ref. 33). Note absence of detectable amounts of desmin in aortic media (*a–c*). A large part, if not all, of the vimentin found in uterine tissue (*d* and *e*) is from blood vessels and connective tissue cells such as fibroblasts (see text).

vimentin (Figs. 2 *a–c* and 3 present examples for man and rat, respectively). Characteristically, vascular vimentin appeared in the form of two isoelectric variants (apparent pI values of 5.30 and 5.29), one of which probably represents phosphorylated vimentin (for comparison see studies in cultured cells in refs. 25 and 37). In all vascular tissues vimentin was a major protein, next to the three actin components separated on these gels (Figs. 2–4) and the tropomyosins. The identity of the vimentin was demonstrated by its enrichment in high-salt-extracted cytoskeletons, by coelectrophoresis with vimentin from purified cytoskeletons of cultured cells of murine, bovine, and human origin (refs. 2, 11, and 22; the major vimentin component comigrated with the less acidic variant present in aortic vimentin), and by the typical "diagonal" series of proteolytic vimentin frag-

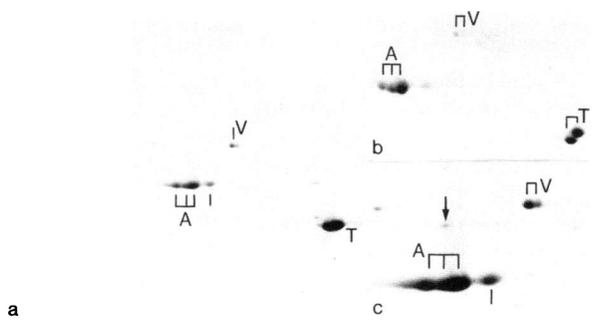

FIG. 3. Two-dimensional gel electrophoresis of rat aorta proteins (same orientation and symbols as in Fig. 2). Note the separation of the three actin types (*b*). Note the occurrence in this tissue of a minor polypeptide (indicated by arrow in *c*) with an electrophoretic mobility similar to that of desmin (compare Figs. 2 *d* and *e* and 4 *a* and *b*). The vertical bar (*a* and *c*) denotes a protein component of similar size as actin that is consistently observed in aortic and venous tissues; whether this is artificially formed from actin or represents a phosphorylated actin (36) remains to be determined.

ments (Figs. 2 *b* and *d* and 3*a*; see also refs. 12 and 34) obtained after extraction in the 9.5 M urea lysis buffer. As in cultured cells and skeletal muscle (12, 34), this degradation by proteases present in smooth muscle tissue was largely inhibited when the sample was briefly denatured by boiling in 1% $NaDodSO_4$ buffer (33), prior to extraction in 9.5 M urea (Fig. 2*c*). In aortic tissue of human, bovine, and porcine origin no protein was detected in the position of desmin (Fig. 2 *a–c*), which appeared in two isoelectric variants of pI values of approximately 5.41 and 5.36 in smooth muscle tissue of uterus, colon, and stomach of all mammalian species examined (Fig. 2 *d* and *e*; cf. Fig. 4). In rat aorta only, we noted a minor component almost comigrating with the less acidic variant of the two desmins (Fig. 3 *a* and *c*). This component, however, occurred in somewhat variable amounts and was not found in extracted cytoskeletons, unlike the typical preparative behavior of desmin in nonvascular smooth muscle (cf. refs. 1–5, 12–17, 19, 22, 24, 25, 34). We further examined the possibility of a selective loss of desmin during the preparation and extraction in sample buffer. However, examination of residual material after extraction of aortic and venous tissues in 9.5 M urea buffer by another one-dimensional (31) or two-dimensional (32) gel electrophoresis did not show desmin material in the nonextracted protein fraction (data not shown). Likewise, cohomogenization and coextraction of cytoskeletal desmin from human or porcine uterus together with aortic tissue showed good recovery of the uterine desmin (Fig. 4). Desmin was not seen on two-dimensional gel electrophoresis of rat vena cava tissue (data not shown).

Immunofluorescence Microscopy. All cells of aortic and venous tissues, including the entire media of arteries and the aorta, were intensely stained with antibodies to vimentin (Fig. 5 *a* and *d–g*). Controls using various nonimmune sera (Fig. 5*c*) or antibodies to prekeratin (not shown) were negative. Antibodies to desmin, which strongly stained smooth muscle cells of various organs in the digestive, respiratory, and urogenital tract of avian and mammalian species (4, 21, 22), did not stain significantly the media of aorta and other arteries of man, cow, and rabbit (Fig. 5*b*); however, some faint staining with desmin antibodies was seen in aorta and arteries of rat (not shown) and considered to be significant because it was diminished after absorption of antibodies on purified desmin and resisted the treatment at 100 mM $MgCl_2$ (see ref. 22). This interspecies difference remains to be explained. Smooth muscle cells of digestive (e.g., muscularis mucosae), respiratory (e.g., trachea), and urogenital (e.g., myometrium) tract were not significantly stained with antibodies to vimentin and were positive after staining with antibodies to desmin (data not shown here; cf. refs. 4 and 8).

Characterization of Actins. Two-dimensional gel electrophoresis of aorta and vena cava proteins revealed three actin components (cf. Figs. 2 *a–c* and 3*b*), classified as α-, β-, and γ-actin (17, 27, 28, 38–41). Typical for all vascular smooth muscle tissues was the predominance of α-actin. This α-actin, however, was shown by amino acid sequence analysis to be different from the α-actins of skeletal and cardiac muscle and to represent a distinct species of smooth muscle α-actin (cf. refs. 27 and 28). By contrast, smooth muscle tissues of digestive tract and uterus showed a large proportion of γ-actin-like smooth muscle-specific actin, similar to the pattern in chicken gizzard (17, 27, 28). We determined the relative amounts of the different actins present in aortic tissues of three different species (man, cow, and rat) and from isolated vena cava of rat, using the electrophoretic separation of their amino-terminal tryptic peptides labeled by [^{14}C]carboxymethylation (for method and demonstration of separation of tryptic peptides of bovine aorta actin see refs. 27 and 28). Table 1 shows that all vascular smooth muscle tissue contained four different actins and that α-type smooth muscle actin was the predominant actin, with β-actin-like nonmuscle

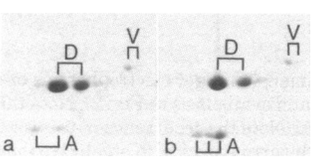

FIG. 4. Two-dimensional gel electrophoresis of proteins of extracted porcine uterus cytoskeleton alone (*a*) or added to rat aortic tissue (as shown in Fig. 3*a*) before homogenization in lysis buffer (*b*). Note the preservation of uterine desmin.

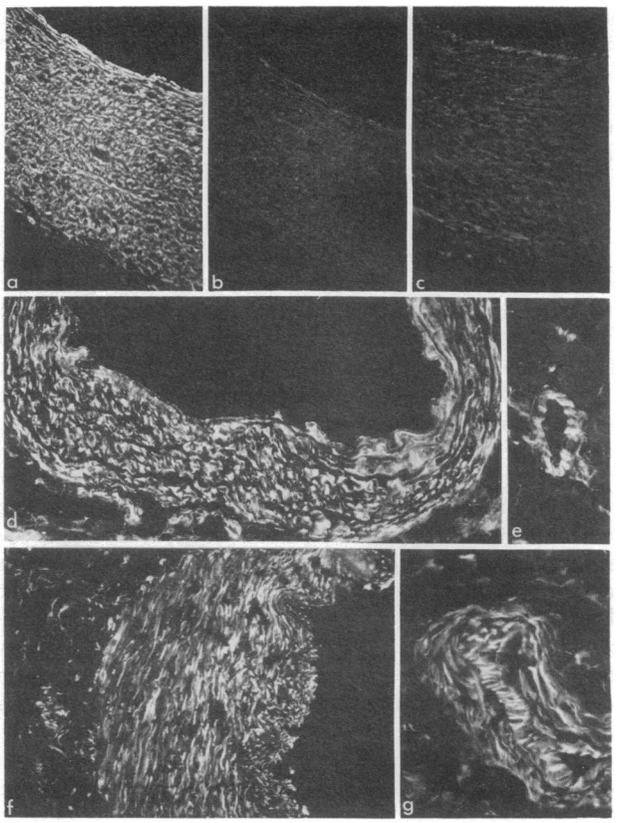

FIG. 5. Indirect immunofluorescence microscopy on frozen sections from rabbit aorta (*a–c*), rat aorta (*d*), coronary arteriole of rat (*e*), human coronary artery (*f*), and small artery of human uterus (*g*) after staining with antibodies to vimentin (*a*, *d–g*), antibodies to desmin (*b*) and preimmune serum (*c*). Strong staining with vimentin antibodies is observed in all regions of arterial walls, primarily in the media, and in connective tissue fibroblasts. (*a–c* ×120; *d–g* ×240.)

Table 1. Relative amounts of different actin types in various vascular smooth muscle tissues

Actin type	Human aorta (media)	Bovine aorta	Rat aorta	Rat vena cava
α smooth muscle	62	66	68	38
γ smooth muscle	4	9	4	14
β nonmuscle	31	18	24	36
γ nonmuscle	3	7	4	12

Results are given in percent of total actin and are based on the quantitation provided by the two-dimensional fingerprint system for separating [^{14}C]carboxymethylated tryptic peptides serving as marker peptides for the different actins (for details see refs. 27 and 28). Greek letters refer to the isoelectric classes (see text). Sarcomeric actins (α-actins) typical for skeletal and cardiac muscles were absent.

actin as the second most frequent actin present. While the relatively high amount of nonmuscle actin in vena cava of rat might simply reflect the lower proportion of smooth muscle cell mass in venous tissues, the high ratio (0.37) of γ-actin-like smooth muscle actin to α-actin-like smooth muscle actin, compared to aortic tissue (0.06), seems to indicate a true difference in actin composition between the two types of blood vessels.

DISCUSSION

Electron microscopy has shown that vascular smooth muscle cells, like other smooth muscle cells, contain abundant intermediate-sized filaments (42, 43). The results of this study using both gel electrophoresis and immunofluorescence microscopy show that the major intermediate filament protein present in vascular smooth muscle cells is vimentin—in contrast to smooth muscle cells of the digestive, respiratory, and urogenital tract, in which intermediate filaments of the desmin type are predominant if not exclusive (4, 5, 14–22). The presence of cytoskeletal filaments of the vimentin type in a muscle cell present *in vivo* has not been demonstrated previously, to our knowledge (the existence of some vimentin at the periphery of Z-disks of skeletal muscle as reported in ref. 12 has not been shown to be associated with intermediate filaments). Our data on vein and arterial smooth muscle of some species, including humans, do not provide any evidence for the existence of desmin in these cells. However, in view of the limitations of the methods used and of some immunologically desmin-related protein detected in arterial wall of the rat, we cannot exclude the presence of low levels of desmin in vascular tissue.

The abundance of vimentin allows one to distinguish vascular smooth muscle cells from the desmin-rich smooth muscle cells associated with the digestive, respiratory, and urogenital tracts and suggests a closer relationship to other mesenchymal cells such as fibroblasts that are also characterized by vimentin as the predominant, if not exclusive, intermediate filament protein (1–5). It has been shown that, when stimulated to move and contract under conditions of tissue remodeling, fibroblasts acquire morphological and functional characteristics of smooth muscle cells (myofibroblasts, ref. 44). Moreover, it has been shown that vimentin filaments appear in nonmesenchymal cells (epithelial and myogenic cells included) when grown in culture (2, 11). These observations may be related to the known proliferative and motile potential of vascular smooth muscle cells as observed in blood vessel differentiation and pathologic situations such as atherosclerotic plaque formation (45).

The biological functions of intermediate filaments are not known (for some hypotheses see ref. 5). The appearance, however, of two different types of predominating intermediate filaments in two groups of structurally and functionally closely similar types of cells—i.e., vascular and nonvascular smooth muscle cells—suggest that: (*i*) the presence of an extended system of vimentin filaments is compatible with the contractile functions of a smooth muscle cell, and (*ii*) the cytoskeletal functions that involve desmin filaments in smooth muscle of the digestive and urogenital tracts are served by vimentin filaments in vascular smooth muscle. This situation is reminiscent of that in myoepithelial cells, which are contractile and contain large amounts of intermediate filaments of the prekeratin type but no detectable desmin (9, 22).

Our data confirm and extend previous observations that two different muscle-type actins occur in smooth muscle cells (28): an α-type actin that is predominant in vascular smooth muscle (this study) and a γ-type that is the predominant form of actin in smooth muscle of digestive tract and uterus (28). As in other cases of demonstrated actin heterogeneity and specificity of gene expression in diverse types of muscle differentiations (27, 28, 38–41, 46), the functional meaning of the presence of different types of actin in different cell types remains unknown. The two forms of actin characteristic for the two types of smooth muscle cells, however, clearly distinguish smooth muscle differentiation from other myogenic differentiations (skeletal and cardiac muscle) as well as from those of other mesenchyme-derived nonmuscle cells. Furthermore, the great difference in the ratio of these two smooth muscle actins (α-type to γ-type) provides another means for distinguishing vascular and nonvascular smooth muscle cells.

The difference between vascular and nonvascular smooth muscle cells shown in this study are in concert with other observations of physiological and pharmacological differences between these two types of smooth muscle. We are hopeful that the demonstration of the conspicuous compositional differences in major cell proteins between vascular and nonvascular smooth muscle cells will help in understanding the biological functions of these tissues and the development of the pathological disorders of vascular smooth muscle cells.

We thank Dr. R. B. Low for critically reading the manuscript and Mr. M. Loehrke for help in tissue preparation. This work was supported in part by the Swiss National Science Foundation (Grant 3.445-079) and the Deutsche Forschungsgemeinschaft.

1. Bennett, G. S., Fellini, S. A., Croop, J. M., Otto, J. J., Bryan, J. & Holtzer, H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4364–4368.
2. Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5034–5038.
3. Lazarides, E. & Balzer, D. R. (1978) *Cell* **14**, 429–438.
4. Schmid, E., Tapscott, S., Bennett, G. S., Croop, J., Fellini, S. A., Holtzer, H. & Franke, W. W. (1979) *Differentiation* **15**, 27–40.
5. Lazarides, E. (1980) *Nature (London)* **283**, 249–256.
6. Franke, W. W., Weber, K., Osborn, M., Schmid, E. & Freudenstein, C. (1978) *Exp. Cell Res.* **116**, 429–445.
7. Sun, T.-T. & Green, H. (1978) *Cell* **14**, 469–476.
8. Franke, W. W., Appelhans, B., Schmid, E., Freudenstein, C., Osborn, M. & Weber, K. (1979) *Differentiation* **15**, 7–25.
9. Sun, T.-T., Shih, C. & Green, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2813–2817.
10. Hynes, O. R. & Destree, A. T. (1978) *Cell* **13**, 151–163.
11. Franke, W. W., Schmid, E., Winter, S., Osborn, M. & Weber, K. (1979) *Exp. Cell Res.* **123**, 25–46.
12. Granger, B. L. & Lazarides, E. (1979) *Cell* **18**, 1053–1063.
13. Bennett, G. S., Fellini, S. A., Toyama, Y. & Holtzer, H. (1979) *J. Cell Biol.* **82**, 577–584.
14. Lazarides, E. & Hubbard, B. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4344–4348.
15. Small J. V. & Sobieszek, A. (1977) *J. Cell Sci.* **23**, 243–268.
16. Cooke, P. (1976) *J. Cell Biol.* **68**, 539–556.
17. Izant, G. I. & Lazarides, E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1450–1454.
18. Bennett, G. S., Fellini, S. A. & Holtzer, H. (1978) *Differentiation* **12**, 71–82.
19. Fellini, S. A., Bennett, G. S., Toyama, Y. & Holtzer, H. (1978) *Differentiation* **12**, 59–69.
20. Campbell, G. R., Chamley-Campbell, J., Gröschel-Stewart, U., Small, J. V. & Anderson, P. (1979) *J. Cell Sci.* **37**, 303–322.
21. Franke, W. W., Appelhans, B., Schmid, E. & Freudenstein, C. (1979) *Eur. J. Cell Biol.* **19**, 255–268.
22. Franke, W. W., Schmid, E., Freudenstein, C., Appelhans, B., Osborn, M., Weber, K. & Keenan, T. W. (1980) *J. Cell Biol.* **84**, 633–654.
23. Lazarides, E. (1978) *Exp. Cell Res.* **112**, 265–273.
24. Gard, L. D. & Lazarides, E. (1980) *Cell* **19**, 263–275.
25. O'Connor, C. M., Balzer, D. R. & Lazarides, E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 819–823.
26. Franke, W. W., Schmid, E., Kartenbeck, J., Mayer, D., Hacker, H.-J., Bannasch, P., Osborn, M., Weber, K., Denk, H., Wanson, J.-C. & Drochmans, P. (1979) *Biol. Cell.* **34**, 99–110.
27. Vandekerckhove, J. & Weber, K. (1978) *J. Mol. Biol.* **126**, 783–795.
28. Vandekerckhove, J. & Weber, K. (1979) *Differentiation* **14**, 123–133.

29. Franke, W. W., Denk, H., Schmid, E., Osborn, M. & Weber, K. (1979) *Lab. Invest.* **40,** 207–220.
30. Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1979) *J. Cell Biol.* **81,** 570–580.
31. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
32. O'Farrel, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
33. Kelly, P. T. & Cotman, C. W. (1978) *J. Cell Biol.* **79,** 173–183.
34. Gard, D. L., Bell, P. B. & Lazarides, E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3894–3898.
35. Ross, R. & Klebanoff, S. J. (1971) *J. Cell Biol.* **50,** 159–171.
36. Steinberg, R. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 910–914.
37. Cabral, F. & Gottesman, M. M. (1979) *J. Biol. Chem.* **254,** 6203–6206.
38. Garrels, J. I. & Gibson, W. (1976) *Cell* **9,** 793–805.
39. Storti, R. V. & Rich, A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2346–2350.
40. Whalen, R. G., Butler-Browne, G. S. & Gros, F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2018–2022.
41. Rubenstein, P. A. & Spudich, J. A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 120–123.
42. Somlyo, A. P. & Somlyo, A. V. (1977) in *Excitation-Contraction Coupling in Smooth Muscle,* eds. Casteels, R., Godfraind, T. & Ruegg, J. C. (Elsevier/North-Holland, Amsterdam), pp. 317–322.
43. Chamley-Campbell, J., Campbell, G. R. & Ross, R. (1979) *Physiol. Rev.* **59,** 1–61.
44. Gabbiani, G., Chaponnier, C. & Hüttner, I. (1978) *J. Cell Biol.* **76,** 561–568.
45. Ross, R., Glomset, J., Kariya, B., Raines, E. & Bungenberg de Jong, J. (1977) in *International Cell Biology 1976–1977,* eds. Brinkley, B. R. & Porter, K. R. (Rockefeller Univ. Press, New York), pp. 629–638.
46. Hunter, T. & Garrels, J. I. (1977) *Cell* **12,** 767–781.