Proc. Natl. Acad. Sci. USA
Vol. 76, No. 8, pp. 3904–3908, August 1979
Cell Biology

Morphological revertants of an avian sarcoma virus-transformed mammalian cell line exhibit tumorigenicity and contain pp60^{src}

(RNA tumor virus/*in vitro* transformation/*src* gene product/*nude* mice)

A. F. LAU*, R. A. KRZYZEK*, J. S. BRUGGE†, R. L. ERIKSON†, J. SCHOLLMEYER‡, AND A. J. FARAS*

*Departments of Microbiology and ‡Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota 55455; and †Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80262

Communicated by Charlotte Friend, May 7, 1979

ABSTRACT **The biological and biochemical properties of Rous sarcoma virus-transformed and revertant field vole cells were investigated. Revertant vole cells appear morphologically similar to normal, uninfected cells, yet, like transformed vole cells, they are fully capable of growing in agar suspension and producing tumors in athymic *nude* mice. These highly tumorigenic, yet morphologically normal appearing, vole cells express viral-specific antigens such as the *gag* gene product (Pr76) but lack the *env* gene protein (gp85). Moreover, they contain the *src* gene protein, pp60^{src}. These results support the concept of the pleiotropic nature of the *src* gene product and in addition suggest that pp60^{src} may have multiple mechanisms of action. With this revertant cell system it may be feasible to distinguish between those biochemical functions of the *src* gene product that are important for tumorigenicity *in vivo* and those that are related to *in vitro* morphological transformation.**

The ability of Rous sarcoma virus (RSV) to transform avian and mammalian cells in culture and to induce tumors in animals has been localized to one of the four genes of the RSV genome, designated *src* (1). Restriction of expression of the RSV genome in infected cells, particularly the *src* gene, has been examined by comparative molecular studies of transformed cells expressing the *src* gene product (pp60^{src}) and their revertant counterparts that have lost the transformed phenotype and appear to be incapable of expressing this particular genetic sequence (2).

For the past several years, we have been studying a mammalian fibroblast cell line (European field vole) that can not only be transformed by RSV but, in some instances, has reverted morphologically to the normal phenotype (3, 4). One of the interesting aspects of this particular cell system is that the reversion phenomenon, unlike that of other transformed mammalian cells, appears not to reflect a consequence of either an alteration in, or the loss of, the *src* gene sequence or its transcription (3). This suggested that posttranscriptional changes must contribute to morphological reversion in these transformed vole cells. Moreover, viral-specific RNA, including *src* RNA, was detected in polyribosomes of revertant vole cells, suggesting that these viral mRNAs are actively translated in these cells. In this communication, we demonstrate that the viral proteins encoded for by these particular viral mRNAs, including pp60^{src}, are present in revertant vole cells. Moreover, we demonstrate that although the presence of pp60^{src} in these cells is not correlated with morphological transformation, it is correlated with tumorigenicity, because these normal-appearing vole cells are capable of producing tumors in athymic *nude* mice.

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.

MATERIALS AND METHODS

Cells. Normal, uninfected field vole (*Microtus agrestis*) fibroblasts, established as described (5), were infected by the Schmidt–Ruppin (SR) (subgroup D) strain of RSV. Several transformed cell lines (clones 1, 21, 22) were isolated by cloning in agar. After four passages in plastic dishes, revertant line 4 was subcloned from transformed line 21 (5). Subsequent recloning of line 4 resulted in revertant subclone 866. Although transformed clones 1 and 22 were employed in these studies because of their availability, more recent studies on transformed clone 21 indicated that it exhibits identical properties, including the presence of pp60^{src}.

Growth Properties. Doubling times were determined by seeding 2–3 × 10^5 cells per 100-mm tissue culture dish and counting the number of trypsinized cells from each of two dishes per group every 12 hr for 4–6 days by using a Coulter counter. Saturation density at confluency was determined by dividing the total number of cells per dish by 78.5 cm^2. Plating efficiencies on plastic, defined as the percentage of the number of cells plated that formed colonies (50–100 cells per colony), were determined 7–11 days after seeding 100 cells into 60-mm dishes. Growth-in-agar studies were performed according to the procedure of Macpherson and Montagnier (6). The number of colonies (>50 cells per colony) formed was determined on a portion of each 60-mm plate 7 days after plating 10^5 cells.

Tumorigenicity Studies. Female athymic *nude* mice (Swiss background) were maintained under strict pathogen-free conditions. Each animal was inoculated subcutaneously with 2 × 10^6 cells in 0.2 ml of sterile 0.85% NaCl and was observed for up to 6 months for tumor development.

Fluorescent Antibody Staining of Actin and Fibronectin. Normal, transformed, and revertant vole cells were grown to approximately 70% confluency on glass coverslips for the visualization of actin and fibronectin networks by indirect immunofluorescence (7). Cells employed for the actin localization studies were made permeable by a series of cold acetone washes [acetone/water (1:1) for 2 min, acetone for 5 min, acetone/water (1:1) for 2 min]. Rabbit antiactin and antifibronectin antibodies were purified by affinity chromatography and were judged >99% monospecific via immunoelectrophoresis (unpublished results). Primary antibodies were mixed with fluorescein isothiocynate-conjugated goat anti-rabbit IgG. Cells were photographed at ×830 with a Zeiss Universal photomicroscope equipped with epi-illumination.

Purification and Radioiodination of Virus Structural Proteins. Virion p27 was isolated from sucrose gradient-puri-

Abbreviations: RSV, Rous sarcoma virus; AMV, avian myeloblastosis virus; gp85, *env* gene protein; CEF, chicken embryo fibroblasts; pp60^{src}, *src* gene product; SR, Schmidt–Ruppin; ASV, avian sarcoma virus; Pr76, *gag* gene precursor polyprotein; RIA, radioimmunoassay.

fied Prague C virus by Sepharose CL-6B column chromatography in 6 M guanidine·HCl (8). Radioiodination ($Na^{125}I$, Amersham) of the p27 was performed by using chloramine-T (9). Radiolabeled product was 83% trichloroacetic acid-precipitable and had an approximate specific activity of 120 μCi/μg (1 Ci = 3.7×10^{10} becquerels). Monospecific goat anti-avian myeloblastosis virus (AMV) p27 serum was capable of precipitating 90% of the ^{125}I-labeled p27. SR-RSV-D *env* gene protein (gp85) was prepared by affinity chromatography on lentil lectin-conjugated Sepharose 4B (10) from sucrose gradient-purified virus. The gp85, radioiodinated in the same manner as p27, exhibited a specific activity of 3.5 μCi/μg and was 80% acid precipitable. Radioiodination of purified AMV reverse transcriptase was conducted according to a modification of the chloramine-T procedure as described by Panet *et al.* (11). Radiolabeled enzyme was 83% acid precipitable, was 66% precipitable by monospecific anti-AMV reverse transcriptase serum, and had a specific activity of approximately 0.40 μCi/μg.

Competition Radioimmunoassay (RIA). Virus structural proteins or cytoplasmic extracts from vole cells and RSV-D-infected chicken embryo fibroblasts (CEF) were prepared as described (11). RIAs consisted of phosphate-buffered saline, bovine serum albumin at 1 mg/ml, 0.1% Nonidet P-40, ^{125}I-labeled antigen (5,000–10,000 cpm), varying amounts of competing cellular cytoplasmic or viral structural protein, and sufficient amounts of the primary antiserum to precipitate the desired fraction of input radioactivity (30–50%). Twenty microliters of the appropriate secondary antiserum was used to precipitate the immune complexes. Radioactivity of the washed pellets was determined in a Beckman gamma counter. One hundred percent precipitation represents the amount of ^{125}I-labeled antigen precipitated in the absence of competing protein. Protein concentrations were determined by the Lowry assay (12) using bovine serum albumin as standard.

Analysis of pp60^{src}, p180, and *gag* Gene Precursor Polyprotein Pr76 from Vole Cells. Antiserum used for the detection of pp60^{src} was generated by inoculation of SR-RSV-D virus into newborn rabbits as described (13). Anti-p27 serum was obtained from adult rabbits that had been inoculated with p27 purified by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (14). [^{35}S]Methionine-labeled immunoprecipitates were prepared and analyzed on sodium dodecyl sulfate-containing 5–15% gradient polyacrylamide slab gels as described (13).

RESULTS

Biological Properties of Transformed and Revertant Vole Cells. A comparison of various growth parameters of normal, RSV-transformed, and revertant vole cells is given in Table 1. Revertant vole cells exhibited a population doubling time similar to that of normal cells (20–24 hr), whereas transformed vole cells demonstrated a faster growth rate (12–16 hr). The saturation density achieved by the revertant subclones was somewhat greater than that of the normal cells and was not significantly different from that of the transformed clones. However, due to decreased substratum adhesiveness of the transformed cells at high cell densities, it was not possible to determine an accurate saturation density for these cells, although values were probably greater than those shown in Table 1.

Transformed cell clones also appeared to be nearly twice as efficient at establishing colonies on plastic substratum compared with normal cells or revertant subclones (Table 1). Furthermore, although we have previously reported that revertant vole cells did not exhibit the capacity to grow in soft agar (3), more recent studies from our laboratory indicated that these cell clones could indeed grow in soft agar if sufficient numbers of cells (i.e., 10^5 cells per 60-mm dish) were employed during plating. Apparently, the lower concentrations of cells (10^3–10^4 cells per 60-mm dish) used in previous studies were inadequate to maintain the revertant cells in agar suspension. This factor, along with the slower growth rate of revertant cells which resulted in colonies that were considerably smaller than those formed by the transformed vole cells, contributed to our previous conception of the nature of the revertant cells.

Despite their morphologically normal appearance, the revertant vole cells were fully capable of inducing tumors in athymic *nude* mice (Table 1). Revertant cell tumors appeared within 7–11 days of inoculation, which was comparable to the 6- to 10-day period required for the appearance of transformed vole cell tumors. Tumors always appeared at the site of inoculation and rapidly grew until the animal died or was killed. Cells recovered from the tumors maintained the morphology of the cell type that was inoculated (unpublished observations). Normal vole cells, which do not grow in soft agar (at 10^5 cells per 60-mm dish), were incapable of producing tumors in *nude* mice even after 6 months of observation.

Both transformed and revertant cells released a proteolytic activity that activated plasminogen, resulting in the lysis of casein in an agar overlay assay (Table 2). The levels of proteolytic activity from transformed and revertant cell clones appeared to be similar to each other and were substantially higher than levels in normal vole cells.

The level of fibronectin and the organization of the actin cytoskeleton were also examined to determine whether they correlated with the observed morphologies of these cells (Fig. 1). The fibronectin extracellular matrix in revertant cells (Fig. 1 *B* and *C*) was somewhat similar to that present in normal vole cells (Fig. 1*A*). However, the level of this protein in transformed cells was markedly reduced (Fig. 1 *D* and *E*). Moreover, indirect immunofluorescence studies of the actin-containing microfilaments indicated that the cytoskeleton is highly organized

Table 1. Growth properties of normal, transformed, and revertant field vole cells

Property	Normal	Revertant subclones		Transformed clones	
		4	866	1	22
Morphology*	Flat	Flat	Flat	Rounded	Rounded
Growth pattern*	Ordered	Ordered	Ordered	Random	Random
Doubling time (hr)	20–24	20–24	20–24	12–16	12–16
Saturation density ($\times 10^5$/cm^2)	1.8	2.8	2.9	2.5	2.5
Plating efficiencies (%)					
Plastic	22.1	20.0	20.0	35.0	40.0
Soft Agar	0.0	2.8	4.2	31.3	3.1
Tumorigenicity†	0/8	6/8	6/6	8/9	5/6

* Cellular morphologies and growth patterns shown here are as described (3).

† Values in the table represent the number of animals developing tumors vs. the number of animals inoculated.

Table 2. Plasminogen activator released by normal, transformed, and revertant field vole cells

Cell type	Serum				
	Chicken	Calf	Human	Pig	Monkey
Normal	0	0	0	0	0/+
Revertant					
Subclone 4	++	+	++++	+	++++
Subclone 866	++	+	++++	+	++++
Transformed					
Clone 22	++++	+	++++	+++	++++
Clone 1	++	+	++++	+++	++++

Plasminogen activator released by vole cells was determined by the casein overlay assay (15). The extent of caseinolysis was measured visually on a scale of 0 to ++++: 0, no lysis; +, mottling; ++, 25% lysis; +++, 50% lysis; ++++, 100% lysis.

in both normal and revertant cells (Fig. 1 *A–C*), whereas no detectable organization of the cytoskeleton components was observed in the transformed vole cell lines (Fig. 1 *D* and *E*).

Presence of Viral Structural Protein Precursors in Transformed and Revertant Vole Cells. Retention of certain transformation-specific functions and the presence of polysomal 21S *src*-specific RNA in the revertant vole cells (16) provided the impetus for further investigation into the possible translation of viral RNA in these cells. Competition RIA with a radioiodinated viral structural protein, p27, as the tracer antigen showed that viral-specific 35S RNA, previously demonstrated to be present in the polysomes of revertant vole cells (16), was indeed translated in these cells (Fig. 2*A*). Furthermore, the concentration of competing material in the revertants was similar to that present in transformed vole cells. Although there was an approximate 5-fold concentration difference between transformed clone 1 and revertant subclone 4, the concentrations of competing protein in clones 22 (transformed) and 866 (revertant) were nearly identical. The concentration of viral structural proteins present in ASV-infected vole cells was 1/15th to 1/80th that in ASV-infected permissive chicken cells, an observation consistent with that of others (17, 18). In contrast to p27, when ^{125}I-labeled gp85 (D subgroup) was used as the tracer antigen in RIAs, no competition was observed with cytoplasmic protein from any of the cell lines (Fig. 2*B*), despite the high concentrations of competing protein used in the RIA (up to 10 mg per reaction). Finally, studies with ^{125}I-labeled reverse transcriptase indicated that similar concentrations of competing protein were present in transformed and revertant cells (Fig. 2*C*). Only about a 2-fold difference in concentration was observed between the cytoplasmic protein of transformed clone 22 and that of revertant clone 4.

Presence of *src* Gene Protein in Transformed and Revertant Vole Cells. The *src* gene product (pp60^{src}), which is a phosphorylated 60,000-dalton protein, was initially identified in RSV-transformed CEF and hamster cells by immunoprecipitation of [^{35}S]methionine-labeled cell lysates with serum from rabbits bearing RSV-induced tumors and analysis of the resultant precipitates on sodium dodecyl sulfate/polyacrylamide gels (13). By employing this procedure, a 60,000-dalton *src* gene-specific protein was also identified in RSV-transformed vole cells (Fig. 3, lanes 7 and 8). We have recently reported (19) that the peptide map of pp60^{src} from transformed vole cells, produced by chymotrypsin and *Staphylococcus aureus* V8 protease, is similar to that of pp60^{src} from RSV-transformed CEF, indicating that pp60^{src} from RSV-infected chicken and vole cells is identical. The revertant vole cells, which appear morphologically like normal, uninfected vole cells, yet exhibit

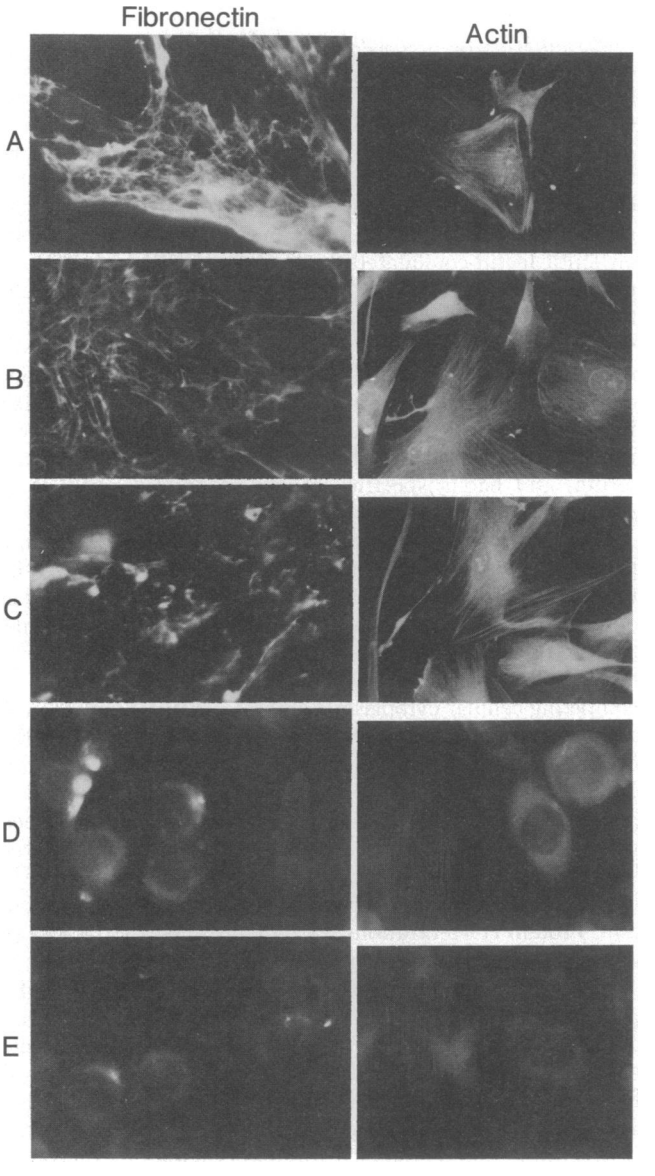

FIG. 1. Fluorescent antibody staining of fibronectin and actin in normal, transformed, and revertant field vole cells. The fibronectin extracellular matrix and actin cytoskeleton were localized as described in *Methods*. (*A*) Normal vole cells. (*B*) Revertant clone 866. (*C*) Revertant clone 4. (*D*) Transformed clone 22. (*E*) Transformed clone 1. (×830.)

similar concentrations of *src* gene-specific polysomal RNA (3, 4), also contained pp60^{src} (Fig. 3, lanes 9 and 10). In addition, the concentration of pp60^{src}, relative to other common bands, appeared to be similar in transformed and revertant cells. However, pp60^{src} was not detected either in total cell lysates from normal, uninfected vole cells (Fig. 3, lane 6) or when the lysates were precipitated with normal rabbit serum (Fig. 3, lanes 1–5).

Fig. 3 also indicated that the *gag* gene precursor polyprotein—Pr76—was present in immunoprecipitates prepared from both transformed and revertant vole cells with serum from tumor-bearing rabbits. Mature low molecular weight group-specific proteins, such as p27, were not detected in these cells despite the 2-hr radiolabeling period (Figs. 1 and 3; ref. 19), which is greater than that required for Pr76 cleavage in infected permissive cells (20). Pr76 was also precipitated from transformed vole clone 1 and SR-RSV-infected CEF with anti-p27

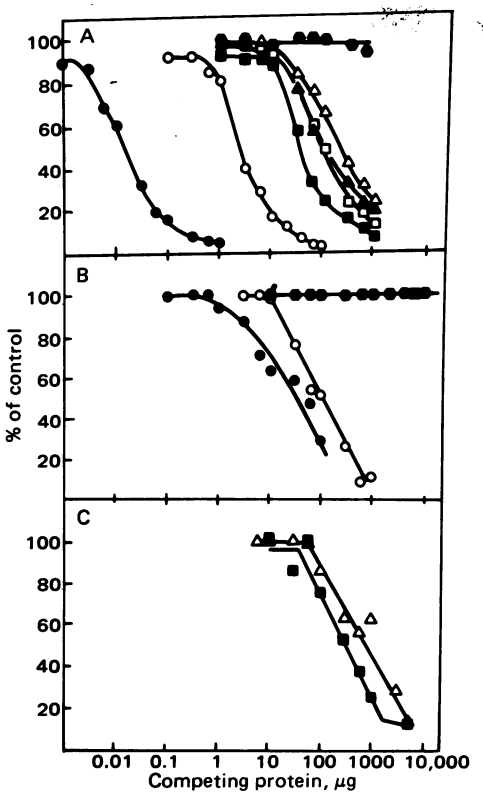

FIG. 2. Quantitation of avian sarcoma virus (ASV) proteins in transformed and revertant vole cells by competition RIA. (*A*) Quantitation of *gag* gene-specific protein. Competing cellular cytoplasmic and viral structural protein were from SR-RSV-D virus (●), RSV-D-infected CEF (○), transformed vole clone 1 (■), transformed vole clone 22 (□), revertant vole subclone 866 (▲), revertant vole subclone 4 (△), and uninfected vole cells (●). (*B*) Lack of gp85. Competing protein was from SR-RSV-D virus (●), cytoplasm of RSV-D infected CEF (○), cytoplasm of uninfected vole cells, transformed clones 1 and 22, and revertant subclones 4 and 866 (●). (*C*) Quantitation of reverse transcriptase-specific protein. Cytoplasmic protein was from transformed clone 22 (■) and from revertant subclone 4 (△).

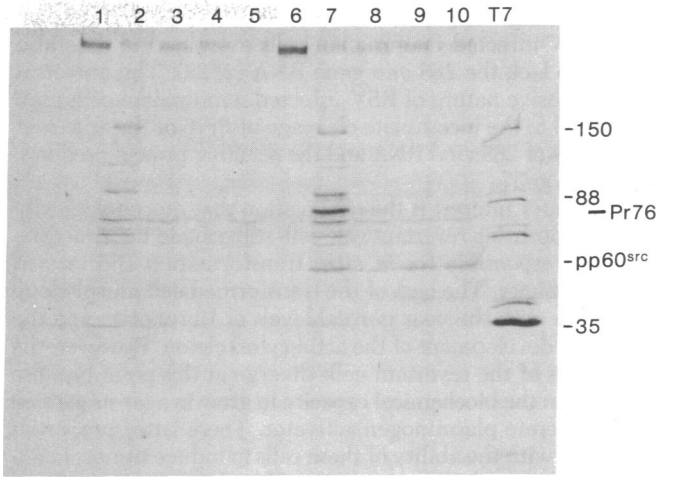

FIG. 3. Presence of pp60^{src} in normal, transformed, and revertant vole cells. The various cell lines grown on 100-mm culture dishes were labeled with 20 μCi of [^{35}S]methionine (New England Nuclear) per ml for 2 hr at 37°C and washed, and cell extracts were prepared as described (13). After immunoprecipitation with either normal rabbit serum (lanes 1–5) or serum from tumor-bearing rabbits (lanes 6–10), samples were subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Lanes: 1 and 6, normal vole cells; 2 and 7, transformed clone 1; 3 and 8, transformed clone 22; 4 and 9, revertant subclone 866; 5 and 10, revertant subclone 4. T7 virion proteins were included as size markers (in kilodaltons).

serum (Fig. 4, lanes 2 and 5). This precipitation was blocked by prior absorption of the antiserum with purified, disrupted RSV-D virus (Fig. 4, lanes 3 and 6). The greater concentration of competing protein found in ASV-infected CEF compared with ASV-infected vole cells, as determined by RIA (Fig. 2*A*), correlates with the amount of Pr76 immunoprecipitated in these cells (Fig. 4). P180, a *gag-pol* gene read-through product (21), was also detected in transformed clone 1 (Fig. 4, lane 5). This band was not detected when the anti-p27 serum was preabsorbed with RSV-D virus (Fig. 4, lane 6).

DISCUSSION

Results of this study further emphasize the unique nature of these ASV-transformed and revertant field vole cells. Unlike RSV-infected revertant hamster cells in which the levels of viral-specific RNA are markedly reduced (2), revertant vole cells contain concentrations of both viral RNA and viral structural protein similar to those of transformed vole cells. Although the production of virion structural proteins in ASV-transformed mammalian cells has been communicated (17, 18), this paper reports the presence of these products in infected yet morphologically normal-appearing cells. However, unlike permissive ASV-infected CEF, competition of vole cell cytoplasmic protein with ^{125}I-p27 is not due to mature p27 itself, but rather to Pr76. Eisenman *et al.* (18) have also observed a complete lack of cleavage of Pr76 in hamster cells infected by the Bryan strain of RSV. Reynolds *et al.* (17) have likewise reported an incomplete, low-level conversion of Pr76 to mature virion structural proteins in RSV-infected rat kidney cells. The lack of gp85 in transformed and revertant cells is consistent with our inability

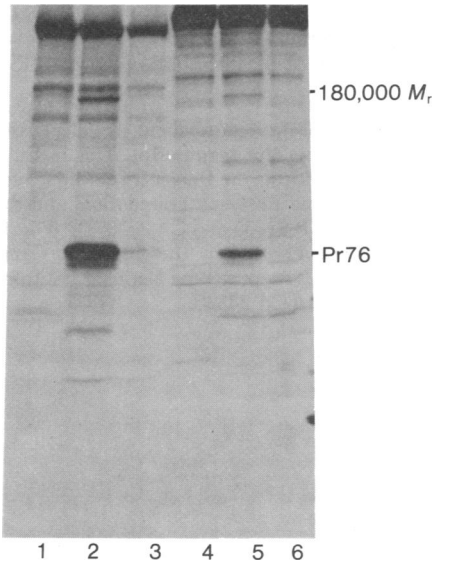

FIG. 4. Presence of P180 and Pr76 in SR-RSV transformed CEF and vole cells. Cells grown in 60-mm dishes were incubated in methionine-free Dulbecco's modified minimal essential medium for 30 min at 37°C. Cells were labeled with [^{35}S]methionine at 100 μCi/ml in phosphate-buffered saline for 15 min. Cells were washed, lysed, and immunoprecipitated with anti-p27 serum as described (13) and immunoprecipitates were electrophoresed. Cells and serum used are as follows. Lanes: 1, SR-CEF and normal rabbit serum; 2, SR-CEF and rabbit anti-p27 serum; 3, SR-CEF and anti-p27 serum preincubated with disrupted SR-RSV; 4, SR-RSV transformed vole clone 1 and normal rabbit serum; 5, transformed clone 1 and rabbit anti-p27 serum; 6, transformed clone 1 and anti-p27 preincubated with SR-RSV.

to detect *env* gene-specific 28S viral RNA in these cells (16). All other RSV-infected mammalian cells examined to date also appear to lack the 28S *env* gene RNA (2, 22). The universal nonpermissive nature of RSV-infected mammalian cells may be related to the incomplete cleavage of Pr76 or the apparent deficiency of 28S viral RNA and the resulting protein products, gp85 and gp37.

Of greater interest is the observation that morphologically normal-appearing revertant vole cells still contain the viral gene product responsible for *in vitro* transformation and *in vivo* tumorigenicity. The lack of the transformed cell morphology correlates with the near normal levels of fibronectin and the extensive development of the actin cytoskeleton. However, the properties of the revertant cells diverge at this point because they retain the biochemical capacity to grow in agar suspension and to secrete plasminogen activator. These latter properties correlate with the ability of these cells to induce tumors in animals. These results are consistent with previous reports (23, 24) that suggest that the fibronectin matrix and actin cytoskeleton are important for normal cellular morphology but can be dissociated from other biological properties such as growth in agar and *in vivo* tumorigenicity. Growth in agar has been found by others to be a reliable indicator of a cell's tumorigenic ability (25); however, plasminogen activator has recently been reported to be clearly dissociable from tumorigenicity (26). These studies described herein suggest that the biochemical effects of the *src* gene product that are involved in morphological changes are inconsequential to malignant conversion. Hence, this cell system may offer the means to distinguish between the biochemical functions of the *src* gene product that are responsible for *in vivo* tumorigenicity and those that are responsible for the morphological changes that accompany *in vitro* transformation. However, it is also conceivable that loss of the transformed cell morphology in the revertant cell may reflect a defect in a host gene product that interacts with the *src* gene protein to facilitate morphological conversion. This alternative may permit the identification of the cellular function(s) involved in morphological transformation of mammalian cells with RSV.

We thank Marianne Baxter for excellent technical assistance and Karen Vaughan for typing this manuscript. This investigation was supported in part by grants from the University of Minnesota Leukemia Task Force, the Leukemia Research Foundation, and the National Cancer Institute (NO1-CP-61055 and CA 26387). A.F.L. is currently a Postdoctoral Fellow supported by the American Cancer Society (Grant PF-1349). R.A.K. is supported by National Institutes of Health Young Investigator Award CA 23833.

1. Hanafusa, H. (1977) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. (Plenum, New York), pp. 401–483.
2. Deng, C.-T., Stehelin, D., Bishop, J. M. & Varmus, H. E. (1977) *Virology* **76**, 313–330.
3. Krzyzek, R. A., Lau, A. F., Faras, A. J. & Spector, D. H. (1977) *Nature (London)* **269**, 175–179.
4. Krzyzek, R. A., Lau, A. F., Vogt, P. K. & Faras, A. J. (1978) *J. Virol.* **25**, 518–526.
5. de La Maza, L. M., Faras, A. J., Varmus, H., Vogt, P. K. & Yunis, J. J. (1975) *Exp. Cell Res.* **93**, 484–487.
6. Macpherson, I. & Montagnier, L. (1964) *Virology* **23**, 291–294.
7. Ali, I. U., Mautner, V., Lanza, R. & Hynes, R.O. (1977) *Cell* **11**, 115–126.
8. Green, R. W. & Bolognesi, D. P. (1974) *Anal. Biochem.* **57**, 108–117.
9. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123.
10. Devare, S. G. & Stephenson, J. R. (1977) *J. Virol.* **23**, 443–447.
11. Panet, A., Baltimore, D. & Hanafusa, T. (1975) *J. Virol.* **16**, 146–152.
12. Lowry, P. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
13. Brugge, J. S. & Erikson, R. L. (1977) *Nature (London)* **269**, 346–348.
14. Erikson, E., Brugge, J. S. & Erikson, R. L. (1977) *Virology* **80**, 177–185.
15. Goldberg, A. R. (1974) *Cell* **2**, 95–102.
16. Krzyzek, R. A., Lau, A. F. & Faras, A. J. (1979) *J. Virol.* **29**, 507–516.
17. Reynolds, F. H., Hanson, C. A., Aaronson, S. A. & Stephenson, J. R. (1977) *J. Virol.* **23**, 74–79.
18. Eisenman, R., Vogt, V. M. & Diggelmann, H. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 1067–1075.
19. Brugge, J., Erikson, E., Collett, M. S. & Erikson, R. L. (1978) *J. Virol.* **26**, 773–804.
20. Vogt, V. M., Eisenman, R. & Diggelmann, H. (1975) *J. Mol. Biol.* **96**, 471–493.
21. Oppermann, H., Bishop, J. M., Varmus, H. E. & Levintow, L. (1977) *Cell* **12**, 993–1005.
22. Brugge, J. S., Purchio, A. F. & Erikson, R. L. (1977) *Virology* **83**, 27–33.
23. Willingham, M. C., Yamada, K. M., Yamada, S. S., Pouyssegur, J. & Pastan, I. (1977) *Cell* **10**, 375–380.
24. Pastan, I. & Willingham, H. (1978) *Nature (London)* **274**, 645–650.
25. Freedman, V. H. & Shin, S. (1974) *Cell* **3**, 355–359.
26. Wolf, B. A. & Goldberg, A. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4967–4971.