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Plasma Membrane Glycoproteins Encoded by Cloned Rauscher and Friend Spleen Focus-Forming Viruses

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Rauscher spleen focus-forming virus (SFFV) was cloned free of its helper virus into normal rat kidney and mouse fibroblasts, and the resulting nonproducer fibroblast clones were analyzed. Our results suggested that Rauscher SFFV encodes a glycoprotein with an apparent M_r of 54,000 (gp54) that reacts with antisera made to the envelope glycoprotein (gp70) of ecotropic murine leukemia viruses, as well as with a rat antiserum that reacts with the gp70's of dual-tropic mink cell focus-inducing and HIX viruses but not with the gp70's of ecotropic viruses. In these respects and in its tryptic peptide map, Rauscher SFFV-encoded gp54 is nearly identical to the gp55 glycoprotein which we previously reported to be encoded by Friend SFFV (Dresler et al., J. Virol. **30**:564-575, 1979). However, gp54 is slightly smaller, and it lacks one methionine-containing tryptic peptide that occurs in gp55. Studies with cytotoxic antiserum in the presence of complement and with a rosetting technique which employed sheep erythrocytes coupled to protein A suggested that the gp54 and gp55 glycoproteins are weakly expressed on the surface membranes of SFFV-infected cells. In addition, the Rauscher SFFV genome also encodes *gag* polyproteins which appear to be identical to the *gag* polyproteins encoded by helper Rauscher murine leukemia virus, but differ from the antigenically related polyproteins encoded by some but not all clones of Friend SFFV. Furthermore, the glycosylated *gag* polyproteins encoded by Rauscher SFFV and by some Friend SFFVs also appear to be expressed on the surface membranes of infected cells. These results suggest that similar *env* gene recombination and partial deletion events were involved in the independent origins of two different strains of acute erythroleukemia virus.

The independently isolated Friend and Rauscher erythroleukemia viruses cause similar diseases and consist of at least two components (8, 19, 37). One component is a replication-defective virus which is responsible for the rapid transformation of erythropoietic stem cells in vivo (1, 42) or in cell culture (7, 9). This component is called the spleen focus-forming virus (SFFV) because of its ability to induce discrete foci of transformed cells in the spleens of infected mice (1). The other component, lymphatic murine leukemia virus (MuLV), is a replication-competent helper virus which by itself causes lymphatic leukemia after a relatively long latent period (8). Recently, nonproducer fibroblasts infected with Friend SFFV (F-SFFV) have been isolated, and their properties have been analyzed (4, 11, 35, 45, 47). Although Rauscher SFFV (R-SFFV) has been investigated less thoroughly, nucleic acid hybridization studies have suggested that R-SFFV may be very similar to F-SFFV (45).

Analyses of nonproducer fibroblasts have demonstrated that the F-SFFV genome encodes a glycoprotein with an apparent M_r of 55,000 (gp55) that is immunologically and structurally related to Friend MuLV (F-MuLV) envelope glycoprotein gp70 (11, 39, 40). However, gp55 also has distinctive structural features that are absent from ecotropic virus gp70's, including antigens that occur in the gp70's of dual-tropic mink cell focus-inducing (MCF) and HIX viruses (25, 39, 40). These results are consistent with the nucleic acid hybridization evidence that F-SFFV is an *env* gene recombinant between ecotropic and xenotropic MuLV's (45, 46). This conclusion is especially intriguing because recently several other highly leukemogenic MuLV's were also found to be dual-tropic *env* gene recombinants (17, 18, 23, 46, 48, 49), and it has been proposed that such recombinant *env* genes or their encoded glycoproteins might be leukemogenic molecules (17, 23, 49). Furthermore, a small proportion of the gp55 made in cells (approximately 3 to 5% of the total) occurs on the cell surface membrane, and this portion contains more highly processed oligosaccharides and is slightly larger than the intracellular gp55 (11, 25, 39, 40). Finally, it has been reported that some but apparently not all F-SFFV genomes encode *gag*

polyproteins which contain p12 and p15 antigens (2, 4, 41, 45).

In this paper we describe the preparation and analysis of R-SFFV-infected nonproducer fibroblasts. Our results suggest that very similar *env* gene recombination events were independently involved in the origins of different acute erythroleukemia viruses.

MATERIALS AND METHODS

Cells and viruses. The F46 erythroleukemia cell line infected with Friend virus was kindly provided by W. Ostertag, Max Planck Institut fur experimentelle Medizin, Gottingen, West Germany. The Rauscher erythroleukemia cell line RVTCT 187GG (16) was provided by A. Fieldsteel, (Stanford Research Institute, Menlo Park, Calif.). The maintenance of these cell lines has been described previously (11, 16, 25). Sc-1 cells derived from a feral mouse embryo were provided under contract E-73-2001-NO 1 with the Special Virus-Cancer Program, National Institutes of Health, through the courtesy of J. Weaver (Cell Culture Laboratory, University of California School of Public Health, Oakland). The Sc-1 cells were maintained as a monolayer culture, as previously described (11).

Normal rat kidney (NRK) cells and the F-SFFV nonproducer NRK clone 1 (45) were obtained through the courtesy of D. Troxler, Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Md. NIH 3T3 cells were kindly provided by S. A. Aaronson, Laboratory of Tumor Virus Genetics, National Cancer Institute. The NRK and NIH 3T3 cells were maintained as monolayers in Dulbecco-modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% complement-inactivated fetal calf serum and antibiotics.

Friend leukemia virus, anemia strain (22), was obtained through the courtesy of C. Friend, Mount Sinai School of Medicine, City University of New York. This virus preparation was clarified by centrifugation at 12,000 × *g* for 10 min with an SS34 rotor in a Sorvall centrifuge. The virus was diluted 1:100 with culture medium, adjusted to 8 μg of polybrene per ml, and overlaid on Sc-1 cells for several hours. The supernatant was then removed, and the cells were grown to confluency in fresh medium containing polybrene.

F-MuLV clone B4, which has been described previously (33), was used for cell infections.

Virus assays. MuLV titers were measured by using a variation of the S^+L^- method of Bassin et al. (3, 11). SFFV was assayed by methods described previously (11).

Virus cloning. Virus was cloned by using a modification of previously described methods (11). A monolayer of NRK and NIH 3T3 cells was overlaid with culture medium from RVTCT 187GG erythroleukemia cells containing 8 μg of polybrene per ml. The cells were infected at an expected multiplicity of infection of 0.25, based on the S^+L^- assay. After 2 h the cells were trypsinized and diluted to 1 to 3 cells per ml, and 0.1 to 0.2 ml was seeded into each of the 96 wells of a Falcon Microtiter II dish. Wells containing a single cell colony were grown to form clonal cell lines. A total of 15 NRK cell clones and 15 NIH 3T3 cell lines were selected for analysis.

Radioactive labeling and extraction of cells. The Friend and Rauscher erythroleukemia cell lines were preincubated for 10 min in methionine-free minimal essential medium (GIBCO Laboratories) and then pulse-labeled for 2 h in methionine-free minimal essential medium supplemented with 50 μCi of L-[^{35}S]methionine per ml. After the labeling medium was removed, the labeled cells were lysed in a volume of immune precipitation buffer (20 mM Tris-hydrochloride [pH 7.5], 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 M NaCl, 1 mM EDTA, 0.2% sodium azide) equal to 20% of the volume of the cell culture medium that had been removed previously from the cells. The cell extracts were centrifuged at 140,000 × *g* for 35 min by using a Beckman type 65 rotor, and the supernatants were recovered.

Monolayer cultures were preincubated in methionine-free minimal essential medium for 10 min before labeling. Pulse-labeling was performed by adding methionine-free minimal essential medium supplemented with L-[^{35}S]methionine (50 to 250 μCi/ml) for 2 h. Cells were labeled with D-[^{3}H]glucosamine by adding the isotope directly to growing cultures (100 μCi/ml) for 7 h. The cells were then lysed as described above.

Immunoprecipitation procedure. The antisera made to Rauscher MuLV (R-MuLV) gp70, p30, and p12 have been described previously (13). The monospecific goat antibody to F-MuLV gp70 was generously provided by J. Collins, Duke University, Durham, N.C. A rat antiserum specific to gp55 was prepared as recommended by Ruscetti et al. (40). The resulting antiserum reacted with gp55 and with the gp70's of the dual-tropic MCF 247 and HIX viruses but not with ecotropic virus gp70's. Immunoprecipitation was performed by using a variation of the fixed *Staphylococcus aureus* (Pansorbin; Calbiochem-Behring Corp., La Jolla, Calif.) procedure described by Kessler (28). The Pansorbin was suspended (10%) in immune precipitation buffer and centrifuged through 1 M sucrose in immune precipitation buffer without NaCl before use. Lysates were preabsorbed with normal goat serum for 1 h at 4°C. Then 10 to 20 μl of Pansorbin per μl of serum was added for 1 h at 4°C.

Electrophoresis. Polyacrylamide (10 to 20%) gels containing sodium dodecyl sulfate were made in the Laemmli buffer system (31, 33). Antigen-antibody complexes were disrupted by incubating the samples in electrophoresis buffer at 100°C for 5 to 10 min. The bacteria were then pelleted by centrifugation, and the supernatants were carefully removed. All gels were processed by fluorography (5). ^{14}C-labeled molecular weight standards (New England Nuclear Corp., Boston, Mass.) were electrophoresed in parallel with the samples. The molecular weights of the standards were 92,500, 69,000, 46,000, 30,000, and 12,300.

Rosette assay for cell surface antigens. Sheep erythrocytes were coupled with *S. aureus* protein A (Sigma Chemical Co.) by the $CrCl_3$ method of Goding (21). Briefly, 50 μl of washed and sedimented erythrocytes was suspended at 25°C in 400 μl of 0.9% NaCl containing 25 μg of protein A. Then, 250 μl of 0.01% $CrCl_3$ which had been prepared as described previ-

ously (21) was slowly added with stirring. After 10 min, the suspension was diluted fivefold with phosphate-buffered saline (GIBCO Laboratories). The erythrocytes were then washed twice with phosphate-buffered saline and suspended in 3 ml of Dulbecco-modified Eagle medium; they were used within 3 days. For rosette analysis, fibroblast monolayer cultures in 25-cm^2 T-flasks were washed and then provided with 5 ml of fresh culture medium containing 10% heat-inactivated fetal calf serum. Cultures were then supplemented with either 2 to 4 μl of antiserum or the same volume of normal goat serum. After 15 min at 37°C, 0.2 ml of the coupled erythrocyte suspension was added to each culture. After 2 h at 37°C, the monolayers were washed with fresh culture medium and examined under a microscope. The antiserum to p30 used for rosetting was monospecific since the assay was blocked completely with 2 μg/ml of F-MuLV p30 that had been purified by phosphocellulose chromatography (43).

Complement- and antibody-dependent cytolysis. Killing of cultured fibroblasts by cytotoxic antibody in the presence of rabbit complement has been described previously (25). This killing was monitored microscopically by observing the rounding of the cells and the condensation of their nuclei, followed by the loss of their adherence to the substratum. In addition, this killing was analyzed quantitatively by measuring the release of incorporated [^{14}C]nicotinamide from the monolayer (25, 30) and the absorbance at 260 nm (A_{260}) which remained adherent to the monolayer as functions of time. Each of these techniques has proven very useful. However, as described previously (25), the rate and time of onset of cell death are highly dependent on the quantity of cell surface antigen. Thus, cells with only a small quantity of surface antigen are often only partially killed after a prolonged 20-h incubation, whereas cells with abundant antigen may be completely killed after 2 h. In our experience, techniques that involve measurement of radioactivity released from cells are less useful for measuring slow cell killing because the radioactivity often is slowly released, even from viable cells, and the sensitivity of the measurements therefore decreases with time. Therefore, for the sensitive detection of weak cell killing, we developed the cell adherence assay. For this assay, multiple cell monolayers in 25-cm^2 T-flasks prepared in parallel with the same cell inoculum are incubated with rabbit complement at 25°C as described previously (25) in the presence of 4 μl of either cytotoxic goat antiserum or normal goat serum. At least two flasks are treated with each serum. After 20 h, the monolayers are washed with phosphate-buffered saline (GIBCO Laboratories). The adherent cells are then dissolved in 1 ml of 0.1% sodium dodecyl sulfate, and the A_{260} is measured. The percentage of lifted cells is calculated as follows: [(A_{260} of flask treated with normal goat serum − A_{260} of flask treated with antibody) × 100]/(A_{260} of flask treated with normal goat serum).

Peptide maps. Tryptic peptide mapping of L-[^{35}S]methionine-labeled proteins was performed by a modification of previously described procedures (12). Radioactive proteins which had been separated by electrophoresis in a polyacrylamide gel were located by autoradiography, and the gel fragments containing these proteins were subsequently washed and trypsinized in situ as described by Elder et al. (12). The tryptic peptides were analyzed on CEL 300 thin-layer plates (20 by 20 cm; thickness 0.25 cm) (Brinkman). The chromatography dimension employed the developing solvent isoamyl alcohol-pyridine-water-ethanol-acetic acid (70:70:60:40:10). Electrophoresis in the second dimension was performed in 28% formic acid (29) at 150 V for the time required for the fast major dye component of acid fuchsin to migrate 15.5 cm (approximately 7 h). The dried thin-layer plates were processed for fluorography as described by Randerath (36).

RESULTS

R-SFFV encodes a gp54 glycoprotein. As shown in Fig. 1, lanes 2 and 4, extracts of L-[^{35}S]methionine-labeled Friend virus-infected cells contained several prominent radioactive components that precipitated with monospecific antiserum made to the R-MuLV envelope glycoprotein gp70. Two of these components, gp70 and $gPr90^{env}$ (the biosynthetic precursor of gp70 and p15E), have been well characterized and are known to be encoded by the MuLV genome (15,

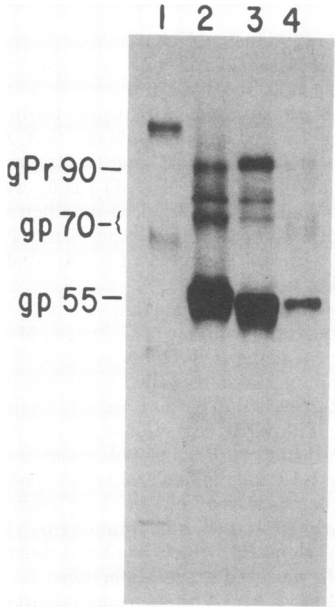

FIG. 1. *Electrophoretic analysis of gp70-related proteins in erythroleukemia virus-infected cells. F46 cells infected with Friend virus, RVTCT 187GG cells infected with Rauscher virus, and Sc-1 cells infected with an anemia-producing strain of Friend leukemia virus were pulse-labeled with [^{35}S]methionine for 2 h. Cellular lysates were immunoprecipitated with antiserum to F-MuLV gp70 and analyzed by electrophoresis on 10 to 20% polyacrylamide gradient gels containing sodium dodecyl sulfate, as described in the text. Lane 1, ^{14}C-labeled protein molecular weight standards; lane 2, F46 erythroleukemia cells; lane 3, RVTCT 187GG cells; lane 4, Sc-1 cells infected with an anemia-producing strain of Friend leukemia virus.*

27, 33, 51). Recently, it has been shown that the major component, gp55, is encoded by the F-SFFV genome (11, 39, 40). Similarly, components which electrophoresed slightly faster than gp55 (with an apparent M_r of 54,000) occurred in Rauscher erythroleukemia cells (Fig. 1, lane 3) and in fibroblasts infected with the anemia-inducing strain of Friend leukemia virus (lane 4). Virus released by these three cell lines caused splenomegaly and erythroleukemia in NIH Swiss female mice. In other studies we have found that these gp54 and gp55 glycoproteins can be readily labeled by incorporation of [^{3}H]-glucosamine and that they are precipitated by our rat antiserum that recognizes antigens on MCF 247 and HIX virus gp70's but does not react with ecotropic MuLV gp70's (see above).

To identify the virus component in the Rauscher virus complex that encodes gp54, we cloned the virus released from Rauscher erythroleukemia cells into NIH 3T3 and NRK fibroblasts at a low multiplicity of infection and examined the resulting fibroblast colonies for virus activities and gp54 synthesis. For example, Fig. 2 shows an analysis of the gp70-related proteins that were immunoprecipitated from [^{35}S]methionine-labeled cellular extracts of 15 of our NIH 3T3 clones. Three of these clones synthesized gp54 (Fig. 2, lanes 7, 12, and 16), whereas one clone contained the MuLV proteins gPr90, gp70, and p15E (lane 10). In agreement with our previous analyses of F-SFFV (11), only the virus regained from clones containing gp54 caused spleen enlargement and erythroleukemic foci.

Figure 3 shows a comparison of NIH 3T3 and NRK cell lines infected with F- and R-SFFVs. The R-SFFV- and F-SFFV-infected nonproducer fibroblasts contained similar glycoproteins (Fig. 3, lanes 3 through 5). However, the gp54 encoded by R-SFFV appeared to be slightly smaller than the gp55 encoded by F-SFFV. Tryptic peptide map analyses of these glycoproteins and of F-MuLV gp70 were highly reproducible (Fig. 4). The peptide maps of the R- and F-SFFV-encoded glycoproteins were nearly identical, except that the R-SFFV gp54 glycoprotein lacked one peptide (Fig. 4, arrows) that occurred in F-SFFV gp55. Moreover, this peptide also occurred in F-MuLV gp70 (Fig. 4) and in R-MuLV gp70 (data not shown). Therefore,

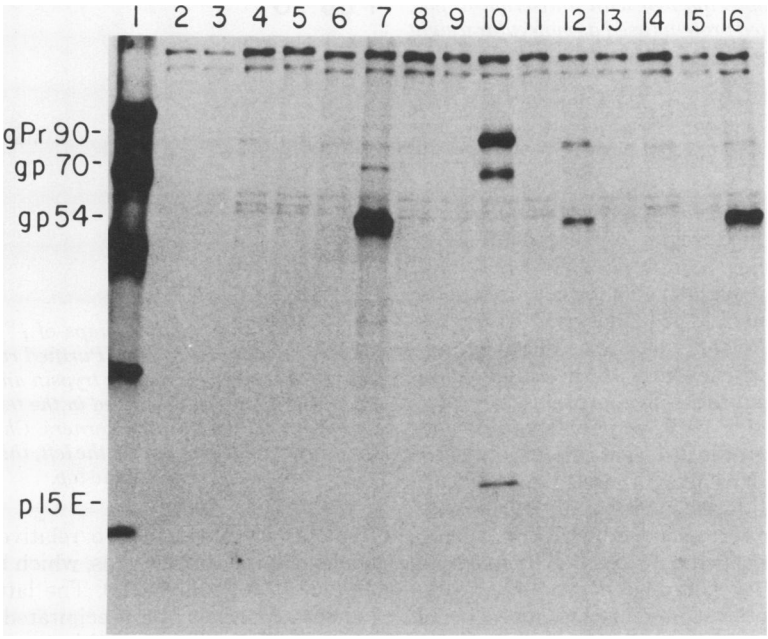

FIG. 2. *Electrophoretic analysis of gp70-related proteins in Rauscher virus clones. Rauscher virus released by the Rauscher erythroleukemia cell line RVTCT 187GG was cloned at a low multiplicity of infection on NIH 3T3 cells. A total of 15 of the resulting clones (designated RV-NIH clone 1 through RV-NIH clone 15) were pulse-labeled with [^{35}S]methionine, and the cellular lysates were immunoprecipitated with antiserum to gp70. The immunoprecipitates were analyzed by electrophoresis in 10 to 20% polyacrylamide gradient gels containing 0.1% sodium dodecyl sulfate. Lane 1, ^{14}C-labeled proteins used as molecular weight standards; lane 2, RV-NIH clone 1; lane 3, RV-NIH clone 2; lane 4, RV-NIH clone 3; lane 5, RV-NIH clone 4; lane 6, RV-NIH clone 5; lane 7, RV-NIH clone 6; lane 8, RV-NIH clone 7; lane 9, RV-NIH clone 8; lane 10, RV-NIH clone 9; lane 11, RV-NIH clone 10; lane 12, RV-NIH clone 11; lane 13, RV-NIH clone 12; lane 14, RV-NIH clone 13; lane 15, RV-NIH clone 14; lane 16, RV-NIH clone 15.*

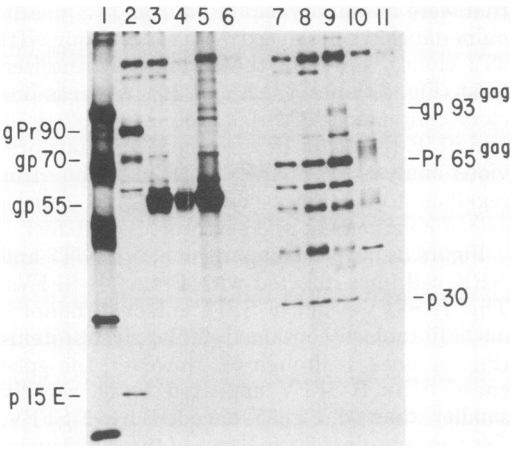

FIG. 3. *Electrophoretic analysis of gp70- and p30-related antigens in cloned Friend and Rauscher virus-infected cells. Rauscher virus- and Friend virus-infected cells were pulse-labeled with [^{35}S]methionine, and then cell extracts were immunoprecipitated with antisera to gp70 and p30. The immunoprecipitates were analyzed by electrophoresis in 10 to 20% polyacrylamide gradient gels containing 0.1% sodium dodecyl sulfate. Lanes 2 through 6 were immunoprecipitated with antiserum to gp70, and lanes 7 through 11 were immunoprecipitated with antiserum to p30. Lane 1, ^{14}C-labeled proteins used as molecular weight standards; lanes 2 and 7, R-MuLV-infected RV-NIH clone 9; lanes 3 and 8, R-SFFV-infected RV-NIH clone 6; lanes 4 and 9, R-SFFV-infected RV-NRK clone 15; lanes 5 and 10, F-SFFV-infected NRK clone 1; lanes 6 and 11, uninfected NRK cells.*

the structural difference appears to be localized to a small ecotropic region of the recombinant-type glycoproteins. As described elsewhere (40), there are also several [^{35}S]methionine-labeled peptides present in gp54 and gp55 which are absent from gp70 and vice versa. These peptide differences are consistent with the antigenic differences between these glycoproteins.

R-SFFV and F-SFFV differ in their *gag* genes. Although the different cell lines infected with cloned SFFVs all synthesized gp54 or gp55 (Fig. 3), they differed substantially in the polyproteins which were encoded by their *gag* genes. When we analyzed the 15 NIH 3T3 clones described above for p30-related proteins, we detected *gag* proteins only in the clones which contain either gp54 or gp70 (Fig. 2, lanes 7, 10, 12, and 16). Figure 3 shows an electrophoretic analysis of the p30-related antigens encoded by the different SFFVs. Whereas all of the R-SFFV-infected nonproducer cells which we isolated synthesized p30-related antigens that were indistinguishable from those encoded by R-MuLV (Fig. 3, lanes 7 through 9), the NRK clone 1 cells which were infected with F-SFFV

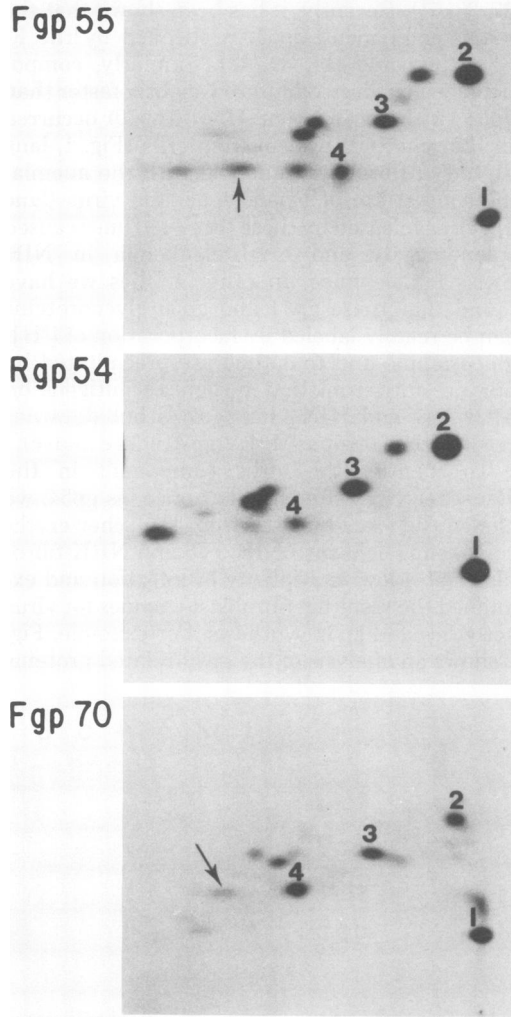

FIG. 4. *Tryptic peptide maps of [^{35}S]methionine-labeled viral glycoproteins. Purified radioactive glycoproteins were treated with trypsin and analyzed on thin-layer plates as described in the text. The origins were near the lower right corners. Chromatography in the first dimension was to the left; this was followed by electrophoresis toward the top.*

(lane 10) synthesized two relatively heterogeneous p30-related antigens, which had apparent M_r's of 50,000 and 70,000. The latter two components could also be precipitated with antiserum specific to p12. In addition, as shown below, the 70,000-molecular-weight component of NRK clone 1 cells (Fig. 3, lane 10) and the 93,000-molecular-weight component of R-SFFV-infected cells (lanes 8 and 9) were glycoproteins, as indicated by their labeling with [^{3}H]glucosamine. Furthermore, we have not been able to detect any *gag*-related proteins in our F-SFFV-infected nonproducer Sc-1 cell lines (unpub-

lished data). Therefore, this result and other evidence (2, 4, 41) suggest that different cloned SFFVs differ in the structure and expression of their *gag* genes.

Cell surface localization of SFFV *gag* and *env* products. Recently, it has been demonstrated by cell surface iodination and by genetic techniques that a small proportion (approximately 3 to 5%) of the gp55 encoded by F-SFFV is expressed on the surfaces of infected cells (11, 25, 40). Whereas the intracellular form of gp55 contains predominantly neutral oligosaccharides which bind tightly to concanavalin A-Sepharose columns, the cell surface molecules are slightly larger and more heterogeneous in size (apparent M_r, 58,000 to 68,000) and they contain large complex sialylated oligosaccharides (M. Ruta, D. Kabat, S. Kornfeld, and R. Kornfeld, unpublished data). Presumably because they contain more carbohydrate and because [^{3}H]glucosamine serves as an efficient precursor of sialic acids, the larger glycoproteins are labeled in a higher proportion with [^{3}H]glucosamine than with [^{35}S]methionine. Figure 5 shows an electrophoretic analysis of the two forms of gp54 and gp55 which occur in [^{3}H]glucosamine-labeled NRK cells infected with F-SFFV (Fig. 5, lane 2) and R-SFFV (lane 3). Although present in both cell lines, the larger putative surface membrane component occurred in a substantially higher proportion in the cells infected with F-SFFV. Figure 5 also shows the glycosylated *gag* proteins in the different SFFV-infected nonproducer cell lines.

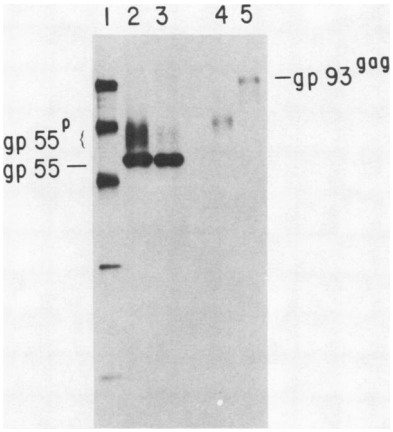

FIG. 5. *Electrophoretic analysis of glycoproteins encoded by cloned F- and R-SFFVs. F- and R-SFFV clones were pulse-labeled with [^{3}H]glucosamine. The lysates were immunoprecipitated with antisera to gp70 and p30. The immunoprecipitates were analyzed by electrophoresis in 10 to 20% polyacrylamide gradient gels containing 0.1% sodium dodecyl sulfate. Lane 1, ^{14}C-labeled proteins used as molecular weight standards; lane 2, F-SFFV-infected NRK clone 1 immunoprecipitated with antiserum to gp70; lane 3, R-SFFV-infected RV-NRK clone 15 immunoprecipitated with antiserum to gp70; lane 4, F-SFFV-infected NRK clone 1 immunoprecipitated with antiserum to p30; lane 5, R-SFFV-infected RV-NRK clone 15 immunoprecipitated with antiserum to p30.*

We obtained evidence for the cell surface localization of SFFV-encoded *gag* and *env* gene products by two techniques in addition to the iodination and genetic methods that have been described elsewhere (25, 32). In one technique, we employed sheep erythrocytes coated with *S. aureus* protein A in order to rosette fibroblasts containing immunoglobulin G bound to cell surface molecules. Figure 6 shows some results obtained with this technique, which demonstrated the surface membrane localization of F-SFFV-encoded gp55 and of MuLV-encoded gp70 and p30 antigens. The rosetting caused by antibody to p30 was completely blocked by purified p30. By this criterion, we also detected gp54, p30, and p12 antigens on the surfaces of R-SFFV-infected cells and p12 antigens on the surfaces of F-SFFV-infected NRK clone 1 cells. In addition, the NRK clone 1 cells appeared to contain a very small amount of surface membrane p30 antigens.

The second technique which we employed involved the killing of SFFV-infected fibroblasts with cytotoxic antisera in the presence of complement. This killing was monitored microscopically by observing the lifting of dead cells from the monolayer, by the [^{14}C]nicotinamide release assay (25, 30), and by measuring the cells remaining adherent to a tissue culture flask (see above). Table 1 shows typical results of the cell adherence assay. These results substantiated the rosette data and demonstrated the presence of both *gag* and *env* gene products on the surfaces of F-SFFV- and R-SFFV-infected nonproducer cells.

DISCUSSION

Proteins encoded by SFFVs. Our results are consistent with the nucleic acid hybridization studies which have suggested that R-SFFV and F-SFFV are similar to each other and are closely related to the dual-tropic MCF viruses which are *env* gene recombinants between ecotropic and xenotropic MuLV's (45, 46). However, these two SFFVs are clearly distinct, not only in certain aspects of pathogenicity (42) but also in genes and gene products (2, 4, 41, 45). For example, our results suggest that R-SFFV contains a recombinant *env* gene which is very similar but not identical to that of F-SFFV. The R-SFFV gp54 glycoprotein is slightly smaller than F-SFFV gp55, and it lacks one methionine-containing tryptic peptide which is common to the

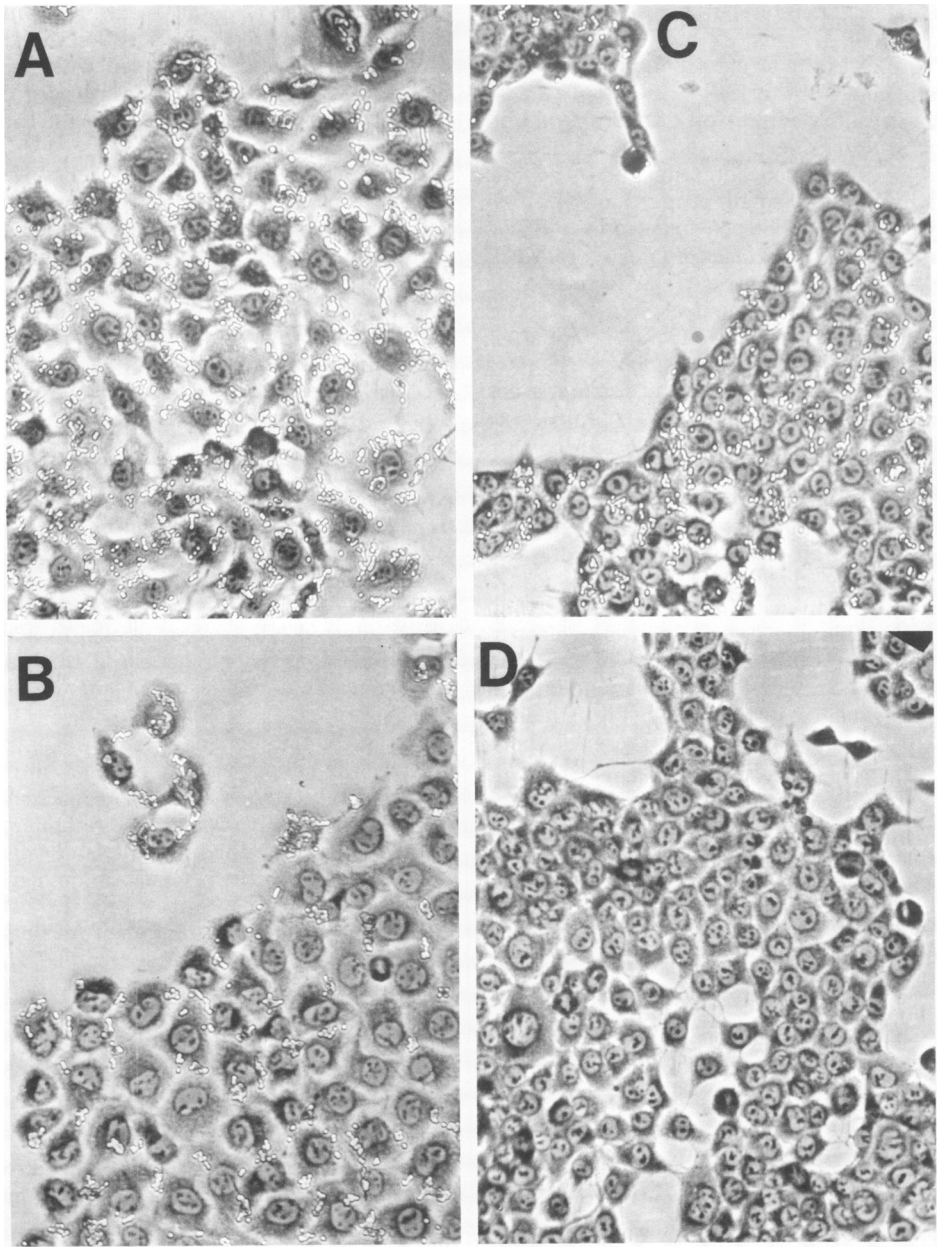

FIG. 6. *Erythrocyte rosette assay for detection of virus-coded cell surface antigens. The binding of erythrocytes coated with protein A to the fibroblast monolayers occurred only in the presence of specific antibodies, which reacted with molecules on the fibroblast surface membranes. (A) F-MuLV-infected (clone F12) NRK fibroblasts reacted with goat antibody to gp70. (B) Cells in (A) reacted with monospecific goat antibody to p30. (C) Rosetting of F-SFFV-infected NRK clone 1 nonproducer cells with goat antibody to gp70. (D) Cells in (C) reacted with nonimmune normal goat serum. The background binding of erythrocytes in the absence of specific antibody was very low. Attached erythrocytes are seen in (A), (B), and (C).*

F-SFFV gp55 and F-MuLV gp70 glycoproteins (Fig. 4). Therefore, we suggest that the structural difference between gp54 and gp55 may be situated in the region of the recombinant glycoproteins which is most closely related to the ecotropic parent. A similar glycoprotein appears to be encoded by an anemia-producing strain of Friend leukemia virus (Fig. 1).

Furthermore, there appear to be differences in the *gag* gene expression of different SFFVs.

TABLE 1. *Cell adherence assay for cell surface antigens*[a]

Expt	Cell line[b]	% of lifted cells[c]		
		Anti-gp70	Anti-p30	Anti-p12
1	F-MuLV-NRK	65		
	R-SFFV-NRK	42		
	NRK	0		
2	F-MuLV-NRK	78		
	F-SFFV-NRK	69		
	R-SFFV-NRK	33		
	NRK	3		
3	F-MuLV-NRK	80		79
	F-SFFV-NRK	77		70
	R-SFFV-NRK	68		57
	R-SFFV-NIH 3T3	69		34
	R-MuLV-NIH 3T3	89		91
4	F-MuLV-NRK		74	86
	F-SFFV-NRK		57	79
	R-SFFV-NRK			86
	NRK		1	0

[a] The cell adherence assay involved measurement of cells remaining attached to the fibroblast monolayer after 20 h of incubation at 25°C in the presence of rabbit complement and either a cytotoxic goat antiserum or normal preimmune goat serum. The adherent cells were measured as the A_{260} which was adherent to the washed monolayer, and measurements were made in duplicate. The cell adherence assay gave a minimum estimate of cell killing because some dead cells and materials adsorbing at 260 nm adhered to the plastic culture dish. A comparison with microscopic estimates suggested that 90% lifting of the A_{260} corresponded to 100% killing.

[b] The NRK fibroblast cell lines used were F12 MuLV-infected cells, clone 15 R-SFFV-infected cells, clone 1 F-SFFV-infected cells, and uninfected NRK cells. The NIH 3T3 fibroblasts used were R-MuLV clone 9 and R-SFFV clone 6.

[c] Percentages of lifted cells were calculated as follows: [(A_{260} of cultures treated with normal goat serum − A_{260} of cultures with antibody) × 100]/(A_{260} of cultures treated with normal goat serum).

For example, we detected *gag* gene products indistinguishable from those encoded by R-MuLV in R-SFFV nonproducer clones (Fig. 3 and 5). However, F-SFFV-infected nonproducer cells contained either smaller *gag* polyproteins (Fig. 3 and 5) or no detectable *gag* gene products, which is in general agreement with previous analyses (2, 4, 41). These results suggest that *gag* gene expression by SFFVs is highly variable and cannot be critical for viral pathogenesis.

Although we found p12- and p30-related antigens in the F-SFFV-encoded *gag* polyproteins with apparent M_r's of 50,000 and 70,000 (Fig. 3 and 5), p30 antigens have not been detected previously in these molecules by radioimmunoassays (2, 45). Presumably, these polyproteins only contain a small proportion of the antigenic sites which are present in complete p30. These few antigenic sites are apparently insufficient for detection by competitive radioimmunoassay but enable the polyproteins to be immunoprecipitated by an excess of our monospecific antiserum to p30. Consistent with this interpretation, it has been reported that Osborne-Mendel rats injected with F-SFFV-infected nonproducer NRK clone 1 cells produce antisera that react with ecotropic MuLV p30 (39).

Surface expression of SFFV proteins. Both humoral and cellular immune processes play a role in host response to tumor virus antigens (10, 20, 24, 34). For example, when mice and rats are inoculated with syngeneic cells containing cloned F-SFFV, both killer T-lymphocytes and SFFV-specific antibodies are elicited (20, 39, 40). In addition, some strains of mice contain lipid factors which specifically inactivate membranes containing xenotropic virus antigens (26). For these reasons, SFFV-encoded surface membrane molecules are likely to be important in pathogenesis.

In this context, it is interesting that the R-SFFV- and F-SFFV-encoded gp54 and gp55 glycoproteins are both weakly expressed on the surface membranes of infected cells (Table 1). However, we have consistently observed that R-SFFV gp54 is expressed relatively less compared with F-SFFV gp55 on the surfaces of infected NRK cells (Table 1) and that these R-SFFV-infected cells also produce only a relatively small amount of the larger sialylated glycoprotein components (Fig. 4) which have been detected previously on the surfaces of F-SFFV-infected cells by cell surface iodination (11, 40) and genetic techniques (25). These observations are consistent with the possibility that R-SFFV-encoded gp54 may be inherently more resistant than F-SFFV gp55 to the metabolic modifications that are required for glycoprotein processing into the plasma membrane. These metabolic modifications are known to include passage through the Golgi apparatus, where peripheral and terminal sugars, including sialic acids, are added onto complex oligosaccharides (38, 44, 51). In addition, our results indicate that *gag* antigens occur in the surface membranes of nonproducer cells infected with isolates of F-SFFV and R-SFFV that encode glycosylated *gag* polyproteins (Fig. 5 and Table 1).

Possible implications concerning SFFV oncogenesis. It has been proposed that the SFFVs may have derived from dual-tropic *env* gene recombinant MCF viruses which form during the process of leukemogenesis by F-MuLV and R-MuLV (49). Consistent with this idea, it has been reported recently that a replication-competent Rauscher MCF virus can cause erythroleukemic spleen foci in mice when it is coinjected with helper ecotropic MuLV (50). Thus, the ability to transform erythroid stem cells may be a property of certain replication-competent MCF viruses which encode recom-

binant gp70's and which contain intact *gag* and *pol* genes. Our results support these ideas since R-SFFV encodes an envelope glycoprotein antigenically related to MCF gp70's, *gag* polyproteins indistinguishable from those of R-MuLV (Fig. 3), and enzymatically active reverse transcriptase (unpublished data). In contrast, partial deletions of *gag* and *pol* genes occur in F-SFFV (Fig. 3 and 5) (4, 6, 14, 41). The encoding of similar gp54 and gp55 glycoproteins by two independently derived SFFVs which differ elsewhere in their genomes suggests that their *env* genes or closely linked genetic information may be important for leukemogenicity. We are currently studying the leukemogenic role of these glycoproteins by isolation and analysis of F-SFFV mutants with defects in gp55 processing (25).

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