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Subcellular Localization of the *env*-Related Glycoproteins in Friend Erythroleukemia Cells

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A scheme was developed for the subcellula fractionation of murine erythroleukemia cells transformed by Friend leukemia virus. The subcellular localization of the *env*-related glycoproteins was determined by immune precipitation with antiserum against gp70, the envelope glycoprotein of the helper virus, followed by gel electrophoresis. In cells labeled for 2 h with [^{35}S]methionine, the glycoprotein encoded by the defective spleen focus-forming virus, gp55^{SFFV}, was found primarily in the nuclear fraction and in fractions containing dense cytoplasmic membranes such as endoplasmic reticulum. A similar distribution was noted for gp85^{env}, the precursor to gp70. The concentration of viral glycoproteins in the nuclear fraction could not be accounted for by contamination with endoplasmic reticulum. In pulse-chase experiments, neither glycoprotein underwent major redistribution. However, labeled gp85^{env} disappeared from intracellular membranes with a half-time of 30 min to 1 h, whereas labeled gp55^{SFFV} was stable during a 2-h chase. In plasma membrane preparations with very low levels of contamination with endoplasmic reticulum, gp70 was the major viral *env*-related glycoprotein detected; a minor amount of gp55^{SFFV} and no gp85^{env} could be detected. The unexpected result of these experiments is the amount of viral glycoproteins found in the nuclear fraction. Presence of viral proteins in the nucleus could be relevant to the mechanism of viral leukemogenesis.

The Friend murine leukemia virus (MLV-F) complex consists of at least two viruses, a highly oncogenic replication-defective spleen focus-forming virus (SFFV) and a less oncogenic helper virus (discussed by Troxler et al. [31]). SFFV is responsible for rapid leukemogenic transformation of erythroid precursor cells. SFFV may be a recombinant in the *env* gene between the MLV-F helper virus and a xenotropic murine leukemia virus (30). Consistent with this genome structure is the synthesis by SFFV of a glycoprotein, gp55^{SFFV}, which shares antigenic determinants and peptides with the helper virus envelope glycoprotein, gp70, and with the gp70 of recombinant mink cell focus-inducing virus and which also contains some unique peptides (6, 23). Although some strains of SFFV synthesize proteins related to virion core (*gag*) proteins, gp55^{SFFV} appears to be the only protein common to all strains and thus is probably involved in leukemogenic transformation (10, 24). Furthermore, in studies with molecularly cloned subgenomic fragments of SFFV, oncogenicity was associated with fragments coding for gp55^{SFFV} and lacking the SFFV *gag* gene (D. L. Linemeyer, S. K. Ruscetti, E. M. Scolnick, L. H. Evans, and P. H. Duesberg, Proc. Natl. Acad. Sci. U.S.A., in press).

gp55^{SFFV} is detectable on the cell surface (but not in virions) by immunological techniques and by surface iodination (10, 23–25). However, most of the surface-labeled gp55^{SFFV} differs from the majority of pulse-labeled gp55^{SFFV} in apparent molecular weight and extent of sialidation (10, 23, 24), suggesting that much of the gp55^{SFFV} may be confined to intracellular membranes. We investigated the subcellular distribution of *env*-related glycoproteins by subcellular fractionation techniques and pulse-chase experiments. The results are consistent with the rapid association of gp55^{SFFV} with the nuclear membrane and endoplasmic reticulum, with little redistribution after this initial association.

MATERIALS AND METHODS

Cells. A cell line established from a murine erythroleukemia induced by MLV-F (GM86, clone 745A) was originally obtained from the Mammalian Genetics Mutant Cell Repository, Camden, N.J. Cells were grown in Dulbecco modified Eagle medium with 10% fetal calf serum. Eveline cells were obtained from A. Langlois, Duke University, Durham, N.C.

Isotopic labeling of cells. Cells in the late log phase of growth (approximately 10^8 cells at 10^6 cells/ml) were washed twice with Dulbecco phosphate-buffered saline and then suspended in 6 ml of medium lacking methionine and incubated 10 min at 37°C.

Cells were sedimented and suspended in 4 ml of methionine-deficient medium containing [^{35}S]methionine (100 μCi/ml, >400 Ci/mmol; New England Nuclear Corp.) and 10% dialyzed fetal calf serum. Incubations were carried out at 37°C for various lengths of time. After a pulse-label, cells were sedimented, either washed twice with ice-cold saline or suspended in medium containing normal levels of methionine, and incubated further at 37°C (pulse-chase) before being washed twice with saline.

Cell fractionation. All procedures were carried out at 0 to 4°C. One-third of the labeled cells were extracted directly for immune precipitation as described below. The remainder were suspended in 10 mM Tris (pH 7.4), 10 mM NaCl, and 1.5 mM $MgCl_2$ (reticulocyte standard buffer) at 2×10^7 to 3×10^7 cells/ml, allowed to swell for 15 min, and then disrupted in a Dounce homogenizer. Cell breakage was monitored by phase microscopy and was always >95% (usually 20 to 30 strokes of the pestle). Less than 10% of nuclei were disrupted. The crude lysate was centrifuged at 250 × *g* for 10 min. The pellet was suspended in one-half its original volume of reticulocyte standard buffer and layered over 45% sucrose (wt/wt in buffer) and centrifuged at 1,600 × *g* for 30 min. The pellet contained the nuclei. The two resulting supernatants were centrifuged separately at 100,000 × *g* for 1 h, and the pellet fractions were suspended in one-half their original volume of reticulocyte standard buffer. A 1-ml portion was layered over a discontinuous sucrose gradient consisting of 1.5 ml each of 55, 45, 40, 35, 29, and 20% sucrose (wt/wt in 1 mM Tris, pH 7.4) and centrifuged for 2 h at 35,000 rpm in an SW41 rotor. Alternatively, a 0.5-ml portion was centrifuged on a similar gradient containing 0.7 ml of each sucrose concentration and centrifuged for 1.67 h at 45,000 rpm in an SW50L rotor. Visible bands were collected, suspended in 10 mM Tris, and pelleted at 100,000 × *g* for 1 h.

Plasma membranes were also isolated by attachment to polycationic polyacrylamide beads as described (3). Briefly, cells were allowed to attach to Affi-gel 731 beads (Bio-Rad Laboratories) in isotonic sucrose-acetate buffer and washed gently to remove unattached cells. The cells were lysed in 10 mM Tris buffer and washed to remove released intracellular contents.

Immune precipitation. Goat antiserum against gp70 of MLV-F was the generous gift of Dani Bolognesi, Duke University. Goat antiserum against disrupted MLV-F virions and goat antiserum against p30 of Moloney MLV were provided through the courtesy of John Cole, Biological Carcinogenesis Branch, National Cancer Institute. Membranes or intact cells were suspended in ice-cold RIPA buffer (10 mM Tris, pH 7.4, 0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM phenylmethylsulfonyl fluoride, 10 mg of bovine serum albumin per ml, 100 kallikrein units of aprotinin per ml [Sigma Chemical Co.], and 1 mM methionine). Extracts of cells and nuclei were centrifuged at 140,000 × *g* for 30 min. A 100-μl portion of RIPA extract was incubated with 10 μl of antiserum for 2 h at 4°C and then with 20 μl of protein A-Sepharose (100 mg per ml of 10 mM Tris; Sigma) for 1 h at 4°C with constant gentle agitation. Both antiserum and protein A-Sepharose were in sufficient excess to precipitate the maximum amount of proteins from an equivalent extract of unfractionated cells. Precipitates were washed six times with 0.5 ml of RIPA buffer without albumin or aprotinin. Precipitates were disrupted and analyzed by SDS-polyacrylamide gel electrophoresis as described (14) in 7.5% polyacrylamide gels unless otherwise noted. Gels were processed for fluorography (1) by using Kodak SB-5 film.

Enzyme assays. Na^+-K^+ ATPase was assayed according to Wallach and Kamat (32). Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome *c* reductase and succinate-cytochrome *c* reductase were assayed according to Sottocasa et al. (27). Protein measurement was according to Lowry et al. (17).

Iodination of membrane proteins. Proteins were solubilized from membranes with the mixture of detergents used for immune precipitation and were reacted with ^{125}I (500 μCi/100 μg of protein) in the presence of 25 μg of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce Chemical Co.) as described (18). The extracts were subjected to immune precipitation and gel electrophoresis. Autoradiography of the gels was enhanced by using intensifying screens.

Electron microscopy. Pellets were combined with 2.5% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate. Primary fixation for 30 min was followed by a wash in three changes of the same buffer and a secondary fixation in phosphate-buffered 1% osmium tetroxide. The samples were dehydrated through a graded series of ethanols and embedded in Epon 812. Thin sections obtained with a diamond knife in a Sorvall MT-2B ultramicrotome were collected on carbon-stabilized, Formvar-coated copper grids. Thin sections obtained were stained with lead citrate and uranyl acetate before viewing in a Philips EM-400 electron microscope.

RESULTS

The GM-86 cells chosen for this study were derived from a MLV-F-induced erythroleukemia. Cells of this type express high levels of gp55^{SFFV} and relatively low levels of helper virus (20, 21). The fractionation procedures used were similar to those used in other virus-cell systems (4, 9, 12, 15, 33) except that the nuclei were further purified by sedimentation through 45% (wt/wt) sucrose (11). Enzymatic markers for cellular membranes included Na^+-K^+ ATPase for plasma membrane (32), NADPH-cytochrome *c* reductase for endoplasmic reticulum, and succinate-cytochrome *c* reductase for mitochondria (27). Table 1 presents the distribution of protein and enzymatic activities among the subcellular fractions obtained. A significant fraction of the markers for plasma membranes, endoplasmic reticulum, and mitochondria was found in the pellet of the initial low-speed centrifugation. These activities could be largely sep-

TABLE 1. *Fractionation of GM-86 cells*

Fraction	Sedimentation,[a] 250 × *g*	Interface[a] (% sucrose)	Nuclei[b] (%)	Protein		Na^+-K^+ ATPase[c]		NADPH-cytochrome *c* reductase[d]		Succinate-cytochrome *c* reductase[d]	
				mg	%	Sp act (increase)[e]	%	Sp act (increase)	%	Sp act (increase)	%
Supernatant, 250 × *g*			1.5	10.2	71.9	0.11	70.5	0.037	82.6	0.067	88.0
Pellet, 250 × *g*			75	3.98	28.2	0.12	29.4	0.020	17.4	0.023	12.0
Nuclear pellet			40	1.60	11.3		<1	0.014(0.4)	6.0	ND[f]	
2′	Pellet	40/45		0.031	0.2		<1	0.135(4.0)[g]	0.9	ND	
3′		35/40		0.058	0.4	0.39(3.5)	1.4	0.075(2.2)	0.9	ND	
4′		29/35		0.043	0.3	2.20(20)[g]	5.9	0.076(2.2)	0.7	ND	
1	Supernatant	45/55		0.164	1.2		<1	0.079(2.3)	2.8	0.274(4.4)	5.8
2		40/45		0.659	4.7		<1	0.115(3.4)[g]	16.5	0.368(5.9)[g]	31.5
3		35/40		0.337	2.4	0.74(6.7)	15.6	0.104(3.1)[g]	7.7	0.179(2.9)	7.9
4		29/35		0.329	2.3	1.51(13.7)[g]	31.1	0.071(2.0)	5.1	0.025(0.4)	1.1
5		20/29		0.233	1.65	0.54(4.9)	7.9	0.048(1.4)	2.4		<1
Total recovery[h] (%)					24.5		61.9		43.4		46.3

[a] A crude lysate of GM-86 cells was centrifuged at 250 × *g* for 10 min. The pellet was suspended and centrifuged over 45% sucrose at 1,600 × *g* for 30 min (nuclear pellet). Membranes from the two supernatants were centrifuged separately on discontinuous sucrose gradients. The fractions at the interfaces were numbered starting from the bottom, with a prime indicating fractions derived from the pellet of the first centrifugation (250 × *g*).

[b] Nuclei were counted in a hemacytometer and are expressed of percentage of nuclei present in this crude lysate.

[c] Expressed as micromoles of ATP hydrolysed per hour per milligram of protein.

[d] Expressed as micromoles of cytochrome *c* reduced per minute per milligram of protein.

[e] Ratio of specific activity in the membrane fraction to that in the crude lysate.

[f] ND, Not determined.

[g] Fractions containing a high concentration of a particular marker enzyme.

[h] Sum of the activities of the nuclear pellet and sucrose gradient fractions as a percentage of the activity of the crude lysate.

arated from nuclei by pelleting the nuclei through 45% sucrose. The properties of cytoplasmic membranes recovered in the pellet from the low-speed centrifugation (2′, 3′, and 4′) did not differ greatly from those of corresponding membranes obtained from the supernatant (2, 3, and 4, respectively; Table 1). Therefore, in some fractionations, the low-speed pellet and supernatant membranes were effectively combined by omitting the initial low-speed centrifugation and centrifuging the crude lysate over 45% sucrose. The activity of the plasma membrane marker was concentrated at the 29/35% sucrose interface (fractions 4 and 4′). The endoplasmic reticulum marker either was distributed evenly (two experiments) between the 35/40% and 40/45% interfaces (fractions 3, 3′, 2, and 2′) or could be found concentrated at one interface or the other (three experiments) (e.g., fraction 2 in Table 1). This heterogeneity may reflect variable factors such as the content of ribosomes in rough endoplasmic reticulum. The mitochondrial marker was concentrated at the 40/45% interface (fractions 2 and 2′). The distribution of marker enzymes was consistent with electron microscopic observations in which mitochondria and rough microsomes were found at the 40/45% interface, both smooth and rough microsomal membranes were found at the 35/40% interface, and smooth membranes were found at the 29/35% interface (data not shown). The nuclear fraction was essentially free of endoplasmic reticulum (Fig. 1). The residual NADPH-cytochrome *c* reductase activity in the nuclear fraction was probably due to the presence of this enzyme in nuclear membranes (11).

Figure 2 illustrates the distribution of viral *env*-related proteins among the subcellular fractions described in Table 1. Table 2 shows the distribution of *env*-related glycoproteins in two experiments quantitated by densitometry. Cells were incubated in the presence of [^{35}S]methionine for 2 h and then fractionated. Each fraction was extracted with detergents, and the *env*-related proteins were immune precipitated with antiserum against gp70, the envelope glycoprotein of the MLV-F helper virus, and analyzed by SDS-gel electrophoresis. The identity of the proteins had been established previously (6, 20, 21, 23) and was confirmed by the observation that they were not precipitable with anti-p30 (Fig. 2, lane A). Some $gp55^{SFFV}$ was found in the light-density membrane fractions containing plasma membranes (4, 5, and 4′), as expected (10). However, most of the $gp55^{SFFV}$ was either in the dense cytoplasmic membrane fractions (2, 2′, 3,

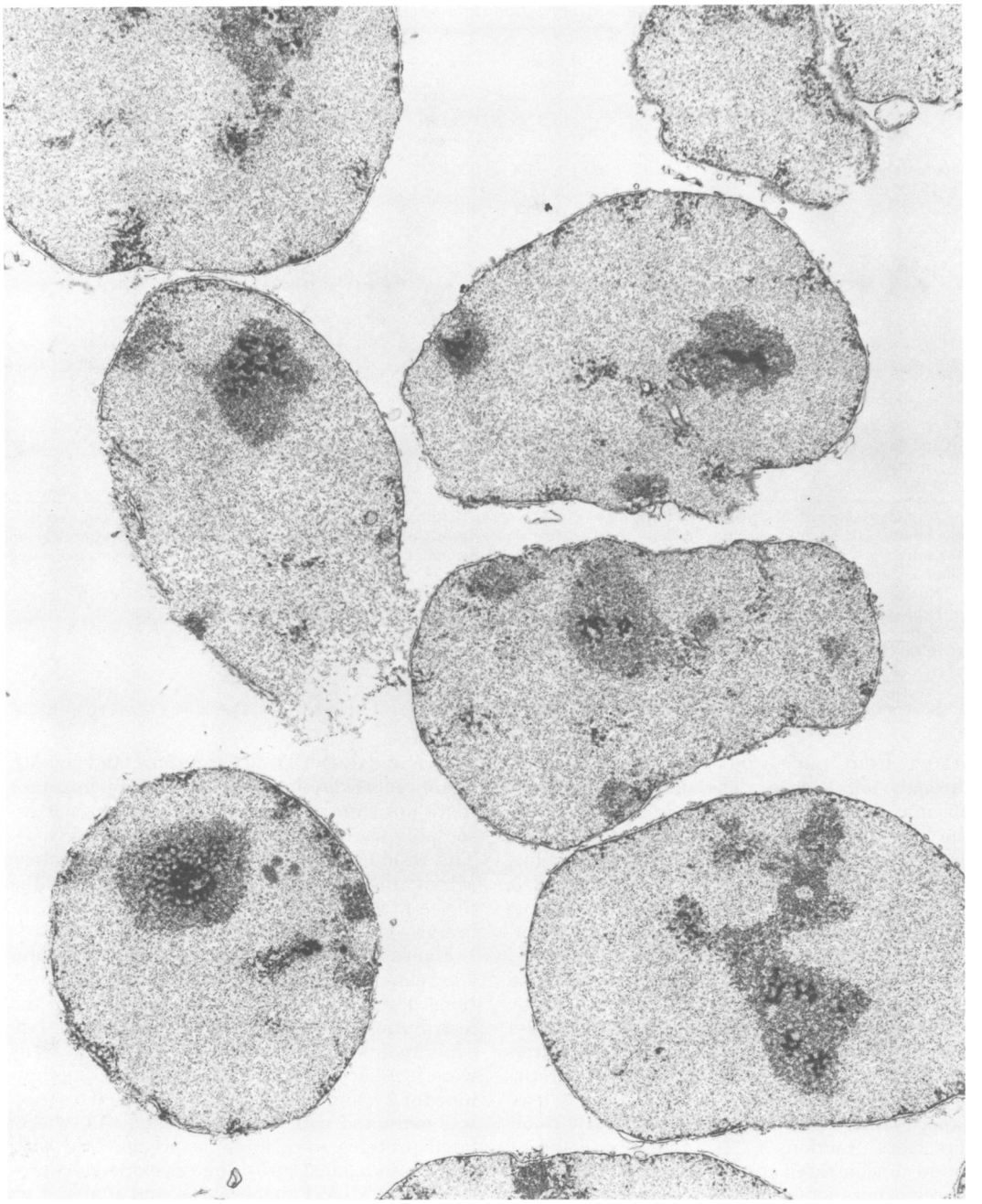

FIG. 1. *Nuclei isolated from GM-86 cells.*

3′) or in the nuclear fraction. The distribution of $gp55^{SFFV}$ between fractions 2 and 3 was variable, being evenly distributed in some experiments (e.g., experiment 1 in Table 2) and favoring one fraction in others. This variability is similar to that observed for the marker enzyme for endoplasmic reticulum (see above). $gp55^{SFFV}$ appeared as a double band of approximately equal intensity in most fractions (discussed below). The slower-migrating form appeared to be in higher concentration than the rapidly migrating form in lighter-density membranes (fractions 4

and 5). The distribution of gp85^{env}, the precursor to the helper virus envelope glycoprotein, was very similar to that of gp55^{SFFV}, whereas gp70, the major cleavage product of gp85^{env}, was present in both dense and light membrane fractions, as observed previously for other MLVs (33). The presence of viral glycoproteins in the nuclear fraction was not due to absorption from membranes in the cytoplasm after cell lysis. Nuclei from unlabeled cells pelleted through cytoplasm from labeled cells did not contain labeled gp55^{SFFV} or gp85^{env} (data not shown).

Because the yields of nuclei and cytoplasmic membrane markers were similar (approximately 40%; Table 1), the experiments in Fig. 2 and Table 2 illustrate the distribution of viral glycoproteins in a semiquantitative manner. To determine the distribution of viral glycoproteins between the nuclear and cytoplasmic membrane fractions in a more quantitative manner, a crude lysate of cells labeled with [^{35}S]methionine for 2 h was layered over 45% (wt/wt) sucrose and

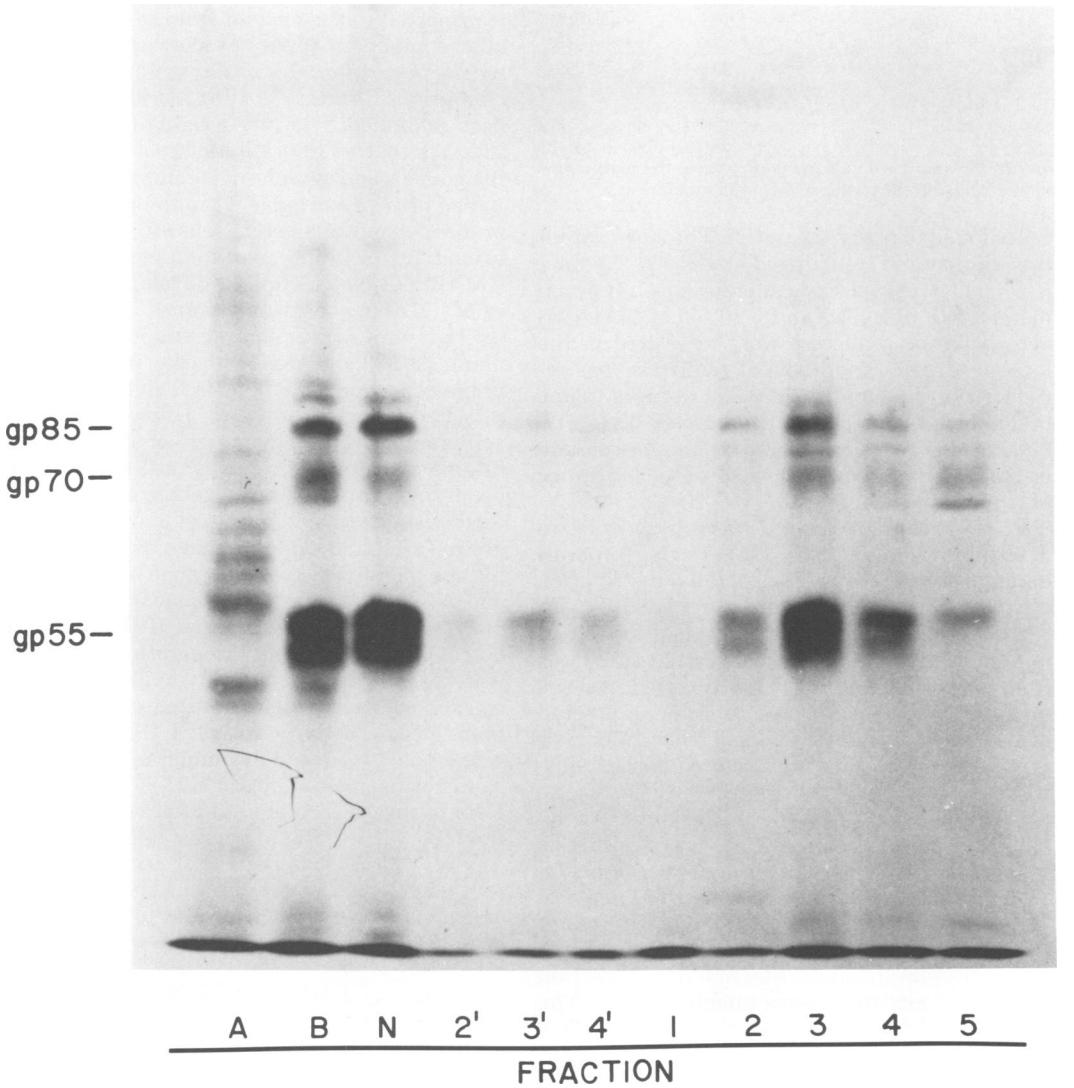

FIG. 2. *Subcellular distribution of env-related glycoproteins in GM-86 cells. Cells were labeled for 2 h with [^{35}S]methionine and were fractionated as described in Materials and Methods. Individual membrane fractions were pelleted, extracted, and subjected to immune precipitation with anti-gp70 and gel electrophoresis. A fluorograph of the dried gel is shown. A, Whole-cell extract precipitated with anti-p30; B, whole-cell extract precipitated with anti-gp70; N, nuclei; fraction numbers are defined in Table 1.*

TABLE 2. *Distribution of env-related proteins in GM-86 cells*

Fraction	% of total protein[a]					
	Expt 1			Expt 2		
	$gp55^{SFFV}$	$gp85^{env}$	gp70	$gp55^{SFFV}$	$gp85^{env}$	gp70
Nuclei	46	35	23	39	43	33
2′	2.8	1.9		2.3		
3′	4.4	3.0		6.0	5.4	4.3
4′	2.2	1.5		3.7	3.2	4.3
1	1.7					
2	15	23	18	7.3	7.5	4.3
3	16	24	21	28	29	28
4	10	9.1	30	10	8.6	11
5	1.7	2.3	7.1	2.8	3.2	15
Total	99.8	99.8	99.1	99.1	99.9	99.9

[a] Percentages were calculated from densitometer tracings of films similar to that shown in Fig. 2. The amounts of a particular glycoprotein in each fraction was divided by the sum of that protein in all fractions to give the percentages shown. The film shown in Fig. 2 is from experiment 2.

centrifuged to pellet the nuclei. The supernatant was diluted and centrifuged at 100,000 × *g* for 1 h to pellet cytoplasmic membranes. Approximately 80% of nuclei and 6 to 8% of NADPH-cytochrome *c* reductase were recovered in the low-speed pellet, and 90% of reductase was recovered in the high-speed pellet. After immune precipitation and gel electrophoresis, 56 to 61% of labeled $gp55^{SFFV}$ recovered by immune precipitation was found in the low-speed pellet (range of three experiments).

Pulse-chase experiments were performed to determine whether the MLV-F glycoproteins undergo changes in their subcellular distribution after their synthesis. Cells were labeled with [^{35}S]methionine for 15 min and then incubated in unlabeled medium for 0, 30, or 120 min. Cells from each time point were fractionated, and the membranes were subjected to immune precipitation with anti-gp70 and gel electrophoresis (Fig. 3). $gp55^{SFFV}$ did not undergo major redistribution during the course of the chase. The amount of $gp55^{SFFV}$ in the nuclear fraction remained constant at about 30 to 40% (in this experiment) of the total throughout the chase, as determined by densitometry of the gel of Fig. 3. A slight shift of $gp55^{SFFV}$ in cytoplasmic membranes to lighter density was detected, which may represent incorporation of a minor fraction of $gp55^{SFFV}$ into the plasma membrane (10). The turnover of $gp55^{SFFV}$ within the cell was slow, with a half-time greater than 2 h (Fig. 3), as noted by others (6, 20, 21, 23). There was also a slight increase in the electrophoretic mobility of $gp55^{SFFV}$ during the chase as observed previously, the nature of which is unknown (20, 21, 23). This shift in mobility may account in part for the appearance of $gp55^{SFFV}$ as a double band after a 2-h pulse (Fig. 2). The distribution of $gp85^{env}$ also did not change dramatically during the chase. However, its turnover had a half-time of 30 min to 1 h as shown by the depletion of labeled $gp85^{env}$ from all fractions after a 2-h chase (Fig. 3). At no time during the pulse or chase was either glycoprotein found in the cytosol fraction (100,000 × *g* supernatant; data not shown). In three initial experiments, it appeared that $gp55^{SFFV}$ in the nuclear fraction was not labeled in a 15-min pulse but was labeled after the 30-min chase, suggesting that $gp55^{SFFV}$ was transported to the nucleus from some other cellular component (data not shown). Despite extensive repetition, this observation has not been confirmed. Shorter labeling periods have not been examined. Thus, the weight of the evidence suggests that equilibration of $gp55^{SFFV}$ between the nucleus and cytoplasm is complete in 15 min.

The presence of $gp55^{SFFV}$ in the plasma membrane of erythroleukemia cells (10, 23) was confirmed by isolating plasma membranes from GM-86 cells by a technique developed by Cohen et al. (5) that yields membranes that are relatively free of contaminating endoplasmic reticulum (3, 5) compared with the plasma membrane-containing fractions in Table 1. Cells are attached noncovalently to polycationic polyacrylamide beads. Upon lysis of the cells, the plasma membrane remains attached to the beads by virtue of the negative charge on its external surface, and other cellular components are released and can be washed away. Yields of plasma membrane markers range from 5 to 20%, and the endoplasmic reticulum marker is usually undetectable (3, 5). Although it is difficult to detect $gp55^{SFFV}$ in such a preparation by labeling with [^{35}S]methionine, $gp55^{SFFV}$ could be detected by labeling detergent-solubilized membranes to high specific activity with ^{125}I (Fig. 4). ^{125}I-labeled $gp55^{SFFV}$ could be immune precipitated from plasma membranes of GM-86 cells, but not from plasma membranes of Eveline cells, which produce MLV-F helper virus but little or no SFFV (7). gp70 was the major *env*-related protein detected in membranes of both cell types, and no uncleaved $gp85^{env}$ could be detected, as described previously (6, 10, 23).

DISCUSSION

The subcellular localization of $gp55^{SFFV}$ is of interest because (i) it may be involved in leukemogenesis by SFFV and (ii) its metabolic behavior is unlike that of other viral glycoproteins studied to date. The available evidence suggests that $gp55^{SFFV}$ is involved in leukemogenic transformation by SFFV. Many strains of SFFV express proteins similar to $gp55^{SFFV}$ (10, 24), and

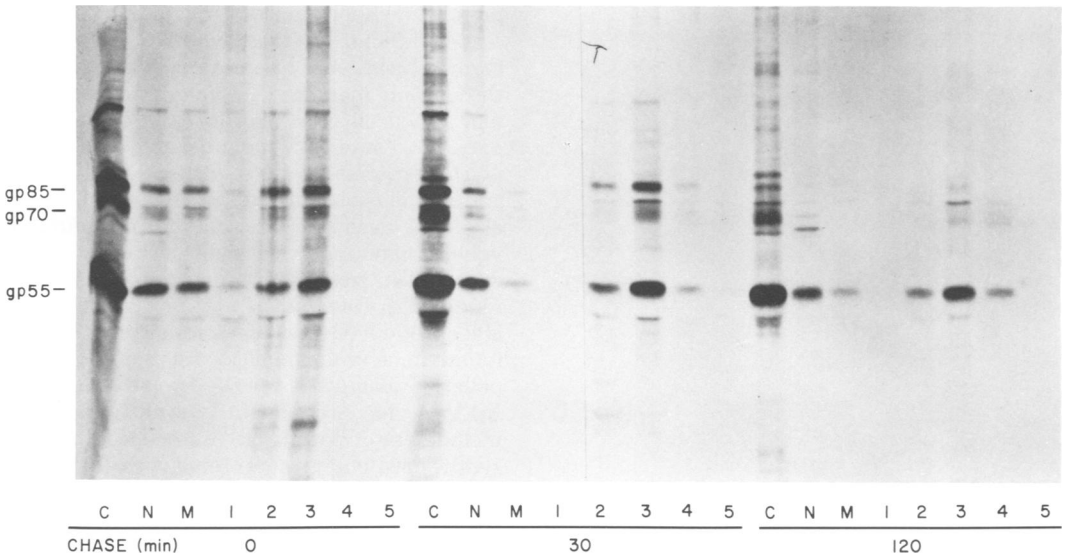

FIG. 3. *Pulse-chase of env-related glycoproteins of GM-86 cells. Cells were labeled for 15 min with [^{35}S]methionine and then incubated with unlabeled methionine for the indicated chase times. Cells from each time point were fractionated, and the fractions were subjected to immune precipitation with anti-gp70 and gel electrophoresis. Fluorographs of the dried gels are shown. C, Whole-cell extract; N, nuclei; M, cytoplasmic membranes in the initial low-speed pellet; fraction numbers are defined in Table 1.*

in some cases gp55^{SFFV} is the only virus-encoded protein that has been detected (25). Furthermore, recombination within the *env* gene between ecotropic and xenotropic MLV, such as that which presumably gave rise to SFFV, is correlated with increased leukemogenicity of helper-independent mink cell focus-inducing viruses as well as the defective SFFV (reviewed by Troxler et al. [31]).

The metabolism of gp55^{SFFV} differs from that of other viral glycoproteins studied to date in that very little is incorporated in the cell plasma membrane, and little or no gp55^{SFFV} is incorporated into virions (10, 23). Furthermore, gp55^{SFFV} does not appear to undergo extensive subcellular redistribution during a pulse-chase, unlike other viral glycoproteins, including the *env* gene product of replication-competent retroviruses, which are transported to the plasma membrane from their site of synthesis in rough endoplasmic reticulum (reviewed by Lenard [16]). This difference in intracellular transport between gp55^{SFFV} and most viral glycoproteins was predicted based on the nature of the oligosaccharides of gp55^{SFFV}, which are predominantly neutral, lacking sialic acid (10), and sensitive to digestion with endoglycosidase H (26).

Oligosaccharides of this type are found on newly synthesized glycoproteins before transport from the endoplasmic reticulum to the Golgi apparatus (discussed by Rothman and Fine [22]). The unexpected observation reported here is that a major portion (30 to 60%) of labeled gp55^{SFFV} is found in the nuclear fraction, with the remainder primarily in fractions containing endoplasmic reticulum. A similar distribution is observed for the *env* precursor glycoprotein gp85^{env}. This observation cannot be accounted for by contamination of the nuclear fraction with endoplasmic reticulum as judged by electron microscopy. Likewise, only 5 to 8% of the endoplasmic reticulum enzyme marker has been found in the nuclear fraction, most of which is probably due to the presence of the enzyme in the nuclear membrane (11). It is likely that both gp55^{SFFV} and gp85^{env} are present in the nuclear membrane rather than in the nucleoplasm. gp85^{env} contains the membrane-binding region of the envelope protein, which upon cleavage may reside either in a 15,000-molecular-weight fragment (p15E; reviewed by Montelaro and Bolognesi [19]) or in a recently described 12,000-molecular-weight fragment (R protein), which is distinct from p15E (28), both of which are located near the carboxyl terminus of gp85^{env}. The nature of the membrane binding of gp55^{SFFV} is not clear, since the SFFV has a deletion in the *env* gene (2) which appears to span the cleavage site between gp70 and p15E (29). Also, gp55^{SFFV} lacks the antigenic determinants of p15E (26). Nonetheless, preliminary experiments (L. Puddington and D. S. Lyles, unpublished data) suggest that gp55^{SFFV} is also localized in the nuclear membrane(s) rather

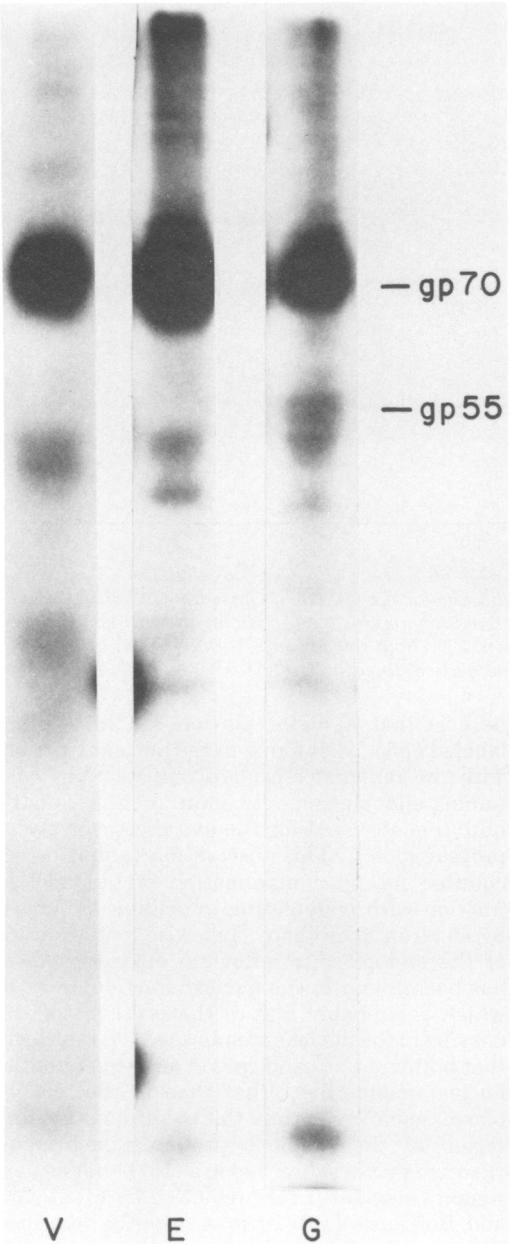

FIG. 4. *Presence of env-related glycoproteins in plasma membranes of MLV-F producing cells. Plasma membranes of GM-86 cells or Eveline cells were isolated as described in Materials and Methods. Membranes and purified virions were solubilized with detergents. The extracts were labeled with ^{125}I and subjected to immune precipitation with anti-gp70 and electrophoresis on 10% polyacrylamide gels. Autoradiographs of the dried gels are shown. V, MLV-F virions; E, plasma membranes from Eveline cells; G, plasma membranes from GM-86 cells.*

than in the nucleoplasm.

It is difficult to assess whether the apparent favored location of Friend virus glycoproteins in the nuclear membrane is unique to this particular virus-cell combination based on available data. Most studies of the subcellular distribution of the proteins of RNA viruses have concentrated exclusively on cytoplasmic membranes or else have used nuclear isolation procedures involving detergent treatments, which would be expected to remove viral glycoproteins. In one report (12), less than 10% of newly synthesized glycoprotein of vesicular stomatitis virus was found in a crude nuclear fraction (low-speed pellet). Possibilities for the favored location of MLV-F glycoproteins in the nuclear fraction include the following. (i) Association of newly synthesized *env* glycoproteins with the nuclear membrane may be a feature unique to retroviruses or to MLV-F in particular. *env* proteins of replication-competent retroviruses would have the structural features necessary to be transported to other membranes, processed, and incorporated into virions. $gp55^{SFFV}$ presumably lacks these structural features and therfore remains associated with the nucleus. (ii) Association with the nuclear membrane may be common to a wide variety of newly synthesized membrane glycoproteins in this cell type, perhaps reflecting a preferential localization of membrane-bound polyribosomes on the outer nuclear membrane rather than endoplasmic reticulum. Such a situation occurs in cell types that have little, if any, endoplasmic reticulum (8), which appears unlikely in the present case. An important question that remains to be answered is whether *env*-related viral glycoproteins are synthesized in association with the nucleus (e.g., on ribosomes bound to the outer nuclear membrane) or whether they are synthesized elsewhere and migrate to the nucleus (e.g., synthesized in the rough endoplasmic reticulum, which is in direct continuity with the nuclear membrane [8]). Results of pulse-chase experiments were ambiguous on this point (see Results). This question may be approached by localizing the mRNA for the viral glycoproteins.

Regardless of the specificity with which the MLV-F *env*-related glycoproteins are associated with the nuclear and cytoplasmic membrane fractions, the localization of these proteins may be involved in the mechanism of leukemogenesis by these viruses. Since the nuclear membrane probably plays a major role in communication between the cytoplasm and nucleus, alterations in this membrane system could lead to oncogenic transformation of cells. If so, the greater oncogenicity of the SFFV compared with replication-

competent MLV may be related to the inability of $gp55^{SFFV}$ to be transported away from the nucleus, causing it to exert a greater influence than glycoproteins whose appearance in the nucleus is transitory. In any case, hypotheses about the mechanism of oncogenic transformation by $gp55^{SFFV}$ must include the possibility of several sites of action within the cell, since this protein is distributed throughout several types of cellular membranes. A similar situation may exist in the case of the *src* gene product of avian sarcoma viruses, which in some cell types is localized at the plasma membrane and in others may be associated with the nuclear membrane and surrounding reticulum (13).

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