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Cell Surface Expression of the *env* Gene Polyprotein of Dual-Tropic Mink Cell Focus-Forming Murine Leukemia Virus

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Differences have been observed in the kinetics of processing of the *env* gene polyprotein of ecotropic, xenotropic, and dual-tropic mink cell focus-forming (MCF) murine leukemia virus. In pulse-chase experiments, the *env* gene polyprotein of the dual-tropic MCF virus exhibits a marked increase in stability relative to that of either ecotropic or xenotropic virus. A comparison of cell surface expression of *env* gene products of ecotropic, xenotropic, and dual-tropic MCF murine leukemia virus has been made. Only gp70 is accessible to lactoperoxidase-catalyzed radioiodination of fibroblasts infected by ecotropic or xenotropic virus, whereas both gp70 and the *env* gene polyprotein are expressed on the surface of dual-tropic MCF virus-infected cells.

The dual-tropic MCF (mink cell focus-forming) class of murine leukemia virus (MuLV), described by Hartley and co-workers (13), has been shown to have several interesting properties attributable to the envelope (*env*) gene. Both the biological and biochemical characteristics of this virus class suggest an etiology involving recombination between an ecotropic and a xenotropic MuLV (7, 9, 13, 26, 29), although the occurrence of this recombination within the lifetime of a single mouse or as an evolutionary event is not established. For example, dual-tropic MCF viruses exhibit the host range of both N-ecotropic and xenotropic MuLV, are interfered with by both virus classes, and are neutralized by antisera specific to either class (13). By serological analysis, it has been shown that two gp70-related cell surface antigens, GRADA1 (induced by N-ecotropic virus infection; 23) and GERLD (induced by xenotropic virus infection; 29; Y. Obata, personal communication), are expressed on fibroblasts infected by many dual-tropic MCF isolates (29; P. V. O'Donnell, personal communication).

Analysis of the tryptic peptides of the gp70 species (9) and envelope antigens (7, 29) of dual-tropic MCF viruses has shown considerable heterogeneity among various isolates, particularly among those dual-tropic MCF viruses isolated from different mouse strains. (The *gag* gene products, on the other hand, appear to be closely related to those of N-ecotropic virus by both tryptic peptide mapping [9] and antigenic analysis [7; P. V. O'Donnell, E. Stockert, Y. Obata, A. B. DeLeo, H. W. Snyder, Jr., and L. J. Old, personal communication).

Characterization of RNase T1-resistant oligonucleotides of certain isolates has demonstrated that whereas the 5' portions of the dual-tropic MCF virus genomes are very similar and related to the N-ecotropic viruses Akv-1 and Akv-2, the 3' portions appear to be only partially shared among the dual-tropic MCF viruses and to be different in large part from those of the Akv-1 and Akv-2 viruses (26).

The significance of these *env* gene properties in relationship to the biological properties of the dual-tropic MCF class of virus in vivo is unknown. It is clear that certain isolates derived from late preleukemic and leukemic thymuses of AKR mice will induce amplified expression of MuLV-related cell surface antigens (16, 17) in the thymus of young AKR mice (E. Stockert, J. W. Hartley, P. V. O'Donnell, W. P. Rowe, Y. Obata, and L. J. Old, personal communication) as well as accelerating the development of leukemia in AKR mice (7, 22; M. Cloyd, J. W. Hartley, W. P. Rowe, E. Stockert, P. V. O'Donnell, and L. J. Old, personal communication). We have undertaken to examine the virus-host interaction of MCF 247 virus in fibroblasts of both mouse and mink origin to ascertain whether any unusual features of protein processing exist. Emphasis has been placed on the processing of the *env* gene products.

MATERIALS AND METHODS

Cells and viruses. Producer lines of MCF 247 virus isolated from the thymus of a 6-month-old AKR mouse by Hartley et al. (13) were constructed in the SC-1 mouse cell line (12) and in the CCL64 mink lung cell line (14) by P. V. O'Donnell of this Center.

The ecotropic virus 69E5 and the xenotropic virus 69X9 used in this study were both isolated from the thymus of the same 6-month-old AKR mouse, cloned, and used to infect SC-1 cells and CCL64 mink cells, respectively (P. V. O'Donnell, personal communication). Cultures were maintained as previously described (24).

Pulse-chase labeling of cell cultures. Pulse-chase labeling of cell cultures was carried out as previously described (10) using 40 μCi of ^{14}C-labeled amino acid mixture (New England Nuclear Corp.) per ml of minimal essential medium containing 10% of normal amino acid concentrations. Cell extracts were prepared by scraping monolayers into cold lysis buffer (0.02 M Tris [pH 7.4], 0.05 M NaCl, 0.5% Nonidet P-40 [Shell Oil Co.], and 0.5% deoxycholate [34]), blending the cell lysate with a Vortex mixer for 1 min, and centrifuging the lysate at 1,500 × *g* for 20 min at 4°C. The clarified supernatants were used as cell extracts. Virus was purified by isopycnic centrifugation as previously described (11).

Radiolabeling of cell cultures with [^{3}H]glucosamine. Confluent monolayers were incubated for 16 h in Dulbecco-modified Eagle minimal essential medium containing 5% fetal calf serum and 70 μCi of D-[6-^{3}H]glucosamine (New England Nuclear Corp.) per ml. Cell extracts and purified virus were prepared as described above.

Radiolabeling of cell surface with iodine-125. Confluent monolayers were washed once with phosphate-buffered saline (calcium and magnesium-free) and scraped into 0.05% EDTA in phosphate-buffered saline. Cells were pelleted at 300 × *g*, washed once with cold phosphate-buffered saline, and resuspended in cold phosphate-buffered saline at a concentration of 15 × 10^6 cells per ml for SC-1 cells and 35 × 10^6 cells per ml for CCL64 mink cells. Cell surface iodination by the lactoperoxidase method was carried out in a 1-ml reaction volume using 2 mCi of ^{125}I (30). Cell extracts were prepared as described above.

Serological procedures. Goat anti-Rauscher-MuLV (R-MuLV) gp70 serum was provided by R. Wilsnack, Huntington Research Center. Rabbit anti-R-MuLV p15(E) serum was provided by E. Fleissner of this Center (15). This serum has been shown to have reactivity to the virus structural protein p15(E) (15) and to the cellular protein LETS (A. Pinter, personal communication), but not to gp70 (25).

Immunoprecipitations of cell extracts were carried out as previously described (10) with the modification that cell extracts were made 0.5% for sodium dodecyl sulfate (SDS) immediately prior to addition of the antiviral serum.

SDS-PAGE. SDS-polyacrylamide gel electrophoresis (PAGE) analysis of virus and immunoprecipitates was carried out using modifications of the procedure of Laemmli (18). Electrophoresis using 7.5% acrylamide slab gels has been previously described (10). Step gradient acrylamide slab gels (1.5 mm thick) were prepared by layering 8 ml of 12.5% acrylamide, 8 ml of 10% acrylamide, and 6 ml of 7.5% acrylamide (a ratio of 30:1.04, acrylamide to bisacrylamide, was used). Stacking gels of 4.5% acrylamide were 1 cm long. Electrophoresis was carried out at 25 mA per gel until a cytochrome *c* marker reached the bottom of the gel.

Slab gels were fixed with a 50% methanol-10% acetic acid solution for 20 min and a 5% methanol-10% acetic acid solution for 30 min (6). The fluorographic procedure of Bonner and Laskey (5) was applied to gels containing ^{14}C- and ^{3}H-labeled proteins. Autoradiography on dried gels was carried out with Kodak XR-2 X-ray film at −70°C. Gels containing ^{125}I-labeled proteins were autoradiographed with Cronex intensifying screens, also at −70°C.

Peptide mapping by limited proteolysis and analysis by SDS-PAGE. Radiolabeled proteins isolated from cell extracts by immunoprecipitation and SDS-PAGE (see above) were subjected to limited proteolysis with *Staphylococcus aureus* V8 protease (Miles Laboratories) according to Cleveland et al. (6). Protease peptides were analyzed with modified Laemmli (18) acrylamide slab gels (mapping gels) consisting of a 15% acrylamide separating gel (acrylamide to bisacrylamide, 30:0.8) of 15-ml volume (1.5 mm thick; approximately 6 cm long) and a 4.5% acrylamide stacking gel (acrylamide to bisacrylamide, 30:0.8) of 10-ml volume (1.5 mm thick; approximately 3 cm long). Both the stacking and separating gels contained 1 mM EDTA.

Immunoprecipitated, radiolabeled proteins were visualized by fluorography (5) for ^{14}C- or ^{3}H-labeled proteins, or by autoradiography with a Cronex intensifying screen for ^{125}I-labeled proteins, and cut from dried polyacrylamide slab gels. (Isolation of proteins from gels which had been subjected to the Bonner and Laskey method [5] did not affect the results of the mapping procedure [unpublished data]). Acrylamide slices were rehydrated by shaking at room temperature in 10 ml of 0.125 M Tris (pH 6.8), 0.1% SDS, and 1 mM EDTA, and loaded into sample wells of mapping gel. Slices were overlaid with 10 μl of buffer containing 0.125 M Tris (pH 6.8), 0.1% SDS, 1 mM EDTA, 0.5% thioglycolate, and 20% glycerol and 10 μl of solution containing 21 U of *S. aureus* V8 protease per ml in 0.125 M Tris (pH 6.8), 0.1% SDS, 1 mM EDTA, and 10% glycerol. Electrophoresis was carried out at 25-mA/gel constant current until a bromophenol blue marker was approximately 2 cm into the stacking gel. Current was then turned off for 30 min, after which electrophoresis was resumed until a cytochrome *c* marker (loaded in a blank well) reached the bottom of the separating gel. Gels were fixed as described above and subjected to fluorography (5) for ^{14}C- or ^{3}H-labeled peptides, or autoradiography with Cronex intensifying screens for ^{125}I-labeled peptides.

RESULTS

A comparison of the kinetics of cleavage and mobility in SDS-PAGE of the *env* gene products of ecotropic, xenotropic, and dual-tropic MCF viruses. Differences were observed in the kinetics of cleavage as well as in the mobility in SDS-PAGE of the *env* gene products of ecotropic virus 69E5, xenotropic virus 69X9, and MCF 247 virus. *env* gene products were examined from extracts of cultures radio-

labeled with ^{14}C-amino acid mixture (New England Nuclear Corp.) in a 15-min pulse or in a 15-min pulse followed by a 3-h chase. Goat anti-R-MuLV gp70 serum was used to identify *env* gene products by the double antibody immunoprecipitation procedure (Fig. 1). In pulse-labeled extracts of cells infected with any one of the three viruses, only the *env* gene polyprotein (PrENV protein) was recognized (Fig. 1A, track 4; Fig. 1B, tracks 1, 3, 5). [Rabbit anti-R-MuLV p15(E) serum also recognized this species (data not shown).] During the 3-h chase period, some cleavage of the PrENV protein to the gp70 species took place in all the infected cultures, but the extent of cleavage varied among the three virus classes. The PrENV protein of the xenotropic virus 69X9 was almost completely processed during a 3-h chase (Fig. 1B, tracks 5 and 6). Scanning densitometer tracings of autoradiographs of gels from two experiments, including the one shown in Fig. 1, indicate cleavage of approximately 90% of PrENV of 69X9 in a 3-h chase. Cleavage of approximately 70 to 75% of PrENV of 69E5 occurred during a 3-h chase (Fig. 1B, tracks 1 and 2), consistent with previously reported data (10). However, little cleavage of the MCF 247 virus PrENV protein took place during a 3-h chase; approximately 30 to 40% of PrENV of MCF 247 virus-infected SC-1 cells (Fig. 1A, tracks 4 and 5) and 15 to 20% of MCF 247 virus-infected mink cells (Fig. 1B, tracks 3 and 4) were cleaved during this chase period.

The mobilities of the *env* gene products of the three virus types are clearly distinguishable by the gel system described in Fig. 1. The PrENV protein (Pr80^{env}) and gp70 of 69E5 have higher apparent molecular weights than the PrENV proteins or gp70's of 69X9 and MCF 247 virus. The mobilities of the *env* gene polyprotein of the latter two viruses are not distinguishable, whereas the gp70 species of MCF 247 virus migrates slightly faster than that of the xenotropic virus. (The PrENV protein of 69X9 and MCF 247 virus will be referred to as Pr76^{env} since it has an apparent molecular weight of 76,000.) Whether the differences observed in the apparent molecular weights of the PrENV and gp70 protein species are a reflection of differences in the length of their polypeptide chain or a reflection of the degree of glycosylation is not known.

To analyze further the stability of Pr76^{env} of MCF 247 virus, a pulse-chase experiment using chase periods of 2, 4, 6, 8, 10, and 12 h was conducted. The data presented in Fig. 2A are the result of analysis by immunoprecipitation with goat anti-R-MuLV gp70 serum of SC-1 cells infected by MCF 247 virus; similar results were obtained when mink cells infected by this virus were analyzed (data not shown). For comparison, Fig. 2B shows an analysis of SC-1 cells infected by 69E5 virus. When several chase periods were examined, the PrENV protein of MCF 247 virus appeared to be considerably more stable than that of 69E5 virus, confirming the results presented in Fig. 1. However, the

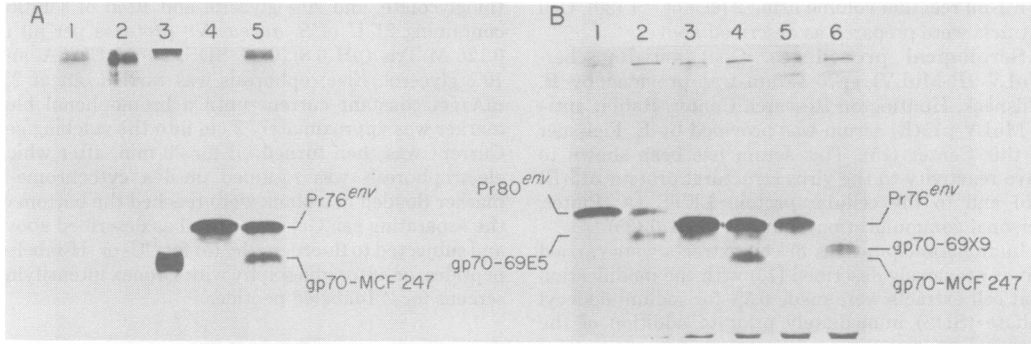

FIG. 1. *SDS-PAGE analysis of immunoprecipitates of pulse-chase-labeled MCF, ecotropic, and xenotropic virus-infected cells. Cytoplasmic extracts were prepared from cultures pulse-labeled for 15 min with ^{14}C-labeled amino acids or pulsed-labeled and chased for 3 h. Equal quantities of protein from each extract were reacted with goat anti-R-MuLV gp70 serum, and equal quantities of the resulting precipitates were subjected to electrophoresis in 7.5% acrylamide slab gels at 25 mA per gel (constant current). Electrophoresis was terminated 1 h after bromophenol blue ran off the gel. (A) Track 1: Uninfected SC-1 cells pulse-labeled for 15 min; track 2: uninfected mink cells pulse-labeled for 15 min; track 3: MCF 247 virus labeled with [^{3}H]-glucosamine; track 4: MCF 247-infected SC-1 cells pulse-labeled for 15 min; track 5: MCF 247-infected SC-1 cells chased for 3 h. (B) Track 1: 69E5-infected SC-1 cells pulse-labeled for 15 min; track 2: 69E5-infected SC-1 cells chased for 3 h; track 3: MCF 247-infected mink cells pulse-labeled for 15 min; track 4: MCF 247-infected mink cells chased for 3 h; track 5: 69X9-infected mink cells pulse-labeled for 15 min; track 6: 69X9-infected mink cells chased for 3 h.*

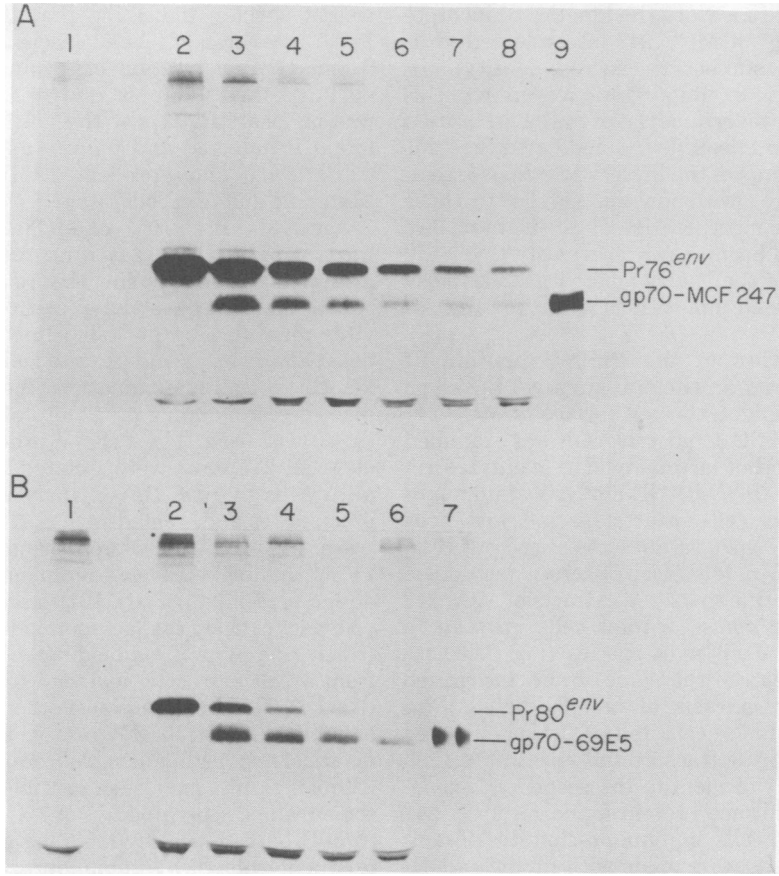

FIG. 2. *SDS-PAGE analysis of immunoprecipitates of pulse-chase-labeled MCF 247 and 69E5 virus-infected SC-1 cells. Cytoplasmic extracts were prepared from infected or uninfected SC-1 cells pulse-labeled for 15 min with ^{14}C-amino acids and chased for various time periods. Equal quantities of protein from each extract were reacted with goat anti-R-MuLV gp70 serum, and equal quantities of the resulting precipitate were subjected to electrophoresis in 7.5% acrylamide slab gels at 25 mA per gel (constant current). Electrophoresis was terminated 1 h after bromophenol blue ran off the gel. (A) Track 1: Uninfected cells pulse-labeled for 15 min; track 2: MCF 247-infected cells pulse-labeled for 15 min; track 3: MCF 247-infected cells chased for 2 h; track 4: MCF 247-infected cells chased for 4 h; track 5: MCF 247-infected cells chased for 6 h; track 6: MCF 247-infected cells chased for 8 h; track 7: MCF 247-infected cells chased for 10 h; track 8: MCF 247-infected cells chased for 12 h; track 11: MCF 247 virus labeled with [^{3}H]glucosamine. (B) Track 1: Uninfected cells pulse-labeled for 15 min; track 2: 69E5-infected cells pulse-labeled for 15 min; track 3: 69E5-infected cells chased for 2 h; track 4: 69E5-infected cells chased for 4 h; track 5: 69E5-infected cells chased for 6 h; track 6: 69E5-infected cells chased for 12 h; track 7: 69E5 virus labeled with [^{3}H]glucosamine.*

kinetics of appearance and disappearance of the gp70 species of MCF 247 and of 69E5 were very similar. The highest intracellular concentration of this cleavage product was found in the 2-h chase, with a roughly linear decay in subsequent chase periods. (gp70 of both MCF 247 and 69E5 is present in a 1-h chase, the shortest chase period examined [data not shown].) The fact that the stability of Pr76^{env} of MCF 247 virus is not accompanied by a delay in the appearance of the gp70 species suggests that only a portion of the PrENV protein may be available for cleavage into the viral envelope glycoprotein and that that fraction of the PrENV protein pool is processed with kinetics similar to those governing the cleavage of the PrENV protein of ecotropic virus. Examination of the ratio of the PrENV protein to its cleavage product shows that Pr80^{env} and gp70 of 69E5 virus are in roughly equivalent amounts in the 2-h chase, with gp70 becoming the major intracellular *env* gene product in subsequent chases, whereas Pr76^{env} of MCF 247 virus is the predominant intracellular *env* gene product in all chase periods examined.

Attempts to measure the kinetics of incorporation of gp70 of MCF 247 into released virus were unsuccessful because sucrose density-purified MCF virus exhibits erratic retention of this molecule. However, analysis of the incorporation of p15(E) into released virus indicated that this cleavage product of the PrENV protein is assembled into virus with kinetics similar to those governing the incorporation of p15(E) into 69E5 virus. [It has been shown previously that gp70 and p15(E) of the N-ecotropic virus WN1802N are incorporated into virions with the same kinetics [10].)

Identification of the PrENV protein of MCF 247 virus on the cell surface. Envelope gene expression on the cell surface of MCF 247 virus-infected SC-1 and mink cells was examined by the method of lactoperoxidase-catalyzed radioiodination. SDS-PAGE analysis of immunoprecipitates of cell extracts reacted with goat anti-R-MuLV gp70 serum was carried out (Fig. 3). Goat anti-R-MuLV gp70 serum recognized two virus-specific species in extracts of MCF 247 virus-infected mouse or mink cells: gp70 and a higher-molecular-weight species (Fig. 3, tracks 3 and 4), whereas the same serum recognized only gp70 in extracts of radioiodinated 69E5 virus-infected SC-1 cells and 69X9 virus-infected mink cells (Fig. 3, tracks 1 and 2).

In an effort to identify the second *env* gene-related cell surface protein found on MCF 247 virus-infected cells, immunoprecipitates of radioiodinated cells were made with rabbit anti-R-MuLV p15(E) serum (Fig. 4). It was found that this serum recognized the higher-molecular-weight species, indicating that this protein is $Pr76^{env}$ or a closely related species (Fig. 4, tracks 2 and 4). Precipitation of a minor amount of gp70 by this serum is due to trapping. The protein near the top of the gel labeled "h" is found in infected and uninfected cells (Fig. 4, tracks 2, 4, 6) and is probably LETS protein (see Materials and Methods).

Analysis of $Pr76^{env}$ of MCF 247 virus by partial protease digestion and SDS-PAGE analysis. To determine the relationship between the *env* gene polyprotein of MCF 247 virus identified by pulse-labeling with radiolabeled amino acids and the *env* gene polyprotein identified by radioiodination, the partial protease digest mapping procedure of Cleveland et al. (6) was used. The PrENV protein and gp70 of MCF 247 virus were isolated from infected mink cells by SDS-PAGE analysis of immunoprecipitates and treated with *S. aureas* V8 protease. Figure 5A shows representative maps of $Pr76^{env}$ radiolabeled in a 15-min pulse with a ^{14}C-amino acid mixture (track 1) or radioiodinated on intact cells by the lactoperoxidase procedure (track 2); a map of radioiodinated gp70 isolated from a cell extract is included for comparison (track 3). The maps of biosynthetically labeled or radioiodinated PrENV protein are essentially identical and distinguishable from those of gp70. [Similar results have been obtained by mapping the envelope gene products of MCF 247 isolated from SC-1 cells and by mapping envelope gene products isolated by immunoprecipitation with rabbit anti-R-MuLV p15(E) serum; data not shown.] These data indicate that there are no

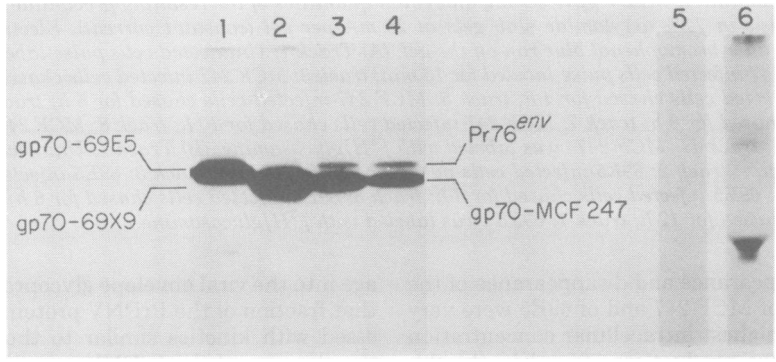

FIG. 3. *SDS-PAGE analysis of gp70 related species on the cell surface of radioiodinated MCF 247, 69E5, and 69X9 virus-infected cells. Cytoplasmic extracts were prepared from intact cells which had been subjected to lactoperoxidase-catalyzed radioiodination. Extracts were reacted with goat anti-R-MuLV gp70 serum, and the resulting precipitate was analyzed on a step gradient polyacrylamide slab gel (7.5%, 10%, and 12.5% acrylamide). Electrophoresis was carried out at 25 mA per gel (constant current) until a cytochrome c marker reached the bottom of the gel. Track 1: 69E5-infected SC-1 cells; track 2: 69X9-infected mink cells; track 3: MCF 247-infected mink cells; track 4: MCF 247-infected SC-1 cells; track 5: uninfected mink cells; track 6: uninfected SC-1 cells. Note that the step gradient slab gel does not clearly resolve the migration differences in the gp70 species of 69E5, 69X9, and MCF 247 viruses demonstrated in Fig. 1 (7.5% slab gel).*

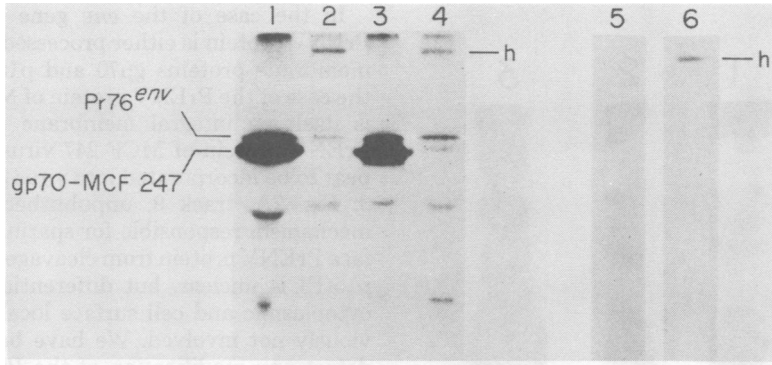

FIG. 4. *SDS-PAGE analysis of gp70 and p15(E) related species on the surface of radioiodinated MCF 247 virus-infected cells. Cytoplasmic extracts were prepared from intact cells which had been subjected to lactoperoxidase-catalyzed radioiodination. Extracts were reacted with either goat anti-R-MuLV gp70 serum or rabbit anti-R-MuLV p15(E) serum, and the resulting precipitates were analyzed on a step gradient polyacrylamide slab gel (7.5%, 10%, 12.5% acrylamide). Electrophoresis was carried out at 25 mA per gel (constant current) until a cytochrome c marker reached the bottom of the gel. Track 1: MCF 247-infected SC-1 cells reacted with anti-gp70 serum; track 2: MCF 247-infected SC-1 cells reacted with anti-p15(E) serum; track 3: MCF 247-infected mink cells reacted with anti-gp70 serum; track 4: MCF 247-infected mink cells reacted with anti-p15(E) serum; track 5: uninfected SC-1 cells reacted with anti-p15(E) serum; track 6: uninfected mink cells reacted with anti-p15(E) serum. See Fig. 3 for anti-gp70 serum reaction with uninfected SC-1 and mink cells.*

major differences between the newly synthesized PrENV protein and the *env* gene polyprotein expressed on the cell surface. To examine further the question of possible modification of $Pr76^{env}$ in the process of its being expressed on the cell surface, a comparison was made between the maps of $Pr76^{env}$ isolated from infected cells after a 15-min pulse and $Pr76^{env}$ isolated from cells which had been pulse-labeled for 15 min and chased for 5 h (Fig. 5B, tracks 1 and 2). No differences in the maps of the PrENV protein radiolabeled by these two protocols were observed. Furthermore, the map of $Pr76^{env}$ isolated from cells after a 16-h exposure to [^{3}H]glucosamine (Fig. 5B, track 3) was very similar to that of ^{14}C-amino acid-labeled or radioiodinated $Pr76^{env}$. The major difference is the absence of bands "a" and "b" in maps of [^{3}H]glucosamine-labeled $Pr76^{env}$, indicating that these fragments do not contain glucosamine. Since the majority of fragments analyzed by this method are glycosylated, it is likely that modifications of the PrENV protein involving changes in the degree of glycosylation would result in changes in the electrophoretic migration of some of the peptide fragments. Such altered migration has not been observed.

DISCUSSION

Identification of the PrENV protein of MCF 247 virus on the cell surface of infected fibroblasts indicates that the primary translational product of the *env* gene is capable of serving two functions: (i) as the precursor to the viral structural components gp70 and p15(E) (10, 21, 35), and (ii) as a virus-induced cell surface component. Dual functioning of an MuLV precursor protein is not unique to the *env* gene polyprotein; the *gag* gene polyprotein has previously been shown to be the precursor to the virion core proteins (1, 2, 27, 33), as well as to an integral cell surface protein (19, 20, 28, 31, 32). The controlling elements which allow the *env* and *gag* gene polyproteins to function as precursors to the virion structural proteins as well as integral cell surface proteins are not yet understood, but we feel that it is likely that different mechanisms govern the compartmentalization of these two gene products. In the case of the *gag* gene product, the major precursor species, $Pr65^{gag}$, is a nonglycosylated, cytoplasmic protein, the cleavage products of which are not found external to the plasma membrane (or virion envelope) (1, 2, 27, 33). The cell surface *gag* polyproteins, $gP85^{gag}$ and $gP95^{gag}$, have been shown to be glycosylated (20, 28, 31), a fact reflected in their estimated molecular weights of 85,000 and 95,000. Specification of a protein as cytoplasmic, on the one hand, or as an integral membrane protein or a secretory protein, on the other, has been hypothesized to reside in the amino acids coded for by the 5′ end of a particular mRNA (signal hypothesis [3, 4]). Thus it is possible that the regulation of expression of these two forms of the *gag* gene product is at the level of mRNA processing.

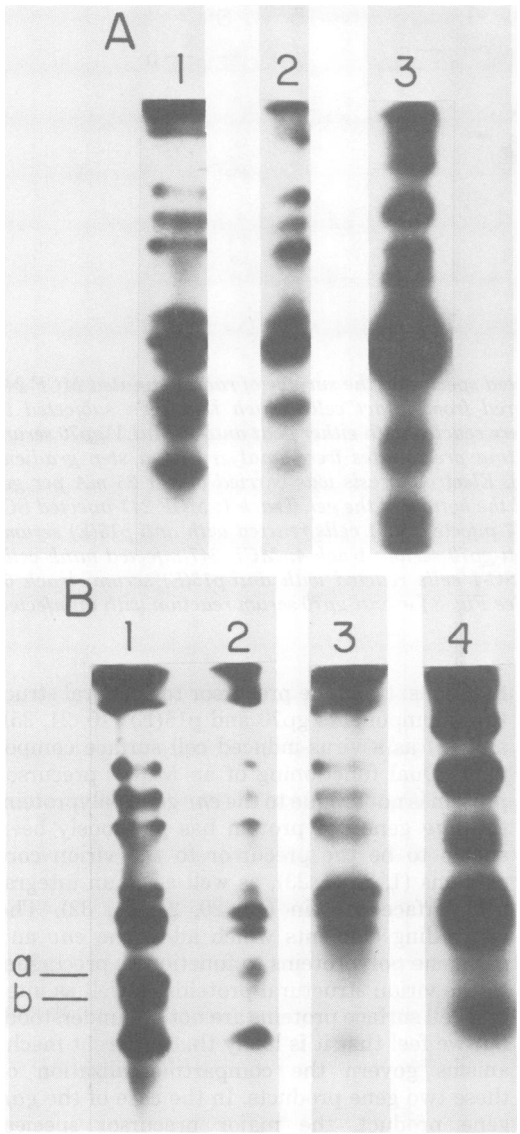

FIG. 5. *Partial digest maps of Pr76^{env} and gp70 of MCF 247 virus. S. aureus V8 protease was used to generate partial digest maps of Pr76^{env} and gp70 of MCF 247 virus; proteins were isolated from infected mink cells by SDS-PAGE analysis of immunoprecipitates made with goat anti-R-MuLV gp70 serum. Partial cleavage peptides were resolved on 15% acrylamide slab gels run at 25 mA per gel (constant current) until a cytochrome c marker reached the bottom of the gel. (A) Track 1: Pr76^{env} radiolabeled with ^{14}C-amino acids in a 15-min pulse; track 2: Pr76^{env} radioiodinated on intact mink cells; track 3: gp70 radioiodinated on intact mink cells. (B) Track 1: Pr76^{env} radiolabeled with ^{14}C-amino acids in a 15-min pulse; track 2: Pr76^{env} radiolabeled with ^{14}C-amino acids in a 15-min pulse followed by a 5-h chase; track 3: Pr76^{env} radiolabeled with [^{3}H]glucosamine for 16 h; track 4: gp70 radiolabeled with [^{3}H]glucosamine for 16 h.*

In the case of the *env* gene products, the PrENV protein is either processed into the viral membrane proteins gp70 and p15(E) or, as in the case of the PrENV protein of MCF 247 virus, is itself an integral membrane protein. (The PrENV protein of MCF 247 virus does not appear to be incorporated into virus [Fig. 1A, track 3; Fig. 2A, track 9; unpublished data].) The mechanism responsible for sparing the cell surface PrENV protein from cleavage into gp70 and p15(E) is unclear, but differentiation between cytoplasmic and cell surface localization is obviously not involved. We have been unable to detect any modification of the PrENV species using partial digest peptide mapping to compare pulse-labeled and pulse-chase-labeled PrENV, or pulse-labeled and cell surface-labeled PrENV. Since the majority of the peptide fragments examined by partial digest mapping are glycosylated, it is likely that we would detect migration differences in some fragments if compartmentalization of the functional precursor molecule and the cell surface species involved major differences in glycosylation. It is interesting that, as is the case for the *env* gene products of ecotropic MuLV (21), the gp70 of MCF 247 virus contains fucose whereas the PrENV species does not (unpublished data). It is possible that the intracellular stability of the PrENV species, i.e., its resistance to cleavage into gp70 and p15(E), might be a reflection of the recombinant nature of the *env* gene of the MCF class of viruses. For example, the Pr76^{env} of MCF 247 virus might represent an unfavorable substrate for the enzyme(s) responsible for processing this species.

The significance of cell surface expression of PrENV protein or the *gag* gene polyprotein, for that matter, is not known. gP95^{gag} and gP85^{gag} are known to carry the determinants of the GCSA antigen (19, 28), a specificity to which mice can respond. Whether or not the PrENV protein carries antigenic determinants unique from those expressed by its cleavage products, gp70 and p15(E), is not known. It is known, however, that leukemias of the AKR mouse express a molecule which, on preliminary analysis, is very similar to the PrENV protein of MCF 247 virus. Examination of an in vitro-cultured AKR leukemia AK2D (8; J. L. Biedler, personal communication) and an in vivo-passaged AKR leukemia AKSL2 (29) have shown expression of a protein of approximately 80,000 daltons which is immunoprecipitated by antisera recognizing gp70 or p15(E). Partial digest mapping of this protein isolated from the AK2D cell line shows it to be very similar but not identical to that expressed on MCF 247-infected fibroblasts (unpublished data). A similar species has been identified on spontaneous AKR leukemias

(J.-S. Tung and E. Fleissner, personal communication).

An important question that remains to be addressed is that of the distribution of the phenotype of cell surface expression of the PrENV species among various MuLV isolates. We have examined other dual-tropic MCF virus isolates derived from preleukemic and leukemic AKR thymus tissue; these isolates are positive for cell surface expression of PrENV (N. Famulari and P. V. O'Donnell, unpublished data). However, it remains to examine dual-tropic MCF isolates derived from mouse strains other than the AKR, as well as a variety of ecotropic, xenotropic, and amphotropic MuLV from different sources, to determine whether cell surface expression of this protein is MCF virus specific and whether its expression correlates with any known biological properties of MuLV such as host range or leukemogenicity.

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LITERATURE CITED

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