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Effects of Monensin on Morphogenesis and Infectivity of Friend Murine Leukemia Virus

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The transport of the gp70 glycoprotein to the cell surface and concomitant release of infectious virus was inhibited by treatment of Friend murine leukemia virus-infected Eveline cells with the sodium ionophore monensin. Virus yields were reduced more than 50-fold by 10^{-5} M monensin, whereas particle production was reduced by 50% in monensin-treated cells. The resulting particles failed to incorporate newly synthesized gp70 and p15(E), whereas the other structural proteins, p30, p15, p12, and p10, were incorporated into virions. However, monensin did not inhibit the incorporation into virions of preformed gp70. A reduction in the efficiency of cleavage of the PrENV glycoprotein precursor and a defect in the processing of simple endo-H-sensitive to complex endo-H-resistant oligosaccharides suggest that intracellular transport of gp70 may be blocked before its entry into the Golgi apparatus. Fewer particles were found to bud from the cell surface, but intracellular vacuoles with budding virions were detected. Ferritin labeling and pulse-chase studies suggested a cell surface origin for these vacuoles. These experiments indicate that monensin inhibits the transport of Friend murine leukemia virus glycoproteins at an early stage, with a resultant block in the assembly and release of infectious virus.

The Friend complex of murine leukemia viruses (F-MuLV) induces a biphasic disease in mice characterized by an early erythroleukemia, followed by a later lymphatic leukemia (10). The two phases of the disease are caused by two different viral components: a replication-defective spleen focus-forming virus responsible for erythroleukemia and a replication-competent helper F-MuLV responsible for either a lymphoid leukemia or, with certain isolates, a rapid leukemia in newborn mice characterized by predominant erythroid involvement (28). Replication-competent MuLV code for a major glycoprotein designated gp70, which is formed by proteolytic cleavage of a glycosylated precursor designated PrENV (24). Studies on the topological arrangement of precursor and product on cell membranes suggest that gp70 but not PrENV is expressed on the cell surface with ecotropic and xenotropic MuLV, whereas gp70 and PrENV are detected on the plasma membrane in cells infected with mink cell focus-forming viruses (8, 36).

The monovalent ionophores monensin and nigericin have been shown to inhibit the transport and surface expression of membrane glycoproteins and the secretion of several secretory proteins (18, 31, 34, 35). The transport of the membrane glycoproteins of enveloped viruses was also reported to be blocked by these ionophores (2, 11, 12), suggesting that they act as generalized inhibitors for the transport of glycoproteins to the cell surface. However, subsequent studies suggest that, at least in some cell types, two distinct intracellular pathways exist for glycoprotein transport that differ in sensitivity to monovalent ionophores (2, 27).

We have investigated aspects of the intracellular transport and the role of viral glycoproteins in morphogenesis and infectivity of F-MuLV. In this report, we show that monensin blocks the transport of gp70 in F-MuLV-infected cells, affecting the release of infectious virions.

MATERIALS AND METHODS

Virus and cells. The Eveline mouse cell line (strain STU) producing F-MuLV was obtained from D. P. Bolognesi and A. J. Langlois, Duke University, Durham, N.C. Mouse SC-1 cells were supplied by the Cell Culture Department of the Naval BioSciences Laboratory, Oakland, Calif. The cells were maintained in Dulbecco medium supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, McLean, Va.). Growth medium was supplemented with dexamethasone (4 to 6 μg/ml, Sigma Chemical Co., St. Louis, Mo.) to enhance virus production.

Virus purification. Confluent monolayers of Eveline cells were radiolabeled with [^{3}H]glucosamine (5 μCi/ml), [^{3}H]leucine (5 μCi/ml), or [^{3}H]uridine (200 μCi/ml) in Eagle minimum essential medium supplemented with 2% fetal calf serum and dexamethasone for 24 h.

The culture fluids were clarified by high-speed centrifugation and purified by banding on discontinuous (20, 45, and 60% [wt/vol]) or linear (15 to 60% [wt/vol]) sucrose density gradients, or both, as described before (16).

Radioimmune precipitation. Radioimmune precipitation was carried out as described by Kessler et al. (17). In brief, labeled cells were extracted into 0.01 M phosphate-buffered saline (pH 7.5) containing 0.5% Nonidet P-40, and the lysates were clarified by centrifugation in a Beckman microfuge for 5 min. Radioimmune precipitation was carried out by using goat anti-gp70 (Rauscher) serum and Formalin-fixed Cowan I strain of *Staphylococcus aureus* to collect antigen-antibody complexes. The complexes were eluted and dissociated by boiling for 2 min in electrophoresis sample buffer (0.625 M Tris-hydrochloride [pH 6.8], 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 10% glycerol, and bromophenol blue) and were subjected to electrophoresis on 10% polyacrylamide gels. Gels were fluorographed by the procedure of Bonner and Laskey (6), dried, and exposed on Kodak X-Omat AR film for autoradiography. To isolate surface membrane glycoproteins, labeled monolayers were reacted with anti-gp70 serum before extraction, and radioimmune precipitation was carried out as described above.

Analysis of glycopeptides. Envelope glycoproteins radiolabeled with [^{3}H]mannose were isolated by radioimmune precipitation of labeled cell extracts with anti-gp70 serum. The proteins were precipitated by ethanol, digested extensively with pronase, and chromatographed on Bio-Gel P-6 columns (Bio-Rad Laboratories, Richmond, Va.) as described previously (13, 16). Glycopeptide peaks were pooled, concentrated, and desalted on Bio-Gel P-2 columns. The fractions containing labeled glycopeptides were lyophilized, dissolved in a small volume of 0.05 M sodium acetate buffer (pH 5.5), and digested with 0.02 U of endo-β-*N*-acetylglucosaminidase H (endo-H; Miles Laboratories, Inc., Elkhart, Ind.) for 24 h at 37°C before rechromatography on a Bio-Gel P-6 column. Glycopeptides from [^{14}C]glucosamine-labeled influenza hemagglutinin were used as an internal standard.

Cell surface iodination. Lactoperoxidase-catalyzed cell surface iodination was carried out by the procedures described by Witte and Weissman (37).

Infectivity assay. The infectivity titer of virus preparations was assayed by an immunofluorescent focus induction assay. Nearly confluent monolayers of SC-1 cells grown in tissue culture chambers or on tissue culture slides (Lab-Tek Products, Naperville, Ill.) were infected with 100 μl of serial 10-fold dilutions of the virus suspensions in the presence of Polybrene (2 μg/ml, Aldrich Chemical Co., Milwaukee, Wis.). The virus was allowed to adsorb for 60 min at 37°C with occasional rocking. Unadsorbed virus was removed by washing, and the cells were fed with fresh growth medium supplemented with dexamethasone. After 48 h, the monolayers were washed and fixed in an ethanol-acetic acid mixture at −20°C for 10 min. The cells were reacted with goat anti-p30 (Rauscher) serum followed by fluorescein-conjugated anti-goat gamma globulin (Antibodies Inc., Davis, Calif.) and examined under a fluorescence microscope. The foci of fluorescent cells were counted, and the titer was expressed as focus-forming units per ml.

Electron microscopy. Monolayers of Eveline cells grown in 60-mm dishes were washed extensively with phosphate-buffered saline and fixed in situ with 1% glutaraldehyde. Cells were postfixed with 1% osmium tetroxide for 30 min and embedded in epoxy resin mixture. Thin sections were cut on a Sorvall MT2B ultramicrotome (Du Pont Co., Wilmington, Del.). The sections were mounted on a 300-mesh copper grid and examined in a Philips EM 301 electron microscope. For surface-labeling studies, monolayers were incubated with cationized ferritin (Miles Laboratories) at a concentration of 100 μg/ml for 60 min at 37°C.

Reagents and isotopes. Monensin was purchased from Calbiochem-Behring Corp., La Jolla, Calif. Goat antiserum to Rauscher MuLV (R-MuLV) gp70 was prepared in this laboratory. Goat antiserum to R-MuLV p30 was obtained from the Office of Program Resources and Logistics of the National Cancer Institute through the courtesy of R. Wilsnack, Huntington Research Center, Brooklandville, Md. ^{125}I (carrier free, as sodium iodide, 16.2 mCi ^{125}I per μg of I), [^{3}H]glucosamine (22.6 Ci/mmol), and [^{3}H]mannose (16 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, Ill.). [^{3}H]uridine (28 Ci/mmol) was obtained from ICN, Irvine, Calif., and [^{3}H]leucine (42 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

Effect of monensin on the release of F-MuLV by Eveline cells. Monolayers of Eveline cells were incubated with different concentrations of monensin (10^{-5} and 10^{-6} M) for a period of 24 h, and the culture fluids were assayed for the presence of infectious virus by the immunofluorescent focus assay. There was a marked reduction in the release of infectious virus in the presence of monensin, and the inhibition was concentration dependent (Table 1). At a concentration of 10^{-5} M monensin, yields of infectious virus were reduced by more than 50-fold. The reduction in the virus yield was not because of toxicity of monensin, since more than 90% of the cells were viable during this period as determined by staining with neutral red, and the incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable fractions was only inhibited by ~30% even at the highest concentrations of monensin tested.

To determine whether the decrease in infectivity was because of a lack of virus particle production in the presence of monensin or because of the release of noninfectious particles, monensin-treated (10^{-6} M) and untreated cells were labeled with [^{3}H]uridine. The virions were purified from the culture fluids and banded on linear sucrose density gradients. Incorporation of [^{3}H]uridine into released virus particles was found to be inhibited by ~50% in monensin-treated cells. The observed reduction in particle production may be due in part to the slight decrease in protein synthesis caused by monen-

TABLE 1. Effect of monensin on yields of infectious F-MuLV and protein synthesis in Eveline cells

Concn of monensin	Virus titer[a]	% Control	Incorporation of [^{3}H]leucine (% control)[b]
0	3.4×10^5	100	100
10^{-6} M	4.1×10^4	12.0	92.2
10^{-5} M	5.6×10^3	1.6	71.2

[a] Monolayers of Eveline cells were incubated in the presence of different concentrations of monensin for 24 h at 37°C. The supernatant fluids were harvested, clarified, and assayed for infectious virus by immunofluorescent focus assay as described in the text. The results are expressed in focus-forming units per ml.

[b] Monolayers of Eveline cells were pretreated with different concentrations of monensin for a period of 24 h, and labeled with [^{3}H]leucine (50 μCi/ml) for 1 h. The monolayers were washed extensively with phosphate-buffered saline and lysed in 0.1 ml lysis buffer; 10-μl samples were spotted on a filter paper and extracted with hot trichloroacetic acid before counting. The control sample contained 199,200 cpm.

sin at the concentration tested. The fact that infectivity titers are decreased by a much greater extent than is particle production indicates that noninfectious particles are produced by monensin-treated cells.

Polypeptide profile of particles released by monensin-treated cells. Virions radiolabeled with [^{3}H]leucine were analyzed by polyacrylamide gel electrophoresis. The polypeptide profiles of virions released from monensin-treated and untreated gels were found to be similar on Coomassie blue-stained gels (Fig. 1C and D) and contained all the viral structural proteins. However, it is evident from the autoradiograms (Fig. 1A and B) that there was a marked reduction in the incorporation of the newly synthesized gp70 glycoprotein and p15E in the presence of monensin, whereas the incorporation of the other major structural proteins was not significantly affected.

Synthesis and transport of viral proteins in monensin-treated cells. To investigate the possible defects leading to failure of incorporation of envelope proteins into virions, labeled cell extracts were analyzed by radioimmune precipitation with antiserum to gp70. Envelope proteins were synthesized in the presence of monensin, but a reduction in the efficiency of cleavage of Pr ENV (Pr84/86) into gp70 and p15E was observed (Fig. 2A and B). Also, at least two to three bands with slightly different molecular weights could be identified in the region of gp70 in monensin-treated cells, with electrophoretic mobilities slightly faster than that of gp70 found in control cells. Eveline cells have been reported to contain two closely related MuLV genomes that code for different sized envelope glycoproteins (20). The multiple bands seen in the gp70 region in lysates of monensin-treated cells may represent these glycoproteins and their partially glycosylated, faster-migrating forms. The slower

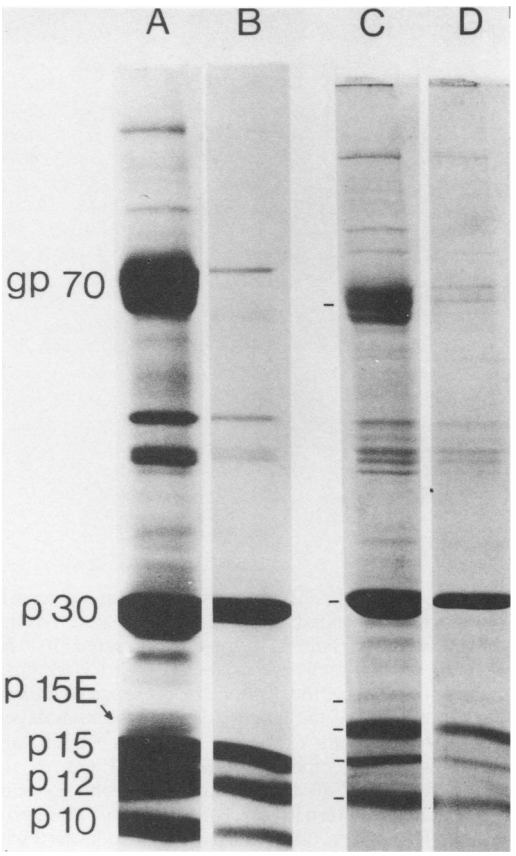

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of F-MuLV proteins from virions grown in the presence or absence of monensin. (Lane A) Autoradiogram showing the polypeptide profile of [^{3}H]leucine-labeled virions released from untreated Eveline cells. (Lane B) Polypeptide pattern of virus particles released in the presence of 10^{-5} M monensin. Lanes C and D show the Coomassie blue-stained gels of virions from untreated (C) and monensin treated (D) Eveline cells, respectively. The position of viral proteins was determined by radioimmune precipitation with monospecific antisera for gp70, p30, p15, and p15E. The proteins p12 and p10 were identified on the basis of their mobility with reference to molecular weight standards. The bands seen just ahead of p30 and those between p30 and gp70 presumably represent contaminating host cell proteins that copurify with MuLV in partially purified preparations. The gels that had been used for fluorography were rehydrated and stained to reexamine the polypeptide profile. The resulting differences in the size of gels before and after fluorography accounts for the differences in the positions of bands in lanes A and B versus C and D.

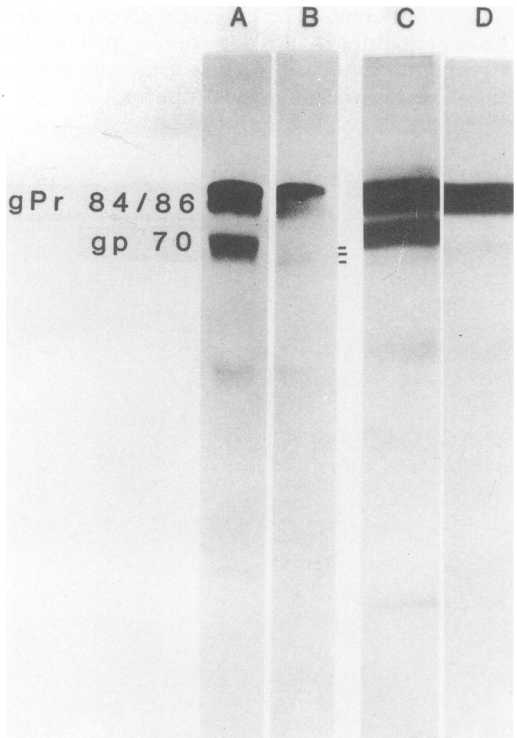

FIG. 2. SDS-PAGE analysis of envelope glycoproteins from untreated and monensin-treated Eveline cells. Untreated (lane A) or monensin-treated (10^{-5} M; lane B) Eveline cells were labeled with [^{3}H]glucosamine for 60 min in the absence or presence of monensin. At the end of the labeling period, the monolayers were washed and extracted into lysis buffer, and radioimmune precipitation was carried out as described in Materials and Methods by using goat-anti gp70 serum. Alternatively, untreated or monensin-treated (10^{-5} M) Eveline cells were pulse-labeled with [^{3}H]glucosamine in the absence (lane C) or presence (lane D) of 10^{-5} M monensin for 10 min and chased for 2 h. At the end of the chase period, cells were lysed and immune precipitation was carried out by using goat-anti gp70 serum.

rate of cleavage of precursor into gp70 was further shown by pulse-chase experiments. During a 60-min chase following a 10-min pulse, most of the label remained in the precursor form in monensin-treated cells, as opposed to controls that showed an equal distribution of label between the precursor and gp70 (Fig. 2C and D).

The slower rate of cleavage and faster electrophoretic mobility of gp70 produced in monensin-treated cells indicate a possible defect in processing or transport of glycoproteins, or both, through intracellular domains where such events occur. To determine whether newly synthesized viral glycoproteins reach the plasma membrane in monensin-treated cells, cells radiolabeled with [^{3}H]glucosamine for 2 h at 37°C were reacted with anti-gp70 before extraction with detergents and radioimmune precipitation. No labeled gp70 was detected at the plasma membrane in monensin-treated cells (Fig. 3A and B).

To determine whether monensin has any effect on incorporation into virions of gp70 which has already arrived at the plasma membrane, preformed gp70 was labeled with ^{125}I by cell surface iodination and chased into virions. Iodinated gp70 could be chased into virions both in

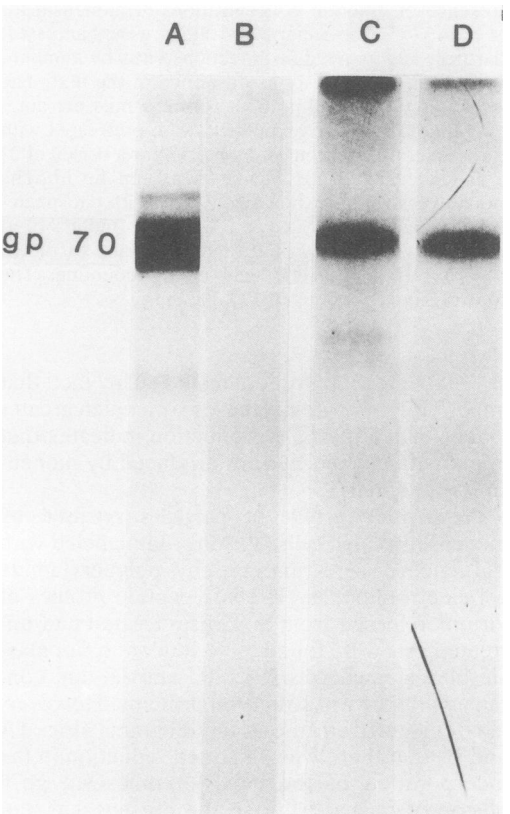

FIG. 3. Analysis of cell surface localization of gp70 in untreated and monensin-treated Eveline cells. Untreated (lane A) or monensin-treated (lane B) Eveline cells were labeled with [^{3}H]glucosamine for 2 h in the absence or presence of 10^{-5} M monensin. At the end of the labeling period, monolayers were washed and incubated on ice with goat-anti gp70 serum for 30 min. Unbound antibodies were removed by washing, the cells were lysed, and the immune complexes were precipitated by using *S. aureus* and analyzed by SDS-PAGE. To investigate the incorporation of preformed gp70 into virions, untreated (lane C) and monensin-treated (10^{-5} M for 3 h) monolayers (lane D) of Eveline cells were subjected to lactoperoxidase-catalyzed cell surface iodination and incubated with fresh medium with or without monensin. Culture fluids were harvested after 16 h and clarified, and released virus was banded on discontinuous sucrose density gradients. The interphase between 20 and 45% sucrose was analyzed by SDS-PAGE.

control and monensin-treated cells, indicating that monensin has little effect on incorporation of gp70 from the plasma membrane into virions (Fig. 3C and D). We have observed that the preformed gp70 pool has a relatively long half-life on infected cell surfaces (unpublished data), which would explain the large amounts of preformed gp70 that can be chased into virions from infected cells pretreated with monensin.

Processing of gp70 in monensin-treated cells. The faster electrophoretic mobility of gp70 in monensin-treated cells suggests possible defects in its oligosaccharide processing. To further investigate this phenomenon, we analyzed the pronase-derived glycopeptides of the envelope glycoproteins. Previous reports from our laboratory (13–16) have shown that pronase digestion of gp70 from ecotropic MuLV yielded four different glycopeptide size classes designated G_1, G_2, G_3, and G_4, with molecular weights of 5,100, 2,900, 2,200, and 1,500, respectively. In contrast, the PrENV precursor protein contained only two glycopeptides that correspond in size to G_3 and G_4. G_1 and G_2 were found to be resistant to digestion with endo-H, whereas G_3 and G_4 were endo-H sensitive.

Control and monensin-treated cells were radiolabeled with [^{3}H]mannose and the envelope glycoproteins (PrENV and gp70) were isolated by radioimmune precipitation as shown in Fig. 2. Gel filtration of pronase digests of the immune precipitate revealed significant differences in the pattern of glycopeptides from control and monensin-treated cells. As reported earlier for other ecotropic MuLVs, glycopeptide analysis of [^{3}H]mannose-labeled *env* glycoproteins from untreated Eveline cells revealed the four size classes G_1 to G_4 (Fig. 4A), of which only G_1 and G_2 were resistant to endo-H (Fig. 4C). In the presence of monensin, oligosaccharides of the G_1 and G_2 size classes were not detected (Fig. 4B), and all the oligosaccharides from monensin-treated cells were completely sensitive to endo-H (Fig. 4D). The large peaks in fractions 70 to 80 in Fig. 4C and D represent the mannose-rich side chains that are cleaved from the glycopeptides by endo-H digestion.

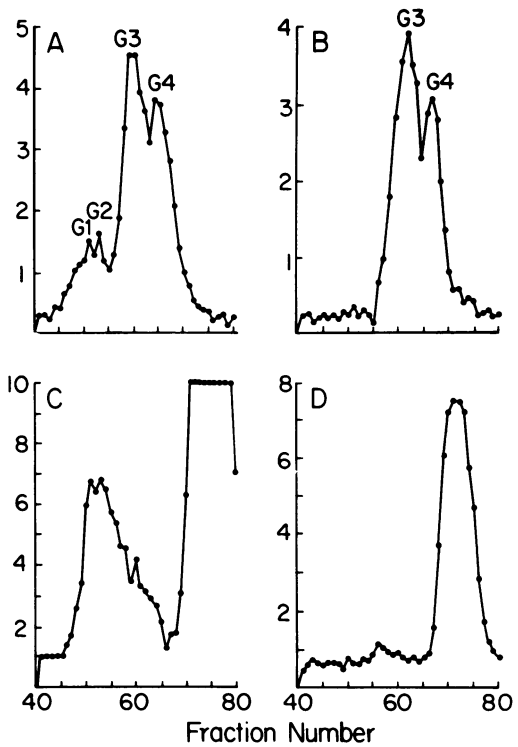

FIG. 4. Analysis of the glycopeptides of F-MuLV envelope glycoproteins from untreated and monensin-treated Eveline cells. Untreated or monensin-pretreated Eveline cells were labeled with [^{3}H]mannose in the absence or presence of 10^{-5} M monensin, and the envelope glycoproteins were isolated by radioimmune precipitation. The glycoproteins were digested extensively with pronase and analyzed by gel filtration on Bio-Gel P-6 columns. One-fourth of the volume from each fraction was used for counting, and the remaining amounts were pooled, concentrated, and desalted before digestion with endo-H. The endo-H digests were chromatographed on Bio-Gel P-6 columns, and the entire fractions were counted. (A) Pronase digests from untreated Eveline cells. (B) Pronase digests from monensin-treated Eveline cells. (C) Endo-H digests of glycopeptides from untreated Eveline cells. (D) Endo-H digests of glycopeptides from monensin-treated Eveline cells.

Morphology of monensin-treated cells. The ultrastructure of monensin-treated and untreated Eveline cells was compared by electron microscopy. Untreated monolayers of Eveline cells revealed extracellular and budding virions (Fig. 5A). The most striking difference in morphology of monensin-treated cells was the appearance of large dilated vesicles (Fig. 5B) similar to those reported previously in monensin-treated cells (2, 11, 33). These vesicles were empty and did not contain any viral particles. However, several vacuoles containing virions were observed (Fig. 5C) which appeared to be distinct from the monensin-induced dilated vesicles. Such virus-containing vacuoles were rarely seen in controls. To investigate the origin of the virus-containing vacuoles, cell surface labeling with cationized ferritin was used. In controls and monensin-treated cells, cationized ferritin showed a patchy distribution on the free apical surface, and passively filled the spaces between cells and the plastic substrate. A large amount of ferritin was also found to be internalized in vacuoles, and most of the virus containing vacuoles also contained ferritin granules (Fig. 5D and E), suggesting a cell surface origin for these

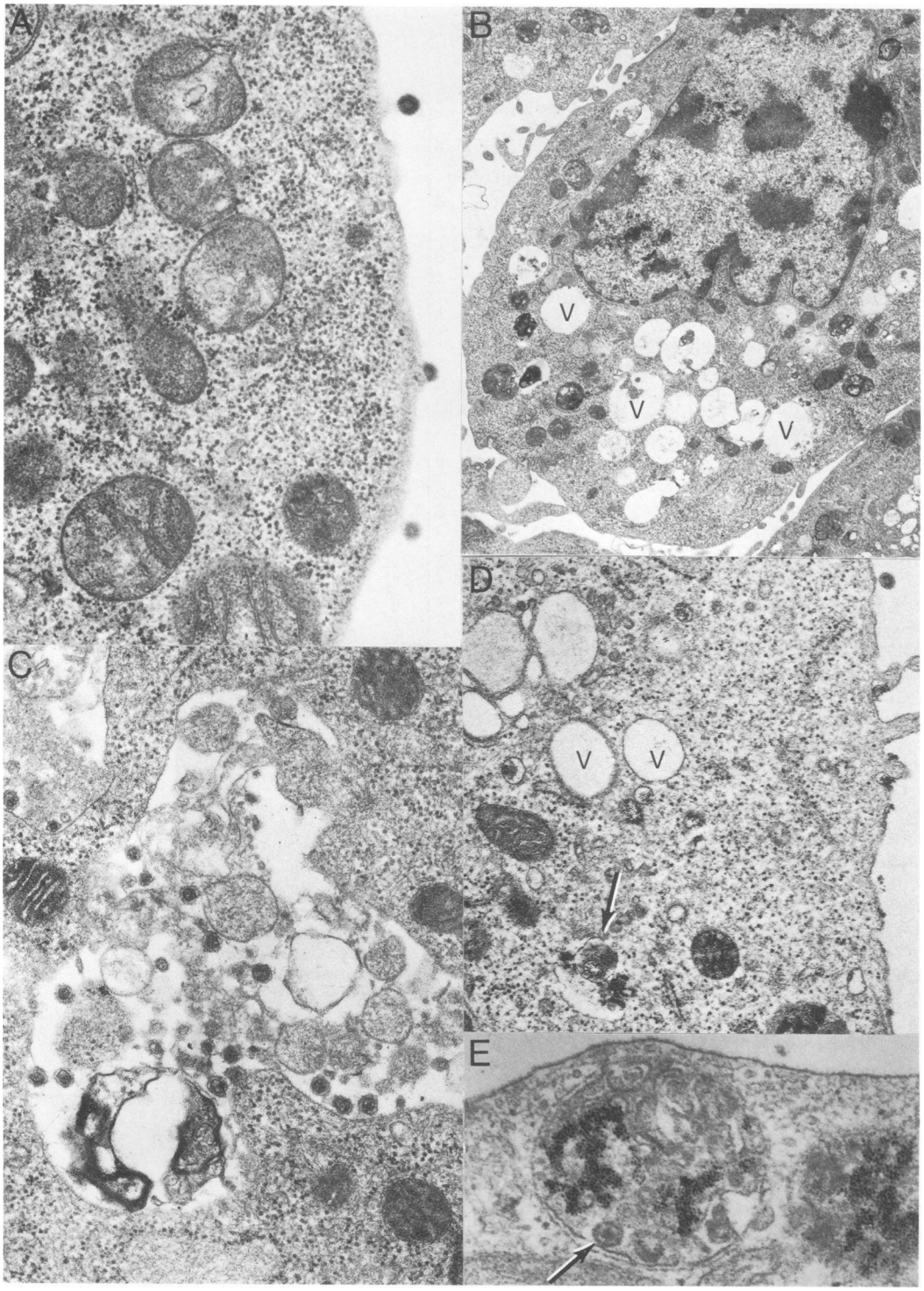

FIG. 5. Electron micrographs of thin sections from untreated and monensin-treated Eveline cells. (A) Untreated Eveline cells showing a normal morphology and virions at different stages of budding. (B) Low-magnification view of monensin-treated Eveline cells showing large, dilated intracytoplasmic vesicles (V). (C) A cytoplasmic vacuole from monensin-treated Eveline cells showing large number of virus particles. (D) Ferritin labeling of monensin-treated cells. Eveline cells were labeled with cationized ferritin (100 μg/ml for 1 h) and incubated in the presence of 10^{-5} M monensin for 3 h. Cells show a patchy distribution of ferritin on the cell surface. Intracellular vacuoles containing virions and ferritin granules are indicated by the arrow. The monensin-induced dilated vesicles (V) are empty. (E) A cytoplasmic vacuole from ferritin-labeled cells, showing ferritin granules and virus particles (indicated by the arrow). Magnification: A, ×21,000; B, ×8,000; C, ×30,000; D, ×23,000; E, ×120,000.

vacuoles. The absence of such vacuoles in untreated cells may be explained if lysosomes which normally fuse with and scavenge the phagocytic vacuoles are nonfunctional in monensin-treated cells because of an increase in pH (3, 21). In support of this, no virus-containing intracellular vacuoles were observed when the effects of monensin were reversed by incubation for 24 h in monensin-free medium. In such cells, all of the effects of monensin were reversed, and the cells displayed a normal morphology with numerous virions budding from the plasma membrane (not shown).

DISCUSSION

The role of gp70 in the morphogenesis of MuLV is not entirely understood. A model for replication of MuLV and other retroviruses suggests that viral glycoproteins are required for the initiation of the budding process (5). However, particles that lack gp70 have been reported to be produced by mutants of Rauscher (19, 25) and Friend (9) strains of MuLV, Gazdar virus (23), and murine sarcoma virus transformed S^+L^- cells (1), suggesting that viral glycoproteins may not be a prerequisite for MuLV morphogenesis. Studies on nondefective viruses cultivated in the presence of glycosylation inhibitors also revealed the production of virus particles that lack viral glycoproteins or their nonglycosylated forms (7, 26, 29). In this study, we have used monensin as a probe to investigate the role of envelope proteins in the morphogenesis and infectivity of F-MuLV.

Release of infectious virus was markedly reduced in the presence of monensin, which we attribute to an inhibition in the transport and subsequent incorporation of gp70 into virions. Metabolic labeling with radiolabeled amino acid and sugar precursors showed that no newly synthesized gp70 or p15E was incorporated into virions in monensin-treated cells. These results are consistent with the concept that gp70 is required for the infectivity of MuLV. Virus particles were still produced in monensin-treated cells, indicating that the decrease in infectivity was not because of a corresponding reduction in the efficiency of particle production. The production of particles in the presence of monensin might suggest that gp70 is not required for morphogenesis. Although iodination of the preformed pool of gp70 on cell surfaces revealed that particles produced in presence of monensin incorporated some prelabeled gp70, stained gels indicate that only low levels of gp70 are detected in such particles.

MuLV envelope glycoproteins formed in the presence of monensin did not contain complex oligosaccharides that are derived by trimming of the initial mannose-rich core and subsequent addition of branch chain sugars by transferases. It has been shown with other systems that monensin does not inhibit the oligosaccharide-processing machinery except for a few terminal events like sialylation and sulfation (31–34). The enzymes responsible for oligosaccharide processing are thought to reside in Golgi membranes (22), and thus it appears that transit of gp70 is blocked before its entry into the Golgi complex in monensin-treated cells. Previous reports suggest that different glycoproteins vary in the site at which their transport is arrested after monensin treatment. In the case of BHK-21 and chicken embryo fibroblasts infected with vesicular stomatitis virus or Sindbis virus, the transport of viral glycoproteins seems to be arrested at a point between the Golgi complex and plasma membrane (2, 11). In cultured hepatoma cells, transport of the vesicular stomatitis virus G protein was reported to be arrested after its acquisition of endo-H resistance, in contrast to transferrin which was inhibited before it acquired resistance to endo-H digestion (31). In human fibroblast cultures, procollagen and fibronectin were reported to accumulate in dilated Golgi cisternae and in dilated vesicles of rough endoplasmic reticulum (35). It has been suggested that accumulation of proteins in the endoplasmic reticulum of monensin-treated cells might reflect either an upstream accumulation of proteins which cannot be accommodated in the altered Golgi compartment or alternatively an additional block in transport at the exit from the endoplasmic reticulum. Our results suggest that the latter possibility may be more likely, since we did not observe the normal oligosaccharide processing for viral glycoproteins in monensin-treated cells, an event which occurs in almost a normal maner even in the altered Golgi compartment of monensin-treated cells (31–34). The factors that govern the site of arrest in the transport of glycoproteins after monensin treatment have not been investigated, and they appear to be distinct for various glycoproteins.

Monensin may be used to reveal distinct intracellular pathways for transport of glycoproteins to the cell surface, which differ in sensitivity to the ionophore (2, 27). The MuLV gp70 glycoprotein has been found free in the sera of certain strains of mice (30). In F-MuLV-infected cells, some gp70 is also found in a free form in the culture fluids (4). It has not been determined whether these molecules represent true secreted proteins or whether they are externalized by release from cell membranes or virions. We did not find any such secreted forms of gp70 from monensin-treated cells (data not shown), suggesting that if these molecules are indeed secreted, they resemble the gp70 molecules on cell membranes in sensitivity to monensin.

Previous studies with ecotropic viruses indicate that only gp70 is expressed on the external surface of the cell membrane, whereas the PrENV precursor is found predominantly in rough and smooth endoplasmic reticulum (36). Analysis of oligosaccharides indicated that carbohydrate processing and proteolytic cleavage of precursor proteins are temporally linked, since no complex oligosaccharides have been observed on the precursors (14, 36). This suggests that cleavage may occur at or before the entry of glycoprotein precursors into the Golgi compartment. The observed reduction in the efficiency of proteolytic cleavage of the precursor in monensin-treated cells may result from a block in transport before the entry of glycoproteins into the Golgi compartment. The limited degree of cleavage observed in our studies may indicate that a small number of molecules are cleaved at the rough endoplasmic reticulum, although cleavage occurs most efficiently at Golgi or plasma membrane domains. Alternatively, this level of cleavage may represent a small proportion of molecules that might have bypassed the monensin block in glycoprotein transport.

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LITERATURE CITED

1. **Aaronson, S. A., R. H. Bassin, and C. Weaver.** 1972. Comparison of murine sarcoma viruses in nonproducer and S^+L^--transformed cells. J. Virol. **9**:701–704.
2. **Alonso, F. V., and R. W. Compans.** 1981. Differential effects of monensin on enveloped viruses that form at distinct plasma membrane domains. J. Cell Biol. **89**:700–705.
3. **Basu, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown.** 1981. Monensin interrupts the recycling of low density lipoprotein receptors in human fibroblasts. Cell **24**:493–502.
4. **Bolognesi, D. P., A. J. Langlois, and W. Schafer.** 1975. Polypeptides of mammalian oncornaviruses. IV. Structural components of murine leukemia virus released as soluble antigen in cell culture. Virology **68**:550–555.
5. **Bolognesi, D. P., R. C. Montelaro, H. Frank, and W. Schafer.** 1978. Assembly of type C oncornaviruses: a model. Science **199**:183–186.
6. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
7. **Chatterjee, S., J. A. Bradac, and E. Hunter.** 1981. Effect of tunicamycin on cell fusion induced by Mason-Pfizer monkey virus. J. Virol. **38**:770–776.
8. **Famulari, N. G., and K. Jelalian.** 1979. Cell surface expression of the *env* gene polyprotein of dual-tropic mink cell focus-forming murine leukemia virus. J. Virol. **30**:720–728.
9. **Fitting, T., M. Ruta, and D. Kabat.** 1981. Mutant cells that abnormally process plasma membrane glycoproteins encoded by murine leukemia virus. Cell **24**:847–858.
10. **Friend, C.** 1957. Cell free transmission in adult Swiss mice of disease having the character of a leukemia. J. Exp. Med. **105**:307–318.
11. **Johnson, D. C., and M. J. Schlesinger.** 1980. Vesicular stomatitis virus and Sindbis virus glycoprotein transport to the cell surface is inhibited by ionophores. Virology **103**:407–424.
12. **Kaariainen, L., K. Hashimoto, J. Saraste, I. Virtanen, and K. Penttinen.** 1980. Monensin and FCCP inhibit the intracellular transport of alphavirus membrane glycoproteins. J. Cell Biol. **87**:783–791.
13. **Kemp, M. C., S. Basak, and R. W. Compans.** 1979. Glycopeptides of murine leukemia viruses. I. Comparison of two ecotropic viruses. J. Virol. **31**:1–7.
14. **Kemp, M. C., N. G. Famulari, and R. W. Compans.** 1981. Glycopeptides of murine leukemia viruses. III. Glycosylation of *env* precursor glycoproteins. J. Virol. **39**:463–470.
15. **Kemp, M. C., N. G. Famulari, P. V. O'Donnell, and R. W. Compans.** 1980. Glycopeptides of murine leukemia viruses. II. Comparison of xenotropic and dual-tropic viruses. J. Virol. **34**:154–161.
16. **Kemp, M. C., K. S. Wise, L. E. Edlund, R. T. Acton, and R. W. Compans.** 1978. Origin of minor glycoproteins of murine leukemia viruses. J. Virol. **28**:84–94.
17. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorbent; parameters of the interaction of antigen-antibody complexes with protein A. J. Immunol. **115**:1617–1624.
18. **Ledger, P. W., N. Uchida, and M. L. Tanzer.** 1980. Immunocytochemical localization of procollagen and fibronectin in human fibroblasts: effects of monovalent ionophore monensin. J. Cell. Biol. **87**:663–671.
19. **Mooren, H. W. D., F. A. Prins, P. Herbrink, and S. O. Warnaar.** 1981. Electron microscopic studies on the role of the envelope antigens of R-MuLV ts-29 in budding. Virology **113**:254–262.
20. **Murray, M. T., and D. Kabat.** 1979. Genetic and sialylation sources of heterogeneity of the murine leukemia virus membrane envelope glycoproteins gp69/71. J. Biol. Chem. **254**:1340–1348.
21. **Ohkuma, S., and B. Poole.** 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc. Natl. Acad. Sci. U.S.A. **75**:3327–3331.
22. **Palade, G. E.** 1975. Intracellular aspects of the process of protein secretion. Science **189**:347–358.
23. **Pinter, A., and E. de Harven.** 1979. Protein composition of a defective murine sarcoma virus particles possessing the enveloped type-A morphology. Virology **99**:103–110.
24. **Racevskis, J., and G. Koch.** 1977. Viral protein synthesis in Friend erythro-leukemia cell lines. J. Virol. **21**:328–337.
25. **Ruta, M., M. J. Murray, M. C. Webb, and D. Kabat.** 1979. A MuLV mutant with a temperature sensitive defect in membrane glycoprotein synthesis. Cell **16**:77–88.
26. **Schultz, A. M., and S. Oroszlan.** 1979. Tunicamycin inhibits glycosylation of precursor polyprotein encoded by *env* gene of Rauscher murine leukemia virus. Biochem. Biophys. Res. Commun. **86**:1206–1213.
27. **Smilowitz, H.** 1980. Routes of intracellular transport of acetylcholine receptor and esterase are distinct. Cell **19**:237–244.
28. **Steeves, R. A., R. J. Eckner, M. Bennet, E. A. Mirand, and P. J. Trudel.** 1971. Isolation and characterization of a lymphatic leukemia virus in Friend virus complex. J. Natl. Cancer Inst. **46**:1209–1217.
29. **Stohrer, R., and E. Hunter.** 1979. Inhibition of Rous sarcoma virus replication by 2-deoxyglucose and tunicamycin: identification of an unglycosylated *env* gene product. J. Virol. **32**:412–419.
30. **Strand, M., and J. T. August.** 1976. Oncornavirus envelope glycoprotein in serum of mice. Virology **75**:130–144.
31. **Strous, G. J. A. M., and H. F. Lodish.** 1980. Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. Cell **22**:709–717.
32. **Tajiri, K., N. Uchida, and M. L. Tanzer.** 1980. Undersulfated proteoglycans are secreted by cultured chondrocytes in the presence of the ionophore monensin. J. Biol.

Chem. **255**:6036–6039.
33. **Tartakoff, A., D. Hoessli, and P. Vassali.** 1981. Intracellular transport of lymphoid surface glycoproteins. J. Mol. Biol. **150**:525–535.
34. **Tartakoff, A. M., and P. Vassali.** 1977. Plasma cell immunoglobulin secretion. Arrest is accompanied by alterations of Golgi complex. J. Exp. Med. **146**:1332–1345.
35. **Uchida, N., H. Smilowitz, and M. L. Tanzer.** 1979. Monovalent ionophores inhibit secretion of procollagen and fibronectin from cultured human fibroblasts. Proc. Natl. Acad. Sci. U.S.A. **76**:1868–1872.
36. **Witte, O. N., A. Tsukamoto-Adey, and I. L. Weissman.** 1977. Cellular maturation of oncornavirus glycoproteins: topological arrangement of precursor and product forms in cellular membranes. Virology **76**:539–553.
37. **Witte, O. N., and I. L. Weissman.** 1976. Oncornavirus budding: kinetics of formation and utilization of viral membrane glycoprotein. Virology **69**:464–473.