JOURNAL OF VIROLOGY, May 1981, p. 770-776
0022-538X/81/050770-07$02.00/0

Vol. 38, No. 2

Effect of Tunicamycin on Cell Fusion Induced by Mason-Pfizer Monkey Virus

SUBHENDRA CHATTERJEE, JAMES BRADAC, AND ERIC HUNTER*

Department of Microbiology, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294

Received 2 December 1980/Accepted 13 February 1981

Mason-Pfizer monkey virus, a D-type retrovirus, has been shown to induce multinucleate cell (syncytium) formation or cell fusion in several normal primate cells. A series of experiments has been carried out to examine whether a glycosylated "fusion-inducing" product is responsible for this biological property of Mason-Pfizer monkey virus. Treatment of rhesus monkey fetal lung cells with different concentrations of tunicamycin, a potent inhibitor of glycosylation, during infection with Mason-Pfizer monkey virus had no effect on cell fusion even though up to 5 μg of the drug per ml was tested. Furthermore, no significant effect on the extent of syncytium formation in rhesus monkey fetal lung cells was observed when the time of addition or duration of treatment with this inhibitor was varied. Nevertheless, tunicamycin was very effective in blocking glycosylation in rhesus cells since virions produced in the presence of this drug completely lacked gp70 and gp20, the two structural glycoproteins of Mason-Pfizer monkey virus. These non-glycosylated virus particles produced in the presence of tunicamycin were noninfectious as determined by a protein A binding assay and were unable to induce syncytium formation when assayed on rhesus cells. These results indicate that glycosylation of the fusion-inducing product is not required for multinucleate cell formation induced by Mason-Pfizer monkey virus.

Several RNA and DNA viruses are known to induce multinucleate cell (syncytium) formation in eucaryotic systems (4-6, 25-27, 40, 41). Two major types of virus-induced cell fusion or syncytium formation have been described; these have been named "fusion from without" and "fusion from within" (2, 20). In the former, the interaction of the surface proteins of incoming virus with the cell membrane is sufficient to induce cell to cell fusion; in the latter, virus fusion proteins produced during replication of the virus induce cell membrane fusion. It has been shown that membrane glycoproteins play an important role in both types of cell fusion (10, 14, 16, 19, 22, 28, 29). Mason-Pfizer monkey virus (M-PMV), a D-type retrovirus isolated from a spontaneous mammary carcinoma (7), has been shown to induce multinucleate cell formation in normal human and nonhuman primate cell lines (1, 4, 9). This property is shared by the other two D-type viruses, Langur virus and Squirrel monkey retrovirus (5). The characteristics and requirements of D-type virus-induced cell fusion previously reported (4, 5) suggest that the mechanism by which multinucleate cell formation is induced by these viruses is different from that reported for other syncytium-forming retroviruses (13, 24) or for the paramyxoviruses (2, 15, 25, 26), in that concentrated virus is unable to induce rapid multinucleate cell formation. De novo protein synthesis, but not virus replication, is apparently required for the induction of cell fusion by M-PMV (4). By using tunicamycin (TM), a glucosamine-containing antibiotic which specifically inhibits glycosylation of proteins in which carbohydrate is linked through asparagine residues (34-38), we report here that the "fusion-inducing protein" involved in M-PMV-mediated cell fusion does not require glycosylation for its activity. Our results show that the addition of TM, under conditions which clearly inhibit the synthesis of virion glycoproteins and infectious virus production, has no effect on M-PMV-induced cell fusion. This is in contrast to Newcastle disease virus- or herpes simplex virus type 1-induced cell fusion which can be inhibited by treatment with 2-deoxy-D-glucose, which although less specific than TM also inhibits glycosylation (10).

MATERIALS AND METHODS

Virus and cell cultures. Normal rhesus monkey fetal lung cells (DBS-FRHL-2) were purchased from Flow Laboratories, Inc., Rockville, Md. MPFS clone 940c3 and MPFS clone 4 (two clones of rhesus foreskin cells transformed by M-PMV) producing infectious M-

PMV particles were provided by M. Ahmed and D. L. Fine. The CMMT cell line (rhesus monkey mammary tumor cells cocultivated with rhesus monkey embryo cells), another source of M-PMV, was provided by M. Ahmed.

DBS-FRHL-2 cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and 0.05% sodium bicarbonate. Both MPFS clone 940.3 and MPFS clone 4 cells were grown in RPMI 1640 medium containing 5% fetal calf serum, 10% tryptose phosphate broth, and 0.05% sodium bicarbonate, whereas CMMT cells were grown in RPMI 1640 medium containing 10% fetal calf serum, 5% tryptose phosphate broth, and 0.05% sodium bicarbonate. All of the sera used were heat inactivated.

Chemicals and radioisotopes. Reagents for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. TM was a generous gift from R. L. Hamill, Lilly Research Laboratories, Indianapolis, Ind. [^{3}H]glucosamine (specific activity, 8.5 Ci/mmol), ^{14}C-amino acid mixture (specific activity, 50 mCi/mmol), and Na^{125}I (specific activity, 17 Ci/mg) were obtained from ICN Pharmaceuticals Inc., Irvine, Calif.

Antisera. The preparation and description of specific antiserum against M-PMV have been published previously (4). Monospecific antisera against M-PMV p27 and against Tween-ether-disrupted M-PMV were obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md.

Assay for cell fusion by M-PMV. The direct syncytia assay for M-PMV has been described previously (4). In brief, monolayers of DBS-FRHL-2 cells in 35-mm dishes were inoculated with 0.25 ml of different dilutions of M-PMV suspension (containing 2 μg of polybrene per ml) in the presence or absence of different concentrations of TM. Some dishes were pretreated with the drug for different time periods as described in the text. Uninfected cells were inoculated with the same amount of growth medium containing polybrene with or without the drug. After 1 h of adsorption at 37°C the cells were overlaid with growth medium with or without the drug and incubated for 48 h at 37°C. Cells were then fixed and stained with May-Grunwald-Giemsa stain (18), and the multinucleate cells were counted.

Harvests from TM-treated cells were assayed in essentially the same manner to determine whether fusion-competent virus was produced in the presence of this drug.

Assay for virus replication by a staphylococcus protein A binding immunoassay. A detailed description of the staphylococcus protein A binding immunoassay will be published elsewhere (E. Hunter, manuscript in preparation); however, a brief outline is presented below. Semiconfluent monolayers of DBS-FRHL-2 cells grown in 96-well microtiter plates (Costar, Cambridge, Mass.) were inoculated with harvests collected from TM-treated and untreated cultures of CMMT cells. After 1 h of adsorption, the cells were washed twice with growth medium and incubated for 8 to 10 days at 37°C. Cells were then washed three times with 25 mM Tris-buffered saline (pH 7.2) and fixed with a mixture of 95% ethyl alcohol and 5% glacial acetic acid at −20°C for 1 h. Fixed cells were washed three times with Tris-buffered saline containing 0.1% bovine serum albumin and 0.1% Triton X-100 and incubated with either goat anti-M-PMV or goat anti-M-PMV p27 antiserum (25 μl to each well) for 1 h at room temperature. Cells were then washed three times with the same buffer, and 5×10^4 cpm of ^{125}I-labeled protein A from *Staphylococcus aureus* (Pharmacia Fine Chemicals, Uppsala, Sweden) was added to each well. After 30 to 45 min of incubation at room temperature, the cells were washed again thoroughly with the same buffer and dried. The viral antigen-positive wells that had bound antibody and protein A were detected by autoradiography for 24 h at −70°C on Kodak XR-2 film with a Du Pont Cronex Hi-Plus intensifying screen.

For quantitative purposes, the cells were disrupted after autoradiography with 1% sodium dodecyl sulfate and counted in a Tracor Model 1190 gamma counter (Tracor Analytic Inc., Atlanta, Ga.).

Preparation of radiolabeled virus and polyacrylamide gel electrophoresis. To determine the effect of TM on the virion polypeptide synthesis, CMMT cells were preincubated for 5 to 6 h in different concentrations of TM and then labeled with [^{3}H]glucosamine (200 μCi/ml) and ^{14}C-amino acid mixture (20 μCi/ml) for 48 h in complete growth medium containing the drug. The culture fluids were clarified by low-speed centrifugation and layered onto a 24 to 48% (wt/wt) discontinuous sucrose gradient; the band at the 24 to 48% interphase was diluted 1:2 and then recentrifuged on a 24 to 48% continuous sucrose gradient as described previously (4). The virus band from the last gradient was collected, diluted, and pelleted by centrifugation at 45,000 rpm for 1 h. Purified virion polypeptides were separated on 12% discontinuous sodium dodecyl sulfate-polyacrylamide slab gels as described previously (17). Radiolabeled polypeptides were localized after Coomassie brilliant blue staining by slicing each lane into 1-mm-thick sections and counting in a toluene-Omnifluor scintillation fluid containing 10% Protosol.

The molecular weights of viral polypeptides were determined by comparing the migration of these proteins with that of protein standards (Bio-Rad) electrophoresed in adjacent wells.

Electron microscopy. TM-treated and untreated cells were fixed and embedded, and thin sections were stained with uranyl acetate and lead citrate as described previously (8). All the preparations were examined under a Philips EM 301 electron microscope.

RESULTS

TM does not inhibit M-PMV-induced cell fusion. Our previous studies indicated that de novo protein synthesis was necessary for M-PMV-induced cell fusion (4). To determine whether glycosylation of this fusion-inducing factor was necessary for its activity, we studied the effect of TM treatment on M-PMV-induced cell fusion. The results of these experiments are summarized in Table 1. Addition of different concentrations of TM (0.25 to 5.0 μg/ml) simultaneously with the virus for up to 24 h postin-

TABLE 1. *Effect of TM on syncytium formation induced by M-PMV in rhesus cells*[a]

Expt no.	Time of treatment (h)	% Syncytia at TM concn (μg/ml): 0	0.25	0.5	1.0	2.0	5.0
1	−12–0	100	−[b]	−	100	104	95
	0–12	100	−	−	97	91	81
	12–24	100	−	−	91	96	88
2	0–12	100	100	106	105	−	−
	12–24	100	100	103	100	−	−
3	0–24	100	−	93	90	89	−
4	0–12	100	−	95	93	87	−
	−2–12	100	−	94	96	91	−

[a] Monolayers of DBS-FRHL-2 cells were pretreated, treated during infection, or posttreated for different time periods with different concentrations of TM as shown in the table. Cells were stained 48 h postinfection. See details in the text.

[b] −, Not tested in this experiment.

fection had no significant effect on multinucleate cell formation, even though the lowest of these concentrations was capable of inhibiting viral glycoprotein synthesis in rhesus cells (see below). Since it was possible that preexisting pools of lipid-sugar precursors might be large enough to allow sufficient glycosylation for cell fusion to occur, cells in one experiment were pretreated with TM for 12 h before infection, at which time the drug was removed (no reversal of TM action was observed within 24 h of removal of the drug), and in a second experiment cells were treated from 2 h before to 12 h after virus addition. In neither case was any effect on cell fusion observed. Although M-PMV from the MPFS clone 4 cell line was used in a majority of the experiments summarized in Table 1, identical results were obtained with M-PMV from different sources (MPFS clone 940_c3 and CMMT cell lines).

TM inhibits the production of infectious and fusion-inducing M-PMV. To confirm that TM, at the levels used in Table 1, could inhibit glycosylation of M-PMV-directed glycoprotein synthesis, experiments were performed to determine the effect of this drug on the synthesis of replicating and fusion-inducing virus. Rhesus monkey (CMMT) cells producing both replicating and fusion-inducing M-PMV were treated with different concentrations of TM for 48 h. The virus harvested from these plates was assayed for replicating virus in a protein A binding immunoassay and for fusion-inducing virus in a direct syncytia assay. For the infectious virus assay, serial dilutions of the harvests were inoculated onto semiconfluent monolayers of DBS-FRHL-2 cells grown in a 96-well microtiter plate. After an adsorption period of 1 h, the cells were washed thoroughly and incubated for 8 to 10 days at 37°C. At this time the plate was processed for the protein A binding assay (Fig. 1 and Table 2). As little as 0.5 μg of TM per ml inhibited synthesis of infectious virus by 88%, and 99% inhibition was observed at 1 μg/ml. Similar results were obtained when the harvests from TM-treated CMMT cells were tested in a direct syncytia assay on DBS-FRHL-2 cells (Table 2); greater than 95% inhibition of cell fusion was observed in harvests from cells treated with 0.5 μg of TM per ml.

Similar effects of TM on virus infectivity have been observed with Rous sarcoma virus and have been correlated to a lack of glycosylated proteins (30, 33). To show directly that the effect of TM on the production of infectious or fusion-inducing virus was at the level of glycoprotein synthesis rather than virus assembly, the following experiments were performed.

M-PMV particle formation occurs in TM-treated cells. To examine whether the formation of virus particles occurs in the presence of TM, rhesus monkey cells (CMMT), producing M-PMV virus were treated with 0, 0.5, and 1 μg of TM per ml for 2 to 4 days. TM-treated and untreated cells were then processed for electron microscopic observation as described above (Fig. 2). The presence of both mature and budding particles can be observed in thin sections of both drug-treated and untreated cells (Fig. 2A and B), indicating that TM does not inhibit the

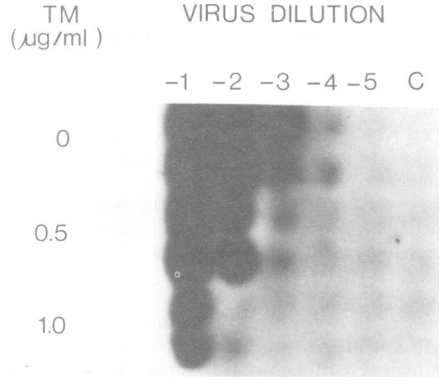

FIG. 1. *Confluent monolayers of CMMT cells producing infectious M-PMV particles were treated with 0, 0.5, and 1.0 μg of TM per ml for 48 h, at which time the culture fluids were harvested. Serial dilutions (100 μl) of these harvests were inoculated onto monolayers of DBS-FRHL-2 cells grown in a 96-well microtiter plate. Duplicate cells were inoculated at each dilution. Cells were assayed for infection by replicating M-PMV in a protein A binding immunoassay as described in the text. C represents uninfected, control wells.*

TABLE 2. *Infectivity of M-PMV released from TM-treated cells as assayed by syncytium formation or replication in the ^{125}I-protein A binding assay*[a]

Concentration of tunicamycin (μg/ml)	Syncytium formation		Replication	
	No. of syncytia/cm^2	% Inhibition	cpm of ^{125}I-protein A bound/well[b]	% Inhibition
0	916	0	1,058	0
0.5	36	96	130	88
1.0	NT[c]	NT	15	99

[a] Confluent monolayers of CMMT cells were treated with the concentrations of TM shown in the table, and the harvests collected were assayed for fusion-inducing and replicative M-PMV as described in the text.

[b] Counts per minute (cpm) bound per well at a 10^{-2} dilution of virus harvest.

[c] NT, Not tested.

formation of M-PMV virus particles.

M-PMV particles produced in the presence of TM lack envelope glycoproteins. The observation of virus particles in thin sections of TM-treated CMMT cells was confirmed in experiments in which the cells were labeled for 48 h with ^{14}C-amino acids and [^{3}H]glucosamine in the presence of the drug, since a peak of radioactive virus was observed at the characteristic density for M-PMV. However, a comparison of the structural polypeptides of these particles with those of virions produced from untreated cells revealed that the prominent peaks of ^{14}C-amino acid and [^{3}H]glucosamine label seen at the positions of gp70 and gp20 in untreated virions were absent in the TM particles (Fig. 3). Not only are the [^{3}H]glucosamine-labeled peaks missing from the TM-treated virion profile, but also no residual or new ^{14}C-amino acid-labeled peaks can be observed—indicating that the viral glycoproteins or their non-glycosylated counterparts are completely absent from these virions. The profile of the major non-glycosylated polypeptides p27, p14, p12, and p10, on the other hand, appears to be the same in virions from both treated and untreated cultures, although a reduction in the total amount of ^{14}C-amino acids incorporated was observed in the presence of TM. This is consistent with the reduction in protein synthesis seen in TM-treated cells (~55%). Thus, virus glycoprotein synthesis is specifically inhibited at concentrations of TM that have no effect on the process of M-PMV-induced cell fusion.

DISCUSSION

Glycosylated polypeptides of a variety of enveloped viruses play a major role in the attachment to and penetration of the host cell by the virus. In some instances, for example, with the paramyxoviruses, the interaction between a glycosylated protein and the cell membrane can result in cell-to-cell fusion and the formation of multinucleate cells or syncytia. The role of the carbohydrate in this process is unclear, but with both the paramyxovirus Newcastle disease virus and the herpesvirus herpes simplex virus type 1 the ability to induce cell fusion from within can be abrogated when glycosylation is blocked (10).

The mechanisms involved in M-PMV-induced cell fusion are unclear. Nevertheless, previous work in this laboratory indicated that fusion was not the result of an interaction between the virion glycoproteins and the cell membrane (4) (i.e., not fusion from without), but rather that de novo protein synthesis during the first 12 h postinfection was necessary for multinucleate cell formation to occur (fusion from within) (4). In other retrovirus systems, murine leukemia virion RNA has been found on polysomes shortly after infection (31), and the major *gag* gene translation product (pr76) of avian myeloblastosis virus can be detected in chicken cells within 3 h postinfection with the virus (11). The fact that the *gag* gene product of both murine leukemia virus and avian myeloblastosis virus could be glycosylated (3, 21, 32) raised the possibility that such a product might mediate M-PMV-induced cell fusion. To examine this possibility we employed tunicamycin, a glycosamine-containing antibiotic produced by *Streptomyces lysosuperficus* (36) and which has been used to determine the biological role of the carbohydrate moiety of several glycoproteins. TM specifically inhibited the formation of the *N*-acetylglucosamine-lipid intermediate which is the first step in the transfer of carbohydrates to the asparagine of newly synthesized protein (39). The experiments reported here show conclusively that glycosylation of the putative fusion-inducing protein was not necessary for its activity. M-PMV-induced fusion thus differs from that of Newcastle disease virus and herpes simplex virus type 1 in which glycosylation is necessary for fusion to occur. The experiments do not, however, rule out the possibility that a glycosylated protein is involved in M-PMV-induced fusion since the unglycosylated G protein of vesicular stomatitis virus, for example, is still active in mediating viral entry into a cell, and we may be observing a similar situation here. Experiments designed to identify M-PMV-specified proteins that are synthesized shortly after infection may provide answers to this question.

The conditions used to block protein glycosylation in these experiments were clearly adequate since at very low concentrations of TM, (0.5 μg/ml) infectious virus production was in-

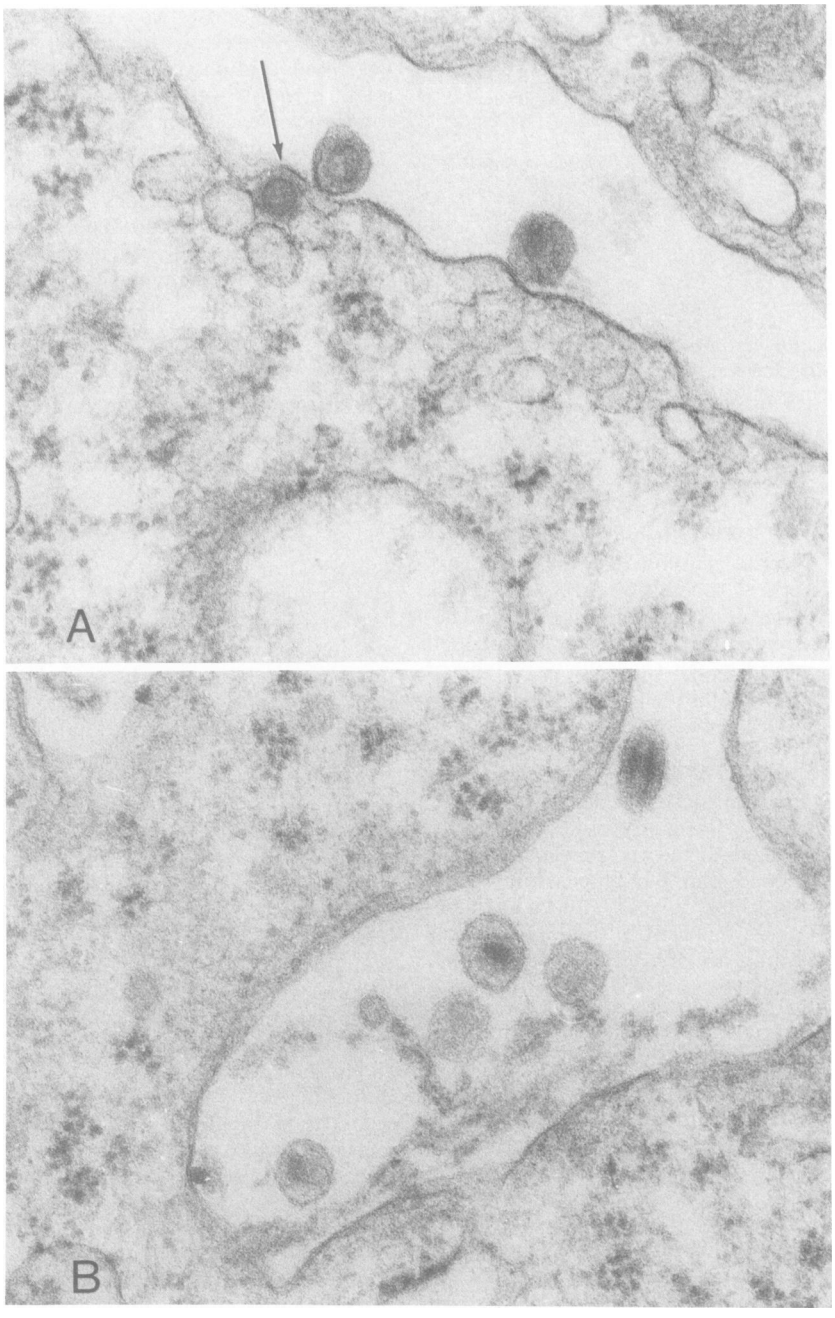

FIG. 2. *Electron microscopic observation of virions present in the thin sections of TM-treated and untreated CMMT cells. (A) Cells were treated with 0.5 μg of TM per ml. Both mature and budding particles (arrow) can be observed. (B) Thin section of the same cell line without drug treatment, also showing mature particles (×75,000).*

hibited almost 90%, and the titer of virus capable of inducing fusion was reduced by 20-fold. As in the case of Rous sarcoma virus, mature M-PMV virus particles which lack any detectable glycoproteins are produced in the presence of TM. Thus, although the viral glycoproteins gp70 and gp20 do not mediate cell fusion, they are required, presumably to allow attachment of the

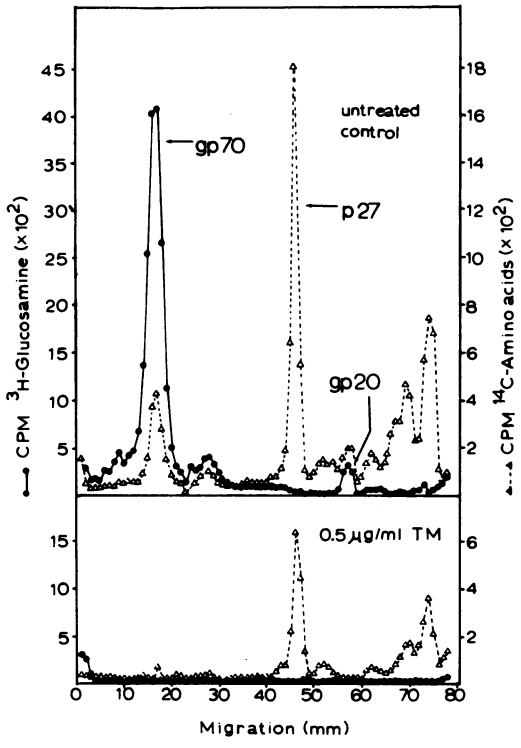

FIG. 3. *Synthesis of M-PMV-glycosylated and non-glycosylated proteins in the presence or absence of 0.5 µg of TM per ml, as analyzed by polyacrylamide slab gel electrophoresis. (A) Polypeptide profile of the virions released from untreated CMMT cells. (B) Polypeptide pattern of the same cell line in the presence of 0.5 µg of TM per ml. Virions synthesized in the presence of the drug lacked gp70 and gp20.*

virus and penetration of the genomic RNA, for fusion to occur.

Particle formation in the presence of TM occurs with several viruses, i.e., influenza virus (23), vesicular stomatitis virus (12), and Rous sarcoma virus (30, 33) but is blocked with others, i.e., Semliki forest and fowl plague virus (30). M-PMV is a D-type retrovirus, and during replication it assembles an intracytoplasmic A-particle which is enveloped during budding from the plasma membrane. The results presented here demonstrate that migration of the A-particle to the outer plasma membrane is not dependent on the presence of a normally glycosylated envelope protein. The production of noninfectious glycoprotein-free virus particles by TM-treated cells cannot, however, be interpreted to mean that virion glycoproteins play no role in virus budding and release, since the nonglycosylated glycoproteins could reach the plasma membrane and be degraded rapidly there. In recent studies with Rous sarcoma virus, in which similar particles are formed, we have found that the non-glycosylated *env* gene product can still migrate to the plasma membrane and may thus still play some role in virus assembly (E. Hunter, unpublished observation). Whether this is also the case for M-PMV remains to be determined.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service contract NO1 CP 7-1013 from the Division of Cancer Cause and Prevention, National Cancer Institute, and Public Health Service grants CA-13148 and CA-27384 from the National Cancer Institute. E.H. is the recipient of Public Health Service research career development award KO4 CA 00685-01 from the National Institutes of Health.

We thank Robert E. Justiss, Jr., and Lawrence R. Melsen for their excellent technical assistance and Dixie W. Robinson for help in preparation of this manuscript.

LITERATURE CITED

1. **Ahmed, M., W. Korol, J. Yeh, G. Schidlovsky, and S. A. Mayyasi.** 1974. Detection of Mason-Pfizer virus infection with human KC cells carrying Rous virus genome. J. Nat. Cancer Inst. **53**:383–387.
2. **Bratt, M. A., and W. R. Gallaher.** 1969. Preliminary analysis of the requirements for fusion from within and fusion from without by Newcastle disease virus. Proc. Natl. Acad. Sci. U.S.A. **64**:536–540.
3. **Buetti, E., and H. Diggelmann.** 1980. Avian oncovirus proteins expressed on the surface of infected cells. Virology **102**:251–261.
4. **Chatterjee, S., and E. Hunter.** 1979. The characterization of Mason-Pfizer monkey virus-induced cell fusion. Virology **95**:421–433.
5. **Chatterjee, S., and E. Hunter.** 1980. Fusion of normal primate cells: a common biological property of the D-type retroviruses. Virology **107**:100–108.
6. **Choppin. P. W., and R. W. Compans.** 1975. Reproduction of paramyxoviruses, p. 95–178. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Publishing Corp., New York.
7. **Chopra, H. C., and M. M. Mason.** 1970. A new virus in a spontaneous mammary tumor of a rhesus monkey. Cancer Res. **30**:2081–2086.
8. **Compans, R. W., K. V. Holmes, S. Dales, and P. W. Choppin.** 1966. An electron microscopic study of moderate and virulent virus-cell interactions of the parainfluenza virus SV5. Virology **30**:411–426.
9. **Fine, D. L., J. C. Landon, and M. T. Kubicek.** 1971. Simian tumor virus isolate: demonstration of cytopathic effects *in vitro*. Science **174**:420–421.
10. **Gallaher, W. R., D. B. Levitan, and H. A. Blough.** 1973. Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and herpes simplex viruses. Virology **55**:193–201.
11. **Gallis, B. M., R. N. Eisenman, and H. Diggelmann.** 1976. Synthesis of the precursor to avian RNA tumor virus internal structural proteins early after infection. Virology **74**:302–313.
12. **Gibson, R., R. Leavitt, S. Kornfeld, and S. Schlesinger.** 1978. Synthesis and infectivity of vesicular stomatitis virus containing nonglycosylated G protein. Cell **13**:671–679.
13. **Harter, D. H., and P. W. Choppin.** 1967. Cell-fusing activity of visna virus particles. Virology **31**:279–288.
14. **Homma, M., and M. Ohuchi.** 1973. Trypsin action on the growth of Sendai virus in tissue culture cells. J. Virol. **12**:1457–1465.
15. **Hosaka, Y., and K. Shimizu.** 1977. Cell fusion by Sendai virus, p. 129–155. *In* G. Poste and G. L. Nicolson (ed.),

Virus infection and the cell surface. Elsevier-North Holland Publishing Co., New York.
16. **Hughes, J. V., B. J. Dille, R. L. Thimmig, T. C. Johnson, S. G. Rabinowitz, and M. C. Dal Canto.** 1979. Neuroblastoma cell fusion by a temperature-sensitive mutant of vesicular stomatitis virus. J. Virol. **30:** 883-890.
17. **Hunter, E., M. J. Hayman, R. W. Rongey, and P. K. Vogt.** 1976. An avian sarcoma virus mutant that is temperature sensitive for virion assembly. Virology **69:** 35-49.
18. **Jacobson, W., and M. Webb.** 1952. The two types of nucleoproteins during mitosis. Exp. Cell Res. **3:**163-183.
19. **Keller, J. M.** 1976. The expression of the *syn*$^-$ gene of Herpes Simplex virus type 1. II. Requirements for macromolecular synthesis. Virology **72:**402-409.
20. **Kohn, A.** 1965. Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. Virology **26:** 228-245.
21. **Ledbetter, J., and R. C. Nowinski.** 1977. Identification of the Gross cell surface antigen associated with murine leukemia virus-infected cells. J. Virol. **23:**315-322.
22. **Manservigi, R., P. G. Spear, and A. Buchan.** 1977. Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. Proc. Natl. Acad. Sci. U.S.A. **74:**3913-3917.
23. **Nakamura, K., and R. W. Compans.** 1978. Effects of glucosamine, 2-deoxyglucose, and tunicamycin on glycosylation, sulfation, and assembly of Influenza viral proteins. Virology **84:**303-319.
24. **Parks, W. P., and G. J. Todaro.** 1972. Biological properties of syncytium-forming ("foamy") viruses. Virology **47:**673-683.
25. **Poste, G.** 1972. Mechanisms of virus-induced cell fusion. Int. Rev. Cytol. **33:**157-252.
26. **Poste, G. H.** 1970. Virus-induced polykaryocytosis and the mechanism of cell fusion. Adv. Virus Res. **16:**303-356.
27. **Rand, K. H., and C. W. Long.** 1973. Fusion of a Rous sarcoma virus transformed human cell line, KC, by RD-114 virus. J. Gen. Virol. **21:**523-532.
28. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57:**475-490.
29. **Scheid, A., and P. W. Choppin.** 1977. Two disulfide linked polypeptide chains constitute the active F protein of paramyxoviruses. Virology **80:**54-66.
30. **Schwarz, R. T., J. M. Rohrschneider, and M. F. G. Schmidt.** 1976. Suppression of glycoprotein formation of Semliki forest, influenza, and avian sarcoma virus by tunicamycin. J. Virol. **19:**782-791.
31. **Shurtz, R., S. Dolev, M. Aboud, and S. Salzberg.** 1979. Viral genome RNA serves as messenger early in the infectious cycle of murine leukemia virus. J. Virol. **31:** 668-676.
32. **Snyder, H. W., Jr., E. Stockert, and E. Fleissner.** 1977. Characterization of molecular species carrying Gross cell surface antigen. J. Virol. **23:**302-314.
33. **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.
34. **Takatsuki, A., K. Khono, and G. Tamura.** 1975. Inhibition of biosynthesis of polyisoprenol sugars in chick embryo microsomes by tunicamycin. Agr. Biol. Chem. **39:**2089-2091.
35. **Takatsuki, A., K.-I. Shimizu, and G. Tamura.** 1971. Effect of tunicamycin on microorganisms: morphological changes and degradation of RNA and DNA induced by tunicamycin. J. Antibiot. **25:**75-85.
36. **Takatsuki, A., and G. Tamura.** 1971. Tunicamycin, a new antibiotic. II. Some biological properties of the new antiviral activity of tunicamycin. J. Antibiot. **24:**224-231.
37. **Takatsuki, A., and G. Tamura.** 1971. Tunicamycin, a new antibiotic. III. Reversal of the antiviral activity of tunicamycin by amino sugars and their derivatives. J. Antibiot. **24:**232-238.
38. **Takatsuki, A., and G. Tamura.** 1971. Effect of tunicamycin on the synthesis of macro molecules in cultures of chick embryo fibroblasts infected with Newcastle disease virus. J. Antibiot. **24:**785-794.
39. **Tkacz, J. S., and J. O. Lampen.** 1975. Tunicamycin inhibition of polyisoprenyl N-acetyl glucosaminyl pyrophosphate formation in calf liver microsomes. Biochem. Biophys. Res. Commun. **65:**248-257.
40. **Wong, P. K. Y., P. H. Yuen, and S. J. Kaufman.** 1977. Induction of syncytia by Moloney murine leukemia virus in myoblasts defective in differentiation. J. Virol. **21:**319-327.
41. **Wong, P. K. Y., P. H. Yuen, and S. J. Kaufman.** 1977. Mechanism of murine leukemia virus-induced fusion in rat myoblasts defective in differentiation. J. Virol. **23:** 768-775.