JOURNAL OF VIROLOGY, July 1993, p. 4415–4420
0022-538X/93/074415-06$02.00/0

Vol. 67, No. 7

Viral Liposomes Released from Insect Cells Infected with Recombinant Baculovirus Expressing the Matrix Protein of Vesicular Stomatitis Virus

YAN LI,[1] LIZHONG LUO,[1] MANFRED SCHUBERT,[2] ROBERT R. WAGNER,[3] AND C. YONG KANG[1]*

Department of Zoology, Faculty of Science, and Department of Microbiology and Immunology, Faculty of Medicine, The University of Western Ontario, London, Ontario, Canada N6A 5B7[1]; National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892[2]; and Department of Microbiology and Cancer Center, University of Virginia School of Medicine, Charlottesville, Virginia 22908[3]

Received 30 November 1992/Accepted 16 April 1993

The matrix (M) protein of vesicular stomatitis virus (VSV) has been found to promote assembly and budding of virions as well as down-regulating of VSV transcription. Large quantities of M protein can be produced in insect cells infected with recombinant baculovirus expressing the VSV M gene under control of the polyhedrin promoter. Analysis by pulse-chase experiments and density gradient centrifugation revealed that the [^{35}S]methionine-labeled M protein synthesized in insect cells is released into the extracellular medium in association with lipid vesicles (liposomes). Electron microscopy and immunogold labeling showed that M protein expressed in insect cells induced the formation on plasma membrane of vesicles containing M protein, which are released from the cell surface in the form of liposomes. The baculovirus vector itself or recombinants expressing VSV glycoprotein (G) or nucleocapsid (N) protein did not produce the formation of vesicles in infected cells. The baculovirus-expressed M protein retains biological activity as demonstrated by its capacity to inhibit transcription when reconstituted with VSV nucleocapsids in vitro. These data suggest that M protein has the capacity to associate with the plasma membrane of infected cells and, in so doing, causes evagination of the membrane to form a vesicle which is released from the cell. This observation leads to the postulate, which requires further proof, that the VSV M protein can induce the formation and budding of liposomes from the cell membrane surface.

The prototype rhabdovirus, vesicular stomatitis virus (VSV), contains a membrane-enclosed ribonucleoprotein (RNP) core with a negative-sense unsegmented RNA genome that codes for five structural proteins (9, 10, 23). Two of the five virally coded proteins are membrane associated: (i) the integral, externally oriented glycoprotein (G) spike is the ligand that recognizes host-cell receptor and is the major type-specific antigen which gives rise to and reacts with neutralizing antibody (12); and (ii) the peripheral matrix (M) protein lines the inner surface of the virion lipid bilayer in close association with the RNP core (22). The three other VSV proteins, comprising the RNP core, are the major nucleocapsid (N) protein and the minor large (L) protein and phosphoprotein (P, formerly designated NS) (10, 23), which collectively serve as the RNA-dependent RNA polymerase activity (7). Also tightly associated by ionic bonds with the RNP core is the M protein, which serves to wind up the RNP core into a tight coil (19). The association of M protein with RNP cores also results in down-regulation of transcription of genomic RNA (3).

Another presumed pivotal function of the M protein is in assembly of the RNP-M complex with the host cell plasma membrane containing the newly inserted G protein, which can also be released from the cell surface by proteolysis (4). The positively charged M protein has been found to interact with and bind to the head groups of negatively charged membrane phospholipids, primarily phosphatidylserine (24). Still another recently described function of the VSV M protein is its late cytopathic effect, probably due to interaction with cytoskeleton, resulting in typical rounding of ordinarily polygonal cells after VSV infection (2). Although it has long been assumed that VSV M protein is the principal factor in promoting virion morphogenesis and budding from the surface membrane (23), no concrete evidence supports this hypothesis. The experiments reported here were designed to determine whether the M protein alone can induce budding of lipid vesicles from the cell surface membrane.

In order to express large amounts of the VSV M protein in SF9 insect cells, the entire M gene sequence was excised from a previously described plasmid, pYL-OM79 (15), with *Bam*HI and *Kpn*I, blunt ended with Klenow polymerase and phage T4 DNA polymerase, and inserted into the *Bam*HI site of baculovirus transfer vector pAcYM1 (18) blunt ended with Klenow polymerase. The resulting plasmid, pAcYM1-M, was cotransfected into SF9 cells with wild-type *Autographa californica* nuclear polyhedrosis virus (AcNPV) viral DNA. A plaque-purified clone of the recombinant baculovirus vAcVSV-M was used to express M protein in infected SF9 cells. The M protein expressed by vAcVSV-M was analyzed at 1, 2, and 3 days after infection of SF9 cells by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie blue staining and by Western blots (immunoblots) of extracted or partially purified M protein electroblotted onto nitrocellulose sheets. Also examined by Western blot analysis was particulate M protein released into the culture medium of SF9 cells infected with vAcVSV-M.

Figure 1 shows the time course of M protein synthesis by vAcVSV-M after SDS-PAGE of infected SF9 cell extracts determined by Coomassie blue staining (Fig. 1A) or by immunoblotting with monoclonal antibody 2 (MAb2) di-

* Corresponding author.

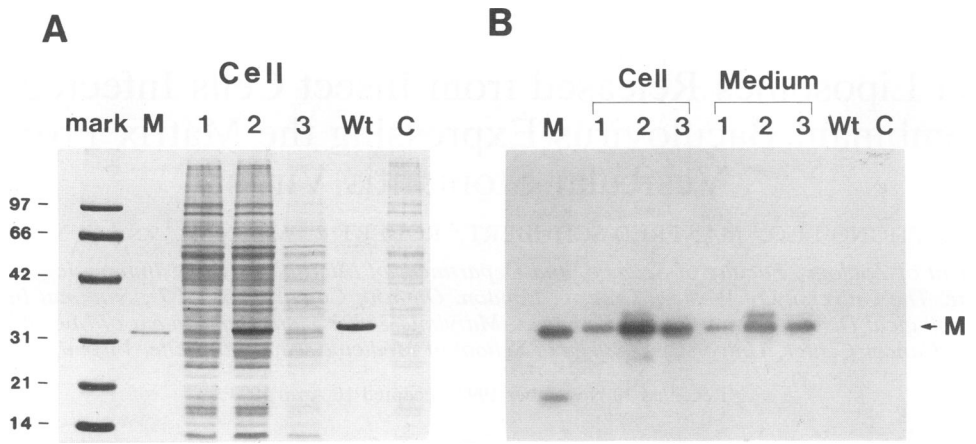

FIG. 1. Electropherograms of daily synthesis of VSV M protein expressed in SF9 cells by recombinant baculovirus vAcVSV-M, detected by Coomassie blue staining of cell lysates (A), Western blot analysis of cell lysates (Cell [B]), and particles in the culture medium (Medium [B]). SF9 cells were infected with vAcVSV-M recombinant or a wild-type baculovirus control (Wt) at multiplicities of infection of 5 PFU per cell. The whole-cell lysates collected at days 1, 2, and 3 p.i. (lanes 1, 2, and 3, respectively) were analyzed by SDS–12% PAGE and detected by staining with Coomassie blue (A) (16) or electroblotted onto a nitrocellulose sheet for immunoblotting with MAb2 (directed to M protein epitope 1) and detected with ^{125}I-labeled staphylococcal protein A (Cell [B]) (15). The presence of M protein in the culture medium at days 1, 2, and 3 p.i. of vAcVSV-M-infected SF9 cells was determined by SDS-PAGE and MAb2 immunoblotting of the pellet after centrifugation in the SW50.1 rotor at 30,000 rpm for 1 h (Medium [B]). Bio-Rad molecular size markers (mark) in kilodaltons are shown at the left, and the VSV virion marker M protein (lanes M) is shown at the right. The controls are mock-infected SF9 cells (C) and cells infected with wild-type baculovirus AcNPV (Wt).

rected to epitope 1 of the M protein (Fig. 1B). As can be seen in Fig. 1A, vAcVSV-M recombinant baculovirus infection of SF9 cells expressed large amounts of a stainable protein that comigrated with virion M protein at ~34 kDa; the amount of stainable M protein synthesized appeared to be maximal at day 2 postinfection (p.i.) and diminished by day 3 p.i., at which time there was a marked decrease in the amount of SF9 cell protein probably due to cell death (Fig. 1A). No stainable bands corresponding to those of M protein in uninfected or wild-type baculovirus-infected SF9 cells were observed after SDS-PAGE (Fig. 1A).

The identity of the M protein expressed in vAcVSV-M-infected SF9 cells was clearly confirmed by reactivity with epitope 1-specific MAb2 by Western blots (Fig. 1B). Once again, maximum synthesis of M protein was observed at day 2 p.i., diminishing by day 3 p.i. It should be noted that expressed recombinant M protein migrated by SDS-PAGE slightly more slowly than the marker virion M protein (lanes M). Moreover, the M protein expressed at day 2 p.i. showed a split band, conceivably due to differences in degree of phosphorylation, as is true for the P (formerly NS) protein in mammalian cells (13).

Of particular interest was the finding that relatively large amounts of M protein were released into the medium from SF9 cells infected with vAcVSV-M (Fig. 1B). It was found that pelleted material present in the medium surrounding vAcVSV-M-infected SF9 cells contained a protein migrating identically with M protein and strongly reactive by Western blot with epitope 1-specific MAb2 (Fig. 1B, Medium). Once again, the largest amount of M protein released into the medium occurred at day 2 p.i. and diminished by day 3 p.i. These results indicate that M protein is extruded from SF9 cells infected with vAcVSV-M expressing the VSV M protein.

The question whether the sedimentable form of M protein released from SF9 cells infected with vAcVSV-M could be due to cell lysates dispersed into the culture medium arose. Light microscopy of these infected cells made cytolysis unlikely as the principal explanation for release of M protein because the cells remained relatively intact until 2 days p.i. when only 20% of the cells had begun to undergo lysis. By the third day p.i., the cytopathic effect was about 70% (data not shown). To shed some light on the question whether sedimentable M protein was gradually released from intact cells infected with vAcVSV-M, a pulse-chase experiment was performed to estimate the rate at which M protein is released. To this end, SF9 cells infected with vAcVSV-M were pulse-labeled with [^{35}S]methionine for 30 min at 16 h p.i.; the cells were then thoroughly washed and incubated in chase medium for 24 h. At intervals after the chase, medium and cells were harvested separately, the cells were lysed, and the cell-free medium and the cell lysate were subjected to immunoprecipitation by adding excess amounts of MAb2 directed to epitope 1 of the VSV M protein.

Figure 2 shows electropherograms of ^{35}S-labeled M protein immunoprecipitated from cell lysates (Fig. 2A) or cell-free culture medium (Fig. 2B) harvested from vAcVSV-M-infected SF9 cells at chase periods of 0.5 to 24 h after pulsing with [^{35}S]methionine. As noted, intracellular radiolabeled M protein was present in large amounts at the 0.5-h chase period after the 16-h pulse; longer chases showed a gradual decline in cell-associated ^{35}S-M protein from 4 to 24 h postchase. By comparison, a small but quite significant amount of M protein was found in extracellular medium by 30 min after chase; thereafter, the amount of M protein gradually accumulated to high levels in the extracellular medium during the 24-h chase period.

These results point to a progressive release of M protein from vAcVSV-M-infected SF9 cells. These data also appear to rule out cell lysis as an important factor contributing to release of M protein into the medium surrounding the infected SF9 cells.

The question whether the sedimentable M protein released from vAcVSV-M-infected SF9 cells is in the form of an

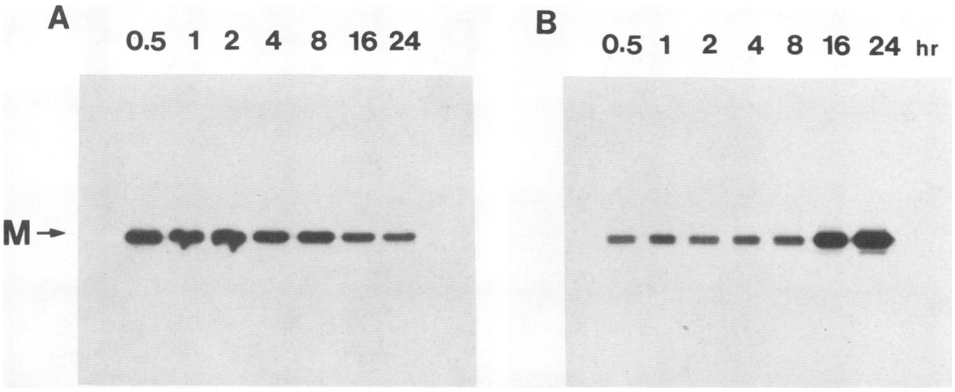

FIG. 2. Pulse-chase analysis of [^{35}S]methionine-labeled M protein synthesized in SF9 cells (A) and released into the cell culture medium (B). SF9 cells were infected with recombinant vAcVSV-M at a multiplicity of infection of 5 PFU per cell. At 16 h p.i., cells were placed in methionine-free Grace medium for 30 min, after which they were pulse-labeled with [^{35}S]methionine (250 μCi/ml) for 1 h. The cells were then washed and chased by incubation in complete medium for different intervals of time up to a period of 24 h. At the chase time intervals indicated for separate cultures, medium and cells were harvested separately and cell lysates were prepared as described by Li et al. (14). Each cell lysate and its corresponding medium were subjected to immunoprecipitation by adding excess MAb2 (directed to M protein epitope 1) to precipitate ^{35}S-labeled M protein. The immunoprecipitated ^{35}S-labeled M protein was analyzed by electrophoresis on SDS–12% polyacrylamide gels and autoradiography. The slab gel in panel B was exposed twice as long as the one in panel A.

insoluble M protein aggregate or is associated with host cell components, one of which could be cell membrane, arose. One method to determine whether the M protein is released from cells in association with membrane lipids is sucrose density gradient centrifugation. To this end, M protein released into the culture medium 48 h after infection of SF9 cells with vAcVSV-M was collected by sedimentation at 81,000 × *g* for 1 h and the pellet was layered over a 20 to 60% discontinuous sucrose gradient and then centrifuged at 120,000 × *g* for 4 h. After centrifugation, three visible bands were discernible at the interfaces of 20/30% sucrose, 30/40% sucrose, and 40/50% sucrose (data not shown). Each of these bands was collected and subjected to electrophoresis on an SDS–12% polyacrylamide gel. The resulting SDS-PAGE proteins were electroblotted onto a nitrocellulose sheet for Western blot analysis using MAb2 directed to epitope 1 of the VSV M protein. Autoradiographs were prepared after exposure to ^{125}I-labeled staphylococcal protein A.

Figure 3 shows the Western blot analysis of the M protein in each of the visible bands in a 20 to 60% discontinuous sucrose gradient after centrifugation. As noted, most of the M protein released from vAcVSV-M-infected SF9 cells was banded at the 30/40% sucrose interface (Fig. 3, lane 4), but a significant amount of M protein was found in association with baculovirus particles, banding at the 40/50% sucrose interface (Fig. 3, lane 5) (16, 17). These data indicate that most of the M protein released from vAcVSV-M-infected cells has a low density consistent with association of the released M protein with membrane lipids in a particulate structure. To test the postulate of an M protein-membrane complex released from cells infected with vAcVSV-M, the material that banded at the 30/40% sucrose interface was subjected to a second round of centrifugation. The purified material that reappeared after recentrifugation at the 30/40% sucrose interface was collected and pelleted by centrifugation at 81,000 × *g*, and the pellet was examined by negative-stain electron microscopy. As shown in Fig. 4B, the low-density fraction of M protein released from vAcVSV-M-infected SF9 cells contains numerous structures closely resembling lipoprotein vesicles.

These data suggest that M protein expressed in SF9 cells is released into the medium in association with cell membrane in the form of liposomes. This observation is compatible with the hypothesis that the VSV M protein induces the formation from host cell plasma membrane of lipoprotein vesicles that are progressively released into the culture medium. The amount of M protein released from vAcVSV-

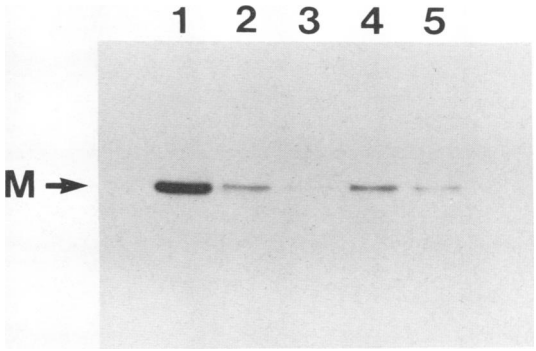

FIG. 3. Western blot analysis of M protein in medium after release from vAcVSV-M-infected SF9 cells after fractionation by centrifugation. SF9 cells infected with vAcVSV-M at a multiplicity of infection of 5 PFU per cell were incubated for 48 h, and the cell-free culture medium was pelleted by centrifugation at 81,000 × *g* for 1 h. The pelleted material was resuspended in cold phosphate-buffered saline (PBS) (500 μl) containing 0.1% Tween 20, 10 μg of aprotinin per ml, and 1 mM phenylmethylsulfonyl fluoride and left at 4°C overnight. The sample was layered on a 20 to 60% discontinuous sucrose gradient and centrifuged in the SW41 rotor at 120,000 × *g* for 4 h at 4°C as described by Luo et al. (16, 17). Three visible bands were collected from the 20/30%, 30/40%, and 40/50% sucrose interfaces, diluted with cold PBS, and repelleted at 81,000 × *g* for 1 h and subjected to electrophoresis on an SDS–12% polyacrylamide gel. After electroblotting onto nitrocellulose sheets, the M protein in each fraction was identified by immunoblotting with MAb2 directed to M protein epitope 1. Autoradiographs were prepared after adding ^{125}I-labeled staphylococcal protein A. Lane 1, purified M protein from VSV virions; lane 2, total pelleted material from vAcVSV-M-infected cell culture medium; lane 3, 20/30% sucrose gradient interface; lane 4, 30/40% sucrose gradient interface; lane 5, 40/50% sucrose gradient interface.

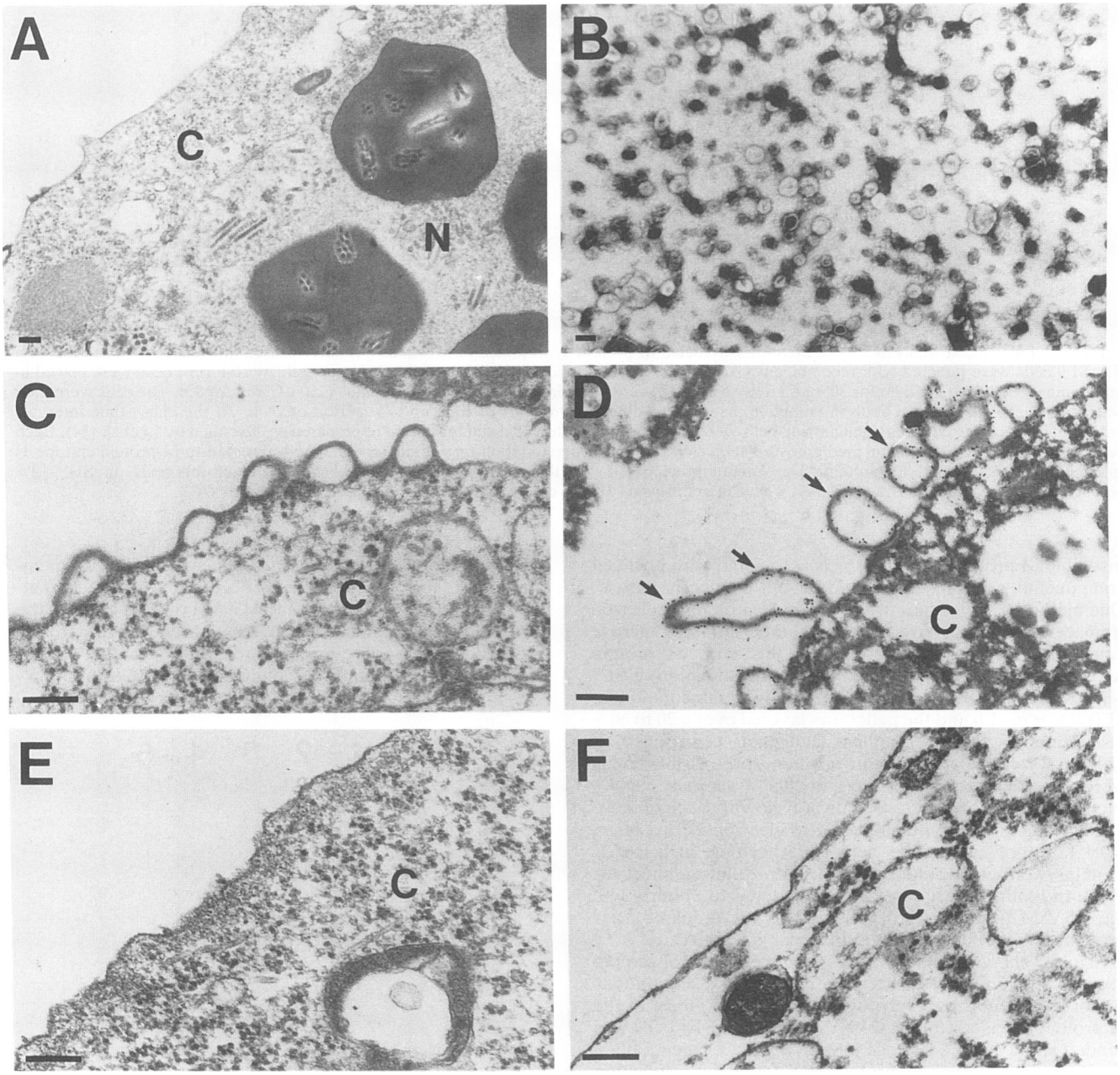

FIG. 4. Electron micrographs of sectioned SF9 cells infected with recombinant baculoviruses and of a negatively stained unsectioned preparation of released vesicles. (A) Cells infected with wild-type baculovirus AcNPV (48 h p.i.); note polyhedrin inclusions bearing progeny AcNPV within enlarged nuclei. (B) Uranyl acetate staining of unsectioned vesicles released from vAcVSV-M-infected SF9 banded by equilibrium gradient centrifugation at the 30/40% sucrose interface shown in Fig. 3, lane 4. (C) Thin-sectioned SF9 cells infected with vAcVSV-M expressing M protein; note formation of vesicles on the cytoplasmic membrane 48 h p.i. (D) Immunogold labeling of sectioned SF9 cells infected with vAcVSV-M expressing M protein and immunodecorated with MAb2 directed to M protein epitope 1 48 h p.i. (E and F) Thin-sectioned SF9 cells infected 48 h previously with recombinant baculoviruses expressing VSV protein G or N, respectively. For immunogold labeling, the thin sections were treated with 1.25% bovine serum albumin in PBS and incubated for 2 h at room temperature with MAb2, which was then identified by conjugation with goat anti-mouse IgG labeled with gold (10 nm; Bio-Rad Laboratories) diluted 10-fold in PBS containing 1% bovine serum albumin. The cell structure in the immunostained section was enhanced with uranyl acetate and lead citrate. All grids were examined in a Philips EM 301 transmission electron microscope. The letters C and N designate cytoplasm and nucleus, respectively. The bar in each panel represents 100 nm.

M-infected SF9 cells by 48 h was estimated at 5 to 8 mg/liter (10^8 cells per liter).

The M protein expressed by vAcVSV-M would appear to be the likely cause for formation of lipoprotein liposomes by infected SF9 cells. The most likely site at which these liposomes are formed would be the cytoplasmic surface membranes, the site for budding of progeny VSV virions (23). To provide some insight into the inducer and morpho-

genesis of the M protein-associated vesicles, we resorted to electron microscopy. By this means, we examined SF9 cells by thin-section transmission electron microscopy at intervals after infection with M protein-expressing vAcVSV-M compared with cells infected with control wild-type baculovirus. As additional controls, SF9 cells were examined by electron microscopy after infection with baculovirus recombinants expressing products of the VSV G gene, YM1VSVG (1), or the VSV N gene, which was constructed by excising the entire coding region of the wild-type VSV N gene from pGN2 (6) with *Xho*I, blunted with Klenow polymerase, and recloning into the blunt-ended *Bam*HI site of pAcYM1. Other cells were examined by thin-section electron microscopy after coinfection with all three baculovirus recombinants individually expressing the VSV G, N, and M genes. To identify VSV protein associated with these vesicles, the sectioned cells were exposed to monoclonal or polyclonal antibodies directed to M, G, or N proteins and then conjugated with goat anti-mouse immunoglobulin G labeled with colloidal gold.

Figure 4 shows the electron micrographs of sectioned SF9 cells infected with wild-type or recombinant baculoviruses, as well as negatively stained vesicles previously described as having been released into the medium from vAcVSV-M-infected SF9 cells (Fig. 4B). The formation of ringlike vesicles emanating from the surface membrane is clearly shown in Fig. 4C, depicting SF9 cells infected with M-protein-expressing vAcVSV-M. In contrast, no vesicle formation is evident on the surface of SF9 cells infected with wild-type AcNPV (Fig. 4A) or on that of SF9 cells infected with baculoviruses expressing VSV G or N proteins (Fig. 4E and F, respectively). These findings appear to indicate that only the M protein causes the production of vesicles, which are then released from vAcVSV-M-infected cells into the surrounding medium (Fig. 4B).

Figure 4D demonstrates that these vesicles induced in vAcVSV-M-infected SF9 cells contain the expressed M protein, as evidenced by labeling of the surface vesicles with MAb2 (directed to epitope 1 of M protein) conjugated with goat anti-mouse immunoglobulin G labeled with colloidal gold. The shape and size of the ringlike vesicles associated with M protein are quite irregular but average about 130 nm in diameter; the immunogold labeling pattern is also quite thick, as evidenced by the density of the ring, suggesting a multimeric layering of the M protein within possibly stacked membrane layers (Fig. 4D). Examination of many sectioned cells failed to show any vesicles associated with M protein within the cytoplasm of vAcVSV-M-infected cells (data not shown). Nor did immunogold labeling provide any evidence for the presence of G or N protein in vesicles formed in SF9 cells coinfected with recombinant baculoviruses expressing M, G, and N proteins (data not shown). These data support the hypothesis that the baculovirus-expressed M protein, in the absence of any other VSV genome products, finds its way to and becomes associated with cell membrane. These results also support data from cross-linking studies showing that M protein interacts with membrane bilayers by electrostatically attaching to negatively charged phospholipid vesicles (24, 25).

It is well documented that binding of wild-type M protein to RNP cores results in down-regulation of transcription (3, 21). It was of interest to determine whether the recombinant M protein expressed by the baculovirus vector in insect cells retained the biological property to inhibit VSV transcription. If so, this would provide large amounts of M protein needed for such studies, which has been a limitation in the past. To this end, the transcription-inhibition activity of M protein extracted from VSV virions and purified by phosphocellulose chromatography (21) was compared with that of recombinant baculovirus-expressed M protein purified by ultracentrifugation on a discontinuous sucrose gradient. Each of these sources of M protein was tested for its capacity to inhibit transcription after reconstitution with VSV RNP cores. When increasing concentrations of virion M protein or baculovirus-expressed M protein were reconstituted with RNP cores (180 μg/ml), transcriptional activity was progressively reduced to a level of 50 to 60% inhibition at M protein concentrations of 200 μg/ml (data not shown). Clearly, purified M protein extracted from virions or expressed by the baculovirus recombinant exhibited almost identical profiles of transcription-inhibition activity. These results demonstrate that the recombinant baculovirus-expressed M protein retains the biological activity of the virion-associated M protein, at least as far as down-regulation of transcription is concerned.

In conclusion, these experiments indicate that the VSV M protein has the intrinsic property of promoting evagination of a circumscribed region of the host cell cytoplasmic membrane, leading to liberation of liposomes. This property is consistent with the recent finding that perhaps 10% of vector-expressed VSV M protein attaches to cytoplasmic membrane and is not readily dissociable (5). Clearly, these functions of the M protein are intrinsic and independent of other VSV proteins. The M protein also binds to RNP cores in vitro (11, 20) and in vivo (5), an event that precedes migration of the RNP-M complex to the surface membrane where the progeny virion buds (23). A logical extension of these findings is that the membrane evagination–vesicle-forming function of the M protein is also the M protein property that causes budding of progeny virions from the surface membrane of VSV-infected cells. Liposomes with associated M protein are also released from mammalian cells transfected with plasmids expressing wild-type VSV M protein (8), indicating that this property is not limited to insect cells. The mechanism by which M protein promotes vesicle formation, and perhaps budding, remains to be determined, but solution of this problem has wide ramifications.

This research was supported by grants from the Medical Research Council of Canada and by USPHS grant R37 AI-11112 from the National Institute of Allergy and Infectious Diseases.

We thank F. Wei for electron microscopy.

REFERENCES

1. **Bailey, M. J., D. A. Mcleod, C. Y. Kang, and D. H. L. Bishop.** 1989. Glycosylation is not required for the fusion activity of the G protein of vesicular stomatitis virus in insect cells. Virology **169**:323–331.
2. **Blondel, D. E., G. G. Harrison, and M. Schubert.** 1990. Role of matrix protein in cytopathogenesis of vesicular stomatitis virus. J. Virol. **64**:1716–1725.
3. **Carroll, A. R., and R. R. Wagner.** 1979. Role of the membrane (M) protein in endogenous inhibition of in vitro transcription of vesicular stomatitis virus. J. Virol. **29**:134–142.
4. **Chatis, P. A., and T. C. Morrison.** 1982. Characterization of the soluble glycoprotein released from vesicular stomatitis virus-infected cells. J. Virol. **45**:80–90.
5. **Chong, L. D., and J. K. Rose.** 1993. Membrane association of functional vesicular stomatitis virus matrix protein in vivo. J. Virol. **67**:407–414.
6. **Emerson, S. U., and M. Schubert.** 1987. Location of the binding domains for the RNA polymerase L and the ribonucleocapsid

template within different halves of the NS phosphoprotein of vesicular stomatitis virus. Proc. Natl. Acad. Sci. USA **84**:5655–5659.
7. **Emerson, S. U., and Y. Yu.** 1975. Both NS and L proteins are required for in vitro RNA synthesis by vesicular stomatitis virus. J. Virol. **15**:1348–1356.
8. **Justice, P., W. Sun, and R. R. Wagner.** Unpublished data.
9. **Kang, C. Y., and L. Prevec.** 1969. Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. J. Virol. **3**:404–413.
10. **Kang, C. Y., and L. Prevec.** 1971. Proteins of vesicular stomatitis virus. III. Intracellular synthesis and extracellular appearance of virus-specific proteins. Virology **46**:678–690.
11. **Kaptur, P. E., R. B. Rhodes, and D. S. Lyles.** 1991. Sequences of the vesicular stomatitis virus matrix protein involved in binding nucleocapsids. J. Virol. **65**:1057–1063.
12. **Kelley, J. M., S. U. Emerson, and R. R. Wagner.** 1972. The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibody. J. Virol. **10**:1231–1235.
13. **Kingsford, L., and S. U. Emerson.** 1980. Transcriptional activities of different phosphorylated species of NS protein purified from vesicular stomatitis virions and cytoplasm of infected cells. J. Virol. **33**:1097–1105.
14. **Li, Y., L. Luo, N. Rassol, and C. Y. Kang.** 1993. Glycosylation is necessary for the correct folding of human immunodeficiency virus gp120 in CD4 binding. J. Virol. **67**:584–588.
15. **Li, Y., L. Luo, R. M. Snyder, and R. R. Wagner.** 1988. Site-specific mutations in vectors that express antigenic and temperature-sensitive phenotypes of the M gene of vesicular stomatitis virus. J. Virol. **62**:3729–3737.
16. **Luo, L., Y. Li, P. M. Cannon, S. Kim, and C. Y. Kang.** 1992. Chimeric *gag*-V3 virus-like particles of HIV induce virus neutralizing antibodies. Proc. Natl. Acad. Sci. USA **89**:10527–10531.
17. **Luo, L., Y. Li, and C. Y. Kang.** 1990. Expression of *gag* precursor protein and secretion of virus-like *gag* particles of HIV-2 from recombinant baculovirus-infected insect cells. Virology **179**:874–880.
18. **Matsuura, Y., R. D. Possee, H. A. Overton, and D. H. L. Bishop.** 1987. Baculovirus expression vectors: the requirements for high level expression of proteins, including glycoproteins. J. Gen. Virol. **68**:1233–1250.
19. **Newcomb, W. W., G. J. Tobin, J. J. McGowan, and J. C. Brown.** 1982. In vitro reassembly of vesicular stomatitis virus skeletons. J. Virol. **41**:1055–1062.
20. **Ogden, J. R., R. Pal, and R. R. Wagner.** 1986. Mapping regions of the matrix protein of vesicular stomatitis virus which bind to ribonucleocapsids, liposomes, and monoclonal antibodies. J. Virol. **58**:860–868.
21. **Pal, R., B. W. Grinnell, R. M. Snyder, and R. R. Wagner.** 1985. Regulation of viral transcription by the matrix protein of vesicular stomatitis virus probed by monoclonal antibodies and temperature-sensitive mutants. J. Virol. **56**:386–394.
22. **Pal, R., and R. R. Wagner.** 1987. Rhabdovirus membrane and maturation, p. 75–128. *In* R. R. Wagner (ed.), The rhabdoviruses. Plenum Press, New York.
23. **Wagner, R. R.** 1987. Rhabdovirus biology and infection: an overview, p. 9–74. *In* R. R. Wagner (ed.), The rhabdoviruses. Plenum Press, New York.
24. **Wiener, J. R., R. Pal, Y. Barenholz, and R. R. Wagner.** 1983. Influence of the peripheral matrix protein of vesicular stomatitis virus on the membrane dynamics of mixed phospholipid vesicles. Biochemistry **22**:2162–2170.
25. **Zakowski, J. J., and R. R. Wagner.** 1980. Location of membrane-associated proteins in vesicular stomatitis virus by use of hydrophobic cross-linking reagents. J. Virol. **36**:93–102.