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Interactions of Normal and Mutant Vesicular Stomatitis Virus Matrix Proteins with the Plasma Membrane and Nucleocapsids

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We demonstrated recently that a fraction of the matrix (M) protein of vesicular stomatitis virus (VSV) binds tightly to cellular membranes in vivo when expressed in the absence of other VSV proteins. This membrane-associated M protein was functional in binding purified VSV nucleocapsids in vitro. Here we show that the membrane-associated M protein is largely associated with a membrane fraction having the density of plasma membranes, indicating membrane specificity in the binding. In addition, we analyzed truncated forms of M protein to identify regions responsible for membrane association and nucleocapsid binding. Truncated M protein lacking the amino-terminal basic domain still associated with cellular membranes, although not as tightly as wild-type M protein, and could not bind nucleocapsids. In contrast, deletion of the carboxy-terminal 14 amino acids did not disrupt stable membrane association or nucleocapsid interaction. These results suggest that the amino terminus of M protein either interacts directly with membranes and nucleocapsids or stabilizes a conformation that is required for M protein to mediate both of these interactions.

Vesicular stomatitis virus (VSV) is an enveloped negative-strand RNA virus containing five proteins. The genomic RNA is encapsidated by the nucleoprotein (N) and is tightly associated with the viral RNA polymerase proteins L and NS(P) (11). This core complex is surrounded by a lipid bilayer derived from the plasma membrane and contains the viral transmembrane glycoprotein (G). The matrix (M) protein of VSV lies beneath the lipid bilayer and presumably bridges between the viral envelope and the nucleocapsid core.

The presence of M protein at the inner surface of the plasma membrane of infected cells (4, 23, 27) and the rapid association of a fraction of M protein with membranes after synthesis on cytosolic polyribosomes (1, 10, 19) have been well documented, but it was not clear whether these were membranes already involved in viral assembly. We recently used subcellular fractionation studies of transfected HeLa cells to show that M protein can associate tightly with membranes in vivo, in the absence of other VSV proteins, but the nature of the membranes to which M protein bound was not determined in that study. Furthermore, membrane-associated M protein was functional in binding nucleocapsid cores to membranes in vitro. On the basis of these findings, we proposed a VSV assembly pathway that could be initiated by membrane-associated M protein (9).

M protein lacks a distinct stretch of hydrophobic residues indicative of a membrane-associating region, and modification by fatty acid has not been demonstrated (22, 29). However, labeling studies with a hydrophobic reagent suggested that the amino-terminal domain penetrates the bilayer (22). Interestingly, a trypsin-resistant core of M protein lacking the amino-terminal 43 residues could also associate with artificial liposomes, suggesting that other regions of M protein may be involved in membrane interaction (26). Here, we report studies using subcellular fractionation to show that M protein expressed in cells binds largely to a plasma membrane fraction rather than nonspecifically to all cellular membranes. Additional studies on truncated M proteins show that the amino-terminal basic domain of M is not required for binding M protein to membranes but is required for stable membrane association. In addition, we demonstrate that this domain is required for membrane-associated M protein to bind nucleocapsid cores in vitro. We propose that this amino-terminal region either interacts directly with membranes and nucleocapsid cores or stabilizes a conformation which is required for M protein to support these interactions.

MATERIALS AND METHODS

Viruses and cell culture. Preparation of radiolabeled VSV (Indiana serotype, San Juan strain) was performed in baby hamster kidney (BHK-21) cells as described previously (9). The recombinant vaccinia virus vTF7-3 (14) was prepared as described by Whitt et al. (35).

Plasmid construction and antibodies. Plasmids pBSG, pBSM, and pBSN, encoding the G, M, and N proteins of VSV (Indiana serotype, San Juan strain), are described elsewhere (9). Plasmid pBSsCD4KDEL containing DNA encoding sCD4KDEL has been described previously (7). Rabbit anti-VSV serum (9) and anti-sCD4KDEL antibody (7) have been described elsewhere. Plasmids pBSMN15 and pBSMC14 were generated by the PCR method, using synthetic oligonucleotide primers and plasmid pARM. Plasmid pARM was generated by cloning a *Bam*HI fragment containing the M protein gene from plasmid pMZ10 (10) into the unique *Bam*HI site of pAR-2529 (14, 30). Plasmid pBSMN15 was made by using one primer (5′-GCGGGATCCATC**ATG**AGTAAGAAATTAGGGA TCGCA-3′) containing a *Bam*HI restriction site (underlined) and an initiation codon (in boldface) followed by sequence corresponding to the DNA encoding residues 18 to 23 of M protein and a second primer (5′-CAACTCAGCTTCCTTT CGGGC-3′) corresponding to downstream vector sequence in pAR-2529. The PCR product was digested with *Bam*HI and ligated into the unique *Bam*HI site of pBS-SK(+) (Stratagene, La Jolla, Calif.). Plasmid pBSMC14 was made by using one primer (5′-ATCGGATCC**TCA**CTTTTTCTCGACAATCAG GCCAAACATTAAGGC-3′) corresponding to DNA encoding residues 205 to 215 of M protein and introducing a stop

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codon (bold letters) to truncate M protein at residue 215, followed by a *Bam*HI restriction site (underlined), and a second primer (5′-TAATACGACTCACTATAGGG-3′) overlapping the sequence encoding part of the T7 RNA polymerase promoter in pAR-2529 located upstream from the *Bam*HI cloning site. The PCR product was digested with *Bam*HI and ligated into the unique *Bam*HI site of pBS-SK(+). DNA sequences were confirmed by dideoxynucleotide sequencing (31) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Expression, radiolabeling, and immunoprecipitation of M proteins. Procedures have been described elsewhere (9). Briefly, HeLa cells (10^6 cells per 3.5-cm-diameter plate) were infected for 30 min at 37°C with vTF7-3 at a multiplicity of infection of 10 in 100 μl of Dulbecco's modified Eagle's medium (DMEM) with the cationic liposome reagent TransfectACE (Bethesda Research Laboratories, Gaithersburg, Md.). At 4 h postinfection, cells were labeled for 1 h with 50 μCi of [^{35}S]methionine per ml of methionine-free DMEM. When indicated, labeling medium was replaced with 2 ml of DMEM supplemented with 2.5 mM unlabeled methionine. Proteins were immunoprecipitated from cell lysates with anti-VSV serum (28). Labeled proteins were resolved by polyacrylamide gel electrophoresis (PAGE) on 10% polyacrylamide gels containing sodium dodecyl sulfate (SDS) (21) and visualized by fluorography (6).

Analysis of membrane-associated M proteins. Procedures have been described elsewhere (9). Briefly, HeLa cells (5×10^6 cells) were infected with vTF7-3 at a multiplicity of infection of 10 for 30 min at 37°C and then transfected with 10 μg of plasmid DNA per 2.5×10^6 cells in 2 ml of DMEM for 4 h. Cells were then labeled for 1 h with [^{35}S]methionine (50 μCi/ml) and chased for 1 h in DMEM supplemented with 2.5 mM unlabeled methionine. Cells were harvested and disrupted in a Dounce homogenizer (9). The postnuclear lysate was adjusted to 80% sucrose and fractionated on an 80%-65%-10% (wt/wt) sucrose step gradient by centrifugation at 35,000 rpm for 18 h at 4°C. Fractions were collected from the top, diluted with detergent solution, and immunoprecipitated with anti-VSV serum (28). Labeled proteins were analyzed by SDS-PAGE (10% gel).

Reconstitution of RNP cores with membranes. Total cellular membranes from transfected and radiolabeled HeLa cells (1.5×10^7 cells) containing associated matrix protein were prepared according to the sucrose membrane flotation gradient method described previously (9). One unit of ribonucleoprotein (RNP) cores, prepared from radiolabeled VSV virions (9), was incubated with the indicated membranes for 1 h at 37°C with periodic shaking. The suspension was then subjected to buoyant density analysis by centrifugation on a continuous 10 to 70% (wt/wt) sucrose gradient at 150,000 × *g* for 45 min at 0°C. Fractions were collected from the bottom, diluted with detergent solution, and immunoprecipitated with anti-VSV serum as described above. Labeled proteins were analyzed by SDS-PAGE (10% gel).

Subcellular fractionation and assays. Total cellular membranes were isolated from the indicated transfected and radiolabeled HeLa cells (1.5×10^7 cells) by the sucrose membrane flotation gradient (9) and then further fractionated according to a method modified from that of Frangioni et al. (13). Isolated membranes were resuspended in 200 μl of 0.25 M sucrose buffer containing 10 mM Tris-HCl (pH 7.4), 5 mM KCl, 1 mM EDTA, and 100 kallikren units of aprotinin per ml. The membrane suspension was applied to the top of a sucrose step gradient consisting of 2.0 M sucrose (bottom), 1.2 M sucrose (center), and 0.25 M sucrose (top), and the gradient was centrifuged at 100,000 × *g* for 2.5 h at 4°C. Fractions were collected from the top. Each fraction was divided in half; one half was immunoprecipitated with the indicated antibody, and labeled proteins were resolved by SDS-PAGE (10% gel). Quantitation of the amount of labeled protein in each fraction was carried out by scanning densitometry of autoradiographs. The other half of the gradient fractions was assayed for α-mannosidase II activity by the method of Storrie and Madden (33).

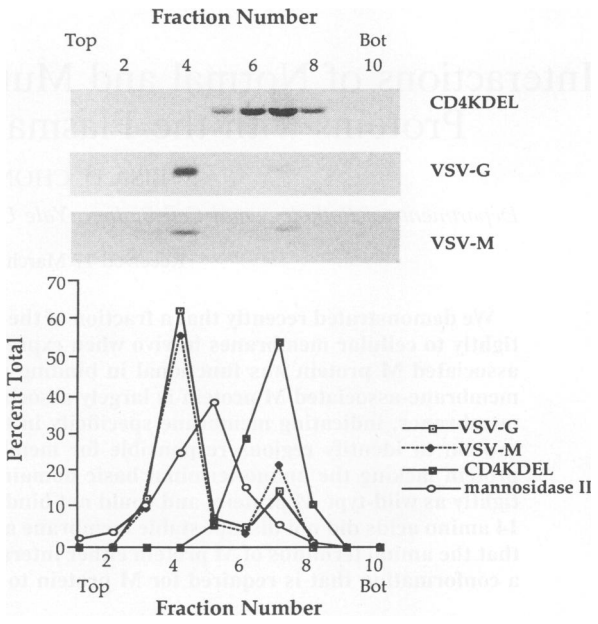

FIG. 1. Fractionation of cellular membranes. Total cellular membranes were isolated from 1.5×10^7 transfected and radiolabeled HeLa cells expressing sCD4KDEL, VSV G protein, or VSV M protein and fractionated on a sucrose gradient by isopycnic centrifugation as described in Materials and Methods. Gradient fractions were collected from the top and divided in half. One half of the gradient fractions was diluted with detergent solution, immunoprecipitated with the appropriate antibody (28), and analyzed by SDS-PAGE (10% gel). Quantitation of total labeled protein in each fraction was carried out by scanning densitometry of autoradiographs. The other half of the gradient fractions was analyzed for α-mannosidase II activity by the method of Storrie and Madden (33). The graph was generated from data collected from two experiments of cells expressing VSV G or sCD4KDEL protein, three experiments of cells expressing VSV M protein, and seven gradients for α-mannosidase II activity. Fractions are numbered from the top (fraction 1) to the bottom (fraction 10).

RESULTS

M protein associates with a plasma membrane-enriched membrane fraction in vivo. Previously, we expressed wild-type M protein in transfected HeLa cells by using a recombinant vaccinia virus/T7 RNA polymerase-based expression system (14) and reported its association with cellular membranes (9). To determine whether there was specificity to the membrane association in vivo, total cellular membranes containing radiolabeled M protein were isolated from transfected HeLa cells by equilibrium membrane flotation in a sucrose gradient (9) and then further separated by isopycnic centrifugation with appropriate markers (13). This fractionation protocol was chosen because it is known to give good separation of membranes

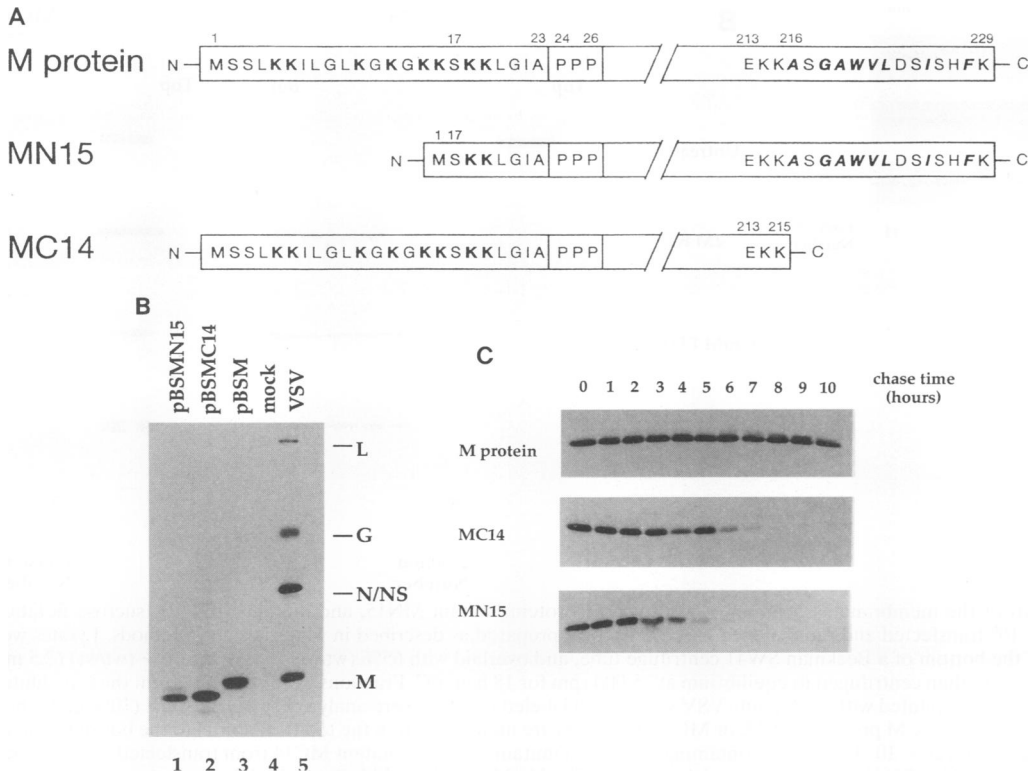

FIG. 2. Construction, expression, and stability of mutated M proteins. (A) Diagram of the N-terminal and C-terminal deletions in M protein. Mutant MN15 lacks the first 15 amino acids following the initial methionine residue. Lysine residues are indicated in boldface. Mutant MC14 lacks 14 residues at the extreme carboxyl terminus. Nonpolar and hydrophobic residues are indicated by boldface italics. (B) HeLa cells were infected with a recombinant vaccinia virus (vTF7-3). After 30 min, infected cells were transfected with 5 μg of pBSMN15 (lane 1), pBSMC14 DNA (lane 2), and pBSM DNA (lane 3) or were mock transfected (lane 4). At 4 h postinfection, cells were labeled for 1 h with 50 μCi of [^{35}S]methionine per ml. Immunoprecipitation of proteins from cell lysates was performed with anti-VSV serum, and labeled proteins were analyzed by SDS-PAGE. VSV protein markers from solubilized virions are shown at the right. Letters indicate the protein designation. (C) HeLa cells were infected with vTF7-3 for 30 min and then transfected with the appropriate plasmid DNA. Cells were pulse-labeled with 50 μCi of [^{35}S]methionine per ml for 1 h and then chased in medium containing excess cold methionine for 1-h intervals up to 10 h. Immunoprecipitation of proteins from cell lysates was performed with anti-VSV serum, and labeled proteins were resolved by SDS-PAGE.

derived from endoplasmic reticulum (ER), Golgi apparatus, and the plasma membrane.

Radiolabeled cells expressing M protein were lysed with a Dounce homogenizer, and nuclei were removed. The supernatant was adjusted to 80% sucrose and placed at the bottom of a sucrose gradient. Membranes were fractionated away from cytosol by equilibrium flotation during ultracentrifugation. Total cellular membranes were collected from the 10%-65% sucrose interface and then subjected to a second separation by isopycnic centrifugation (13).

The extent of enrichment for ER, plasma membrane, and Golgi apparatus in the gradient fractions was determined by assaying for specific markers. The protein sCD4KDEL, which accumulates in the ER of transfected HeLa cells as a result of a specific retention signal (7), was used as a marker for the ER. When expressed in HeLa cells and analyzed by this method, sCD4KDEL was found predominantly in the high-density region near the 1.2 M-2.0 M sucrose interface (Fig. 1). In contrast, the transmembrane glycoprotein G of VSV expressed at the surface of transfected HeLa cells was used as a marker for plasma membrane. The majority of the total G protein localized to the 0.25 M-1.2 M interface (Fig. 1), although a small amount was found at the higher-density interface. The nature of this higher-density fraction containing G is not clear, but it might represent G in endosomes. Distribution of α-mannosidase II activity, a marker for the Golgi membranes, was between the ER and plasma membrane markers, as would be expected for these membranes of intermediate density (Fig. 1).

When we examined the subcellular localization of membrane-bound M protein under the same conditions, it showed a distribution virtually identical to that of G protein, with the majority in the light membrane fraction and a small amount in the high-density fraction. These results indicate that the fraction of M protein that binds membranes is specifically targeted to the plasma membrane rather than binding membranes nonspecifically.

We also used indirect immunofluorescence microscopy to attempt to examine the localization of M protein. Although many cells appeared to have some M protein bound to the plasma membrane, the high background of cytosolic M protein made definitive localization impossible. However, others have successfully shown M protein staining at the plasma membrane of infected cells by alternative cell disruption methods (23) as well as quick-freezing and deep-etch replica methods (25), but we have not applied these methods to transfected cells.

Expression of two truncated forms of M protein. To define the sequence requirements for the membrane association and nucleocapsid binding of M protein, we decided to generate mutants. Although the three-dimensional structure of M protein is not known, computer modeling of the structure predicts

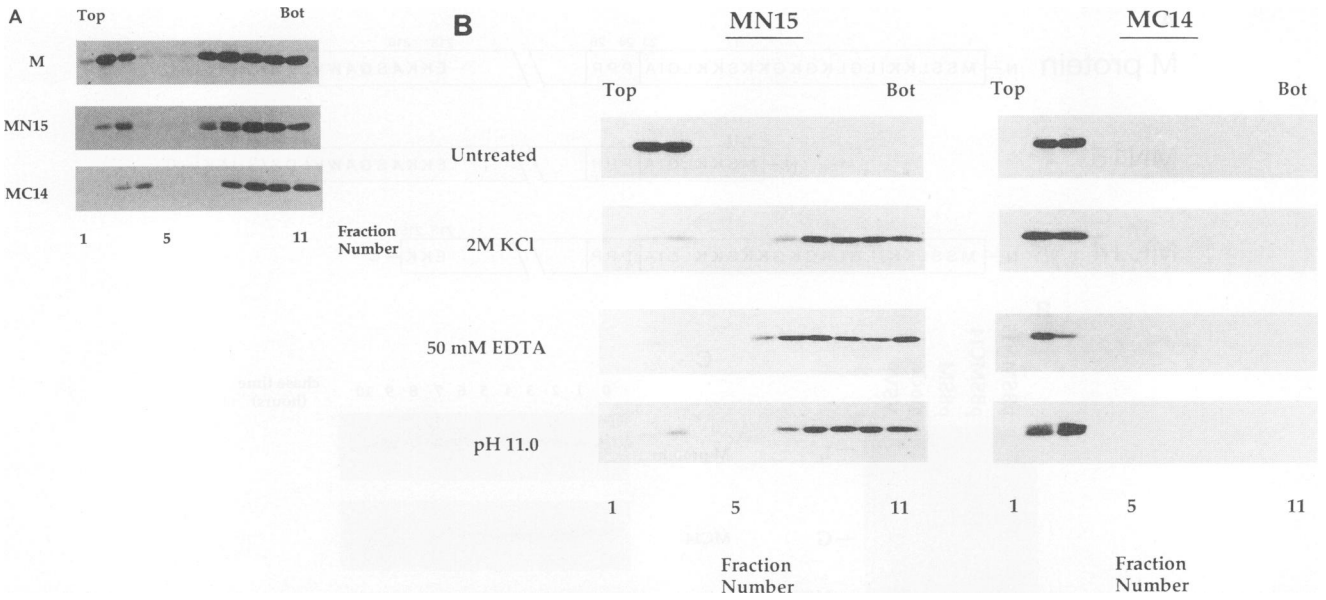

FIG. 3. Analysis of the membrane association of wild-type M protein, mutant MN15, and mutant MC14 by sucrose flotation gradients. (A) Lysates from 5×10^6 transfected and radiolabeled HeLa cells were prepared as described in Materials and Methods. Lysates were made to 80% sucrose, placed at the bottom of a Beckman SW41 centrifuge tube, and overlaid with 65% (wt/wt) (5 ml) and 10% (wt/wt) (2.5 ml) sucrose layers. The step gradients were then centrifuged to equilibrium at 35,000 rpm for 18 h at 4°C. Fractions were collected from the top, diluted with detergent solution, and immunoprecipitated with rabbit anti-VSV serum, and labeled proteins were analyzed by SDS-PAGE (10% gel). Shown are gradients from cells expressing wild-type M protein, MN15, or MC14. Fractions are numbered from the top (fraction 1) to the bottom (fraction 11). (B) Total cellular membranes from 1.5×10^7 HeLa cells containing associated mutant MN15 or mutant MC14 from transfected and [^{35}S]methionine-labeled HeLa cells were prepared by the sucrose flotation method as described in Materials and Methods. Membranes were treated with 2 M KCl–10 mM Tris-HCl (pH 7.4) or 50 mM EDTA–Tris-HCl (pH 7.4) for 1 h at 25°C, extracted with carbonate buffer (pH 11.0) for 30 min at 0°C (15), or left untreated. Samples were made to 80% sucrose, and membranes were reisolated on a second sucrose flotation gradient. Fractions were collected from the top, diluted with detergent solution, immunoprecipitated with rabbit anti-VSV serum, and analyzed by SDS-PAGE. Fractions are numbered from the top (fraction 1) to the bottom (fraction 11).

a globular core with very little secondary structure at its amino or carboxyl terminus. We reasoned that if both termini were free, they might constitute domains interacting with the nucleocapsid and the membrane. The amino-terminal 19 residues contain eight lysines and are followed by a triple-proline region, suggesting that they define a separate domain (29). The carboxyl terminus is somewhat hydrophobic when analyzed by the program of Kyte and Doolittle (20). We therefore constructed two plasmids, pBSMN15 and pBSMC14, which differed from the complete M gene clone pBSM by deletions of 15 amino acids at the amino terminus and 14 amino acids at the carboxyl terminus of M protein. A schematic representation of the truncated proteins is presented in Fig. 2A.

Both mutant proteins were expressed in HeLa cells, as shown by immunoprecipitation from lysates of transfected and radiolabeled cells with anti-VSV serum (Fig. 2B). Both mutant MN15 and mutant MC14 had mobilities on SDS-PAGE consistent with the deletion sizes (Fig. 2B, lanes 1 and 2). To compare the stability of mutant proteins MN15 and MC14 with that of wild-type M protein, a pulse-chase experiment was conducted in which transfected HeLa cells were labeled with [^{35}S]methionine for 30 min and then incubated in medium supplemented with unlabeled methionine for a period of 1 h to 10 h (Fig. 2C). Wild-type M protein was stable through 10 h of chase time. However, mutant MC14 exhibited a half-life of 5 to 6 h, and mutant MN15 exhibited a half-life of 3 to 4 h. A chase time of 1 h was chosen in subsequent experiments so that similar levels of labeled wild-type and mutant proteins could be studied.

Membrane association of mutants MN15 and MC14. We expressed mutants MN15 and MC14 in HeLa cells and analyzed membrane association by subcellular fractionation. Total cell lysates were prepared from transfected and radiolabeled cells expressing M protein, mutant MN15, or mutant MC14, and membranes were fractionated from cytosolic material by equilibrium flotation during ultracentrifugation as previously described (9). Approximately 10% of the total M protein, MN15 protein, or MC14 protein was associated with cellular membranes (Fig. 3A). This result indicated that despite deletions at either terminus, both mutant proteins were capable of membrane association.

The amino terminus of M protein is required for stable membrane association. Membranes containing radiolabeled mutant MN15 or MC14 were isolated from transfected HeLa cells by the sucrose flotation gradient method as previously described (9). Membrane samples were then extracted with 2 M KCl, 50 mM EDTA, or pH 11.0 carbonate buffer and subjected to fractionation on a sucrose flotation gradient (Fig. 3B). All of these conditions removed mutant MN15 protein from cellular membranes, and the dislodged MN15 protein was detected at the bottom of the gradient. However, all of these conditions failed to release mutant MC14 from membranes, similar to what was previously observed with membrane-associated wild-type M protein (9).

The amino terminus is required for membrane-associated M protein to bind RNP cores. To determine whether the deletions affected the ability of MN15 or MC14 to bind RNP cores in vitro, membranes containing radiolabeled M protein, mutant MN15, or mutant MC14 were isolated from transfected HeLa cells by the sucrose flotation gradient method. Mem-

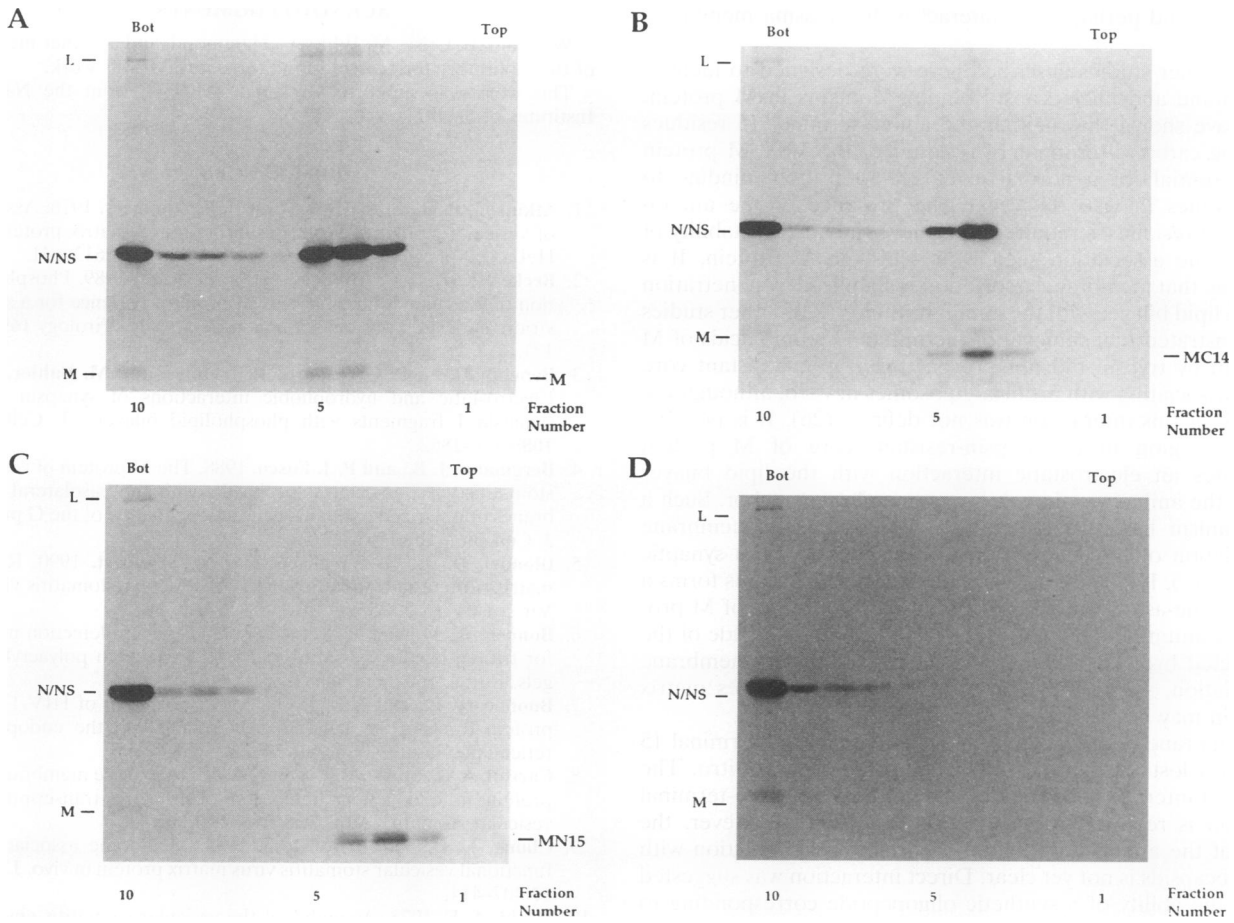

FIG. 4. Analysis of the interaction of RNP cores with membrane-associated M protein, mutant MC14, and mutant MN15 by sucrose density gradients. Membranes were isolated from 1.5×10^7 transfected and radiolabeled HeLa cells expressing M protein (A), mutant MC14 (B), or mutant MN15 (C) or from mock-transfected HeLa cells (D) as described in Materials and Methods. Membranes were incubated with 1 U of RNP cores for 1 h at 37°C. Each mixture was then subjected to gradient analysis by centrifugation on a continuous 10 to 70% (wt/wt) sucrose gradient at 150,000 $\times$ *g* for 45 min at 0°C. Fractions were collected from the bottom, diluted with detergent solution, and immunoprecipitated with anti-VSV serum. Labeled proteins were resolved by SDS-PAGE. Fractions are numbered from the bottom (fraction 10) to the top (fraction 1).

brane samples were then incubated with RNP cores prepared from radiolabeled VSV virions. As observed previously, these RNP cores contained L, N, and NS proteins and trace amounts of M protein (8, 26). Binding of RNP cores to membranes was assayed by the colocalization of RNP core proteins with membranes on sucrose density gradients. RNP cores associated with membranes containing wild-type M protein (Fig. 4A) or mutant MC14 (Fig. 4B) but not with membranes containing mutant MN15 (Fig. 4C). RNP cores did not bind to membranes lacking VSV M protein (Fig. 4D), indicating that the trace amount of nucleocapsid-bound M protein did not facilitate association with membranes. Because MN15 protein remained associated with membranes after RNP binding was attempted (Fig. 4C), we concluded that lack of binding was not due to displacement of the MN15 protein from membranes during the assay.

DISCUSSION

Our earlier study on membrane binding of the VSV matrix protein in vivo (9) showed that about 10% of the VSV matrix protein expressed in VSV-infected cells or expressed in cells in the absence of VSV proteins was very tightly associated with membranes and had the characteristics of an integral membrane protein. In addition, membranes containing M protein were able to bind VSV nucleocapsids in vitro. Furthermore, we suggested that the membrane-bound M protein fraction might be required to initiate the budding process by tethering nucleocapsids to the membrane and perhaps also interacting with G.

The experiments reported here were designed to address the specificity of M protein's membrane association, because M protein involved in viral assembly would be expected to localize at the plasma membrane. These membrane fractionation studies, carried out with markers for ER, plasma membrane, and Golgi membranes, showed that membrane-bound M protein had a distribution virtually identical to that of a plasma membrane marker. It is therefore reasonable to speculate that this M protein fraction might direct viral assembly at the plasma membrane. The basis for the membrane binding specificity is not known because the nature of the membrane attachment itself is unclear. M protein does not contain any significant hydrophobic amino acid domains that resemble membrane-spanning domains, nor does it have any known lipid modification. Our current model is that a fraction of M protein may fold into a conformation that is capable of membrane

insertion and perhaps also interact with a plasma membrane component.

The other studies described here were designed to identify membrane and nucleocapsid binding domains in M protein. We have shown that neither the amino-terminal 15 residues nor the carboxyl-terminal 14 residues of the VSV M protein are essential for a normal extent of M protein binding to membranes in vivo. However, the presence of the amino-terminal residues is required to achieve the normal stability of membrane association seen with wild-type M protein. It is possible that membrane association is stabilized by penetration of the lipid bilayer with the amino terminus (22). Other studies demonstrated that removal of the initial 43 amino acids of M protein by trypsin did not prevent the trypsin-resistant core from associating with artificial liposomes in vitro, although the nature of this interaction was not defined (26). It is possible that a region in the trypsin-resistant core of M protein mediates an electrostatic interaction with the lipid bilayer while the amino terminus acts as a membrane anchor. Such a mechanism has recently been proposed for the membrane association of the neuronal protein synapsin I with synaptic vesicles (3). It is also possible that the amino terminus forms a membrane-associating domain with another region of M protein. A mutation in measles virus matrix protein outside of the predicted hydrophobic carboxyl terminus affected membrane association, suggesting that other regions in measles matrix protein may be involved in membrane binding (16).

Membrane-bound M protein lacking the amino-terminal 15 residues lost the ability to bind nucleocapsids in vitro. The simplest interpretation of these data is that the amino-terminal domain is required for nucleocapsid binding. However, the role of the amino terminus of M protein in interaction with nucleocapsids is not yet clear. Direct interaction was suggested from the ability of a synthetic oligopeptide corresponding to the first 20 amino acids of M protein to prevent transcription inhibition by M protein in vitro (32). Kaptur et al. (18) suggested that the amino terminus of M protein is exposed when bound to nucleocapsids because protease could cleave nucleocapsid-bound M protein at positions 19 and 20. However, it is not clear from that experiment whether residues preceding position 19 interact with the nucleocapsid. The inability of MN15 protein to bind nucleocapsids would also be consistent with the suggestion by Kaptur et al. (18) that proteolytic cleavage at the amino terminus may cause a conformational change that is disruptive to a downstream nucleocapsid binding region (26, 32). Removal of critical phosphorylation sites in the amino terminus or the alteration of other phosphorylation sites in M protein due to a conformational change may affect nucleocapsid and/or membrane association (2, 17).

Although the carboxyl-terminal 14 residues of M protein are slightly hydrophobic, their removal had no effect on the extent of stability of membrane binding in vivo or nucleocapsid binding by membranes containing the truncated M protein. This region therefore may not be critical to assembly, although other studies have indicated that the C terminus may be required for the cytopathic effects caused by M protein (5, 24).

The association of M protein with the plasma membrane in vivo and the ability of membrane-bound M protein to interact with nucleocapsid cores in vitro (9), taken together, support a model of VSV assembly in which membrane-bound M protein could nucleate sites for viral assembly at the cell surface (9). The cell-free system in which nucleocapsid cores can bind to membrane-bound M protein may therefore be useful for further investigation of the budding process.

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