JOURNAL OF VIROLOGY, Dec. 1993, p. 7246–7253
0022-538X/93/127246-08$02.00/0

Vol. 67, No. 12

Vesicular Stomatitis Virus M Protein May Be inside the Ribonucleocapsid Coil

ANNIE BARGE,[1] YVES GAUDIN,[2] PATRICE COULON,[2] AND ROB W. H. RUIGROK[1]*

EMBL Grenoble Outstation, c/o ILL, BP 156, F-38042 Grenoble Cedex 9,[1] and Laboratoire de Génétique des Virus, Centre National de la Recherche Scientifique, F-91198 Gif sur Yvette Cedex,[2] France

Received 7 June 1993/Accepted 2 September 1993

Vesicular stomatitis virus is an enveloped virus with an external glycoprotein G and a nucleocapsid that form, together with the M protein, a tight helically coiled structure: the skeleton. Negative staining and immunoelectron microscopy studies on skeleton preparations were performed to determine the localization of the M protein. These studies have resulted in a new model for the structure of rhabdoviruses in which the nucleocapsid is wound around a core containing the M protein. This model predicts contact between M and lipid only at the extreme ends of the skeleton, which is confirmed by skeleton-liposome binding studies.

Vesicular stomatitis virus (VSV), the prototype rhabdovirus, is an enveloped virus with a negative-strand RNA genome. The RNA is associated with the polymerase (L), a polymerase cofactor (NS, or also called P), and the nucleocapsid protein (N) to form a nucleocapsid. Each virion contains about 50 copies of L, 450 copies of NS, and 1,250 copies of N (25). Together with the so-called matrix protein (M; 1,800 copies), this nucleocapsid forms a tight, helically coiled structure called the skeleton. This skeleton is surrounded by the viral lipid membrane in which are embedded about 400 trimers of the external viral glycoprotein, G (2, 15, 25). The generally accepted view is that M is responsible for the condensation of the nucleocapsid and that its position is more or less between the nucleocapsid coil and the membrane, making the contact between the skeleton and the cytoplasmic tails of G.

Influenza virus, another negative-strand virus, also has an M protein with a molecular weight similar to that of the VSV M protein. Both M proteins are located inside the viral membrane, inhibit in vitro RNA transcription, and show some sequence homology (3, 22, 29). The influenza virus M protein is also thought to line the inside of the viral membrane (17, 23), agreeing with the above model for the localization of VSV M. The rhabdovirus structure has been studied by cross-linking experiments on intact virus, looking at the interactions of M with lipid and of M with G (5, 12). Apart from these experiments with virus, there have been many studies on the in vitro interaction of M with liposomes (9, 20, 27, 28; reviewed in reference 18).

However, there are few hard data on the VSV structure, and Brown and Newcomb have suggested that the nucleocapsid coil contains an "axial channel," probably filled with M (2). In this paper we have studied the localization of M in VSV by using negative staining and immunoelectron microscopy (immuno-EM). In agreement with the suggestion of Brown and Newcomb, we propose a new model for the structure of VSV in which the nucleocapsid is wound around a core containing M protein and where the only place of contact between M and the lipid is at the extreme ends of the skeleton. This last point is supported by studies on skeleton-liposome interactions.

* Corresponding author.

MATERIALS AND METHODS

Virus, skeletons, and nucleocapsids. The Indiana Laboratory strain (Orsay) of VSV was grown in BSR cells (a clone of BHK 21) at 37°C in minimal essential medium supplemented with 2% calf serum. At 24 h postinfection, cell fragments were removed from the culture fluid by centrifugation, and virus particles were pelleted through 30% glycerol in 10 mM Tris-HCl (pH 7.5)–50 mM NaCl–1 mM EDTA and resuspended in TD (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 25 mM Tris-HCl [pH 7.5], 10 mM EDTA). Virus was labeled by adding [^{35}S]methionine (20 μCi/ml of Eagle medium) 6 h postinfection.

Skeletons and nucleocapsids were isolated by octyl glucoside extraction as previously described (14). Both preparations were dialyzed against Tris buffer (TB; 10 mM Tris-HCl [pH 7.4]) at 4°C and used within 4 days.

Antibodies against N and M proteins. (i) Polyclonal anti-M antibodies. M protein (50 μg), purified by a previously described method (11), was emulsified with an equal volume of Freund's complete adjuvant and injected intraperitoneally (i.p.) into a BALB/c mouse previously stimulated by i.p. injection of 2,6,10,14-tetramethyl-pentadecane (Pristane; Sigma). The rest of the protein was frozen and conserved at −80°C to be injected after emulsion with an equal volume of Freund's incomplete adjuvant 2 and 4 weeks later. Six weeks after the first injection, 10^6 myelomas were injected i.p. after checking that the mice had been immunized correctly (by immunofluorescence of VSV-infected and control cells). Ascitic fluid was collected 2 weeks later.

(ii) Anti-M MAbs. Three mouse monoclonal antibodies (MAbs), MAbs 2, 3, and 25, that react with epitopes 1, 2, and 3 of M protein (19) were kindly provided by R. R. Wagner. An MAb against N protein (epitope unknown) was a kind gift of R. A. Weiss.

Specificity and cross-reactivity of all antibodies used were checked by Western immunoblot analysis after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) or by direct dot blotting of purified proteins on Immobilon-P transfer membrane (Millipore). All antibodies were found to be monospecific.

Liposome binding to skeletons. Commercial preparations of phospholipids (phosphatidylcholine and phosphatidylserine) and cholesterol (Sigma) dissolved in organic solvents (1 mg of lipid total) were dried in vacuo (for the liposome-skeleton cosedimentation experiments, 1 μCi of tritiated cholesterol was added). After addition of 1 ml of TB plus 50 mM NaCl, the

mixture was sonicated six times for 5 min (each). The liposome suspension was clarified at 3,500 × *g* for 5 min, and the supernatant containing mainly small unilamellar vesicles (as checked by EM) was used for the experiments.

For binding experiments, skeletons or nucleocapsids dialyzed against TB were added to radioactive liposomes. After 30 min of incubation at room temperature, the mixture was layered on top of a 30% glycerol solution in 10 mM Tris-HCl (pH 7.5)–50 mM NaCl–1 mM EDTA and centrifuged for 1 h at 35,000 rpm in an SW41 rotor (Beckman). The fraction of liposomes associated with skeletons and nucleocapsids in the pellet was determined by liquid scintillation counting. In the absence of nucleocapsids or skeletons, less than 0.2% of the total radioactivity was found in the pellet. EM observations were done directly on the pellet material by using cold lipids.

Triton X-114 phase separation. One volume of ^{35}S-labeled virus in TD was added to 1 volume of 2% Triton X-114 in TD or 1 M KCl. The mixture was incubated for 3 h on ice before phase separation (1). Proteins in the aqueous phase were trichloroacetic acid precipitated before analysis by SDS-PAGE. In order to determine the quantities of G, N plus NS, and M in the detergent phase, the proteins were separated by PAGE, the bands cut out and solubilized in H_2O_2 at 80°C for 16 h, and the radioactivity was counted.

EM. For negative staining, samples in TD (virus) or TB (skeletons) were adsorbed onto glow-discharged carbon-coated grids and stained with 1% aqueous sodium silicotungstate (SST), without prior salt treatment.

For immuno-EM, samples were adsorbed to Formvar-coated nickel grids and floated onto a solution of TB or TB plus 0.5 M NaCl (TBS), with 2% bovine serum albumin and 0.05% Tween 20. They were then treated with diluted anti-M antibodies (MAb2, 1/100; MAb3, undiluted; and MAb25, 1/10; polyclonal antibody, 1/300 [dilutions were in TB or TBS]) or with the mouse anti-N MAb (1/50). Controls were done with ascitic fluid (BALB/c control ascites fluid MA; Cedarlane Laboratories, Hornby, Ontario, Canada) used at the same dilution in TB or TBS. The grids were then rinsed and floated onto a diluted solution (1/10 in TB) of goat anti-mouse antibody conjugated with 5-nm gold particles (Auroprobe TM EM, RNP 424; Amersham). The samples were fixed for 20 min in 0.1% glutaraldehyde and, after a washing, were negatively stained with 1% SST. Immuno-EM preparations in TB and TBS were called low- and high-salt conditions, respectively. EM was carried out with a JEOL 1200 EXII under low-dose conditions. Magnification was calibrated by using negatively stained catalase crystals (26).

For thin sections, virus was fixed with 2% glutaraldehyde in cacodylate buffer (pH 7.4), postfixed in 1% OsO_4, dehydrated with ethanol, infiltrated with London Resin White (Agar Scientific Ltd.), and polymerized. Thin sections were stained with 2% uranyl acetate and then by lead citrate.

RESULTS

Morphology of isolated skeletons. In EM after negative staining, VSV shows the characteristic shape of a rhabdovirus: a bullet-shape particle covered with spikes (trimers of the glycoprotein G) (Fig. 1A, left). When stain has penetrated the virion, the helically coiled nucleocapsid is revealed (Fig. 1A, right), as already described (13). After negative staining of isolated skeletons, without prior salt treatment, we observed tightly coiled nucleocapsid skeletons similar to those previously described (14) (Fig. 1B). The mean diameter of the isolated skeletons is 46.7 nm compared with 53.8 nm of the skeletons in virus (Table 1). This smaller diameter of isolated skeletons was observed before by Newcomb and Brown (14), who also found that, apart from being thinner, isolated skeletons were also somewhat longer and had a slightly increased distance between the striations (14). A similar difference in diameter was observed with cryo-EM of samples in frozen, hydrated state (Table 1), indicating that it is not an artifact of the negative staining procedure.

In some particles, the stain reveals the presence of amorphous material inside the coiled nucleocapsid, the "axial channel material," which can sometimes be observed protruding from one or both extremities of the skeletons where the nucleocapsid is unwound (Fig. 1C, arrowheads). This axial channel material can sometimes be found completely free of the nucleocapsid (Fig. 1D, arrows), having the same morphology and diameter as the structures extending from the coils (26 nm; Table 1). Because of its shape, we will call this structure the cigar.

However, one needs to be careful in interpreting the two-dimensional images obtained in EM and translating them into a three-dimensional structure, i.e., is the cigar really inside the nucleocapsid coil? The images shown in Fig. 1E and F of negatively stained skeletons seen end on and of thin-sectioned virus (see also Fig. 14.1 and 14.5 in reference 2) strongly suggest that there is indeed material inside the coil, and we assume that this material is the cigar.

The nature of these cigars and the localization of M were subsequently tested by using immunogold labeling with specific anti-M and anti-N antibodies.

Immunogold labeling suggests that M is inside the nucleocapsid coil. In low salt, the skeletons appear very compact, like those in Fig. 1B, and immunogold labeling with polyclonal antibodies and all three anti-M MAbs resulted in weak labeling of the extremities of the skeletons only (not shown). We performed the same experiment under high-salt conditions (0.5 M NaCl), which led to partial unwinding of the coil. Using both polyclonal antibodies (Fig. 2A) and MAbs (Fig. 2B), we observed strong labeling at the skeleton extremities, where the ends of the cigar are exposed, and on free cigars (arrowheads), suggesting that M is located in the cigars. No anti-M label was associated with the sides of the skeleton where the striations could be observed.

Incubating skeletons under low-salt conditions with the MAb directed against N protein resulted in uniform labeling of the skeletons (Fig. 2C). Labeling was never very dense, as was also previously observed by using a polyclonal antibody (16). Probably the tight coiling of the nucleocapsid obscures some of the epitopes on N. In high salt, the gold particles were again found along the sides of the skeleton and associated with unwound nucleocapsid at the coil ends but never on the exposed bits of cigar (Fig. 2D, arrowheads).

Analysis of purified skeletons by SDS-PAGE shows that they contain the L, N, NS, and M proteins in the same proportions as found in intact virus (Fig. 3). We wanted to know whether the cigars consisted purely of M or of a coassembly of M with NS and maybe L, by isolating cigars and analysis via SDS-PAGE. In order to remove the nucleocapsid, we treated isolated skeletons with a range of NaCl concentrations. This treatment either had no effect on the skeletons or led to disruption of the cigar and to the apparent solubilization of M, as judged from analysis on glycerol gradients (data not shown). We never succeeded in isolating intact cigars, and the fact that free cigars can be observed in EM is probably due to the stabilization of this structure by contact with the EM carbon support film.

Skeletons bind negatively charged liposomes. It has been well documented that M interacts with lipid membranes,

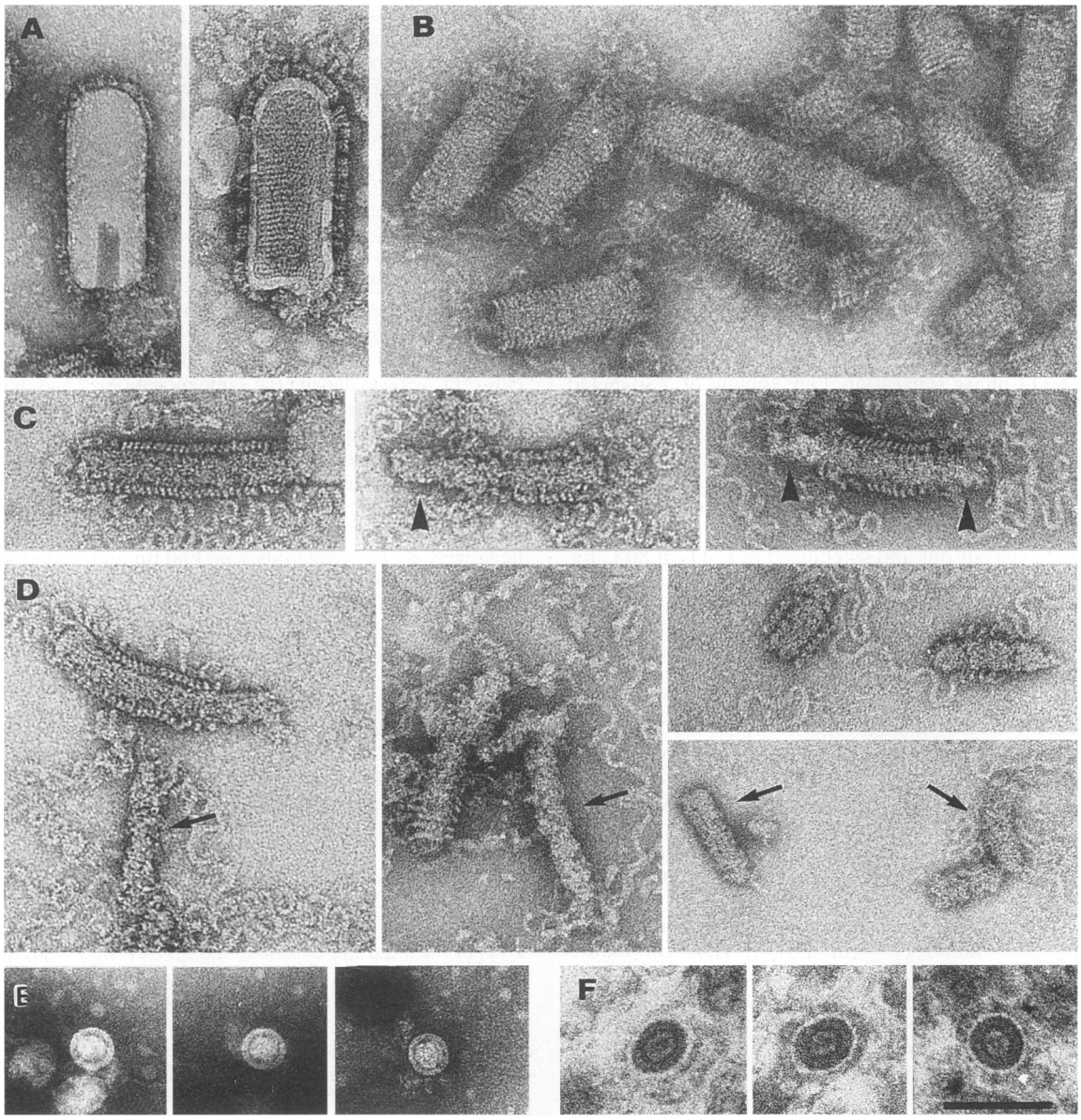

FIG. 1. EM of VSV structure. Negative staining was done with 1% SST. (A) Intact VSV. The particle on the left has an intact membrane, although membrane invagination has occurred as a result of the negative staining procedure (24). The particle on the right shows the internal skeleton structure visible because of stain penetration through the viral membrane. (B) Isolated skeletons showing tightly coiled structures. (C and D) Isolated skeletons in the process of unwinding and revealing their internal structure, the cigar. This cigar extends from the ends of unwinding skeletons (C, arrowheads) and can be found lying free on the support film (D, arrows). The method of preparation of the particles in panels B, C, and D was the same. The difference in degree of unwinding is probably caused by intrinsic variations in the negative staining procedure due to differences in local hydrophilicity of the support film, sample concentration, room temperature, and humidity. (E) Gallery of negatively stained skeletons in end-on view. (F) Transversally cut sections of VSV showing material inside the nucleocapsid coil. All micrographs have the same magnification. (F) Bar, 100 nm.

notably those which are negatively charged (9, 18, 20, 28). We set out to investigate whether intact skeletons or nucleocapsids free of M were able to bind liposomes with different charges. Whereas purified nucleocapsid did not significantly bind liposomes, whatever the charge (Fig. 4A), skeletons clearly bound negatively charged liposomes much more strongly than neutral liposomes (Fig. 4A). This suggests that the M protein and/or the integrity of the skeleton structure is necessary for interaction between skeletons and negatively charged liposomes. When the skeleton-liposome pellets were studied by negative-stain EM, we found, when using neutral lipids, very few

TABLE 1. Mean diameter (in nanomoles) of skeleton in virus and of free skeleton

Sample	Diam (± SD [*n*])	
	Negative stain with SST	Frozen hydrated[a]
Skeleton in virus	53.8 ± 3.2 (15)	53.6 ± 1.2 (21)
Isolated skeleton	46.7 ± 2.3 (16)	44.1 ± 2.1 (16)
Cigar in skeleton	26.3 ± 2.0 (19)	ND[b]
Isolated cigar	25.7 ± 2.5 (19)	ND

[a] The skeletons in the frozen hydrated sample were found in a fresh and untreated virus preparation (24).
[b] ND, not done.

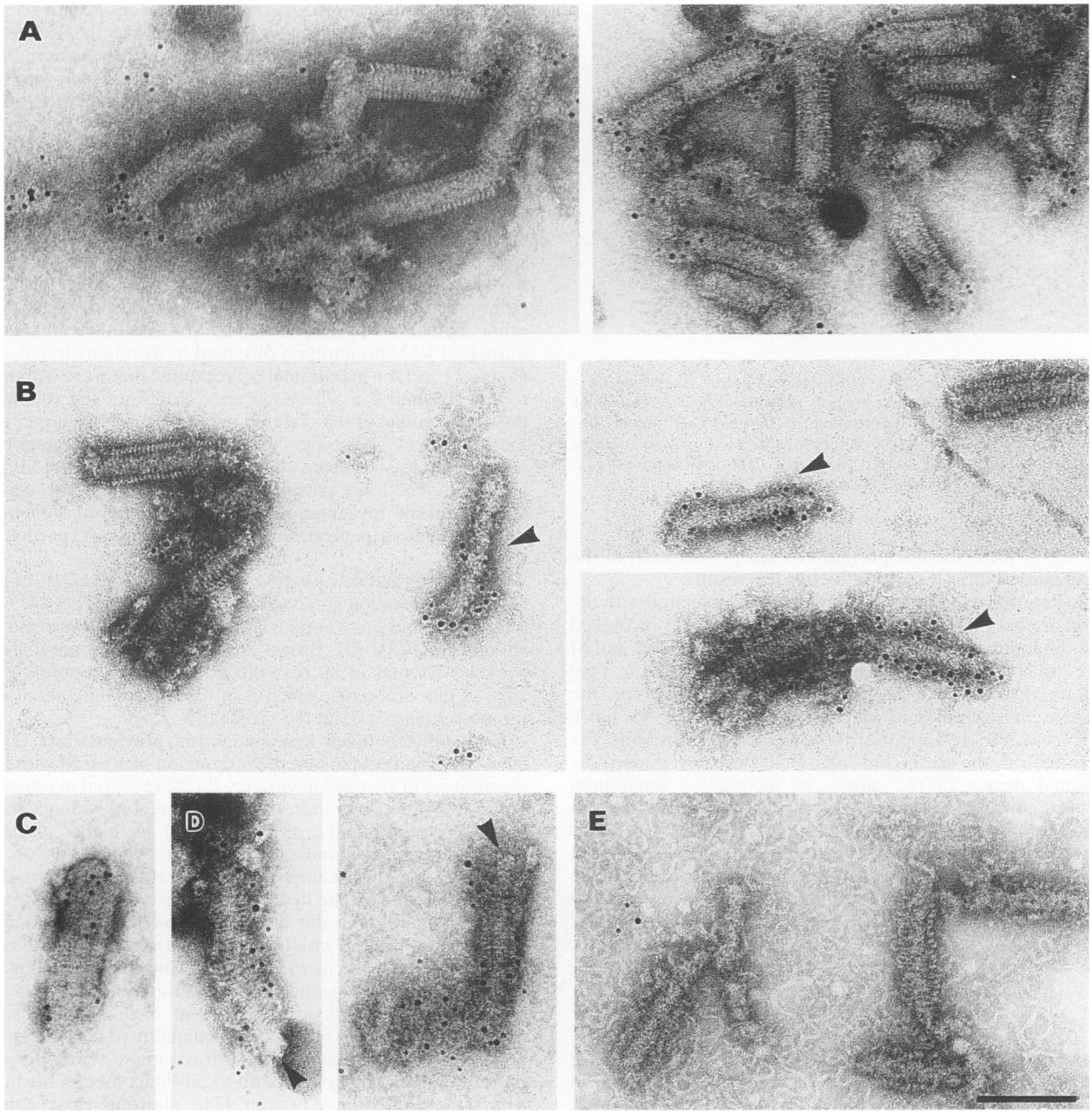

FIG. 2. Immunogold labeling plus negative staining of isolated VSV skeletons. (A) Polyclonal anti-M antibody; (B) anti-M MAb (MAb 25); (C and D) anti-N MAb; and (E) control labeling with BALB/c ascites fluid. (C) Labeling was done at 0 M NaCl; (A, B, D, and E) labeling was at 0.5 M NaCl. Arrowheads point at cigar ends extending from skeletons or free-lying cigars. (E) Bar, 100 nm.

liposomes associated with skeletons, and those were in a random fashion (Fig. 4B). With negatively charged liposomes, the association was much more extensive and was observed predominantly at both extreme ends of the skeletons (Fig. 4C). More than 80% of these liposomes were associated with the ends, and no liposomes were found touching well-defined and tightly wound sides of coils.

VSV M protein in virus is hydrophilic. A recent paper on VSV M protein expressed in HeLa cells (4) shows that 10% of M is associated with cell membranes and this subpopulation has the characteristics of an integral membrane protein. However, in Fig. 3 it is clear that, under low-salt conditions, octyl glucoside treatment of virus does not lead to removal of M from the skeleton, since there is no M in the supernatant in which G is present. In order to investigate the character of M in the virus, we performed Triton X-114 phase separation on ^{35}S-labeled virus. This extraction is commonly used to characterize integral membrane proteins or lipid-anchored proteins by providing phase partitioning of lipophilic from hydrophilic proteins. The content of each phase was analyzed by SDS-PAGE, and the results are shown in Fig. 5. The detergent phase contained the glycoprotein (G) and only small amounts

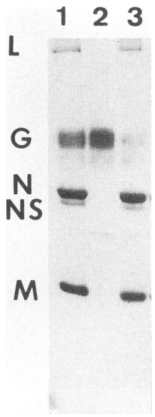

FIG. 3. SDS-PAGE of VSV virion and skeleton. Skeletons were prepared via octyl glucoside extraction as previously described (14). Lanes: 1, intact virus; 2, supernatant of detergent-extracted virus containing only G; 3, pellet of detergent-extracted virus containing skeletons consisting of M, N plus NS, and L. The gel contained 12% acrylamide and was stained with Coomassie G250.

of M and N plus NS. The presence of salt (0.5 M KCl) in the solubilization buffer did not influence the results.

Calculations of the proportion of protein associated with the detergent phase were made assuming that all G was recovered in the detergent phase and by comparing the ratio of G and M (or G and N plus NS) in the detergent phase with that in a gel of intact virus. We found that about 5% of M was in the detergent phase compared with about 6% of N plus NS. Since both N and NS are supposed to be hydrophilic proteins, the 6% found in the detergent phase is probably caused by artifactual mixing of the phases or incomplete separation. Therefore, taking the amount of N plus NS as an internal control for mixing artifacts, we find that little if any M is present in the detergent phase, meaning that virtually all M in the virus is hydrophilic. This agrees well with the results of the skeleton-liposome binding experiments that suggested an electrostatic interaction between M and negatively charged lipids.

DISCUSSION

New model for the structure of VSV. VSV has a helical nucleocapsid, and inside the axial channel of this nucleocapsid we found material which we called the cigar. Using immunogold labeling, we have shown that M protein can be found in these cigars, and we did not find anti-M label attached to the outside of the nucleocapsid helix. We therefore propose a new model for the structure of rhabdoviruses in which M forms a scaffold, around which the nucleocapsid is wound. The only contact area between M and the viral membrane is at one or both ends of the nucleocapsid coil. In vitro it has been shown that M interacts with negatively charged but not with neutral lipids (reviewed in reference 18). We show here that isolated skeleton behaves in the same manner and that the binding of negatively charged liposomes occurs predominantly at the extreme ends of the skeleton where the cigar is exposed.

However, both the lipid binding and the antibody labeling experiments cannot exclude the possibility that, apart from M being located in the cigar, some fraction of M might also be on the outside of the nucleocapsid coil (epitopes or lipid binding domains could be hidden). Another caveat we need to add is that, in the preparations we used, the skeletons do not seem to have one rounded end and one blunt end like the skeleton in intact virus. We do not yet understand this observation, but it could mean that our finding that liposomes associate with both ends of the skeleton does not necessarily reflect the situation in virus.

Packing of M in virus and cigar. The inside diameter of free skeletons, 26 nm, is the same as the width of the cigars. Inside a free skeleton with a length of 200 nm (14), there is enough space to accommodate the 1,800 copies of M present in the virus (25) (assuming a cubic packing symmetry and a partial specific volume for M of 0.73 ml/g). However, free skeletons have a smaller diameter than skeletons in intact virus, as determined here (Table 1) and as previously shown (14). This same diameter was also observed for free skeletons in an untreated VSV preparation observed in frozen, hydrated state (Table 1) and for membraneless skeletons that were beginning to bud through the cell membrane (16). Since the volume of the axial channel of the skeleton in virus is 1.7 times larger than that of isolated skeletons, the packing of M inside intact virus is probably less compact than that in isolated skeletons and free cigar. The loose packing of M in virus could also explain the membrane invaginations frequently observed with negatively stained virus particles (like that in Fig. 1A, particle on the left).

Our results indicate that M is situated in the cigar, but we have no information about whether cigars contain only M or M plus other proteins (notably NS and L). In a review on the structure of VSV (2), Brown and Newcomb cite unpublished work by Thomas et al. that the axial channel contains 10 to 15% of the viral mass, which is in reasonable agreement with the percentage of M in the virus (18% [25]).

No conflict between new model and previous data. There have been many papers on the interaction of VSV M with lipid membranes in vivo and in vitro (9, 20, 28; reviewed in reference 18). All results clearly indicate association of M with negatively charged phospholipids, most likely via its highly positively charged amino terminus (9, 27). Our own results on the binding of negatively charged liposomes at the extreme ends of the skeleton and on the hydrophilic nature of M in virus are in good agreement with this literature. However, the amount of M in virus associated with lipid has been estimated to be more than can be expected from our model. Such quantification was done via cross-linking studies with hydrophobic probes. These kinds of experiments are difficult to evaluate because of specific reactivities of the probes, availability of reactive groups on the protein, and state of the virus sample (virus preparations often contain particles with a still-intact membrane but in which the skeleton is broken [24], allowing close contact between newly formed ends of skeleton and membrane).

If our model is right and if there is no M on the outside of the nucleocapsid coil, then the contact between the nucleocapsid and the cytoplasmic tails of the glycoprotein G is probably made via the N protein. However, direct contact between M and G at the ends of the skeleton cannot be excluded, since, in cryo-EM of VSV, G can be seen along the sides of the virion bullet but also at the top and at the blunt end (24). Interaction between G and M has been suggested on the basis of protein cross-linking studies with intact virus (5, 12), although a G-N complex was also found (12). Studies on the fate of M and G in infected cells (8) and after coexpression in HeLa cells (4) suggest that a strong G-M interaction does not exist. Further, although the amount of G relative to other viral proteins in a virus suspension may vary with time postinfection (10), careful determination of the stoichiometry of viral proteins by using scanning transmission EM (in which only intact bullet shapes

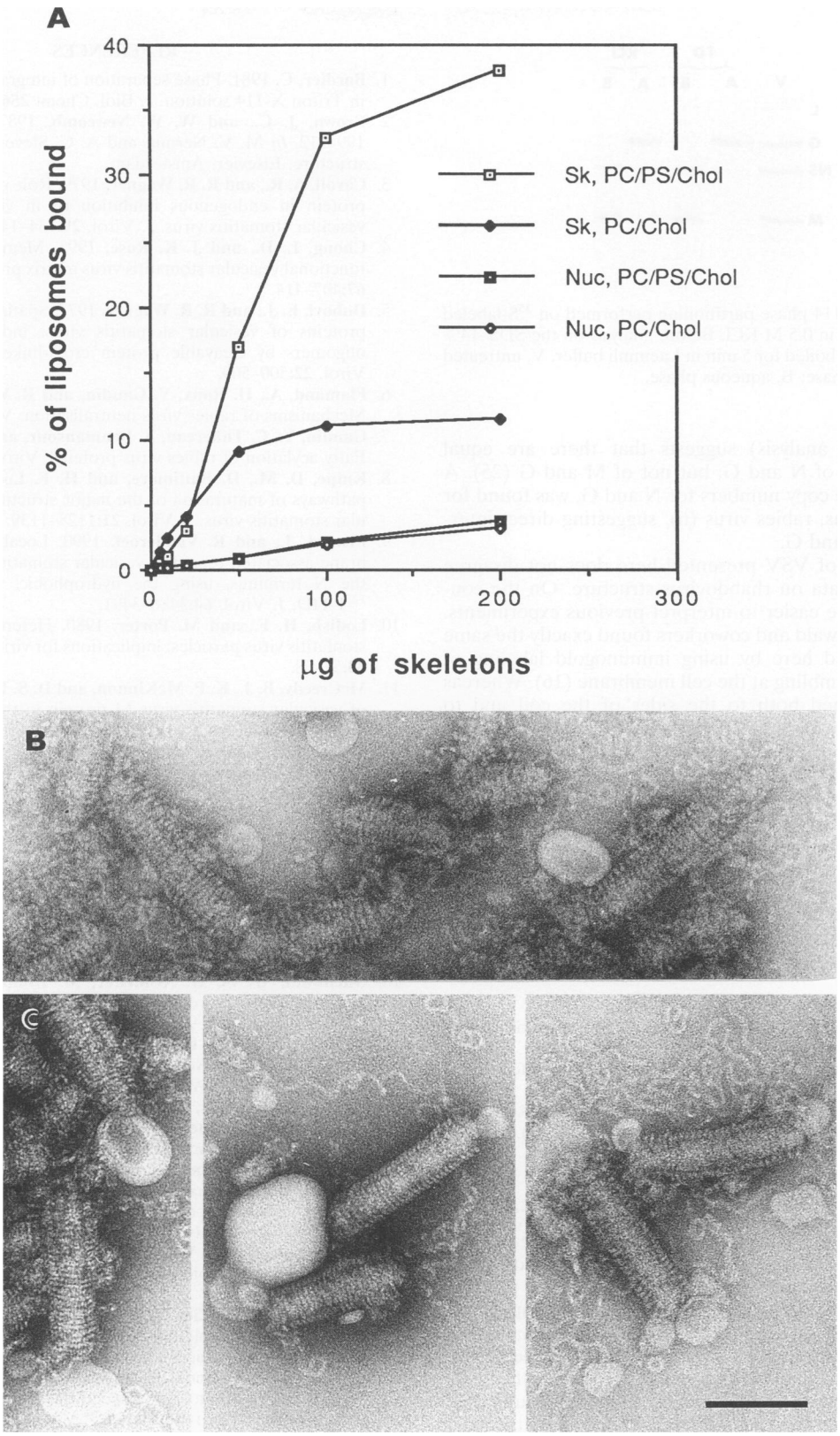

FIG. 4. Binding of liposomes to skeletons and nucleocapsids free of M. (A) Tritiated liposomes (15 µg) of two compositions, phosphatidylcholine-cholesterol (PC/Chol, 3/1 [wt/wt], neutral) or phosphatidylcholine-phosphatidylserine-cholesterol (PC/PS/Chol, 3/3/2, negatively charged), were mixed with increasing amounts of skeleton (Sk) or nucleocapsid (Nuc) and pelleted through 30% glycerol. The fraction of liposomes bound was determined by measuring the amount of radioactivity in the pellet. (B and C) EM of negatively stained skeleton-liposome pellets, corresponding to the neutral and negatively charged liposomes, respectively. (C) Bar, 100 nm.

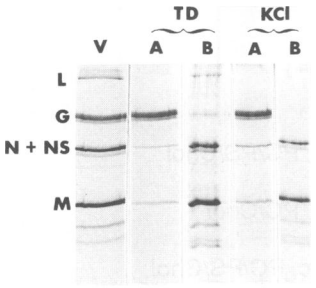

FIG. 5. Triton X-114 phase partitioning performed on ^{35}S-labeled virus in TD buffer or in 0.5 M KCl. Before analysis on the SDS–14% PAGE, samples were boiled for 5 min in Laemmli buffer. V, untreated virus; A, detergent phase; B, aqueous phase.

were selected for analysis) suggests that there are equal numbers of copies of N and G, but not of M and G (25). A similar result, same copy numbers for N and G, was found for another rhabdovirus, rabies virus (6), suggesting direct interaction between N and G.

The new model of VSV presented here does not disagree with the existing data on rhabdovirus structure. On the contrary, it may now be easier to interpret previous experiments. In particular, Odenwald and coworkers found exactly the same results as presented here by using immunogold labeling of VSV skeletons assembling at the cell membrane (16). Whereas anti-N label attached both to the sides of the coil and to unwound nucleocapsid, anti-M label attached only to unravelling ends of skeletons but not to the sides.

Finally, rabies virus M protein does not have a positively charged amino terminus (21), making it impossible for this protein to bind to acidic phospholipid headgroups as proposed for the VSV M protein. However, rabies virus M is palmitoylated in the virus, not for 100% like the glycoprotein in the same virus preparation, but for only 10% (7), suggesting that in a matured virion, not all, but only a subpopulation, of rabies M is anchored in the viral membrane. We suggest that it is the M that is situated at the ends of the skeleton.

ACKNOWLEDGMENTS

We thank R. R. Wagner, R. A. Weiss, and P. Naporowski for kind gifts of MAbs, Anne Flamand for her interest and support, Bernard Jacrot for critical comments on the text, and J. Bénéjean for expert technical assistance.

Y.G. is a postdoctoral fellow of the Fondation pour la Recherche Médicale. This work was supported by CNRS (UPR 02431).

ADDENDUM

After submission of this article we learned from J. C. Brown (University of Virginia Health Sciences Center, Charlottesville, Va.) that, although they never published their findings, he and W. W. Newcomb have done experiments similar to those we describe in our report, with similar results. Using negative-stain EM, they have also observed that the axial channel of VSV is filled and that the filling has the shape of a pole or rod, sometimes lying free, sometimes sticking out of the axial channels. Using immunogold labeling, they also found that skeletons label only at the ends with anti-M and along the sides with anti-N antibodies (W. Newcomb and J. Brown, unpublished results). STEM measurements showed that the axial pole has a mass of at least 30 MDa (W. Newcomb, J. Wall, A. Steven, and J. Brown, unpublished results). They also concluded that M is probably located inside the nucleocapsid coil, as they mentioned previously (2).

REFERENCES

1. **Bordier, C.** 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. **256:**1604–1607.
2. **Brown, J. C., and W. W. Newcomb.** 1987. Rhabdoviridae, p. 199–212. *In* M. V. Nermut and A. C. Steven (ed.), Animal virus structure. Elsevier, Amsterdam.
3. **Caroll, A. R., and R. R. Wagner.** 1979. Role of the membrane (M) protein in endogenous inhibition of in vitro transcription by vesicular stomatitis virus. J. Virol. **29:**134–142.
4. **Chong, L. D., and J. K. Rose.** 1993. Membrane association of functional vesicular stomatitis virus matrix protein in vivo. J. Virol. **67:**407–414.
5. **Dubovi, E. J., and R. R. Wagner.** 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. J. Virol. **22:**500–509.
6. **Flamand, A., H. Raux, Y. Gaudin, and R. W. H. Ruigrok.** 1993. Mechanisms of rabies virus neutralisation. Virology **194:**302–313.
7. **Gaudin, Y., C. Tuffereau, A. Benmansour, and A. Flamand.** 1991. Fatty acylation of rabies virus proteins. Virology **184:**441–444.
8. **Knipe, D. M., D. Baltimore, and H. F. Lodish.** 1977. Separate pathways of maturation of the major structural proteins of vesicular stomatitis virus. J. Virol. **21:**1128–1139.
9. **Lenard, J., and R. Vanderoef.** 1990. Localization of the membrane-associated region of vesicular stomatitis virus M protein at the N terminus, using the hydrophobic, photoreactive probe ^{125}I-TID. J. Virol. **64:**3486–3491.
10. **Lodish, H. F., and M. Porter.** 1980. Heterogeneity of vesicular stomatitis virus particles: implications for virion assembly. J. Virol. **33:**52–58.
11. **McCreedy, B. J., K. P. McKinnon, and D. S. Lyles.** 1990. Solubility of vesicular stomatitis virus M protein in the cytosol of infected cells or isolated from virions. J. Virol. **64:**902–906.
12. **Mudd, J. A., and R. E. Swanson.** 1978. *In situ* cross-linking of vesicular stomatitis virus proteins with reversible agents. Virology **88:**263–280.
13. **Nakai, T., and A. F. Howatson.** 1968. The fine structure of vesicular stomatitis virus. Virology **35:**268–281.
14. **Newcomb, W. W., and J. C. Brown.** 1981. Role of the vesicular stomatitis virus matrix protein in maintaining the viral nucleocapsid in the condensed form found in native virions. J. Virol. **39:**295–299.
15. **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.
16. **Odenwald, W. F., H. Arnheiter, M. Dubois-Dalcq, and R. A. Lazzarini.** 1986. Stereo images of vesicular stomatitis virus assembly. J. Virol. **57:**922–932.
17. **Oxford, J. S., and D. J. Hockley.** 1987. Orthomyxoviridae, p. 213–232. *In* M. V. Nermut and A. C. Steven (ed.), Animal virus structure. Elsevier, Amsterdam.
18. **Pal, R., Y. Barenholz, and R. R. Wagner.** 1987. Vesicular stomatitis virus membrane proteins and their interactions with lipid bilayers. Biochim. Biophys. Acta **906:**175–193.
19. **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.
20. **Pepinsky, R. B., and V. M. Vogt.** 1979. Identification of retrovirus matrix proteins by lipid-protein cross-linking. J. Mol. Biol. **131:**819–837.
21. **Rayssiguier, C., L. Cioe, E. Withers, W. H. Wuller, and P. Curtis.** 1986. Cloning of rabies virus matrix protein mRNA and determination of its amino acid sequence. Virus Res. **5:**177–190.
22. **Rose, J. K., R. F. Doolittle, A. Anilionis, P. J. Curtis, and W. H. Wunner.** 1982. Homology between the glycoproteins of vesicular stomatitis virus and rabies virus. J. Virol. **43:**361–364.
23. **Ruigrok, R. W. H., L. J. Calder, and S. A. Wharton.** 1989. Electron microscopy of the influenza virus submembranal structure. Virology **173:**311–316.
24. **Ruigrok, R. W. H., and E. A. Hewat.** 1991. Comparison of negatively stained and frozen hydrated samples of influenza viruses A and B and of vesicular stomatitis virus. Micron Microsc. Acta **22:**423–434.

25. **Thomas, D., W. W. Newcomb, J. C. Brown, J. S. Wall, J. F. Hainfeld, B. L. Trus, and A. C. Steven.** 1985. Mass and molecular composition of vesicular stomatitis virus: a scanning transmission electron microscopy analysis. J. Virol. **54:**598–607.
26. **Wrigley, N. G.** 1968. The lattice spacing of crystalline catalase as an internal standard of length in electron microscopy. J. Ultrastr. Res. **24:**454–464.
27. **Zakowski, J. J., W. A. Petri, and R. R. Wagner.** 1981. Role of matrix protein in assembling the membrane of vesicular stomatitis virus: reconstitution of matrix protein with negatively charged phospholipid vesicles. Biochemistry **20:**3902–3907.
28. **Zakowski, J. J., and R. R. Wagner.** 1980. Localization of membrane-associated proteins in vesicular stomatitis virus by use of hydrophobic membrane probes and cross-linking reagents. J. Virol. **36:**93–102.
29. **Zvonarjev, A. Y., and Y. Z. Ghendon.** 1980. Influence of membrane (M) protein on influenza A virus transcriptase activity in vitro and its susceptibility to rimantadine. J. Virol. **33:**583–586.