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Distribution of virus structural proteins and protein–protein interactions in plasma membrane of baby hamster kidney cells infected with Sindbis or vesicular stomatitis virus

(enveloped viruses/membrane glycoproteins/membrane biogenesis)

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ABSTRACT **The plasma membrane of baby hamster kidney (BHK-21) cells infected with either Sindbis or vesicular stomatitis virus was isolated by a technique involving the ingestion of latex beads by the cells. Plasma membrane isolated from Sindbis virus-infected cells contained only one (E_1) of the three (E_1, E_2, and C) structural proteins of this virus. When the latex beads were pretreated with either polylysine or DEAE-dextran, plasma membrane obtained from Sindbis virus-infected cells contained all three structural proteins and PE_2, a precursor to one of the structural proteins. In pulse-chase radiolabeling experiments with Sindbis virus-infected cells, it was possible to follow the appearance of the precursor protein (PE_2) in the plasma membrane and its eventual conversion to E_2. The appearance of Sindbis virus membrane proteins PE_2 and E_1 in the purified plasma membrane was not affected by the drug tunicamycin, an inhibitor of glycosylation. These experiments imply the following: (*i*) Cleavage of the Sindbis virus precursor polypeptide PE_2 to E_2 is not a prerequisite for its transport to the cell plasma membrane; (*ii*) transport of virus membrane proteins to the cell surface does not depend on glycosylation; and (*iii*) although all Sindbis virus structural proteins are associated with the plasma membrane, a generally accepted pairing of PE_2–E_1 or E_2–E_1 in the plasma membrane either does not exist or, if it does exist, involves a very weak interaction. The procedures used in this study also resulted in the successful isolation of plasma membrane from vesicular stomatitis virus-infected cells containing the glycoprotein, the matrix protein, and the nucleocapsid protein, a result that suggests that these proteins are located on the media side of baby hamster kidney cells grown in monolayer.**

Membrane-limited RNA-containing viruses such as the alphatogaviruses and the rhabdoviruses achieve maturity through the envelopment of a ribonucleoprotein complex by a fragment of cell plasma membrane that has been modified by virus-specified glycoproteins (1).

In the case of the alphavirus Sindbis or the closely related Semliki Forest virus, important steps in processing the virus membrane proteins—e.g., proteolytic cleavage (2, 3), glycosylation (4), and lipidation (5)—take place during maturation. Mature infectious Sindbis virions contain a capsid or core structure composed of multiple copies of a single virus-coded protein (C) and the infectious (+) single-stranded RNA (6). The capsid is, in turn, surrounded by a membrane bilayer containing two virus-specified proteins (7) that are glycosylated by host enzymes (8). The membrane lipids are of host origin (6). In the mature virion, the membrane proteins are paired (9); there is indirect evidence suggesting that this pairing is established soon after the integration of polypeptides E_1 and PE_2, the precursor to E_2, into membranes of the rough endoplasmic reticulum (3, 10–13). There is also indirect evidence suggesting that the proteolytic cleavage of polypeptide PE_2 to polypeptide E_2 is intimately involved in the envelopment (budding) of the virions from the cell plasma membrane and that this cleavage occurs after polypeptide PE_2 reaches the cell surface (11–13). Experiments with Sindbis virus temperature-sensitive mutants defective in conversion of PE_2 to E_2 suggest that PE_2 can be transported to the cell surface without processing to E_2; however, PE_2 cannot be detected in the cell membrane by a number of procedures that readily demonstrate the presence of E_2 (11).

The presence or absence of the precursor polypeptide PE_2 in the plasma membrane and the pairing relationship between this protein (and the cleavage product E_2) and protein E_1 are important considerations in understanding the molecular events that take place as the modified cell membrane is converted into the virus envelope. We have investigated the composition of cell plasma membrane from cells prepared by a technique in which the ingestion of latex beads gives relatively pure "inside out" plasma membrane; our experiments were primarily carried out with Sindbis virus-infected cells, although some basic experiments were also performed with vesicular stomatitis virus (VSV) to test the generality of the observations.

MATERIALS AND METHODS

Cells, Viruses, and Media. Baby hamster kidney (BHK-21) cells were grown in Eagle's minimal medium as described (11, 14). The heat-resistant strain of Sindbis virus, which served as wild-type virus, and the Indiana serotype of VSV were gifts from Elmer Pfefferkorn (Dartmouth Medical School). The characteristics and growth of these viruses have been described (15).

Infection of Cells and Labeling Procedures. Subconfluent monolayers of BHK-21 cells grown in 100-mm petri dishes were infected with either Sindbis virus or VSV at a multiplicity of infection of 50–100 plaque forming units per cell after a 1-hr pretreatment with actinomycin D at 4 μg/ml.

Radiolabeling of the virus proteins was carried out by starving infected cells in methionine-free medium for 1 hr and then adding [^{35}S]methionine (1000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels; Amersham Searle) to a final concentration of 20 μCi/ml at 5 hr postinfection. Labeling was carried out for 1 hr. In pulse-chase experiments, labeling was for 5 min. Chase pe-

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Abbreviations: BHK, baby hamster kidney; VSV, vesicular stomatitis virus.

riods followed the pulse after protein synthesis had been terminated by addition of cycloheximide (50 μg/ml) and unlabeled methionine (10 times the concentration in normal medium).

In experiments in which protein glycosylation was blocked with tunicamycin (a gift of Eli Lilly), the drug was added either immediately or 2 hr after infection to a final concentration of 1 μg/ml. This concentration has been shown by Leavitt *et al.* (4) to completely block glycosylation reactions, an observation confirmed by us (data not shown).

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. The polypeptide compositions of the cells, viruses, and plasma membrane were analyzed on 14-cm vertical slab gels by using a discontinuous system modified from Laemmli (16). The resolving gel contained 10.8% acrylamide, and the stacking gel contained 3% acrylamide.

Samples for electrophoresis were pelleted by centrifugation, and the pellets were dissolved in buffer (1% NaDodSO$_4$/1.5% dithiothreitol/0.25 M Tris, pH 6.8) and heated to 100°C before electrophoresis.

Gel slabs were impregnated with diphenyl oxazole by the method of Bonner and Laskey (17). Each gel was dried and exposed to x-ray film (Kodak, X-Omat RP) to produce a fluorogram.

Preparation of Plasma Membrane. Virus-infected BHK-21 cell plasma membrane was prepared by the isolation of latex beads ingested into cell cytoplasms by endocytosis. The procedure was a modification of that of Wetzel and Korn (18) that was adapted to cells in culture by Charalampous *et al.* (19).

A suspension of polystyrene beads (0.7 μm in diameter; Sigma) in Eagle's minimal medium was added to infected cell monolayers to a final concentration of 4×10^3 beads per cell (concentrations of stock suspensions of beads were determined by using a Coulter counter and determining their optical density at 350 nm). The bead suspension was sonicated for 2 min in a Branson sonicator just before the addition to ensure the ingestion of single unclumped beads. The monolayers and the bead suspension were incubated together at 37°C for 30 min with gentle rocking. This short incubation period was chosen to reduce the probability of fusion of the phagosomes with lysosomal membranes (19). Ingestion of the beads was terminated by rapidly cooling the cells to 0°C. The monolayers were washed 4 times with ice-cold 0.3 M sucrose. The cells were suspended in phosphate-buffered saline and then broken in a Dounce homogenizer at 0°C. The beads released from the cell cytoplasm were separated from the homogenate by centrifugation on a sucrose step gradient. The homogenate was mixed with an equal volume of 60% (wt/vol) sucrose in phosphate-buffered saline and placed in the bottom of a 39-ml cellulose nitrate centrifuge tube. The layer containing the homogenate was overlaid sequentially with 25%, 20%, and 10% sucrose solutions (wt/vol in phosphate-buffered saline), and the gradient was centrifuged at 23,000 rpm for 90 min by using a Sorvall AH 627 rotor. The membrane-coated beads were recovered at the 10% to 20% interface. The band containing the membrane-coated beads was purified by repeating this procedure, and its composition was then determined.

In certain experiments, the surface properties of the beads were altered by treatment with DEAE-dextran, polylysine (Sigma), or serum before the beads were added to infected cells. In such cases, a 1-ml sample of concentrated bead suspension was treated with 20 μl of a 0.1% solution of polylysine or DEAE-dextran or with 200 μl of calf serum. Addition of this altered bead suspension to the cell monolayers to produce the required concentration (4×10^3 beads per cell) reduced the polylysine or DEAE-dextran concentration in the final suspension to 0.0003%. Pretreatment of the beads did not affect the amount of lipid recovered.

RESULTS

Isolation of Cell Plasma Membrane. When the homogenate derived from Sindbis virus-infected cells that had ingested polystyrene beads was subjected to step-gradient centrifugation, the radioactivity of the [^{35}S]methionine-labeled proteins was located primarily in the load zone (fractions 1–10) and in fractions 14–16 (where the band containing the beads could be identified by inspection) (Fig. 1). However, when the homogenate of virus-infected cells that had not been treated with beads was subjected to step-gradient centrifugation, all the label remained in the load zone.

Similar results were obtained when cells infected with VSV were used (data not shown). These experiments show that purification of latex beads from cells infected with Sindbis or VSV leads to the separation of radioactive proteins from the cell homogenate. In this system, the shut-off of host cell protein synthesis is such that these labeled proteins are nearly all of virus origin; however, nonspecific association between cell-associated labeled virus proteins and the beads during processing can be ruled out by the following control experiments.

(*i*) Radiolabeled virus-infected cells were disrupted by homogenization and mixed with an amount of beads equal to that used in a typical experiment, and the mixture was then purified by step-gradient centrifugation.

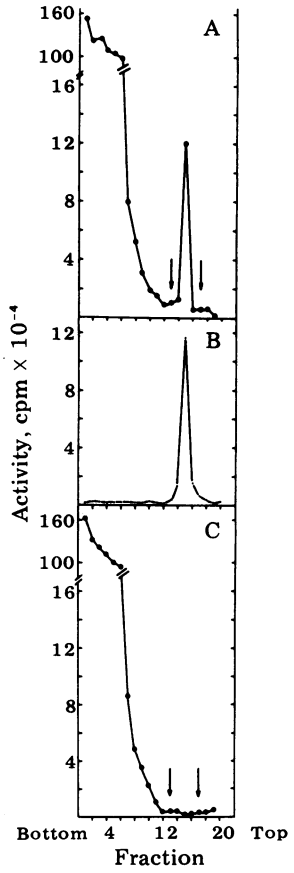

FIG. 1. Purification of latex beads containing phagosomes from Sindbis virus-infected cells. Cells were infected, radiolabeled with [^{35}S]methionine, and disrupted. Distribution of radioactivity in a step gradient: (*A*) Homogenate of Sindbis virus-infected cells with ingested latex beads. (*B*) Fractions 14–16 of the gradient shown in *A* run in a second identical step gradient. (*C*) Homogenate of Sindbis virus-infected cells without latex beads.

(*ii*) Virus-infected unlabeled cells that had ingested beads were mixed with an equal number of virus-infected radiolabeled cells that had not ingested beads, and the mixture was homogenized and centrifuged.

(*iii*) Uninfected unlabeled cells that had ingested beads were mixed with an equal number of radiolabeled virus-infection cells that had not ingested beads, and the mixture was homogenized and centrifuged. The amount of label found in all of these experiments was only 2–10% of that found in experiments in which beads had been ingested by virus-infected radiolabeled cells. Analysis of the radioactive material associated with the beads in the controls by polyacrylamide gel electrophoresis suggested no preferential association of proteins with beads (data not shown). This fraction contained residually synthesized host and all identifiable virus proteins, both in very low concentrations. These results were not altered by treating the beads with polylysine, DEAE-dextran, or serum and show that the radioactive protein associated with the beads (Fig. 1) is specifically associated during phagocytosis of the beads by the cells and that cell-associated radioactivity is not associating with the noningested beads (control *i*) or with membrane-coated beads (controls *ii* and *iii*) during cell breakage.

Protein Composition of Bead-Associated Plasma Membranes. The composition of plasma membrane purified from cells infected with Sindbis virus (or VSV) by using beads treated in various ways but under identical conditions of labeling and processing is shown in Fig. 2. In each experiment, both the plasma membrane derived from the cells and the cells themselves (load zone, Fig. 1) were analyzed. Only one protein profile of the cells is shown for each virus infection as there was no difference in the total radiolabeled protein content of any of the infected cells.

The results shown in Fig. 2 can be summarized as follows. (*i*) Plasma membrane isolated from Sindbis virus-infected cells after ingestion of untreated beads contained only polypeptide E_1, even though the cells from which the beads were isolated contained the membrane proteins PE_2, E_1, E_2 and the capsid protein C (tracks 3 and 2). Identical results were obtained when beads pretreated with serum were used (track 4). (*ii*) Plasma membrane recovered from beads that had been treated with either DEAE-dextran or polylysine contained virus proteins PE_2, E_1, E_2, and *C* (tracks 5 and 6). (*iii*) Plasma membrane prepared from VSV infected cells contained proteins G, M, and N, regardless of the pretreatment of the beads used (tracks 8 and 9). The composition of the isolated plasma membrane was similar to that of the VSV infected cells from which it was derived (track 7).

We also examined the protein composition of plasma membrane derived by using this procedure and labeled mock-infected cells (track 11) and compared the composition of this fraction with that of the whole cells (track 10). The plasma membrane was enriched in some proteins.

Effect of Tunicamycin on Incorporation of Sindbis Virus Proteins into Plasma Membranes. The composition of the plasma membrane isolated after radiolabeling in the presence of tunicamycin is shown in Fig. 3. The analysis of the proteins of the Sindbis virus-infected cells showed that tunicamycin treatment prevented cleavage of PE_2 to E_2, as has been shown by Leavitt *et al.* (4) (tracks 2 and 3)—that is, the nonglycosylated forms of PE_2 and E_1 (PE_2*, E_1*) migrate faster in a polyacrylamide gel than their glycosylated counterparts. Analysis of the plasma membrane from Sindbis virus-infected cells treated with tunicamycin showed that this drug did not block the association of virus membrane proteins with the plasma membrane, although the amount of virus-specific label incorporated into the plasma membrane was generally only 50–60% that of the non-drug-treated control experiment, which reflects the fact that tunicamycin reduces total protein synthesis (4). Plasma membrane associated with untreated beads contained only nonglycosylated E_1 (tracks 4 and 5), whereas plasma membrane associated with beads pretreated with polylysine or DEAE-dextran contained nonglycosylated PE_2 and E_1 (track 7; only polylysine shown). The results were not affected by the time of addition of drug (tracks 4, 5, 7, and 8). Thus, addition of tunicamycin immediately or 2 hr after infection (when the cell surface is modified by glycosylated virus proteins; ref. 20) did not prevent the incorporation of newly synthesized nonglycosylated proteins into the plasma membrane.

Appearance of Sindbis Virus Proteins in Cell Plasma Membranes. Pulse-chase protein-labeling experiments were carried out on Sindbis virus-infected cells, and the plasma membrane was isolated from the cells at various times after labeling to determine the appearance and processing of virus proteins in the cell plasma membrane.

Sindbis virus-infected actinomycin D-treated cells were labeled at 5 hr postinfection for 5 min with [^{35}S]methionine and

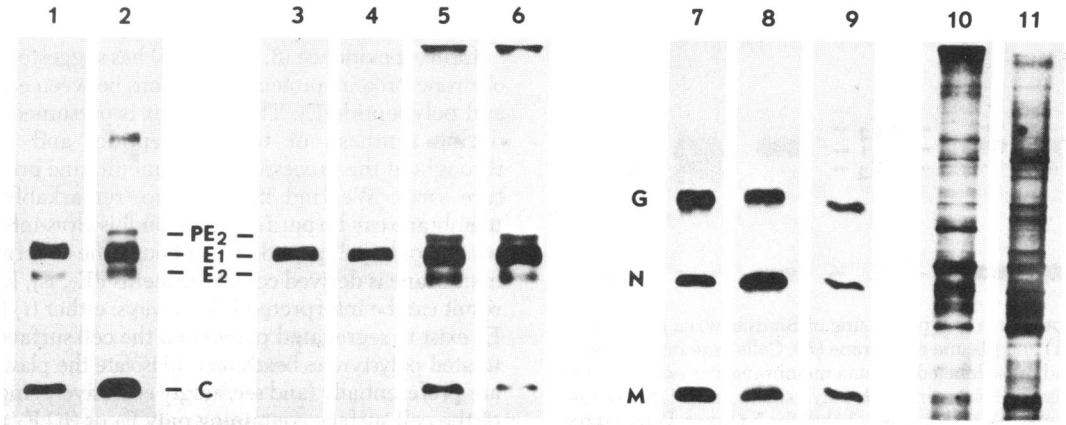

FIG. 2. Protein composition of latex beads containing phagosomes prepared from BHK-21 cells infected with Sindbis virus or VSV. The protein profile of the cells was always derived from the cells from which the plasma membrane was prepared (load zone of the first step gradient). Track 1, ^{35}S-labeled Sindbis marker virus; track 2, Sindbis virus-infected cells; track 3, plasma membrane from Sindbis virus-infected cells (track 2); track 4, plasma membrane isolated with serum-treated latex beads; track 5, plasma membrane isolated with polylysine-treated latex beads; track 6, plasma membrane isolated with DEAE-dextran-treated beads; track 7, cells infected with VSV; track 8, plasma membrane isolated with untreated latex beads and VSV-infected cells (track 7); track 9, as in track 8, except for use of serum-treated beads; track 10, plasma membrane from mock-infected cells; and track 11, mock-infected cells from which plasma membrane (track 10) was derived.

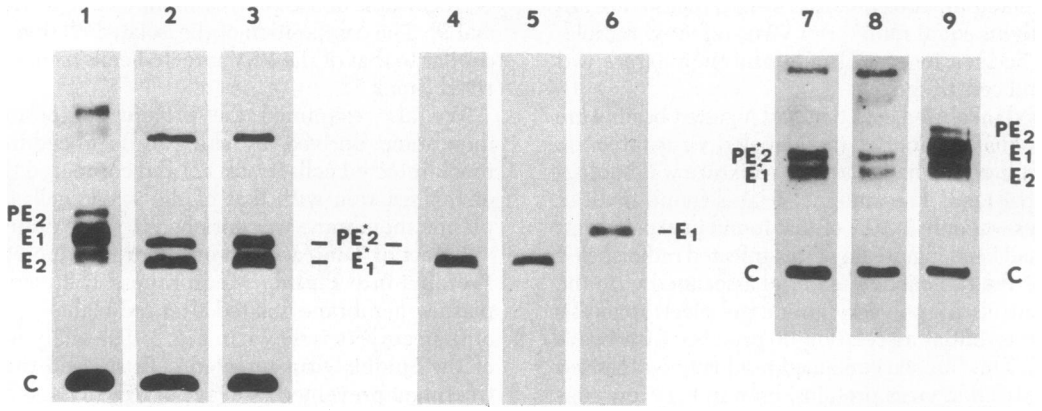

FIG. 3. Protein composition of plasma membranes from BHK-21 cells infected with Sindbis virus in the presence or absence of tunicamycin. The protein profile of the cells was derived from the same ones from which the plasma membrane was isolated. Track 1, infected cells; track 2, infected cells treated with tunicamycin immediately after infection; track 3, infected cells treated with tunicamycin 2 hr after infection; track 4, plasma membrane isolated from cells (as in track 2) with untreated latex beads; track 5, plasma membrane isolated from infected cells (as in track 3) with untreated latex beads; track 6, plasma membrane isolated from infected cells (as in track 1) with untreated latex beads; track 7, plasma membrane isolated from cells (as in track 2) with polylysine-treated latex beads; track 8, plasma membrane isolated from cells (as in track 3) with polylysine-treated latex beads; and track 9, plasma membrane isolated from cells (as in track 1) with polylysine-treated latex beads. PE_2* and E_1* represent the unglycosylated forms of proteins PE_2 and E_1.

chased (Fig. 4). In these experiments, bead ingestion was allowed to occur for a period of 25 min at 37°C. This time period must be added to the chase time; consequently, the shortest chases used were 25 min long. The protein compositions of the infected cells (*A*) and the isolated plasma membrane (*B*) are shown in Fig. 4. The experiments were carried out with polylysine-treated beads to see the presence of all virus structural proteins in the plasma membrane. Plasma membrane after a 25-min chase period contained primarily PE_2, E_1, and C. As the chase period was extended to 55 and 85 min, PE_2 was converted quantitatively to E_2; a similar conversion took place in the cells (tracks 1–3), although some PE_2 could still be detected in the cell extract 85 min after the pulse (track 3). Experiments in which techniques of isolating total cellular membranes were used (data not shown) suggest that this residual PE_2 may not be membrane associated. Treatment of the infected cells with the latex beads did not affect the incorporation of label into mature virus (data not shown).

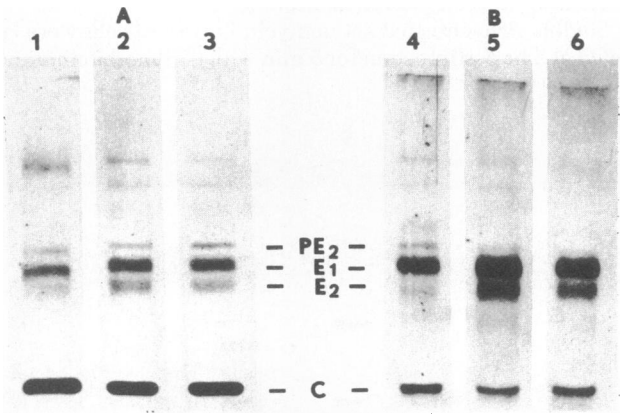

FIG. 4. Appearance and processing of Sindbis virus proteins in BHK-21 cells (*A*) and plasma membrane (*B*). Cells were infected with Sindbis virus and pulse labeled. Plasma membrane was isolated after appropriate chase by treatment with polylysine-treated latex beads. Track 1, virus-infected cells pulse-labeled for 5 min and chased for 25 min; track 2, virus-infected cells pulse-labeled for 5 min and chased for 55 min; track 3, virus-infected cells pulse-labeled for 5 min and chased for 85 min; track 4, plasma membrane from cells in track 1; track 5, plasma membrane from cells in track 2; and track 6, plasma membrane from cells in track 3. Cell protein profiles were derived from the load zones after the first runs in the first step gradients (see Figs. 2 and 3). Equal counts per minute were run in each track.

DISCUSSION

The technique of isolating cell plasma membranes free of other cellular contaminants by endocytosis on latex beads was developed by Wetzel and Korn for use with phagocytic protozoans and adapted to mammalian cells grown in culture by Charalampous *et al.* (19) in 1973. Recently, this technique has been used to demonstrate an asymmetric distribution of plasma membrane proteins (21) and the transmembranyl configuration (22) of some plasma membrane proteins from cultured mammalian cells.

Analysis of the plasma membrane isolated from Sindbis virus-infected cells after ingestion of polylysine or DEAE-dextran-treated polystyrene beads suggests the presence of polypeptide PE_2 in the plasma membrane. Pulse-chase experiments show that this polypeptide is converted to polypeptide E_2 in the plasma membrane, which discounts the possibility that PE_2 reaching the plasma membrane is defective and not suitable for further processing.

Indirect evidence (3, 10–12, 15) has suggested the existence of strong protein–protein interactions between either E_2 or PE_2 and polypeptide E_1. This complex is presumed to be formed during synthesis of the polypeptides and is maintained throughout the processing of the membrane proteins into mature virus. We find it, therefore, remarkable that plasma membrane can be purified from Sindbis virus-infected cells that contain only polypeptide E_1, although the cells from which this membrane is derived contain proteins PE_2, E_1, E_2, and C. This result can be interpreted in two ways: either (*i*) E_1 and PE_2 or E_2 exist in segregated patches on the cell surface, and the untreated polystyrene beads used to isolate the plasma membrane are preferentially (and seemingly exclusively) ingested at areas of the cell surface containing only E_1 or (*ii*) E_1 and PE_2 or E_2 are intermixed on the cell surface but, as the beads are ingested, PE_2, E_2, or both are excluded from the membrane region forming the vesicle so that only E_1 is recovered in the plasma membrane surrounding the beads. The latter possibility seems more likely, which suggests that, if pairing exists between PE_2 or E_2 and E_1 in the plasma membrane, the protein–protein

interactions forming the pairs are weak. Stronger interactions of these components may occur as a final step in the assembly of the virus envelope during the actual budding process. Polypeptide C was never found associated with plasma membrane containing only E_1 but was readily recovered from plasma membrane containing PE_2, E_1, and E_2. This observation supports our opinion that the protein sequences of the PE_2 and E_2 polypeptides are the essential components in the association of the nucleocapsids and the modified plasma membrane and may contain the binding site for the capsid (11).

Tunicamycin (an inhibitor of glycosylation) did not prevent the appearance of the precursor protein PE_2 and the protein E_1 in the plasma membrane nor did it alter the capability of our experimental method to separate them from each other during membrane purification. A previously reported inability to detect virus protein on the surface of cells by using lactoperoxidase-mediated radioiodination (4) probably was due to the inability of this procedure to detect virus membrane protein in the absence of E_2 production (10, 11). Plasma membrane from tunicamycin-treated cells containing E_1 but not PE_2 also contains no capsid protein. Plasma membrane containing PE_2 and E_1 also contains capsid, suggesting that PE_2 alone is capable of binding nucleocapsids, as predicted (11). These observations support a model for Sindbis virus maturation in which the modified plasma membrane contains PE_2 and E_1. If these components are paired, the association is very weak. Conversion of PE_2 to E_2 occurs at the plasma membrane. Glycosylation is essential for the conversion of PE_2 to E_2 but not for transport of the membrane proteins to the cell surface or for the binding of nucleocapsids. The formation of the complex of nucleocapsid and PE_2 in the plasma membrane is followed by interaction with polypeptide E_1, and the subsequent cleavage of PE_2 to E_2 initiates the budding process and establishes a tight pairing of E_2 and E_1 in the developing virus membrane.

Experiments in which plasma membrane from VSV-infected cells was prepared by our procedures showed that plasma membrane contained the three major virus proteins (G, M, and N), regardless of pretreatment. It has been suggested that, when Madin–Darby canine kidney cells are grown in monolayer and infected with VSV, virus maturation occurs only on the surface of the cell in contact with the supporting substrate and virus antigens are located only on this basal surface (23). These observations, based primarily on electron microscopy data, were interpreted as suggesting that special cellular mechanisms direct virus proteins to specified regions of the cell surface. In our experiments, plasma membrane isolated from BHK cells growing in monolayer contained most of the VSV proteins essential for maturation. These membranes are most likely derived from the free (apical) surface of the cell. These contrasting observations may result simply from differences between canine kidney and BHK cells. It is also possible that virus membrane proteins such as those of VSV are integrated uniformly into cell surfaces and then separated by lateral diffusion into regions of the cell in contact with solid surfaces such as plastic or glass growth substrates or the polystyrene (plastic) beads used in this study.

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1. Compans, R. W. & Klenk, H. D. (1979) *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum, New York), Vol. 13, pp. 293–407.
2. Schlesinger, S. & Schlesinger, M. J. (1972) *J. Virol.* **10**, 925–932.
3. Schlesinger, M. J. & Schlesinger, S. (1973) *J. Virol.* **11**, 1013–1016.
4. Leavitt, R., Schlesinger, S. & Kornfeld, S. (1977) *J. Virol.* **21**, 375–385.
5. Schmidt, M. F. G., Bracha, M. & Schlesinger, M. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1687–1691.
6. Pfefferkorn, E. R. & Shapiro, D. (1974) *Comprehensive Virology* (Plenum, New York), Vol. 2, pp. 171–230.
7. Schlesinger, M. J., Schlesinger, S. & Burge, B. W. (1972) *Virology* **47**, 539–541.
8. Grimes, W. J. & Burge, B. W. (1971) *J. Virol.* **7**, 309–313.
9. Ziemiecki, A. & Garoff, H. (1978) *J. Mol. Biol.* **122**, 259–269.
10. Sefton, B. M., Wickus, G. G. & Burge, B. W. (1973) *J. Virol.* **11**, 730–735.
11. Smith, J. F. & Brown, D. T. (1977) *J. Virol.* **22**, 662–678.
12. Bracha, M. & Schlesinger, M. J. (1976) *Virology* **74**, 441–449.
13. Jones, K. J., Scupham, R. K., Pfeil, J. A., Wan, K., Sagik, B. P. & Bose, H. R. (1977) *J. Virol.* **21**, 778–787.
14. Eagle, H. (1959) *Science* **130**, 432–437.
15. Renz, D. & Brown, D. T. (1976) *J. Virol.* **19**, 775–781.
16. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
17. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
18. Wetzel, M. G. & Korn, E. D. (1969) *J. Cell Biol.* **43**, 90–104.
19. Charalampous, F. C., Gonatas, N. K. & Melbourne, A. D. (1973) *J. Cell Biol.* **59**, 421–435.
20. Birdwell, C. R. & Strauss, J. H. (1973) *J. Virol.* **11**, 502–507.
21. Evans, R. M., Ward, D. C. & Fink, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6235–6239.
22. Evans, R. M. & Fink, L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5341–5344.
23. Roth, M. G., Fitzpatrick, J. P. & Compans, R. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6430–6434.