A Fluorescence Photobleaching Study of Vesicular Stomatitis Virus Infected BHK Cells. Modulation of G Protein Mobility by M Protein†

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ABSTRACT: The mobility of vesicular stomatitis virus (VSV) G protein on the surface of infected BHK cells was studied by using the technique of fluorescence photobleaching recovery. The fraction of surface G protein that was mobile in the time scale of the measurement (minutes) was at least 75%, a relatively high value among cell surface proteins so far observed. For studies of the effect of an internal viral protein (M protein) on G protein mobility, cells infected with wild-type VSV were compared with those infected with temperature-sensitive VSV mutants of complementation group III, which contain lesions in the M protein. At the permissive temperature, a pronounced decrease in the mobile fraction of surface G was observed for each of three mutants studied, while mobility of surface G at the nonpermissive temperature was indistinguishable in mutant and wild-type infected cells. A significantly lower mobile fraction of G protein was also observed in SV40 transformed 3T3 cells infected with wild-type VSV, but not in 3T3 or chick embryo fibroblast cells similarly infected. None of the variables tested had a measurable effect on the lateral diffusion coefficient of the mobile G protein. These results are interpreted as modulation of the mobility of a specific cell surface protein by a specific intracellular protein.

Many types of enveloped viruses mature by budding from the plasma membrane of an infected host cell. The virions contain protein and RNA, which are virally coded, and a lipid bilayer derived from host cell lipids [reviewed by Lenard & Compans (1974); Lenard, 1978]. A special advantage of using cells infected with enveloped viruses to study surface organization is the small number of viral proteins and the availability of genetically well-characterized mutations in the different proteins.

In the present paper, we report observations made on cells infected with vesicular stomatitis virus (VSV).[1] VSV is a bullet-shaped enveloped virus, classified as a rhabdovirus. This virus has a single glycoprotein (G), which can constitute the major surface protein in infected cells (Knipe et al., 1977a). The matrix protein (M) is situated inside the lipid envelope of the mature virion where it interacts with the viral nucleocapsid. The M protein is considered to be indispensable to the budding process, since viral buds may lack any of the other viral proteins but always contain M protein (Schnitzer & Lodish, 1979; Schnitzer et al., 1979; Weiss & Bennett, 1980). M protein is thought to organize the nascent viral bud by specific interactions with the other viral components, viz., the viral nucleocapsid and a portion of the host cell plasma membrane containing G protein. However, the nature of these interactions remains largely unknown.

We have measured the lateral mobility of G protein on the surface of BHK cells infected with either wild-type (wt) VSV or VSV containing lesions in M protein (complementation group III; Lafay, 1974; Pringle, 1975; Knipe et al., 1977c) in order to assess the role of M protein on G protein mobility. We find that all the temperature-sensitive (ts) mutations in M protein induce profound alterations in the mobility of the protein on the infected cell surface.

Measurements were made by using the technique of fluorescence photobleaching recovery (FPR), a technique that permits the determination of two parameters which characterize the mobility of a fluorescently labeled cell surface component—the fraction that is capable of undergoing lateral diffusion (the "mobile fraction", *Rf*) and the lateral diffusion coefficient, *D*, characterizing that mobile fraction (Schlessinger & Elson, 1980). Interaction of a mobile cell surface component with a large, immobile structure such as a viral bud would be detected as a reduction in the size of the mobile fraction without a change in the value of *D*, provided the interaction is long-lived compared to the measured time for diffusional fluorescence recovery. If the interaction lifetime is short compared to the recovery time, a decrease in *D* without a change in *Rf* would be observed (Elson & Reidler, 1980).

Materials and Methods

Cells and Viruses. BHK 21F cells, 3T3 cells, and SV3T3 cells were maintained in Dulbecco's Modified Eagles's medium (ME medium) supplemented with 10% fetal calf serum. Secondary chick embryo fibroblasts, prepared by standard procedures (Rein & Rubin, 1968), were grown in the same medium supplemented with 4% calf serum, 1% chicken serum, and 10% tryptose phosphate broth.

The wt VSV, Indiana serotype (Birmingham strain), was used in these experiments. The ts mutants of complementation group III were plaque purified and tested for temperature sensitivity before use.

Fluorescein-Labeled Monovalent Antibody Fragments (Fab). The specific anti-G antibody used was the early antibody described by Miller et al. (1980). The crude globulin was isolated by precipitation in 50% ammonium sulfate. To the resuspended protein (20 mg/mL) in 0.15 M NaCl and 0.05 M sodium phosphate, pH 7.2, was added 0.1 volume of 0.5

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[1] Abbreviations used: VSV, vesicular stomatitis virus; FPR, fluorescence photobleaching recovery; *D*, lateral diffusion coefficient; *Rf*, the "mobile fraction", capable of undergoing lateral diffusion; wt, wild type; ts, temperature sensitive; ME medium, Eagle's minimum essential medium; Fab, monovalent antibody fragments; moi; multiplicity of infection; pfu, plaque-forming units.

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M carbonate, pH 9.6, and fluorescein isothiocyanate (15 μg/mL, Sigma). This was stirred for 60 min at 31 °C, and free reagent was removed by gel filtration on a Sephadex G-25 column equilibrated with 0.15 M NaCl and 0.05 M sodium phosphate, pH 7.2. After dialysis against 0.005 M sodium phosphate, pH 7.5, the labeled antibody was applied to a DEAE-Sephadex A-50 column in the same buffer. Stepwise elutions were carried out by using 0.03 M sodium phosphate, pH 7.5 and the same buffer with added NaCl in 0.1 M increments from 0.1 to 0.5 M. The fluorescein to protein ratio in each fraction was calculated from the relationship $F/P = (2.87 \times E_{495}/E_{280}) - (E_{495} \times 0.35)$. The fraction eluting with 0.4 M NaCl gave a F/P ratio of about 3.5 and was used for all experiments.

Fab were prepared by hydrolysis of the labeled antibody with papain (0.875 mg of crystalline papain in 35 mL of solution, containing 2.5 mg of antibody/mL in 0.002 M EDTA, 0.01 M cysteine, and 0.9% NaCl). Polyacrylamide gel electrophoresis failed to detect any intact immunoglobulin heavy chain remaining in the solution after papain digestion.

FPR Technique and Mobility Measurements. The FPR technique has been described in detail previously (Axelrod et al., 1976). D and Rf can be determined from this measurement. The technique measures the rate at which new fluorescent molecules diffuse into an illuminated region following a brief, intense bleaching pulse of focused laser light in that region. A krypton laser (Coherent) at a wavelength of 476 nm was used to excite individual VSV-infected cells in a Zeiss Universal microscope equipped for epiillumination and imaged via a Zeiss Photometer 01 onto a sensitive photomultiplier (RCA C31-034). Excitation laser light was filtered by using a dichroic mirror (Zeiss FL-500) and a barrier filter (Shott Optical Glass OG-530). A chopper wheel with a 7% light cycle minimized bleaching during measurements. The laser beam was typically attenuated 1000 × during measurements (compared to bleaching) with a neutral density filter. To bleach the cells, this filter was removed briefly from the path of the laser beam. Measurements on cells in vitro at the permissive (31 °C) or restrictive (39 °C) temperatures were accomplished by using a water-jacketed temperature-control stage that housed the tissue culture dish (Reidler, 1979). The temperature was monitored continuously in the culture dish with a miniature probe (Baily Instruments). In varied less than ±0.1 °C during any single bleaching experiment and less than ±0.5 °C for all bleaches. Fluorescence intensities were collected by using photon counting electronics (Princeton Applied Research 1190) and recorded simultaneously with the temperature on a dual-pen chart recorder.

Measurements of G Protein Mobility on Infected Cell Surfaces. VSV was adsorbed onto confluent BHK cells in 35-mm culture dishes at room temperature for 30 min in 0.2 mL of phosphate-buffered saline containing 1% fetal calf serum. Unadsorbed virus was removed by washing, and the cells were overlaid with ME medium containing 4.5 g/L glucose and then incubated at either 31 or 39 °C. Culture dishes were removed at intervals, washed 3 times with phosphate-buffered saline, and labeled with 0.1 mL of fluorescein-labeled Fab fragments of anti-G antibody at a concentration of 1 mg/mL for 15 min in the temperature stage. Unattached antibody was removed by washing 3 times with phosphate-buffered saline.

Measurements of fluorescence intensities were made at 0.4- or 0.8-s intervals for 30 or 60 ms, respectively. The bleaching pulse also lasted for 30 or 60 ms. Typically, two bleaches in one spot per cell were carried out. Tissue culture dishes for measurement were replaced at least once each hour. The data were analyzed by using a three-point fitting procedure (Axelrod et al., 1976). The results were pooled and compared by using a t test for statistical significance.

Table I: Effect of moi on Lateral Mobility of VSV G protein in BHK Cells Infected with wt VSV

moi	Rf (%)	D (cm²/s) × 10^{10}
	39 °C Infection	
5, 10	80 ± 13 (10)[a]	7.4 ± 3.2 (10)
33, 50	72 ± 13 (25)	6.7 ± 3.2 (25)
100, 300	70 ± 12 (17)	5.4 ± 3.2 (17)
	31 °C Infection	
10	62 ± 4 (3)	5.5 ± 0.7 (3)
50, 100	76 ± 13 (16)	4.3 ± 1.3 (16)

[a] Numbers on parentheses represent the number of measurements.

Table II: Effect of Time Postinfection on Lateral Mobility of VSV G protein[a]

hours after infection	Rf (%)	D (cm²/s) × 10^{10}
	39 °C Infection	
5–6	77 ± 7 (13)[b]	5.5 ± 3.0 (13)
6–7	74 ± 15 (9)	8.2 ± 2.8 (9)
7–8	71 ± 13 (10)	4.9 ± 1.7 (10)
8–10	71 ± 17 (10)	6.5 ± 3.5 (10)
average over all times	74 ± 13 (42)	6.2 ± 2.2 (42)
	31 °C Infection	
9–10	78 ± 14 (6)	4.9 ± 1.0 (6)
10–11	72 ± 13 (13)	4.3 ± 1.4 (13)
average over all times	74 ± 13 (19)	4.5 ± 1.3 (19)

[a] Infection of BHK cells by wt VSV (see Materials and Methods). [b] Numbers in parentheses represent the number of measurements.

Estimation of Viral Protein Concentrations by Radioactive Labeling. Confluent monolayers of BHK cells (35-mm dishes containing ca. 1×10^6 cells) were infected at a multiplicity of 50 pfu/cell and incubated in ME medium containing 10 μg/mL of actinomycin D at either 31 or 38.5 °C. After 6 h, the medium was replaced by 0.5 mL of methionine-free ME medium containing 10 μCi of [^{35}S]methionine, and the plates were returned to the appropriate temperature for 2 h. Cell protein was solubilized in sodium dodecyl sulfate solution and precipitated with trichloroacetic acid as described by Miller et al. (1980). Polyacrylamide gels were run by the procedure of Laemmli (1970). Radiolabeled protein bands were detected by radioautography on Kodak X-omat R film, and integrated intensities of the viral protein bands were measured with a Joyce–Loebl densitometer.

Results

Lateral Mobility of Surface G Protein Is Independent of Multiplicity of Infection (moi) or of Time after Infection. Varying the moi induced no significant changes in the mobility of G protein in wt infected BHK cells (Table I). Neither Rf nor D differed significantly at multiplicities of wt VSV from 5 to 300 pfu/cell at 30 °C or from 10 to 100 pfu/cell at 31 °C.

Observations at various times after infection with wt VSV revealed no significant alterations in the mobility of surface G protein (Table II). No significant differences were found in either the mobile fraction or diffusion coefficient between 5 and 10 h postinfection at 39 °C or between 9 and 11 h postinfection at 31 °C.

Table II shows also the values of D and Rf, averaged over all multiplicities and times, for wild-type G protein at each

Table III: Mobility of Cell Surface G Protein after Infection of BHK Cells with wt or ts M Protein Mutants of VSV

temp (°C)	diffusion coefficient (cm^2/s) × 10^{10} wild type	tsO23	tsG33	tsO89
39	6.3 ± 3.0 (48)[a]	5.7 ± 2.2 (8)	8.3 ± 2.4 (7)	5.4 ± 1.7 (16)
31	4.5 ± 1.3 (19)	6.9 ± 2.1 (16)	4.9 ± 1.6 (20)	7.3 ± 4.1 (7)
	mobile fraction (%)			
39	73 ± 13 (52)	74 ± 17 (8)	75 ± 12 (5)	74 ± 11 (14)
31	74 ± 13 (19)	58 ± 12 (18)	48 ± 13 (18)	37 ± 4 (8)

[a] Numbers in parentheses represent the number of measurements.

Table IV: Rate of Virion Production after Infection with wt or ts M Protein Mutants at 31 °C[a]

cell type	virus	number of infectious units produced (×10^{-8}) 3-6 h	6-9 h	9-12 h
BHK	wild type	1.5	1.5	10
	tsO23	0.05	0.7	8
	tsG33	1.2	2	3
	tsO89	0.001	0.07	0.6
3T3	wild type	0.01	0.1	0.3
SV3T3	wild type	0.2	1	1.3

[a] Each value is the average of plaque assays performed on two dishes.

Table V: Effect of Cell Type on Lateral Mobility on VSV G Protein

cell type	*Rf* (%)	*D* (cm^2/s) × 10^{10}
BHK (39 °C)	73 ± 13 (52)	6.3 ± 3.0 (48)
CEF (39 °C)	65 ± 9 (5)	7.2 ± 1.6 (5)
3T3 (37 °C)	68 ± 16 (15)	5.4 ± 1.1 (5)
SV3T3 (37 °C)	23 ± 21 (5)	6.7 ± 1.9 (5)

Table VI: Relative Incorporation of [^{35}S]Methionine into BHK Cells Infected with wt or ts M Protein Mutants of VSV[a]

virus	G/M
wt	1.09
tsO23	0.81
tsO89	1.15
tsG33	1.26

[a] Cells infected at 31 °C labeled 6-8 h postinfection and harvested 8 h postinfection. Ratios obtained from integrated intensities of scan of autoradiogram made from polyacrylamide gel (Laemmli, 1970).

of the two temperatures studied. The size of the mobile fraction is independent of temperature within experimental error and is about 75% of the total G protein on the cell surface. This is a relatively high value compared to other surface membrane proteins so far studied (Elson & Schlessinger, 1979).

Uninfected cells, cells that had been infected less then 4 h, or cells infected with the G protein mutant ts45 at 39 °C showed only background fluorescence after labeling with the fluorescent Fab fragments from anti-G antibody, thus demonstrating that the cell surface G protein was being specifically labeled. The fluorescence intensity on the surface of the infected BHK cells increased only slowly as moi increased, suggesting that the concentration of cell surface G did not increase linearly with multiplicity. In general, cells infected at 31 °C showed lower fluorescence intensities than those infected at 39 °C, indicating that less G protein was on the cell surface at the lower temperature.

Effects of M Protein on G Protein Mobility. For evaluation of the role of M protein on the lateral mobility of surface G protein, ts mutants with defects in M protein (Group III) were used to infect BHK cells at permissive (31 °C) and restrictive (39 °C) temperatures. The mobile fraction of surface G is similar at 39 °C for wt and for each of the ts mutants tested (Table III). However, cells infected with the mutants at 31 °C showed a pronounced decrease in the mobile fraction. Diffusion coefficients for G protein were not significantly different between mutants and wt at either temperature (Table III).

Virus production occurs at a normal rate from the wild type infection at 39 °C, while negligible virus production occurs from infection by any of the mutants, even though the mobility measurements are similar (Table III). Cells infected with M protein mutants at 31 °C produce virus at a comparable or slower rate than do cells infected with wt virus (Table IV). While ts33 produces virions at a comparable rate to wild type, ts89 produces them at a rate that is much lower, even though infection by either mutant results in a decreased mobile fraction (Table III). Although the observed differences in mobile fraction between the wild-type and the M protein mutants must be related to the M protein lesion in these mutants, that change is not directly reflected in the rate of budding (see Discussion).

Mobile Fraction of Surface G Protein Differs in Different Cell Types. The mobility of surface G in cells of different kinds infected with wild-type VSV is shown in Table V. Although BHK, CEF, and 3T3 cells all show similar, large fractions of mobile G, the mobile fraction is strikingly reduced in 3T3 cells transformed with SV40. It is not known whether the reduction in G protein mobility in SV3T3 cells arises from an overproduction of M protein, from a specific interaction with some SV3T3 cell component, or from the accumulation of a budding intermediate (see Discussion). In any case, the reduced mobility is compatible with a high rate of virus production (Table IV).

Estimation of Viral Protein Concentrations in Mutant and wt Infected Cells by Radioactive Labeling. Estimates of the intracellular concentration of each viral protein were made by labeling the cells infected by wt or mutant VSV with [^{35}S]methionine 6-8 h postinfection, i.e., during the period when the photobleaching observations were made. While this approach can only yield very approximate values for the relative concentrations of each viral protein, certain conclusions became evident. First, no significant differences were observed in the M/G ratio in wild-type and mutant infected cells grown at 31 °C (Table VI). Thus the decreased mobile fraction of G found in mutant infected cells at 31 °C (Table III) does not appear to arise from a large excess of M protein relative to G in the infected cells. Second, although the overall rate of radioactive incorporation was somewhat less at the nonpermissive than at the permissive temperature, in both mutant and wild-type infected cells at the nonpermissive temperature, substantial amounts of labeled M protein were formed. In fact, the ratio of M to G was generally somewhat higher than that

found at 31 °C. Thus, the high level of G protein mobility displayed by the mutant-infected cells at nonpermissive temperature does not appear to result from lack of M protein.

Discussion

A long-range objective of these studies is to obtain a molecular description of the interactions that drive virus assembly. In this report, we focus on interactions between the M and G proteins of VSV.

Our results demonstrate the modulation of the lateral mobility of a specific cell surface component (G protein) due to mutation of a specific intracellular protein (M protein). The fraction of G protein that is mobile on cells infected with wt virus is essentially the same at 31 and 39 °C (Tables I and II). Moreover, there seem to be no gross differences in the relative amounts of M and G protein produced in mutant as opposed to wild-type infection at permissive or nonpermissive temperatures (Table VI). It therefore appears that the immobilization of G protein observed in mutant infected cells at 31 °C results from functional differences between mutant and wild-type M proteins rather than from a temperature-dependent behavior of the G protein itself or from a change in the ratios of G and M protein in infected cells.

We do not know whether the functionally altered M protein exerts its effect on G protein mobility by a direct interaction with G or indirectly by affecting some other cellular component. Since M and G proteins are known to be closely associated in the mature virion, however, and since there is no evidence that mutant and wild-type M proteins have significantly different effects on cell structure at the permissive temperature, it seems most likely that the immobilization of G protein results from a direct interaction between it and M protein.

Interpretation of our results in terms of a simple, economical, but not unique, model yields interesting conclusions about the relative rates of various processes along the assembly pathway. (1) In cells infected by wt virus, the formation of (immobile) M–G complexes in the plasma membrane appears to be a slow, probably rate-limiting step along the pathway of virus assembly. This is deduced from the following observations: (A) M and G proteins are present in roughly comparable amounts in cells infected with mutant and wt virus at both 31 and 39 °C (Table VI). (B) Nevertheless, the amount of G protein immobilized on cells in which wild-type virus are being rapidly produced at 39 °C is no greater than the "background" level of immobile G seen on the cells infected by mutant virus in which no progeny are produced at this temperature (Table III). Therefore immobile M–G complexes that are intermediates in the formation of viral buds must be present at low concentrations (compared to the total amount of cellular M and G) even on cells rapidly producing virus. Hence, we conclude that the rate of formation of these complexes must be slow compared to subsequent steps along the assembly pathway. This conclusion is consistent with results obtained previously by others using different methods (Knipe et al., 1977d).

(2) In cells infected with M protein mutants at 31 °C, the rate of formation of immobile M–G complexes (or some prior step) is no longer rate limiting. Rather, a later step becomes slower. This is deduced from the following observations: (A) Immobile M–G complexes accumulate on cells infected with M protein mutants at 31 °C (Table III). (B) The rate of virus production is equal to or less than that observed in cells infected by wt virus under the same conditions (Table IV). If the rate of formation of immobile M–G complexes were limiting under these conditions, we would expect cells with larger amounts of these complexes to produce virus more rapidly.[2] That this was not observed indicates that the rate of formation of M–G complexes is no longer rate limiting for virus production from mutant-infected cells.

Alternatively, immobilization of G protein on the surface of mutant-infected cells at 31 °C could result from defective association, i.e., one that is inappropriate for viral budding. Then this immobile fraction would correspond to a dead-end population of G protein molecules, which are not incorporated into virions, or are incorporated at a decreased rate. In this case, the immobile fraction would be a competitor to, rather than a component of, the pathway of viral budding. While this possibility cannot be excluded on the basis of the data reported here, it is less attractive because it is more complicated; i.e., it postulates a larger number of independent consequences arising in the M protein molecule in common in all the mutants studied from different single mutational events (see below). Investigation of the effect of mutations in the N protein on G protein mobility should distinguish between these two alternatives.

Consistent with the former possibility, however, independent evidence indicates that the association of the M–G complex with the viral nucleocapsid may be impaired in cells infected with M protein mutants even at 31 °C. Results in the following paper of this issue (Wilson & Lenard, 1981) show that the affinity between nucleocapsids and mutant M protein in vitro is decreased compared with wt M protein (Wilson & Lenard, 1981). A similar M–nucleocapsid association could be the later step in the assembly pathway, the retardation of which is responsible for the accumulation of M–G complexes in the plasma membrane and the reduced rate of production of some of the mutant viruses at 31 °C.

This analysis implies that the similar G protein mobilities in wild-type and mutant infected cells at 39 °C arise in quite different ways. The M protein is functional in wt-infected cells at 39 °C; then the M–G interaction is rapidly followed by association with nucleocapsid and budding of normal virions. On the other hand, the M protein in the mutant-infected cells at 39 °C is nonfunctional and has lost its ability to associate with G protein in the normal way.

The measured mobile fraction for G protein in wild-type infection or in mutant infection at 39 °C is about 75%, which is in the upper range of that typically found for other cell surface proteins (Elson & Schlessinger, 1979). This value represents, for technical reasons, a lower limit; the true fraction of mobile G protein may be substantially higher. The possibility must be considered, however, that the measured immobile fraction of ~25%, rather than being a technical artifact, represents a distinct population of G protein which is solely responsible for viral budding. The mobile G protein would then be on a dead-end pathway, not destined for incorporation into virions. The presence of a similar immobile fraction on cells infected by mutant virus at the nonpermissive temperature argues against this possibility, as do the pulse–chase experiments of Knipe et al. (1977a). The latter demonstrated that more than 50% of surface G protein can eventually be chased into virus particles from the surface of infected CHO cells. Unfortunately, we have not measured the size of the mobile fraction in these cells.

The arguments presented here favor the interpretation that the mobile G protein molecules on the surface of cells infected with wild-type VSV are incorporated into virions. This precludes any model of virus assembly that requires G protein

[2] It is possible that the greater immobilization of G protein on SV3T3 as opposed to 3T3 cells infected with wt (Table III) is associated with a faster rate of virus production (Table IV) for this reason.

to be on the surface of the infected cell in a largely immobile state, e.g., due to self-association or interaction with host cell components. This conclusion is consistent with pulse–chase studies, which have shown that G protein arrives at the cell surface independently of other viral components (Atkinson, 1978; Knipe et al., 1977b; Rothman & Fine, 1980).

Further, our results are consistent with a model in which the G protein molecules, after insertion into the plasma membrane, diffuse away from their sites of insertion and become randomly distributed over the cell surface. Subsequent accumulation of G protein into patches would arise from interaction with other viral components, most notably M protein (Atkinson, 1978). Analyses of the results presented here are consistent with the idea that M–G interaction at the plasma membrane is the rate-limiting step for budding to wt VSV, while subsequent interaction with nucleocapsid becomes the rate-limiting event for mutant VSV budding.

In contrast, Sindbis virus has recently been found to assemble by a different mechanism. Sindbis virus glycoproteins are essentially immobile on the cell surface, and evidence has been suggesting that they arrive at the cell surface already in association with nucleocapsids (J. C. Johnson, E. L. Elson, and J. Schlesinger, unpublished results).

References

Atkinson, P. H. (1978) *J. Supramol. Struct. 8*, 89–109.
Axelrod, D., Ravdin, P., Koppel, D. E., Schlessinger, J., Webb, W. W., Elson, E. L., & Podleski, T. R. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4594–4598.
Elson, E. L., & Schlessinger, J. (1979) in *The Neurosciences, Fourth Study Program* (Schmitt, F. O., & Worden, F. G., Eds.) pp 691–701, MIT Press, Cambridge, MA.
Elson, E. L., & Reidler, J. A. (1980) *J. Supramol. Struct. 12*, 481–489.
Knipe, D. M., Lodish, H. F., & Baltimore, D. (1977a) *J. Virol. 21*, 1121–1127.
Knipe, D. M., Baltimore, D., & Lodish, H. F. (1977b) *J. Virol. 21*, 1128–1139.
Knipe, D. M., Lodish, H. F., & Baltimore D. (1977c) *J. Virol. 21*, 1140–1148.
Knipe, D. M., Baltimore, D., & Lodish, H. F. (1977d) *J. Virol. 21*, 1149–1158.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lafay, F. (1974) *J. Virol. 14*, 1220–1228.
Lenard, J. (1978) *Annu. Rev. Biophys. Bioeng. 7*, 139–165.
Lenard, J., & Compans, R. W. (1974) *Biochim. Biophys. Acta 344*, 51–94.
Miller, D. K., Feuer, B. I., Vanderoef, R., & Lenard, J. (1980) *J. Cell Biol. 84*, 421–429.
Pringle, C. R. (1975) *Curr. Top. Microbiol. Immunol. 69*, 85–116.
Reidler, J. A. (1979) Ph.D. Thesis, Cornell University, Ithaca, NY, pp 134–139.
Rein, A., & Rubin, H. (1968) *Exp. Cell Res. 49*, 666–678.
Rothman, J. E., & Fine, R. E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 780–784.
Schlessinger, J., & Elson, E. L. (1980) in *Biophysical Methods* (Ehrenstein, G. & Lecar, H., Eds.) Academic Press, New York (in press).
Schnitzer, T. J., & Lodish, H. F. (1979) *J. Virol. 29*, 443–447.
Schnitzer, T. J., Dickson, C., & Weiss, R. A. (1979) *J. Virol. 29*, 185–195.
Weiss, R. A., & Bennett, P. L. P. (1980) *Virology 100*, 252–274.
Wilson, T., & Lenard, J. (1981) *Biochemistry* (following paper in this issue).

Interaction of Wild-Type and Mutant M Protein of Vesicular Stomatitis Virus with Nucleocapsids in Vitro†

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ABSTRACT: We have characterized the interactions between mutant or wild-type M protein and nucleocapsids of vesicular stomatitis virus (VSV) by assaying for inhibition of in vitro transcriptase activity. The interactions are primarily electrostatic in nature: high concentrations of NaCl or poly(L-glutamic acid) reverse the inhibition. These interactions are much weaker in each of the four M protein mutants (complementation group III) tested than in wild-type VSV. Temperature-insensitive revertants were selected from each of the M protein mutants studied. The salt-dependent inhibitory profiles of all the revertants resemble that of wild-type VSV, suggesting that M–nucleocapsid interactions are integrally related to the temperature-sensitive phenotype of group III mutants. These results are discussed in relation to the accompanying paper [Reidler, J. A., Keller, P. M., Elson, E. L., & Lenard, J. (1981) *Biochemistry* (preceding paper in this issue)] which shows that interaction between M protein and infected cell membranes is increased in all group III mutants studied.

The matrix (M)[1] protein of vesicular stomatitis virus (VSV), a small nonglycosylated polypeptide (M_r 29 000; Bishop & Smith, 1978), is one of the three major structural proteins of the virion. Recent studies have elucidated two functions of M protein, one structural and one regulatory. Experiments utilizing VSV temperature-sensitive (ts) mutants and viral pseudotypes have shown that M protein is essential for the budding of virions or virus-like particles from the plasma

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[1] Abbreviations used: wt, wild type; VSV, vesicular stomatitis virus; M protein, matrix protein; ts, temperature sensitivie; wtO, wild-type VSV, Orsay variant; wtG, wild-type VSV, Glasgow variant; DTT, dithiothreitol; poly(Glu), poly(L-glutamic acid); RNP, ribonucleoprotein; NC, nucleocapsid; pfu, plaque-forming unit; HS, high salt; LS, low salt.

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