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arrive at the cell surface already in association with nucleocapsids (J. C. Johnson, E. L. Elson, and J. Schlesinger, unpublished results).

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

Tazewell Wilson‡ and John Lenard*

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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membrane of an infected cell (Schnitzer & Lodish, 1979; Schnitzer et al., 1979; Weiss & Bennett, 1980). It has also been shown that M protein has a regulatory role in virus-directed RNA synthesis in infected cells (Clinton et al., 1978; Martinet et al., 1979) and acts as an inhibitor of VSV in vitro transcription (Carroll & Wagner, 1979; Combard & Printz-Anê, 1979; cf. Perrault & Kingsbury, 1974).

For fulfillment of these roles, M protein must interact directly with both the viral nucleocapsid and that portion of the host cell plasma membrane that will become the viral envelope. At present, relatively little is known about the interactions between these structural components of the VS virion and M protein or about the biochemical characteristics of M protein itself, because M protein is quite intractable in an isolated form (Carroll & Wagner, 1979; unpublished observations). In this and the preceding paper in this issue, we have used group III ts mutants of VSV, which produce defective M protein, to study the interactions of M with each of the other major viral components. The present study utilizes M protein mediated inhibition of in vitro transcriptase activity as an assay to characterize the interactions between M and nucleocapsids.

Materials and Methods

Cells and Virus. BHK 21-F cells were used for growth of virus stocks, and Vero cells were used for all plaque assays. Cells were maintained as described previously (Miller & Lenard, 1980). The orsay (wtO) and Glasgow (wtG) variants of VSV Indiana were obtained from A. Huang, and the group III mutants were obtained from A. Huang and A. Flamand. Temperature-insensitive revertants of each of these mutants were isolated by the following procedure. Individual plaques were picked from assays of group III mutants performed at 39 °C and resuspended in Dulbecco's phosphate-buffered saline. Dilutions (100-fold) of these suspensions were then used to infect Vero cells, and, following a 2-day incubation at 39 °C, the cell supernatants of these cultures were collected and assayed at 31 and 39 °C. If a culture had comparable titers at the two temperatures, it was considered to be a revertant, and plaques were picked from the 39 °C assays. Suspensions of these plaques were again tested for temperature sensitivity and were used as seed for stock cultures of the revertants.

All virus stocks, wild types, mutants, and revertants, were prepared from plaque-purified inocula; the methods for virus growth and purification were described previously (Miller & Lenard, 1980). Immediately prior to use in in vitro assays, purified virus was concentrated by centrifugation [(3.6×10^6)g·min] and resuspended in the appropriate buffer solution.

UV-inactivated virus was irradiated for 8.0 min with 2500 ergs s^{-1} cm^{-2} at 254 nm with a germicidal lamp (Sylvania G30T8). This treatment resulted in >95% reduction of activity in in vitro transcription assays.

Disruption and Fractionation of Virus. Purified virus was resuspended at 10 mg/mL in 10 mM Tris, pH 8.0, 1.5 mM dithiothreitol (DTT), 10% glycerol, and either 10 mM NaCl (low salt) or 250 mM NaCl (high salt). Disruption was achieved by the addition of Triton X-100 (final concentration: 0.5% v/v) with gentle mixing. Disrupted virus was fractionated on discontinuous glycerol gradients as described previously (Carroll & Wagner, 1979). The viral fractions used in in vitro transcription assays were dialyzed against 10 mM Tris, pH 8.0, 1.5 mM DTT, 10% glycerol, and 0.5% Triton X-100. Virus to be used for polyacrylamide gel electrophoresis analysis was treated similarly, except that the nucleocapsid (NC) fraction was simply pelleted rather than fractionated on glycerol gradients.

In Vitro Transcription Assays. A slightly modified version of the method of Carroll & Wagner (1978 a,b) was used to assay viral transcription. Briefly, 1 volume of virus in RSB (10 mM NaCl, 1.5 mM $MgCl_2$, and 10 mM Tris, pH 7.4) and 10% glycerol was treated with 1 volume of disruption mixture (18.7% glycerol, 3.74% Triton X-100, 4.4 mM KCl, 1.2 mM $MgSO_4$, and 4.4 mM Tris, pH 8.0) containing the appropriate concentration of NaCl (0.2–1.44 M) for 5 min at 0 °C. Triton concentrations in the disruption mixture were varied appropriately for the experiments shown in Figure 4. Three volumes of RSB–20% glycerol, plus or minus poly(L-glutamic acid) (poly(Glu), type I, Sigma Chemical Co.), and 5 volumes of pre-reaction mix (1.4 mM each ATP, GTP, and CTP, 0.14 mM [^{3}H]UTP, 750 μCi/μmol, 8 mM magnesium acetate 1.3 mM DTT, and 50 mM Tris, pH 8.0) were then added. Duplicate samples (110 μL each) were incubated in capped tubes at 31 °C. RNA synthesis was assayed by removing either 50- or 100-μL samples to iced tubes containing 67 μM sodium pyrophosphate and 2 mg/mL yeast RNA at the appropriate times (30 min and/or 60 min), followed by trichloroacetic acid precipitation and filtration. Radioactivity was measured by liquid scintillation counting.

Assays of disrupted virus fractions were performed under similar conditions, except that the solubilizer was omitted and 2.5 M NaCl was added to the nucleocapsid samples to give the appropriate final concentration.

Results

Carroll & Wagner (1979) have demonstrated that a suspension of purified M protein inhibits in vitro transcription by VSV ribonucloprotein (RNP) cores. The interpretation of these results is complicated, however, by the facts that (1) purified M protein is not soluble under most conditions and (2) the level of inhibition observed was considerably lower than that observed with whole virus. We have attempted to circumvent these problems by disrupting and fractionating virus under different ionic conditions and comparing the inhibitory activity of the soluble extracts containing both the viral glycoprotein G and M protein with those containing only G. Figure 1 shows the $NaDodSO_4$–polyacrylamide gel patterns of supernatants and pellets of wild-type (wt) VSV disrupted in low (0.01 M NaCl) or high (0.25 M NaCl) ionic strength. In low salt, VSV is fractionated into a soluble component containing only G protein and lipid (LS supernatant, lane 5) and a fast sedimenting fraction containing the RNP core proteins and M protein (LS-NC, lane 4). Under high-salt conditions, most of the M protein is found in the soluble fraction (HS supernatant, lane 2) while the sedimentation profiles of the other viral proteins are unchanged (HS-NC, lane 1).

Following dialysis to remove NaCl, the in vitro transcriptase activity of the fractions was assayed. In the absence of added NaCl, only the HS-NC fraction shows appreciable RNA synthesizing capacity, and this activity is independent of NaCl concentration (closed circles, Figure 2a,b). Addition of NaCl to the reaction mixture restores transcriptase activity to the LS-NC fraction in a manner similar to that observed with disrupted whole virus. Figure 2b shows the transcriptase activity of HS-NC mixed with either the low salt or the high salt supernatant fractions. Addition of the HS supernatant, which contains both G and M proteins, inhibits transcriptase activity in a manner similar to that seen in the LS-NC or whole virus assays, while the LS supernatant containing only G does not.

Because earlier work indicated that group III ts mutants of VSV are less sensitive to M protein mediated inhibition of

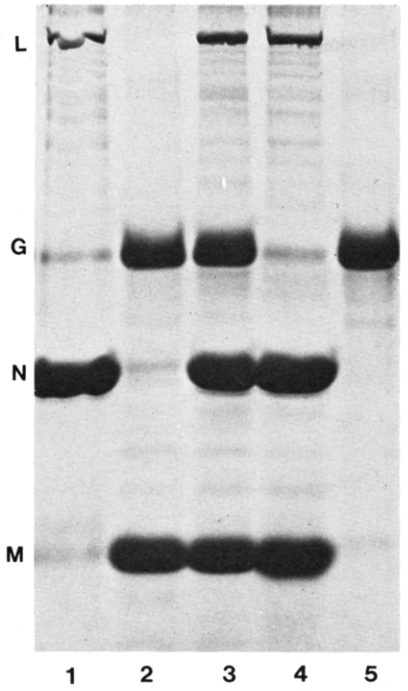

FIGURE 1: Fractionation of VSV under different ionic condtions. VSV (wtO) was disrupted and fractionated in the presence of 0.01 M NaCl (LS) or 0.25 M NaCl (HS) as described under Materials and Methods. Fractions were concentrated and analyzed on polyacrylamide gels as described previously (Miller et al., 1980). (Lane 1) HS-NC; (lane 2) HS supernatant; (lane 3) unfractionated VSV; (lane 4) LS-NC; (lane 5) LS supernatant.

in vitro transcription (Carroll & Wagner, 1979), we also tested tsO23 (III) in this manner. Extraction of the virus under high- or low-salt conditions gives gel patterns similar to those seen in Figure 1 for wtO VSV (data not shown). The results from in vitro transcriptase assays of these fractions are shown in Figure 2c,d. In the absence of NaCl, the results are quantitatively similar to those obtained with wtO; that is, only HS-NC shows transcriptase activity. The inhibition observed with tsO23 LS-NC and whole virus is, however, much more readily reversed by low concentrations of added NaCl (0.0625 M, Figure 2c). Similarly, less inhibition of HS-NC activity is conferred by addition of mutant than of wt HS supernatant. This is most evident when activity is assayed at 0.0625 M NaCl, but is significant also at 0.125 M NaCl (Figure 2d). The LS supernatant from tsO23, like that from wt, does not significantly inhibit in vitro transcription. When tsO23 HS supernatant is mixed with wtO HS-NC, the mutant pattern of salt-dependent reversal of inhibition is observed (data not shown). This result was expected because the RNP cores of wt and group III mutant virions should be identical.

In combination with the earlier work of Carroll & Wagner (1979), our results suggest that inhibition of in vitro transcription provides a sensitive assay for the interaction of M protein with the RNP cores of VSV. We first attempted to characterize this interaction by studying the effect of ionic strength on the disruption of whole virus. Figure 3 shows a comparison of the NaCl dependence of in vitro RNA synthesis in several group III ts mutants (tsO23, tsO89, tsG31, tsG33) and their parental wt strains (wtO, wtG). Although there are small quantitative differences between the different mutants and between the wild types, all the mutants differ significantly from the wt with regard to both their sensitivity to NaCl concentration and their overall ability to incorporate [^{3}H]UTP. These differences are even more dramatic in the presence of poly(Glu) (Figure 3), which significantly enhances transcriptase activity in whole virus but not in RNP cores (Carroll & Wagner, 1978b).[2] It should be noted that although the experiments illustrated in Figure 3 were carried out at high viral protein concentrations (1 mg/mL), identical patterns of NaCl dependence were observed at lower virus concentrations (0.5 or 0.25 mg/mL). In general, the group III mutants do not exhibit the concentration-dependent inhibition of in vitro transcription observed for wt VSV under standard (high salt) assay conditions (Carroll & Wagner, 1979; unpublished observations).

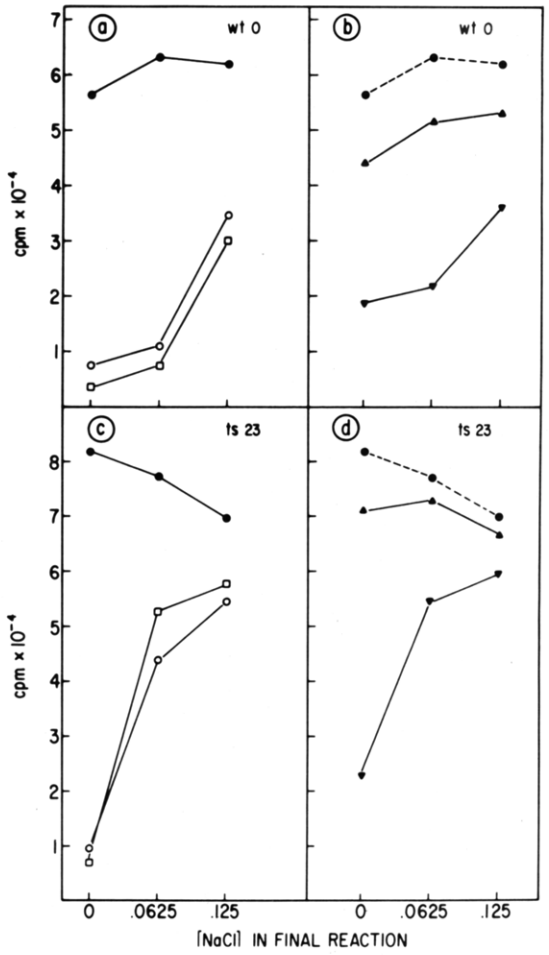

FIGURE 2: In vitro transcriptase activity of fractionated VSV, wtO (a,b) or tsO23 (c,d). Concentrated virus (10 mg/mL) was disrupted and fractionated as described under Materials and Methods. Dialyzed fractions were adjusted so that the final reaction mixtures contained the appropriate concentrations of NaCl and the following concentrations of viral protein: HS-NC, 0.15 mg/mL; LS-NC, 0.3 mg/mL; HS sup, 0.3 mg/mL; LS sup, 0.15 mg/mL. The final concentration of whole virus in the controls was 0.5 mg/mL. Assays were performed as described under Materials and Methods. (a) (●) HS-NC; (○) LS-NC; (□) whole virus. (b) (●) HS-NC; (▲) HS-NC + LS supernatant; (▼) HS-NC + HS supernatant. (c) (●) HS-NC; (○) LS-NC; (□) whole virus. (d) (●) HS-NC; (▲) HS-NC + LS supernatant; (▼) HS-NC + HS supernatant.

We have also examined the effect of detergent concentration on the in vitro transcriptase reaction (Figure 4). Under standard assay conditions (e.g., 0.144 M NaCl), RNA synthesis is significantly inhibited only when the detergent concentration is extremely low (0.001% Triton, or 10^2-fold less than in standard assays). Furthermore, no differences in

[2] Enhancements by poly(Glu) shown in Figure 3 are smaller than those found by Carroll & Wagner (1978a,b). Enhancements similar to those reported by these authors were observed by us in experiments by using the Birmingham variant of VSV.

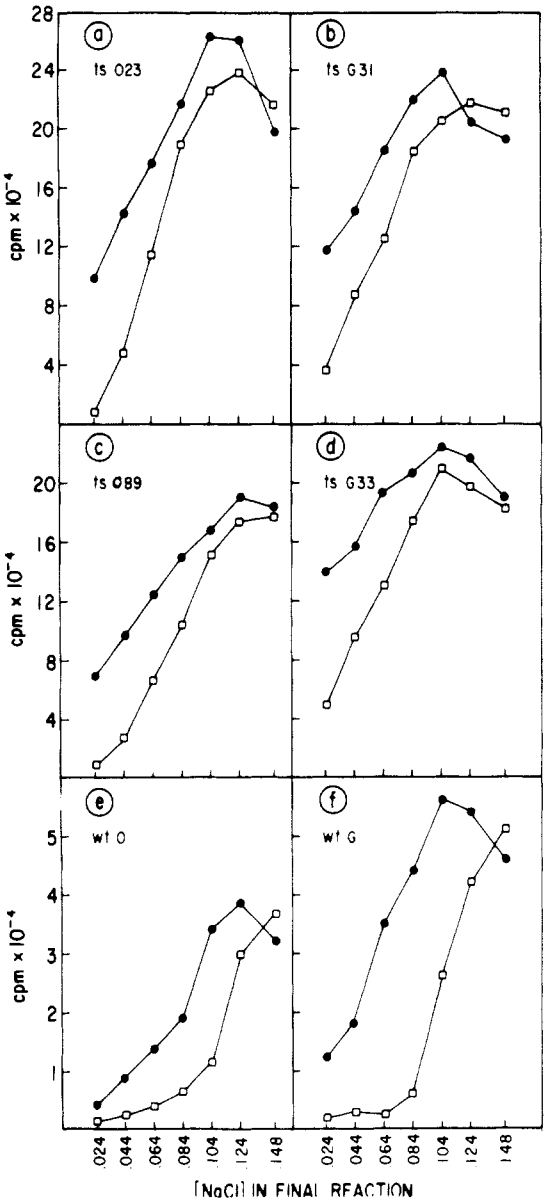

FIGURE 3: NaCl dependence of in vitro transcription: group III mutants and parental wt. Concentrated virus was disrupted in the presence of the appropriate concentration of NaCl, and in vitro transcriptase activity was assayed either in the presence (circles) or in the absence (squares) of poly(Glu) (final concentration 3 mg/mL), as described under Materials and Methods. The final concentration of virus in individual assays was 1 mg/mL; the final NaCl concentrations indicated result from variations in the NaCl present in the disruption mixture.

detergent dependency are observed when the mutants and their parental wild types are compared. It is noteworthy that activity is essentially unimpaired at Triton concentrations well below the critical micelle concentration (0.0162%; Helenius & Simons, 1974), suggesting that the detergent is not involved in the transcription reaction itself but acts only to break down the permeability barrier of the viral envelope.

A mixing experiment similar to that described by Perrault & Kingsbury (1974) was performed by using live tsO23 test virus and UV-inactivated tsO23 or wt VSV as a source of inhibitor. The results illustrated in Figure 5 show that irradiated wt VSV is an effective inhibitor of in vitro transcription at either high or low salt concentrations. Irradiated tsO23 is essentially ineffective as an inhibitor at high salt; under low salt conditions, tsO23 does have inhibitory activity, but it is much lower than that observed for wt VSV. These results indicate that both mutant and wt M protein can equilibrate

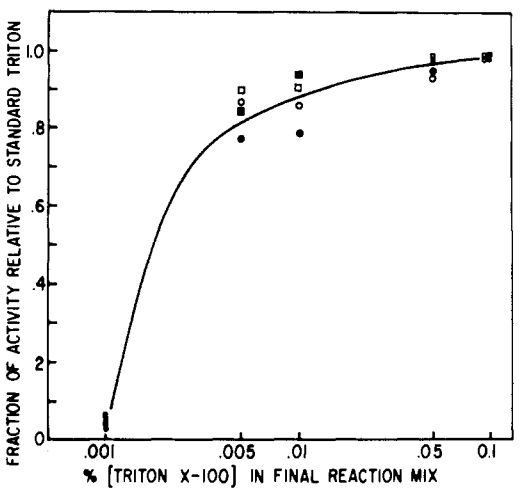

FIGURE 4: Effect of detergent concentration on in vitro transcription. Concentrated virus was disrupted in the presence of the appropriate concentration of Triton X-100 and assayed as described under Materials and Methods (final NaCl concentration 0.144 M; final virus concentration 1 mg/mL). (○) wtO; (●) tsO23; (□) wtG; (■) tsG31.

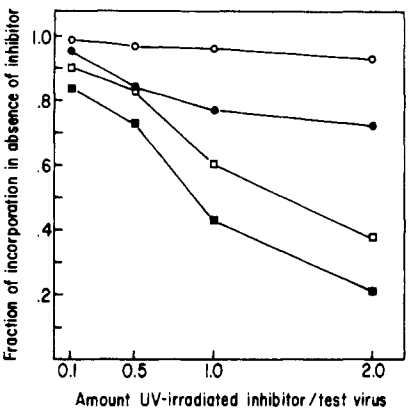

FIGURE 5: Inhibition of in vitro transcription by UV-irradiated virus. Active tsO23 was mixed with different amounts of UV-irradiated tsO23 (circles) or wtO (squares) and assayed for in vitro transcriptase activity. The final NaCl concentration in the assay mixtures, either 0.044 M (closed symbols) or 0.124 M (open symbols), reflects the concentration of NaCl in the disruption mixture.

between different nucleocapsids even at low ionic strength, conditions under which electrostatic interactions between the viral components should be strongest and M protein is least soluble (Figure 1).

The in vitro comparisons of wt and group III mutants described above show a consistent pattern of altered interaction between M protein and viral nucleocapsids. They do not, however, indicate any necessary correlation between this property and the in vivo temperature-sensitive phenotype. A series of temperature-insensitive revertants were isolated from four group III mutants in order to see if the temperature sensitivity of the group III mutants is linked to M protein–nucleocapsid interactions. The in vivo phenotypes of selected revertants and their parental strains are described in Table I. The NaCl dependence of in vitro transcriptase activity was then determined for each of these revertants; the results are shown in Figure 6. All of the revertants show a profile of salt dependence resembling that of wt VSV. Thus a reversion of the group III temperature-sensitive phenotype appears always to be accompanied by a reversion of the NaCl-dependent profile of M protein inhibition of transcriptase activity, suggesting that these two properties are integrally related. Results shown in Figures 3 and 6 can be expressed in terms of the salt concentrations required for 50% of maximum ac-

Table I: Temperature Sensitivity of Group III Mutants and Revertants[a]

	titer	
	31 °C	39 °C
tsO23	1.5×10^{10}	7×10^{5}
O23r5	6×10^{9}	2×10^{9}
O23r7	5.5×10^{9}	8.5×10^{8}
tsO89	3×10^{9}	7.5×10^{5}
O89r5	8×10^{8}	1.5×10^{8}
O89r7	2.5×10^{9}	8×10^{8}
tsG31	9×10^{9}	1×10^{5}
G31r4	4.5×10^{9}	9×10^{8}
G31r8	2×10^{9}	9×10^{8}
tsG33	7.5×10^{9}	3×10^{5}
G33r1	1.5×10^{8}	3×10^{7}
G33r5	3×10^{9}	1×10^{9}

[a] Log dilutions of purified stocks of group III mutants and revertants were assayed in duplicate on monolayers of Vero cells at the temperatures indicated. Following a 1-day incubation, cultures were stained, and plaques were counted. All titers are expressed in plaque forming units (pfu)/mL.

Table II: NaCl Dependence of in Vitro Transcription: VSV wt, Group III Mutants, and Revertants

	[NaCl] required for 50% of maximum activity	
	−poly(Glu)	+poly(Glu)
wtO	0.11	0.08
tsO23	0.06	0.04
tsO23r5	0.13	0.07
tsO23r7	0.11	0.09
tsO89	0.08	0.04
tsO89r5	0.11	0.05
tsO89r7	0.12	0.05
wtG	0.10	0.06
tsG31	0.06	0.02
tsG31r4	0.09	0.04
tsG31r8	0.10	0.05
tsG33	0.05	<0.02
tsG33r1	0.09	0.04
tsG33r5	0.11	0.06

tivity and are presented in this way in Table II.

Discussion

We have confirmed the findings of Carroll & Wagner (1979) that the M protein of VSV inhibits in vitro viral transcription and that M protein from wild-type virus is a much more effective inhibitor than M derived from group III mutants (Figure 2). These results are consistent with recent in vivo studies showing that cells infected with group III mutants have an altered pattern of virus-directed RNA synthesis (Martinet et al., 1979; Clinton et al., 1978) and, at the nonpermissive temperature, produce virus-like particles that contain envelope components but lack nucleocapsids (Schnitzer & Lodish, 1979). Taken together, these results suggest that there is a specific interaction between wild-type M protein and viral nucleocapsids and that this interaction is decreased in group III mutants.

In the present study, we have characterized the interactions between wild-type or mutant M protein and nucleocapsids by assaying for inhibition of in vitro transcription under different conditions. Viral fractionation studies indicate that M is dissociated from nucleocapsids only in the presence of high salt (Inblum & Wagner, 1974; Figure 1), suggesting an electrostatic component to M–nucleocapsid interactions. Because the standard conditions for the disruption and assay of in vitro transcription prescribe high ionic strength, we examined the effects of lowering the salt concentration in the

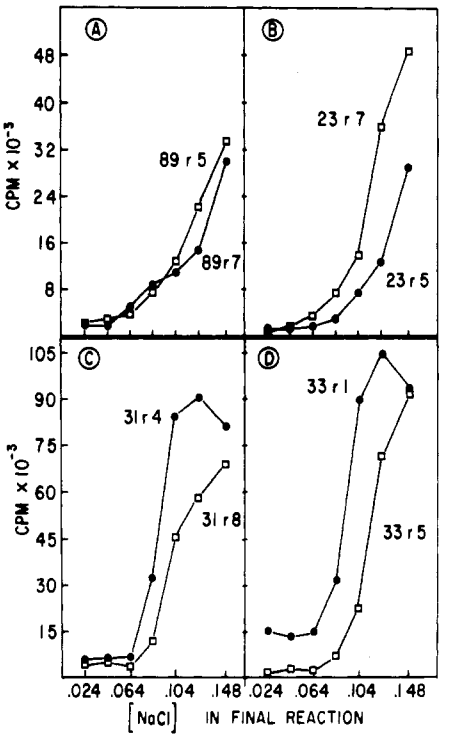

FIGURE 6: NaCl dependence of in vitro transcription: group III revertants. Concentrated virus was disrupted in the presence of the appropriate concentration of NaCl, and in vitro transcriptase activity was assayed as described under Materials and Methods. The final concentration of virus in the assays was 1 mg/mL.

disruption mixture (Figure 3). Under conditions of low ionic strength, both wild-type and mutant M proteins effectively inhibit in vitro RNA synthesis. As the concentration of NaCl is gradually increased, mutant M becomes a less effective inhibitor while wild-type M protein inhibition is significantly reversed only at relatively high ionic strength. Addition of poly(Glu) to the in vitro reaction mixture significantly increased the level of transcription, particularly at the lower salt concentrations. However, even under optimal conditions for dissociation of M from nucleocapsids in the reaction, wild-type M shows significant inhibitory capacity relative to mutant M. Our interpretation of the NaCl and poly(Glu) effects observed is that the interaction between M protein and nucleocapsids that results in inhibition of transcription is largely electrostatic in nature. Further, in all the group III mutants examined, these electrostatic interactions are weakened, allowing the dissociation of M protein and nucleocapsids under conditions of lower ionic strength.

Several pieces of evidence suggest that hydrophobic interactions do not play a significant role in M protein mediated regulation of RNA synthesis. The pattern of detergent dependence in in vitro transcription assays is identical for M protein mutants and their parental wild types (Figure 4), and no significant inhibition is observed until the detergent concentration is >100-fold lower than that used in standard assays and >3-fold lower than the critical micelle concentration. Furthermore, when the detergent concentration is sufficiently low to show inhibition, the reaction is 90% inhibited, suggesting that this may have been due to insufficient disruption of the virus particles.

Ideally, we would like to determine the stoichiometry of M–nucleocapsid interactions by using purified components. Thus far, however, it has not been possible to obtain a fully functional, monomeric preparation of pure M protein (Carroll & Wagner, 1979; unpublished observations). Although Carroll & Wagner (1979) have shown that a suspension of pure, but

insoluble, M protein has some inhibitory activity in in vitro transcription assays, the level of inhibition is significantly lower than that observed with whole virus, even when M protein is present in 5-fold excess. Our results indicate that the addition of equimolar amounts of UV-irradiated wild-type virus to in vitro assays of tsO23 inhibits transcription by about 50% (Figure 4), as might be expected if the functional (wild type) M protein is randomly associating with nucleocapsids during disruption. We also found that a crude virus fraction containing M protein, G protein, and lipid effectively inhibits in vitro transcription at concentrations roughly equivalent to those present in whole virus (Figure 2). At present, it is not clear whether the differences between the activity of the pure protein and that of a crude extract are simply a reflection of the solubility properties of M protein under different conditions or if the G protein is also affecting viral RNA synthesis, as was suggested by the in vivo studies of Martinet et al. (1979).

Because our studies comparing the effects of wild-type and mutant M proteins were performed at the permissive temperature for group III mutants (31 °C), it was necessary to demonstrate that the reduced M–nucleocapsid interaction observed in vitro is functionally related to the in vivo temperature-sensitive phenotype. Following isolation of temperature-insensitive revertants from each of the group III mutants studied, several of these revertants were characterized with regard to their in vitro transcriptase activity. Every revertant exhibited a profile of salt-dependent M protein inhibition of transcriptase activity that resembled the wild-type profile far more closely than the equivalent profile of the mutant from which the revertant was derived (Figure 6, Table II). It therefore appears that the alterations observed in M–nucleocapsid interactions are not an incidental property but are integrally related to the temperature-sensitive phenotype.

In the preceding paper in this issue, Reidler et al. (1981) present evidence that mutant M protein immobilizes a significantly larger fraction of the G molecules in the plasma membrane of infected cells than does wild-type M protein. This is consistent with other evidence from this laboratory obtained by labeling purified virions with the hydrophobic, photoreactive probe iodonaphthyl azide. It was found that the M protein of group III mutants was labeled to about twice the extent of M proteins of wt or revertant virions (D. Mancarella and J. Lenard, unpublished results). There are several ways in which such a coordinate effect—increased interaction of M with membrane and reduced interaction of M with nucleocapsid—might arise from a single mutation. The mutation might specifically alter the segment of the polypeptide chain that interacts with nucleocapsids into a segment that interacts with membranes. This is unlikely, however, because such a change might be expected to produce a totally nonfunctional M protein and because it would not be expected that every mutation would result in such a similar effect on both interactions, as is observed. A second possibility is that the mutation causes a conformational change that affects two different regions of the protein in a coordinate manner. It has recently been reported that M protein exists in at least two phosphorylated states (Clinton et al., 1978). While phosphorylation of M protein has not yet been shown to have any functional significance, it is possible that such a coordinate conformational change could arise from a mutation affecting a phosphorylation site.

There is, however, an additional interpretation of the observed changes in M protein interactions. The coordinate effect can be explained even if the mutation in M protein involves a single amino acid change in only one of two independent binding sites; e.g., the binding with nucleocapsid is decreased by substitution of a basic amino acid residue. Then, even if the membrane binding region of the polypeptide chain is completely unaffected by the mutational change, association with membranes would increase purely on the basis of mass action:

$$(\text{M-NC} \rightleftharpoons \text{M}_{\text{sol}} \rightleftharpoons \text{M-Memb})$$

This interpretation predicts that the effect of an M protein mutation *must* be coordinate even if the binding sites for nucleocapsid and for membranes are completely independent. This could provide an explanation for the surprising observation that each of the M protein mutants studied shows similar alterations in both nucleocapsid and membrane binding properties (cf. Reidler et al., 1981; D. Mancarella and J. Lenard, unpublished results).

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