Phosphorylation of NS Protein by Vesicular Stomatitis Virus Nucleocapsids: Lack of Effect during RNA Synthesis and Separation of Kinase from L Protein

DOUGLAS M. MASSEY, NANCY DEANS, AND JOHN LENARD*

*Department of Physiology and Biophysics, University of Medicine and Dentistry of New Jersey,
Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854-5635*

Received 25 January 1990/Accepted 3 April 1990

The relationship between NS protein phosphorylation and RNA polymerase activities was determined in nucleocapsids purified from vesicular stomatitis virus grown in BHK cells. Phosphate incorporation into endogenous NS protein under transcription conditions reached a maximum value of 0.06 mol/mol of NS within 20 to 30 min, while RNA synthesis remained linear for 90 min. Phosphate incorporation into NS increased further upon addition of kinase-free NS protein but not upon addition of nucleocapsid kinase (prepared as described below), indicating that cessation of NS phosphorylation under transcribing conditions was due to substrate exhaustion. When NS was phosphorylated with ^{32}P , <8% of the radiolabel was lost during subsequent transcription, indicating that this phosphate did not turn over. Treatment of nucleocapsids with 5'-*p*-fluorosulfonylbenzoyl adenosine resulted in >90% inhibition of NS phosphorylation but had no effect on RNA polymerase activity. Fast protein liquid (Superose-6) chromatography of a nucleocapsid (L+NS) fraction resulted in complete separation of the viral (L+NS) protein from NS-phosphorylating activity. The addition of this kinase-free (L+NS) fraction to a kinase-deficient N-RNA fraction reconstituted an active RNA polymerase containing <20% of the original NS-phosphorylating activity. These results demonstrate that NS-phosphorylating activity is unnecessary during vesicular stomatitis virus RNA synthesis and indicate that all of the protein kinase(s) present in purified nucleocapsids is probably of cellular rather than viral origin.

The RNA polymerase activity of vesicular stomatitis virus (VSV) resides in a complex (the nucleocapsid) containing a single-stranded, negative-sense, 11-kilobase (kb) RNA molecule, associated with three of the five viral proteins (1). These proteins, designated N, NS, and L, are all essential for activity (11, 24). N protein (49 kilodaltons [kDa], 1,258 molecules per virion [28]) is tightly associated with the RNA genome to form the N-RNA template. L protein (241 kDa, 50 molecules per virion [28]) is thought to act as the enzyme, conferring the following activities on the complex: transcription of the five viral mRNAs from the N-RNA template, including initiation, elongation, and termination; polyadenylation; capping; methylation; and replication of full-length positive- and negative-sense strands from the N-RNA template (2). NS protein (>25 kDa, 466 copies per virion [12, 13, 28]) is a phosphoprotein that is required for RNA polymerase activity, although its precise function remains unknown. A small amount of viral M protein and some protein kinase activity are also present in purified nucleocapsids.

The protein kinase activity present in purified VSV preparations has been studied extensively. Various investigators have suggested that this kinase is derived from the host cell (4, 8, 14, 17, 18), although copurification of kinase activity with L protein led to the suggestion that phosphorylation of NS might be specifically catalyzed by L (25). We have previously shown, however, that about 95% of the NS-phosphorylating activity associated with purified L protein could be removed by gel filtration (20).

The NS protein in mature virions is highly phosphorylated (15). At least 21 different serine residues can be phosphorylated, but surprisingly, the actual stoichiometry of NS phosphorylation appears never to have been determined (16). The

extent of NS phosphorylation has a profound effect on RNA polymerase activity: the more highly phosphorylated form(s) generally confers greater activity, and treatment with phosphatase causes substantial inactivation (2, 16, 19, 27). In addition, site-directed mutagenesis at selected phosphorylation sites in the NS molecule causes loss of most RNA polymerase activity (7).

The role of protein kinase(s) during the course of the RNA polymerase reaction (as opposed to their role in determining the phosphorylation state of NS) remains unclear. It has been suggested that protein phosphorylation might be required to "prime" RNA synthesis in some way, although this has been questioned by others (2). In this article we show that the protein kinase activity (against NS protein) present in transcribing nucleocapsids is able to act on <10% of the NS present and may be inactivated without effect on transcription. Furthermore, the small amount of NS phosphorylation that does occur is stable, i.e., it does not turn over during transcription. The kinase activity was completely separated from a viral (L+NS) preparation by gel filtration on fast protein liquid chromatography (FPLC), indicating that it is probably of cellular rather than viral origin.

MATERIALS AND METHODS

Cells, viruses, and nucleocapsids. VSV Indiana, Birmingham strain, was originally supplied by David H. L. Bishop (National Environment Research Council, United Kingdom). Virus was grown in BHK21F monolayers, infected at a multiplicity of infection of 1, harvested after 24 h, and gradient purified as described previously (23). Virus composed of radioactively labeled viral proteins was prepared as described above except that virus was initially grown on methionine-deficient medium with [^{35}S]methionine added 1 h

* Corresponding author.

after infection. Nucleocapsids were prepared from purified detergent-disrupted VSV by centrifugation on discontinuous glycerol gradients (6). Protein concentration was determined by a modified Lowry procedure (5).

NS phosphorylation. NS phosphorylation was measured in 100 μ l of the following solution: 50 mM Tris (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, 4 mM dithiothreitol (DTT), 5 μ M ATP, and 20 to 30 μ Ci of [γ - ^{32}P]ATP (4,500 Ci/mmol). Substrate for the reactions was either phosphocellulose column chromatography-purified NS protein (25) or FPLC-purified (L+NS) fraction, prepared as described below. Reaction mixes were incubated for 20 min at 37°C, and reactions were terminated by addition of 50 μ g of bovine serum albumin (BSA) and 20 μ l each of 0.1 M EDTA and 20 mM ATP. Proteins were precipitated with 20% trichloroacetic acid, pelleted, suspended in gel electrophoresis sample buffer (0.0625 M Tris hydrochloride [pH 6.8], 2% SDS, 10% glycerol, 5% 2-mercaptoethanol), placed in a boiling-water bath for 3 min, and then separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The labeled band of NS protein was excised, and the ^{32}P content was determined by scintillation counting.

Transcription and NS phosphorylation assay. Measurement of NS phosphorylation throughout the course of transcription was carried out with 40 μ g of nucleocapsid in 200- μ l reaction mixes containing 50 mM Tris (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, 4 mM DTT, and either 1 mM ATP, 1 mM GTP, 1 mM CTP, 0.1 mM UTP, and 10 μ Ci of [3H]UTP (35 Ci/mmol) or 1 mM [γ - ^{32}P]ATP (25 Ci/mmol), 1 mM GTP, 1 mM CTP, and 0.1 mM UTP. Reaction mixes were incubated for a total of 90 min at 30°C, and 5- μ g samples were removed at various times. Reactions measuring transcription were stopped by addition of 100 μ l of yeast RNA (2 mg/ml) in 0.067 M sodium pyrophosphate. RNA was precipitated with trichloroacetic acid and filtered on Whatman GF/C filters, and radioactivity was counted. Reactions measuring phosphorylation of NS were analyzed as described above.

Pulse-labeling of NS protein. Purified nucleocapsid (40 μ g) was preincubated at 30°C for 30 min with 11 μ M ATP and 150 μ Ci of [γ - ^{32}P]ATP (4,500 Ci/mmol) in 50 mM Tris (pH 8.0)-5 mM $MgCl_2$ -100 mM NaCl-4 mM DTT. The transcription reaction was then initiated by adding the remaining components to yield a final concentration of 50 mM Tris (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, 4 mM DTT, 1 mM ATP, 1 mM GTP, 1 mM CTP, 0.1 mM UTP, and 10 μ Ci of [3H]UTP. The reaction mixture was then incubated at 30°C for 90 min, and duplicate 24- μ l samples were removed at appropriate times. The samples were analyzed for RNA synthesis and phosphorylation of NS protein as described above.

Inhibition of kinase activity by FSBA. Nucleocapsids (40 μ g) were incubated in the presence or absence of 0.8 mM 5'-*p*-fluorosulfonylbenzoyl adenosine (FSBA) at 32°C for 30 min in 50 mM Tris (pH 8.0)-5 mM $MgCl_2$ -100 mM NaCl. Phosphorylation was then initiated by adding the remaining components to yield a final concentration of 50 mM Tris (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, and 30 μ Ci of [γ - ^{32}P]ATP (4,500 Ci/mmol). The reaction mixture was then incubated at 32°C, and 25- μ l samples were removed at appropriate times. The reactions were terminated and analyzed for NS phosphorylation as described above.

FSBA effect on in vitro transcription. Nucleocapsids (20 μ g) were incubated at 32°C in the presence or absence of 0.8 mM FSBA in 50 mM Tris (pH 8.0)-5 mM $MgCl_2$ -100 mM NaCl. After 30 min, the reaction mixture was centrifuged at

FIG. 1. Time course of RNA synthesis (●) and NS phosphorylation (■) by purified nucleocapsids (NCC) under standard transcription conditions.

16,000 $\times$ *g* for 20 min at 4°C. The pellet was suspended and assayed for transcription as described above.

Dissociation of nucleocapsids in high salt. Nucleocapsids were treated with 0.8 M NaCl and centrifuged on discontinuous glycerol gradients (10). The high-salt extract remaining at the top of the gradient consisted of the L and NS (L+NS) proteins, residual M protein, and 43% of the NS-phosphorylating activity of the starting nucleocapsids. N-RNA template, at the 30 to 100% glycerol interface, was further purified by two additional gradient centrifugation steps (10); it retained 35% of the original nucleocapsid NS-phosphorylating activity.

FPLC. Superose-6 FPLC columns were treated with 10 mM Tris (pH 8.0)-0.8 M NaCl-0.6 mM DTT-2.0% BSA. Running buffer was identical except that it contained 0.2% BSA. Samples, in running buffer minus BSA, were concentrated to an appropriate volume (<200 μ l) with Amicon Centricon 10's and loaded onto the column. The FPLC flow rate was 0.2 ml/min. Fractions (1 ml) were collected, and the ^{35}S content was determined by scintillation counting. Each fraction was diluted twofold with column running buffer minus NaCl, concentrated, and then tested for NS phosphorylation activity or for transcription activity in a reconstituted system.

Polyacrylamide gel electrophoresis. NS phosphorylation reactions were analyzed on 10% SDS-polyacrylamide slab gels overlaid with a 4% polyacrylamide stacking gel. Following electrophoresis, gels were stained with Coomassie blue, dried, and autoradiographed. NS-containing bands were excised from the gels, and radioactivity was determined in a Beckman liquid scintillation counter.

Reconstitution of RNA synthesis. Reconstitutions were performed with 15 μ g of total protein under the transcription conditions described above. The amounts of FPLC-purified N-RNA (10.6 μ g) and L+NS (4.4 μ g) used were based on a published determination of virion composition (28).

RESULTS

NS phosphorylation of nucleocapsid kinases does not affect most of the NS present. RNA was synthesized by nucleocapsids in a continuous, linear fashion for at least 90 min (Fig. 1). In contrast, phosphorylation of NS under the same

FIG. 2. Time course of ^{32}P incorporation into NS protein of purified nucleocapsids under standard transcription conditions (■). After 45 min of incubation (arrow), kinase fraction (●) from Superose-6 FPLC or L+NS fraction from Superose-6 FPLC (○) was added (see Fig. 7 for chromatographic profile).

conditions was complete after 20 to 30 min. Quantitation of the amount of ^{32}P incorporated into NS showed that the maximum value corresponded to 0.06 mol of phosphate incorporated per mol of NS, indicating that no more than 6% of the NS molecules present could have acquired one additional phosphate group. It is noteworthy that the rate of RNA synthesis did not change perceptibly during or after completion of NS phosphorylation.

Cessation of NS phosphorylation after 20 to 30 min could arise either from exhaustion of substrate or from inactivation of kinase. To distinguish between these possibilities, the experiment shown in Fig. 2 was performed. Nucleocapsids were incubated under normal transcribing conditions for 45 min in the presence of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. This time was sufficient for phosphorylation of NS to reach maximal levels (Fig. 2). After 45 min, a sample of either kinase-free viral L+NS proteins or nucleocapsid-associated kinases devoid of viral L+NS proteins (prepared as described below) was added. As shown in Fig. 2, addition of substrate but not of enzyme resulted in enhanced NS labeling. The rapid cessation of NS phosphorylation during transcription must therefore be due to exhaustion of NS substrate. This finding, together with the very low level of NS phosphorylation, indicates that NS protein is already nearly completely phosphorylated at all of its susceptible sites before the start of transcription.

FIG. 3. Prelabeling of NS protein. NS protein was phosphorylated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and then incubated under transcribing conditions as described in Materials and Methods. Symbols: ●, ^{32}P content of NS protein, expressed as picomoles of ^{32}P per milligram; □, $[\text{H}]\text{UTP}$ incorporated into RNA, expressed as counts per minute.

FIG. 4. FSBA inhibition of NS phosphorylation. Nucleocapsids were incubated with (lanes 1 to 3) or without (lanes 4 to 6) FSBA, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was added for the times indicated (in minutes), and NS phosphorylation was measured as described in Materials and Methods.

Nucleocapsid-catalyzed phosphorylation of NS is stable. The low levels of phosphorylation of NS observed during transcription (Fig. 1 and 2) could represent a steady-state level arising from a rapid rate of phosphorylation and breakdown. This possibility was tested by a chase experiment. NS protein was phosphorylated with low concentrations of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (10 μM), and maximal phosphorylation of ca. 0.03 mol/mol of NS protein was obtained. Transcribing concentrations of the nucleoside triphosphates (including 1 mM ATP; see Materials and Methods) were then added. As shown in Fig. 3, transcription was linear for at least 60 min, while loss of label from the NS protein was 0.002 mol/mol, or ca. 7% of the total label. Over 90% of the phosphate added by the nucleocapsid-associated kinases thus remained on the NS proteins throughout the transcription reaction.

Inactivation of nucleocapsid-associated kinases by FSBA. Brief incubation of nucleocapsids with FSBA (30 min at 32°) resulted in nearly complete inhibition of NS-phosphorylating activity (Fig. 4) without any effect on transcription (Fig. 5). Counting of radiolabeled NS bands showed that phosphorylation of NS was reduced 93% by FSBA treatment. Thus, the presence of NS-phosphorylating activity is not necessary to sustain normal rates of transcription by nucleocapsids.

Preparation of kinase-free L+NS proteins and reconstitution of kinase-deficient transcription from purified compo-

FIG. 5. Effect of FSBA on in vitro transcription. Nucleocapsid (40 μg) was incubated in the presence (■) or absence (○) of 800 μM FSBA and then pelleted and tested for RNA polymerase activity as described in Materials and Methods. Results are from three experiments $\pm$ standard deviations.

FIG. 6. Fractionation scheme for purification of nucleocapsid components. Amount of NS-phosphorylating activity, relative to whole nucleocapsids, is shown in parentheses. NCC, Nucleocapsids, purified as described in Materials and Methods.

nents. Fractionation of nucleocapsid components was carried out in order to further characterize the nucleocapsid kinase activity. As shown in Fig. 6, nucleocapsids were first disrupted in high salt, and the components were separated by published procedures (10). The L+NS fraction prepared by density gradient centrifugation was then subjected to FPLC Superose-6 chromatography. As shown in Fig. 7, the L and NS proteins eluted together (fractions 14 to 17), presumably as a complex, while all measurable NS-phosphorylating activity was recovered in fractions 18 to 20. Autoradiographs of SDS gels of column fractions (not shown) revealed that the small amount of ^{35}S present in fractions 18 and 19 arose from M protein, which is normally

present in various amounts in nucleocapsid preparations (28). We have previously shown that ca. 95% of the total nucleocapsid NS-phosphorylating activity is separated from phosphocellulose-purified L protein in two distinct fractions, which differ in their casein-phosphorylating activity (20). These results confirm, therefore, that NS-phosphorylating activity is not intrinsic to L protein but arises from other proteins, presumably cellular in origin.

Gradient-purified N-RNA from nucleocapsids still contained about 35% of the original nucleocapsid-associated NS-phosphorylating activity (Fig. 6). In order to develop a reconstituted transcription system containing the lowest possible amount of kinase, the gradient-purified N-RNA fraction was subjected to Superose-6 chromatography (not shown). Although NS-phosphorylating activity coeluted with the N-RNA, specific activity was reduced to about half of its original value (Fig. 6). Similar reductions could be achieved by heating the N-RNA at 60°C for 30 s (data not shown).

Transcription was reconstituted by adding together col-

FIG. 7. Superose-6 chromatography of a [^{35}S]methionine-labeled nucleocapsid high-salt extract. Symbols: Δ , [^{35}S]methionine cpm; $\blacksquare$, NS-phosphorylating activity. From 90 to 95% of radiolabel and >100% of NS-phosphorylating activity were recovered from the column.

TABLE 1. NS phosphorylation and RNA synthesis in reconstituted transcription systems

Prepn	Phosphate (mol/mol of NS)	UTP (pmol/mg of protein)
Purified nucleocapsids	0.036 (100%) ^a	500 (100%) ^a
Unfractionated L+NS + unfractionated N-RNA	0.028 (77.8%)	88 (17.6%)
Fraction 14 (L+NS) + Superose-purified N-RNA	0.007 (18.5%)	363 (72.6%)
Fraction 14 (L+NS) + fraction 18 (kinase) + Superose-purified N-RNA	0.02 (56.3%)	337 (67.4%)

^a Percentage of value with purified nucleocapsids.

umn-purified L+NS, which was essentially free of NS-phosphorylating activity, and column-purified N-RNA, which was reduced in NS-phosphorylating activity (Table 1). Most of the RNA polymerase activity was recovered in this reconstituted system, in which NS phosphorylation was reduced by >80%. Polymerase activity was not augmented by additional NS-phosphorylating activity.

Reconstitution with gradient-purified fractions, without further column purification, showed reduced polymerase activity (Table 1). Presumably, an inhibitor was introduced during gradient purification which was removed by the Superose-6 chromatography.

DISCUSSION

Beckes et al. (3) have recently reported that viral NS protein was phosphorylated to the extent of ca. 0.4 mol/mol by protein kinases present in an unfractionated preparation of purified, detergent-disrupted VSV. Earlier work, however, showed that at least 85% of total viral protein kinase was membrane associated and could be removed during the preparation of actively transcribing nucleocapsids (27, 30). Any NS-phosphorylating activity that might be required for transcription would be expected to remain associated with nucleocapsids. In this article we have therefore characterized the nature of nucleocapsid-associated NS phosphorylation and its relationship to transcription activity.

The possibility that RNA synthesis by VSV polymerase might require prior or concomitant phosphorylation of NS protein was first suggested by Watanabe et al., who used unfractionated disrupted virions as the source for viral polymerase (29). However, Sinacore and Lucas-Lenard prepared fully active nucleocapsids from which >95% of the casein-phosphorylating activity had been removed and showed that adding kinase back to these nucleocapsids did not increase transcribing activity (27). These workers did not determine NS-phosphorylating activity in their experiments, raising the possibility that the relevant, i.e., NS-specific, kinase(s) might still be present; our previous experiments, for example, showed that BHK-grown VSV possesses at least two NS-phosphorylating kinases, separable by chromatography on Superose-12, of which only one has appreciable casein-phosphorylating activity (20).

The present study provides three different lines of evidence that phosphorylation of NS by nucleocapsids is not a necessary step during, or part of the mechanism of, RNA transcription. First, inactivation of NS-phosphorylating activity by FSBA had no effect on RNA polymerase activity (Fig. 4 and 5). Second, the extent of NS phosphorylation in actively transcribing nucleocapsids was quite low, only 0.06 mol/mol of NS protein (Fig. 1), and this phosphate did not undergo turnover during transcription (Fig. 3). At least 94% of total nucleocapsid NS thus remains unmodified by kinase during the transcription reaction, presumably because it has already been fully phosphorylated (Fig. 2); thus, either 94% of the NS present is unnecessary for transcription, or else phosphorylation of the remaining 6% is not required. Finally, about 80% of NS-phosphorylating activity could be chromatographically removed from nucleocapsid components (Fig. 6) with little or no effect on the reconstituted transcription activity of the purified components (Table 1). These findings in no way contradict the abundant evidence that the phosphorylation state of NS determines its transcriptional competence (16, 19, 22). They do indicate, however, that the NS proteins in virions are already in a transcriptionally competent phosphorylation state, presum-

ably as a result of prior phosphorylation in the infected cell. This state is then stably maintained throughout transcription (Fig. 3).

The nucleocapsid-associated NS-phosphorylating activity seems almost certain to be cellular in origin. The sequence of the entire VSV genome is known (2, 12, 26), and recognizable ATP-binding sequences are found only in L protein (21). Consistent with the sequence data, L protein but not NS could be photoaffinity labeled with 8-azido-ATP (25). Since L protein has now been shown to lack NS-phosphorylating activity entirely (Fig. 7) (20), this activity must be derived from the infected cell. It remains to be determined whether the nucleocapsid-associated NS-phosphorylating enzymes possess unique specificities for making active forms of NS, or whether they are simply adventitiously bound proteins of no special functional significance. Our observation that these kinases cannot be chromatographically removed from whole nucleocapsids, even under high-salt conditions (data not shown), however, suggests that only certain cellular kinases may associate specifically with the intact complex. The fact that the kinases in the L+NS fraction chromatographed into two distinct peaks differing in substrate specificity (20) also suggests that only certain cellular kinases are bound. Finally, it should be noted that the VSV used in this study was grown in BHK cells; VSV grown in different cells possesses different envelope-associated kinases (18) and probably possesses different nucleocapsid-associated kinases as well.

ACKNOWLEDGMENTS

The technical assistance of Roger Vanderoef is gratefully acknowledged. We are indebted to Mitsuo Tagiya for suggesting the use of FSBA in these experiments.

This work is supported in part by Public Health Service grant AI-13002 from the National Institutes of Health.

LITERATURE CITED

1. Banerjee, A. K. 1987. The transcription complex of vesicular stomatitis virus. *Cell* 48:363-364.
2. Banerjee, A. K. 1987. Transcription and replication of rhabdoviruses. *Microbiol. Rev.* 51:66-87.
3. Beckes, J. D., L. C. Childers, and J. Perrault. 1989. Phosphorylation of vesicular stomatitis virus M protein: evidence for a second virion-associated protein serine kinase activity. *Virology* 169:161-171.
4. Bell, J. C., E. G. Brown, D. Takayasu, and L. Prevec. 1984. Protein kinase activity associated with immunoprecipitates of the vesicular stomatitis virus phosphoprotein NS. *Virology* 132:229-238.
5. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* 70:241-250.
6. Carroll, 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.
7. Chattopadhyay, D., and A. K. Banerjee. 1987. Phosphorylation within a specific domain of the phosphoprotein of vesicular stomatitis virus regulates transcription *in vitro*. *Cell* 49:407-414.
8. Clinton, G. M., N. G. Guerina, H. Guo, and A. S. Huang. 1982. Host-dependent phosphorylation and kinase activity associated with vesicular stomatitis virus. *J. Biol. Chem.* 257:3313-3319.
9. Colman, R. F. 1983. Affinity labeling of purine nucleotide binding sites in proteins. *Annu. Rev. Biochem.* 52:67-92.
10. De, B. P., and A. K. Banerjee. 1984. Specific interactions of vesicular stomatitis virus L and NS proteins with heterologous genome ribonucleoprotein template lead to mRNA synthesis *in vitro*. *J. Virol.* 51:628-634.
11. Emerson, S. U., and Y. H. Yu. 1975. Both NS and L protein are required for in vitro RNA synthesis by vesicular stomatitis virus. *J. Virol.* 15:1348-1356.
12. Gallione, C. J., J. R. Greene, L. E. Iverson, and J. K. Rose.

1981. Nucleotide sequences of the mRNAs encoding the vesicular stomatitis virus N and NS proteins. *J. Virol.* **39**:529–535.
13. Gill, D. S., and A. K. Banerjee. 1985. Vesicular stomatitis virus NS proteins: structural similarity without extensive sequence homology. *J. Virol.* **55**:60–66.
14. Harmon, S. A., L. L. Marnell, and D. F. Summers. 1983. The major ribonucleoprotein-associated protein kinase of vesicular stomatitis virus is a host cell protein. *J. Biol. Chem.* **258**:15283–15290.
15. Hsu, C., and D. W. Kingsbury. 1985. Constitutively phosphorylated residues in the NS protein of vesicular stomatitis virus. *J. Biol. Chem.* **260**:8990–8995.
16. Hsu, C., E. M. Morgan, and D. W. Kingsbury. 1982. Site-specific phosphorylation regulates the transcriptive activity of vesicular stomatitis virus NS protein. *J. Virol.* **43**:104–112.
17. Ikeuchi, T., M. Yokoyama, and K. Ohtsuki. 1988. Characterization of a polypeptide-dependent membrane protein kinase that specifically phosphorylates NS protein of vesicular stomatitis virus *in vitro*. *Tohoku J. Exp. Med.* **155**:41–56.
18. Imblum, R. L., and R. R. Wagner. 1974. Protein kinase and phosphoproteins of vesicular stomatitis virus. *J. Virol.* **13**:113–124.
19. Kingsford, L., and S. U. Emerson. 1980. Transcriptional activities of different phosphorylated species of NS protein purified from vesicular stomatitis virions and cytoplasm of infected cells. *J. Virol.* **33**:1097–1105.
20. Massey, D. M., G. M. Clinton, and J. Lenard. 1989. Evidence that the protein kinase activity of vesicular stomatitis virus nucleocapsids is heterogeneous and cellular in origin, p. 146–151. *In* B. W. J. Mahy and D. Kolakofsky (ed.), *Genetics and pathogenicity of negative-strand viruses*. Elsevier Biomedical Press, New York.
21. Massey, D. M., and J. Lenard. 1987. Inactivation of the RNA polymerase of vesicular stomatitis virus by *N*-ethylmaleimide and protection by nucleoside triphosphates. *J. Biol. Chem.* **262**:8734–8737.
22. Masters, P. S., and A. K. Banerjee. 1986. Phosphoprotein NS of vesicular stomatitis virus: phosphorylated states and transcriptional activities of intracellular and virion forms. *Virology* **154**:259–270.
23. Miller, D. K., and J. Lenard. 1980. Inhibition of vesicular stomatitis virus infection by spike glycoprotein. *J. Cell Biol.* **84**:430–437.
24. Naito, S., and A. Ishihama. 1976. Function and structure of RNA polymerase from vesicular stomatitis virus. *J. Biol. Chem.* **251**:4307–4314.
25. Sanchez, A., B. P. De, and A. K. Banerjee. 1985. *In vitro* phosphorylation of NS protein by the L protein of vesicular stomatitis virus. *J. Gen. Virol.* **66**:1025–1036.
26. Schubert, M., G. G. Harmison, and E. Meier. 1984. Primary structure of the vesicular stomatitis virus polymerase (L) gene: evidence for a high frequency of mutations. *J. Virol.* **51**:505–514.
27. Sinacore, M. S., and J. Lucas-Lenard. 1982. The effect of the vesicular stomatitis virus-associated protein kinase on viral mRNA transcription *in vitro*. *Virology* **121**:404–413.
28. 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.
29. Watanabe, Y., S. Sakuma, and S. Tanaka. 1974. A possible biological function of the protein kinase associated with vaccinia and vesicular stomatitis virions. *FEBS Lett.* **41**:331–334.
30. Witt, D. J., and D. F. Summers. 1980. Relationship between virion-associated kinase-effected phosphorylation and transcription activity of vesicular stomatitis virus. *Virology* **107**:34–49.