The Major Ribonucleoprotein-associated Protein Kinase of Vesicular Stomatitis Virus Is a Host Cell Protein*

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(Received for publication, June 6, 1983)

Ribonucleoprotein particles (RNPs) of vesicular stomatitis virus (VSV) were fractionated by column chromatography through Fractogel TSK HW-55F and by centrifugation through KCl sucrose. Analyses of fractions for protein content and for protein kinase activity indicated that the major peak of kinase activity did not correspond to a viral protein and that the VSV-specific proteins. Neither anti-NS nor anti-M IgG preparations inhibited protein kinase activity, and IgG did not act as an exogenous phosphate acceptor. Reconstitution of an RNP-enzyme complex did not result in a restoration of protein kinase activity. In vitro translation of VSV-specific poly(A)-containing RNA did not result in any detectable production of kinase activity. Thus, the major RNP-associated kinase is a host cell protein which is tightly bound to the RNP particle.

VSV1 has a virion-associated protein kinase activity which is cAMP independent and has been suggested to be a host-derived protein (6, 7, 24). In an in vitro reaction, the VSV-associated kinase phosphorylates mainly the virion proteins NS and M and to a lesser extent proteins L and G, as well as nonviral proteins (7, 39). The function of the VSV-associated kinase is not known, but there is some evidence (6, 20, 39) suggesting that a functional relationship exists between protein kinase activity and transcription. However, no direct correlation between phosphorylation and transcription was established by these investigations. In contrast, other recent studies by Sinacore and Lucas-Lenard (34) indicated that phosphorylation of ribonucleocapsid-associated proteins in vitro does not play a necessary role in VSV primary transcription.

An electron microscopic study of VSV virions following phosphorylation in vitro indicated a possible relationship between phosphorylation and virion uncoating (38).

Clinton et al. (6), using immunological techniques, tentatively identified one protein kinase associated with VSV as pp60 src. Other protein kinases are probably associated with VSV since the reaction products obtained from in vitro and in vivo reactions differ (7, 15, 24).

The above studies which have attempted to define the possible role(s) of the one or more kinases associated with the

* This research was supported by National Institutes of Health Grant 5 ROI AI 12316 and National Science Foundation Grant PCM 81-10986. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: VSV, vesicular stomatitis virus; RNP, ribonucleoprotein; BSA, bovine serum albumin; DTT, dithiothreitol; RNP-N, ribonucleoprotein particle containing only N protein plus the RNA template; PAGE, polyacrylamide gel electrophoresis.

VSV virion and RNP particle have only indirectly implicated this enzyme activity as a possible means of regulating the virus-specific RNA polymerase during replication. We still lack direct evidence for this function, and we do not know the number and location of the phosphate groups on the NS protein and the difference(s) in phosphorylation sites on active or inactive polymerase. It is clear that other negative strand viruses have virion-associated kinase activity and/or phosphoproteins as do viruses such as Rous sarcoma (4) and SV40 (36). In all cases our understanding of the role(s) of the phosphoproteins in regulation of virus replication is still unclear.

The purpose of this study was to develop methods for purifying the L/NS VSV RNA polymerase and RNP-N to decide if the RNP-associated kinase was a VSV protein. Furthermore, the purified enzyme subunits from our strain of VSV Indiana would then be amenable to structural analyses such as the topology of the phosphopetides in active versus inactive NS protein. Since published methods (9, 25) for enzyme purification failed to produce pure L/NS enzyme subunits or pure RNP-N template, we have established the purification procedure reported here. Our results indicate that the RNP-associated kinase is a host cell protein which is tightly bound to the RNP particle.

MATERIALS AND METHODS

Cells and Virus—HeLa S3 cells and VSV Indiana were grown as previously described (17) with the exception that 10% calf serum (Irvine Scientific) was used in the medium instead of fetal calf serum. Baby hamster kidney cells were grown as the HeLa cells except for the addition of 2% fetal calf serum and 10% tryptose phosphate broth (29.5 g/liter). Purified virus preparations were stored at -70 °C in 10% dimethyl sulfoxide.

Isolation of RNPs from Virions—RNPs were isolated by mixing virions with an equal volume of lysis solution (3.8% Triton N 101, 0.8 M NaCl, 1.2 mM DTT) and subsequent centrifugation through a discontinuous glycerol gradient (12). The pellet of RNPs was resuspended in 10 mM Tris, pH 8.0 containing 5 mM DTT.

Fractionation of RNPs—The RNPs, which contained the proteins L, NS, N, and some M, were made 2 M CaCl2 and 10 mM Tris, pH 7.4, and were incubated for 20 min at 20 °C. The suspension of RNPs proteins and molecular weight marker proteins were layered onto a column (20 x 540 mm) containing Fractogel TSK HW-55F (EM Reagents) which had been equilibrated with 1 M KCI, 10 mM Tris, pH 8.0, 1 mM DTT, and 15% sucrose. The column was eluted at 20 °C at a flow rate of 0.5 ml/min. Fractions of 1 ml were collected and stored at 4 °C until assayed.

Protein peaks, identified by Coomassie blue or silver nitrate staining of the proteins separated in polyacrylamide gels (23), were dialyzed against 10 mM Tris, pH 8.0, 1 mM DTT, and concentrated by placing the dialysis tubing in crystalline sucrose until the volume was reduced by the desired amount.

The L and NS proteins, which eluted from the column together as a complex, were further separated by layering the dialyzed proteins over a 5-25% sucrose gradient containing 1 M KCI, 10 mM Tris, pH 8.0, 1 mM DTT, with a 0.2-mI cushion of 80% sucrose and centrifuging...
at 35,000 rpm for 20 h at 4 °C using an SW 60 rotor. Nineteen fractions were collected from each gradient and were assayed for protein content by polyacrylamide gel electrophoresis and silver nitrate staining and for protein kinase activity.

The Fractogel peak fraction which corresponded to partially purified RNPs was purified further by isopycnic centrifugation. The fractions were combined and centrifuged through either 26–33% or 46–70% CsCl and 15284

Centrifugation of intracellular RNPs from VSV-infected cells in a Renografin column was used for these experiments was isolated by isopycnic centrifugation. The band of RNPs was collected, diluted with 10 mM Tris, pH 8.0, and centrifuged at 40,000 rpm for 2 h at 4 °C using an SW 41 rotor. The pellet of RNPs was resuspended in 10 mM Tris, pH 8.0, containing 5 mM DTT, and stored at −70 °C.

Protein kinase activity was assayed according to previously described methods (24, 39). In addition to aliquots of column or gradient fractions, the reaction mixture (100 μl) contained 10 mM Tris, pH 8.0, 10 mM MgCl₂, 5 mM DTT, 1 × 10⁻⁴ M ATP, 6 μCi of [γ-³²P]ATP (New England Nuclear; specific activity, 6.7 Ci/mmol), and 20 μg of casein as an exogenous phosphate acceptor (8). After incubation of the protein kinase reaction mixtures at 37 °C for 45 min, aliquots were precipitated with 10% trichloroacetic acid containing 50 mM monosodium phosphate and 50 mM sodium pyrophosphate, precipitates were collected on GF/C Whatman filters, and radioactivity was determined in a Beckman liquid scintillation spectrometer. The protein kinase activity of column or gradient fractions, the reaction mixture was mixed with gel sample buffer (20% glycerol, 2% DTT, 6% SDS, 0.125 M Tris, pH 6.8, and 0.004% bromphenol blue) and boiled for 1 min.

Samples were applied to discontinuous Tris-glycine-buffers SDS-polyacrylamide gels consisting of a 10% resolving gel and a 4% stacking gel (12). Gels were dried and exposed to Kodak SB-5 film at −70 °C.

Protein Quantitation—Proteins were quantified by densitometric scanning of silver stained bands in a polyacrylamide slab gel, or protein concentration were determined colorimetrically with BioRad protein dye reagent measuring absorbance at 595 nm, using ovalbumin as a standard.

Reconstitution of Purified L and NS Protein with the RNP-N Template—Purified L and NS proteins from sucrose gradients were mixed with RNPs-N template in quantities which were determined to be saturating for bound L/NS enzyme. The protein kinase activity of this reconstituted RNP particle was assayed as described above using casein as an exogenous phosphate acceptor. Two major and one minor peak of protein kinase activity were observed (Fig. 1B). The relative heights of the peaks of kinase activity varied (Fig. 1A), the first peak of this activity eluted after the L/NS complex and corresponded to the RNP-N template. The second small peak around fraction 42 corresponded to the L/NS complex as determined by a silver-stained polyacrylamide gel. The third and largest peak of protein kinase activity did not correspond precisely with any of the peaks of VSV-specific proteins; the peak of this activity eluted after the L/NS complex and slightly before the peak of M protein. There were no other detectable silver-stained proteins visible in this region.

The relative heights of the peaks of kinase activity varied somewhat from experiment to experiment and depended on the initial amount of RNPs used as starting material. When the RNP concentration was very dilute, stripping of the template appeared to be more efficient and the peak of kinase activity associated with the RNP-N template decreased with a concomitant increase in the third peak of kinase activity.

Effect of Monospecific Anti-VSV Protein IgG Preparations on the RNP-associated Protein Kinase Activity—Since the Fractogel peak fraction which gave maximum protein kinase activity (see Fig. 1B) eluted at a position in between the L/NS complex and M, it was tested further to determine if specific antiseraum against the VSV proteins, NS and M, had any effect on protein kinase activity.
of anti-M IgG and anti-NS IgG were mixed with a constant amount of protein from the Fractogel peak; and protein kinase activity was assayed in duplicate reactions except that casein was omitted from one set of reactions. Fig. 2A shows the Coomassie blue-stained polyacrylamide gel of the kinase reactions, and Fig. 2B shows the autoradiogram of the products on the same gel. In reactions where casein was omitted, the protein kinase activity was not above background since IgG did not act as an exogenous phosphate acceptor in this reaction. In no case did either anti-M IgG or anti-NS IgG have any effect on the RNP-associated protein kinase activity when casein was used as an exogenous phosphate acceptor.

These results strongly support the notion that the major protein kinase activity is a host protein which is present at levels too low to be detected by the silver-staining technique.

Effect of Isopycnic Centrifugation of Partially Purified RNPs in CsCl or in Renografin—The Fractogel peak fraction which corresponded to partially purified RNPs and which contained significant protein kinase activity was centrifuged either in CsCl (3 successive gradients) or in Renografin. Virion RNPs, purified by either procedure, still contained small amounts of L and NS in addition to N protein but showed no protein kinase activity above backgrounds (data not shown). Therefore, the kinase activity associated with the RNP fraction from the Fractogel column was dissociated with further purification and likely represents incomplete stripping of contaminating proteins by the high salt treatment. This interpretation is confirmed by the presence of small amounts of L, NS, and M proteins in the preparation (Fig. 1A) and the variability of the amount of kinase activity recorded as a function of
RNP concentration.

Separation of L and NS Proteins—Since small amounts of kinase activity were associated with the NS and L complex, this complex was dissociated and the separate proteins analyzed for kinase activity. The fractions from the column containing the L and NS complex were combined, dialyzed, and concentrated. They were then applied to a 5–25% sucrose gradient containing 1 M KCl, 10 mM Tris, pH 8.0, and 1 mM DTT. After centrifugation, fractions were collected, and each fraction was analyzed for protein content and for protein kinase activity.

Fig. 2. Effect of monospecific anti-VSV protein IgG preparations on protein kinase activity. A, Coomassie blue-stained SDS-polyacrylamide gel of protein kinase reaction mixes. V is purified VSV; L, G, NS, N, and M are VSV proteins. Lanes 1–7, no exogenous casein; lanes 8–14, casein used as an exogenous phosphate acceptor. Lanes 1 and 8, no IgG preparation; lanes 2 and 9, 5 µl (17.5 µg) of anti-NS; lanes 3 and 10, 10 µl of anti-NS; lanes 4 and 11, 25 µl of anti-NS; lanes 5 and 12, 5 µl of anti-M; lanes 6 and 13, 10 µl of anti-M; lanes 7 and 14, 25 µl of anti-M. B, autoradiogram of the same SDS-polyacrylamide gel in A. V is purified VSV; L, G, NS, N, and M are VSV proteins. Lanes 1–7, casein used as an exogenous phosphate acceptor; lanes 8–14, no exogenous casein. Lanes 1 and 8, 25 µl of anti-M; lanes 2 and 9, 10 µl of anti-M; lanes 3 and 10, 5 µl of anti-M; lanes 4 and 11, 25 µl of anti-NS; lanes 5 and 12, 10 µl of anti-NS; lanes 6 and 13, 5 µl of anti-NS; lanes 7 and 14, no IgG preparation. The order of the lanes in A and B was reversed through photography. 180K, for example, 180,000.
kinase activity. Fig. 3A shows a silver nitrate stain of an SDS-polyacrylamide gel of the sucrose gradient fractions and demonstrates the complete separation of the L (fractions 1-4) and NS (fractions 9-12) proteins. Each of these fractions was then analyzed for protein kinase activity; Fig. 3B shows that the major protein kinase activity was found in fractions 10-15, as determined using casein as an exogenous phosphate acceptor. This latter broad peak of protein kinase activity did not correspond precisely with the peak of NS protein; the maximum kinase activity was always found in fractions representing material of smaller size in SDS gels than NS protein. No other silver-stained bands were visible in the region of this peak from the gradient.

A small amount of protein kinase activity was also found in fractions 1-4 which contained L protein. This kinase activity represented only 1-2% of the total kinase activity of the fractions from Fractogel chromatography. It may represent protein aggregates which sediment with L protein. Since it was such a small percentage of the total activity, no attempt has been made to characterize it further.

The Effect of Reconstitution on Protein Kinase Activity—Since the capping, methylation, transcription, and polyadenylation reactions carried out by the L/NS enzyme require RNP-N template (10), it was conceivable that there also is a VSV-specific kinase activity which requires the presence of L/NS enzyme and RNP-N template to function. To test this hypothesis, a reconstitution experiment was performed.

Table I shows the protein kinase activities of purified L and NS proteins and mixtures of purified L and NS proteins combined with RNP-N template. These activities were compared with the protein kinase activity of detergent-solubilized VSV using an amount of VSV which was similar in protein content to the purified preparations of the proteins. All mixtures containing NS protein possessed slight protein kinase activity; however, this activity did not increase when NS and L protein were mixed with RNP-N template using conditions which resulted in reassociation of these proteins with the template, indicating that formation of an RNP-enzyme complex did not enhance kinase activity.

In Vitro Translation of VSV Poly(A)-containing RNAs—In order to further test whether the VSV-associated kinase activity was a VSV protein or a host protein, VSV-specific poly(A)-containing RNA was purified from infected baby hamster kidney cells and was then translated in a rabbit reticulocyte lysate mixture containing 164 µg of RNA. We reasoned that the poly(A)-containing VSV mRNA, which would direct the in vitro synthesis of at least microgram amounts of VSV proteins in the reticulocyte lysate, might be detectable as an increase in the kinase activity of the lysate if one of these four VSV proteins was a protein kinase. Fig.
TABLE I

Protein kinase activities of purified proteins

RNP-N was purified by banding RNP s from VSV-infected cells in Renografin as described in the text. NS and L proteins were prepared by Fractogel column chromatography and 1 M KCl sucrose centrifugation as described in the text. Protein kinase activities were determined by using casein as an exogenous phosphate acceptor and 0.4 μg of RNP-N, 0.6 μg of NS protein, and 0.6 μg of L protein per reaction. After incubation for 45 min at 37 °C, duplicate samples were trichloroacetic acid-precipitated, filtered, and radioactivity determined. Activity of each sample was compared with solubilized VSV virions of similar protein content.

| Component of protein kinase reaction mixture | Per cent of solubilized VSV activity |
|---------------------------------------------|-------------------------------------|
| Solubilized VSV virions                     | 100                                 |
| RNP-N alone                                 | 1.8                                 |
| NS alone                                    | 6.1                                 |
| L alone                                     | 1.4                                 |
| RNP-N + NS                                  | 5.8                                 |
| RNP-N + L                                   | 1.4                                 |
| NS + L                                      | 6.7                                 |
| RNP-N + NS + L                             | 6.1                                 |

Fig. 4. Autoradiogram of an SDS-polyacrylamide gel of in vitro translation. A, no added RNA; B, poly(A)-containing RNA from VSV-infected cells was added. 47K, for example, 47,000.

4 shows that the major proteins translated were the VSV-specific proteins NS, N, and M with a small amount of unglycosylated G. Since reticulocyte lysates have endogenous kinase activity (data not shown), the proteins of the in vitro translation reaction were fractionated on an agarose poly-L-lysine column. Control experiments showed that M protein elutes from this column at 0.5 M KCl, and L, N, and NS protein elute between 0.7 and 1.5 M KCl, while 90% of total HeLa cell proteins elute between 0 and 0.5 M KCl. This column was used, therefore, as a crude means of separating proteins in the reticulocyte lysate. Either reticulocyte lysate alone (Fig. 5A) or lysate containing translated VSV protein (Fig. 5B) was fractionated on agarose poly-L-lysine; the pooled fractions were dialyzed and tested for kinase activity using casein as a phosphate acceptor. There was no increase in total activity in the lysate that contained in vitro translated VSV proteins, and the fractions that were enriched for VSV proteins showed less activity. The products of these reactions were also resolved on SDS-polyacrylamide gels, and there were no detectable differences in the phosphorylated proteins from the fractions shown in Fig. 5, A and B (data not shown). Thus, there was no evidence of a VSV-specific kinase activity among the in vitro translated VSV proteins.

DISCUSSION

The RNP of VSV is a large enzyme-template complex and has been shown previously to be involved in transcription (10, 12), in the synthesis and addition of the cap group and methylation of each of the VSV-specific mRNAs (1, 2), and in replication (13, 26, 33, 37). In addition, there is an associated diphosphokinase activity (29, 35) and a CAMP-independent protein kinase activity (18, 24, 39). The switch from transcriptive functions to replication probably involves some as yet undefined modification of the L/NS enzyme complex and a probable role for host factors in this switch (26, 33) which leads to a suppression of transcription and associated capping and methylation to synthesis of full length (+) and (−) strands assembled into RNP particles. Direct evidence that the L/NS enzyme complex is involved in both transcription and replication was recently obtained through the use of monospecific antisera against the enzyme subunits which were shown to affect both in vitro transcription and replication (12, 14). This change in RNA products brought
about by regulation of L/NS enzyme molecules also results in
the abolition of the recognition of the two base intercistronic
stop signals by the enzyme and the synthesis of complete
complementary strands which are predominantly (−) strand
(32).

A possible mechanism proposed for L/NS modification was
the phosphorylation of the L/NS enzyme by the RNP-asso-
ciated protein kinase (39), although Schnitzlein and Reich-
mann (31) have suggested that the L/NS subunit ratio was
increased under conditions which favored transcription over
replication.

To test the possibility that protein phosphorylation regu-
lated L/NS enzyme function, we wanted to determine whether
the protein kinase activity was one of the functions of the
virus RNP proteins or the result of an associated host protein.
Published methods (9, 16, 22, 25) for purifying the VSV RNP
proteins did not, in our hands, yield proteins of sufficient
purity, when analyzed by silver nitrate staining, to allow us
to determine this. Previous methods have used high salt and
Renografin to remove L and NS proteins from the RNP
template and column chromatography with DEAE-Sephadex
and phosphocellulose and centrifugation to separate and pu-
ry the VSV RNP proteins. When we isolated virion RNPs
by centrifuging virions, lysed by Triton X-100, in a high salt
buffer through glycerol, the RNPs were not completely free
of M protein (12). In addition, application of high salt-dissoc-
iated proteins to DEAE-Sephadex or phosphocellulose col-
umns did not result in pure preparations of the individual
RNP proteins, using RNPs prepared from our strain of VSV.

In view of this, we have developed an alternative purifica-
tion method for our strain of VSV using Fractogel TSK which
is comprised of a hydrophilic vinyl polymer which is stable
in high concentrations of salt (11). We applied RNPs to a column
of Fractogel, using a KC1 concentration which was sufficiently
high to dissociate most of the L and NS proteins from the
RNP-N template but not high enough to cause release of N
protein from the RNP-N template. In 1 M KC1 the Fractogel
column allowed separation of partially purified RNPs, an L/
NS complex, and M protein (see Fig. 1A). The L/NS complex
thus obtained was greater than 95% pure, as determined by
silver nitrate staining. The L and NS proteins were subse-
sequently separated from each other by high speed centrifuga-
tion through sucrose gradients containing 1 M KC1 (see Fig. 3A).

Using proteins with known molecular weights as standards,
the apparent molecular weight of the L/NS complex, deter-
mimed by elution from the Fractogel column, was approxi-
ately 75,000. This apparent molecular weight was obviously
surprising since the molecular weight of L protein alone is
about 180,000. We could not determine the number of NS
molecules per L molecule in this complex because of the small
amount of material in our preparations. Naito and Ishihama
(25) showed that the active transcriptase probably was a
L:NS, heterodimer, but did not attempt to determine the
molecular weight of this complex. The apparently low mol-
ecular weight that we observed for our L/NS complex was not
due to degradation of either subunit since SDS-polyacrylam-
ide gels of the complex components (Fig. 1A) never showed
breakdown. This aberrant elution pattern is likely due to one
or more factors such as enzyme configuration in 1 M KC1,
charge effects of the Fractogel column, etc.

After obtaining purified enzyme subunits, we could then
assay these purified VSV proteins for protein kinase activity
plus and minus RNP-N template. We showed that the major
peak of protein kinase activity did not correspond exactly
with any of the VSV-specific proteins either eluted from the
columns or isolated on sucrose gradients. The kinase activity
associated with the RNP-N template was probably the result
of the same kinase since the decrease of activity associated
with RNP-N template resulted in a concomitant increase in
this major peak of kinase activity.

Reconstitution of purified L and NS proteins with the
RNP-N template did not restore protein kinase activity indi-
cating that formation of an RNP-enzyme complex was not
necessary for kinase activity (Table I). These data strongly
support the notion that the major VSV-associated protein
kinase activity is a host-derived protein, as was suggested
previously (18, 22). This was further substantiated by the
results showing no increase over background in protein kinase
activity when VSV-specific mRNAs were translated in vitro
to produce VSV proteins (Fig. 5).

Whatever the nature of this cellular protein kinase, it is
present in small amounts since we were unable to detect a
polypeptide which corresponded with the activity by silver-
staining polycrylamide gels of active preparations. In addi-
tion, it was tightly associated with the virion RNP since
considerable kinase activity was always found in the partially
purified RNP peak (Fig. 1) even after incubation with 2 M
CsCl and subsequent fractionation over Fractogel in the pres-
ence of 1 M KC1. Whether or not this association is a specific
one is impossible to determine at this time.

The protein kinase activity does not use IgG as a phosphate
acceptor (Fig. 2). IgG preparations specific for NS and M
proteins had no effect on either endogenous protein kinase
activity or activity with casein as an exogenous phosphate
acceptor. Phosphorylation of the src protein, pp59, also was
not inhibited by incubation with specific antisera (8, 21).

Since NS protein has been shown to be phosphorylated
shortly after synthesis in infected cells and is packaged into
infectious virus as a phosphoprotein, we found in rather
surprising that the RNP-associated kinase activity was, in all
likelihood, a host cell protein. This finding seemed puzzling
particularly since VSV seems to replicate quite well in such a
broad spectrum of vertebrate and invertebrate cells. This
broad host cell range, albeit not a natural in vivo range, would
suggest that such cell kinases, if necessary to VSV enzyme
function, are ubiquitous. Perhaps this matter could be par-
tially resolved by further examination of kinase activity as-
associated with VSV RNPs derived from a broad spectrum of
cells in culture.

With enzyme subunits and RNP-N template greater than
95% pure we have begun fine structural studies of the L/NS
enzyme to determine whether or not phosphorylation of one
or both subunits is required for enzyme regulation and/or
activation (5, 15, 20, 25, 34). We are presently utilizing our
purified enzyme preparations to establish the exact number
of phosphate residues per NS and L proteins and their loca-
tions on the NS molecule. These proteins were derived from
virion RNPs, actively transcribing RNPs, intracellular pools of
free NS proteins, etc.

This L/NS complex involved in VSV transcription and
replication which shows remarkable changes in template rec-
cognition and product formation very likely has enzyme coun-
terparts in all (−) strand animal virus systems such as influ-
enza, measles, arenaviruses, bunyaviruses, etc., which all con-
tain large multifunctional RNA polymerase molecules. Un-
derstanding the means by which these enzymes are modified
and regulated in transcription and replication will be impor-
tant to our understanding of the pathogenic mechanisms of
these agents.

These large multifunctional enzymes are also somewhat
similar to the well studied fatty acid synthase system of
animal cells which is a seven-enzyme complex, and the first
three enzymes responsible for the synthesis of orotic acid of
the pyrimidine biosynthetic pathway in mammals (19) which is a trimer of a 200,000-dalton protein. The VSV RNA polymerase, which is a heterodimer, is unique in the sense that the enzyme in transcriptive mode possesses five or more activities all of which are suppressed by an as yet undetermined mechanism to switch to a replicative enzyme. These many activities are likely directed by active sites located on two polymerase subunits, L and NS, a fact which implies an extreme example of the conservation of genetic information.

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The major ribonucleoprotein-associated protein kinase of vesicular stomatitis virus is a host cell protein.
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J. Biol. Chem. 1983, 258:15283-15290.

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