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Specific Interactions of Vesicular Stomatitis Virus L and NS Proteins with Heterologous Genome Ribonucleoprotein Template Lead to mRNA Synthesis In Vitro

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Two dissociable proteins, L and NS, and N-RNA template were purified from two serologically distinct vesicular stomatitis viruses, Indiana [VSV(IND)] and New Jersey [VSV(NJ)]. Requirements for RNA synthesis in heterologous reconstitution reactions in vitro were studied. The L and NS proteins of VSV(NJ) failed to synthesize full-length leader RNA and mRNAs in vitro when reconstituted with N-RNA(IND) template. However, when purified homologous NS(IND) was added to the reaction mixture, mRNA synthesis ensued. The requirements for transcription of N-RNA(NJ) template were different from those for N-RNA(IND). For RNA synthesis, transcription specifically required L(NJ), but the NS(NJ) and NS(IND) proteins were interchangeable. This suggests that there are specific domains on the L(NJ) protein, at which NS proteins of both serotypes may interact to form an active RNA polymerase complex, whereas L(IND) lacked such domains for interaction with NS(NJ). The function of the L protein appeared primarily to initiate RNA chains, and the NS protein was required for chain elongation. The results of these in vitro complementation experiments are discussed in light of previous in vivo complementation studies.

The transcribing ribonucleoprotein (RNP) of vesicular stomatitis virus (VSV) is a complex consisting of the single-strand genome RNA tightly associated with the nucleocapsid protein (N) and two soluble and easily dissociable proteins, L and NS (34). Transcription in vitro can also be achieved by the addition of dissociated L and NS proteins to purified transcriptionally inactive N-RNA complex (14). A requirement for both L and NS proteins has been demonstrated in this in vitro reconstitution reaction for mRNA synthesis (15, 25). In addition, monospecific antisera against L and NS proteins were shown to inhibit RNA synthesis by RNP in vitro (18, 19). From these studies, it has been envisaged that the template for RNA synthesis both in vitro and in vivo is the N-RNA complex, whereas the soluble proteins L and NS constitute the transcriptase and interact specifically with the template to initiate, elongate, and terminate five monocistronic mRNAs and a leader RNA (2). It is still unclear, how L and NS proteins recognize specific sites on the template and which of the above steps in RNA synthesis are mediated by these proteins. In addition, the precise roles of the N protein, which maintain the functional integrity of the template genome RNA in the transcription process, have not yet been established.

To determine the functions of the viral polypeptides L and NS in the transcription process, we have purified these proteins to study their interactions with the N-RNA complex leading to RNA synthesis in vitro (32). In the present studies, we used two serotypes of VSV, Indiana (IND) and New Jersey (NJ). Although these two viruses are serologically distinct, some relatedness in cross-reactivity of their RNPs has been reported (6). Biochemical studies have revealed that the sequences of leader RNA templated by the 3′-end of the genome RNA of these serotypes are very similar (11, 20): there is one base change in the first 24 bases and 9 base changes in the last 23 bases. Moreover, the 5′ terminal-capped AACAG sequence is conserved in all five mRNAs (16), and extensive homology is observed in the polyadenylic acid adjacent sequences of the N-mRNA of the two serotypes (29). Despite this homology in the important domains on the genome template which are involved in RNA polymerase binding (21), transcription initiation (13) (leader RNA and mRNAs), and termination (polyadenylic acid adjacent sequence), there appears to be less sequence homology in corresponding full-length mRNAs of the two serotypes (10). The recent complete nucleotide sequence of N-mRNA of VSV(NJ) (A. K. Banerjee, D. P. Rhodes, and D. S. Gill, Virology, in press) indicates that ca. 68% homology exists between the corresponding N protein sequence of VSV(IND) (17). Since interaction of N-RNA template with the soluble transcriptase results in RNA synthesis, heterologous in vitro transcription reconstitution systems offer an excellent system to study the specificity of such interactions of L and NS proteins of two serotypes with the heterologous N-RNA complex.

In this communication, we demonstrate that L and NS proteins of New Jersey serotype fail to transcribe N-RNA(IND), but require in addition, homologous NS protein for RNA synthesis. In contrast, N-RNA(NJ) requires homologous L protein and NS protein from either serotype for transcription of its genome RNAs in vitro. These results have given us an insight into the functions of L and NS proteins in mRNA synthesis in vitro.

MATERIALS AND METHODS

Purification of VSV. VSV(IND) (Mudd-Summers strain) and VSV(NJ) (Ogden strain) were grown in roller bottles containing monolayers of baby hamster kidney cells (BHK-21) and purified as previously described (3).

Isolation and purification of L and NS proteins. Purified VSV (4 mg at 500 μg/ml) of both serotypes was disrupted in 10 mM Tris-hydrochloride (pH 8.0) containing 5% (vol/vol) glycerol, 0.4 M NaCl, 1.85% Triton X-100, and 0.6 mM dithiothreitol (final concentrations) (buffer A). The RNP was sedimented by centrifugation in an SW60 rotor at 45,000 rpm

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for 2 h, through 30% (vol/vol) glycerol onto a 100% glycerol cushion. The RNP was collected from the cushion and diluted fivefold with buffer containing 10 mM Tris-hydrochloride, pH 8.0, and 1 mM EDTA. An equal volume of 2× buffer A containing 0.8 M NaCl (without Triton X-100) was added and kept on ice for 1 h. The mixture was centrifuged through 30% glycerol as described above. For RNP(NJ), disruption was carried out in the same buffer except that the Triton X-100 concentration was 0.1%. The released L and NS proteins were recovered from the top (ca. 2 ml) of the centrifuge tube, dialyzed against phosphocellulose-column buffer (PC buffer) containing 20 mM Tris-hydrochloride (pH 7.4), 10% glycerol, 0.1% Triton X-100, and 0.3 mM dithiothreitol for 12 h at 4°C. The dialysate was loaded onto a phosphocellulose column (Whatman) (1.5 by 3.5 cm) equilibrated with PC buffer. The unbound fraction, containing NS protein, was further washed with 8 ml of PC buffer and concentrated with an Amicon concentrator. The column was then washed with a two column volume of PC buffer containing 0.5 M NaCl. The L protein was eluted with a two column volume of PC buffer containing 1 M NaCl. The L protein fraction was dialyzed against 10 mM Tris-hydrochloride (pH 8.0) containing 10% glycerol, 0.2 M NaCl, and 0.3 mM dithiothreitol for 12 h at 4°C. The dialysate was concentrated as described above. The final protein concentrations of the L and NS fractions were 160 and 100 μg/ml, respectively. Both fractions were kept in −70°C before use.

Purification of N-RNA template. N-RNA template was collected in a glycerol pad after removal of the L and NS proteins by 0.8 M NaCl as described above. The template was treated again with buffer A containing 0.8 M NaCl, recovered by centrifugation through 15% (wt/vol) Renografin solution and collected on a 76% Renografin pad. The centrifugation was carried out in an SW60 rotor at 45,000 rpm for 2 h. The template was collected from the pad, diluted to 4 ml by the addition of buffer containing 10 mM Tris-hydrochloride (pH 8.0) and 1 mM EDTA, and further centrifuged and collected over a 30% glycerol pad. The template was recovered and kept at −70°C. The final concentration of N-RNA template was 750 μg of N protein per ml.

Homologous and heterologous reconstitution reactions. The reaction mixture (0.2 ml) contained 50 mM Tris-hydrochloride (pH 8.0), 5 mM $MgCl_2$, 4 mM dithiothreitol, 0.05 mM CTP, 1 mM each of ATP, GTP, and UTP, 20 μCi of [α-^{32}P]CTP (specific activity 410 Ci/mmol) (Amersham Corp., Arlington Heights, Ill.), 5 μl of N-RNA template of either serotype as described in the figure legends, 10 and 15 μl of L and NS fractions, respectively, of either serotype as described in individual experiments in each figure. These amounts of L and NS proteins, with respect to the amount of template used, were found to be saturating for optimal RNA synthesis in vitro. The NaCl concentration in each reaction was adjusted to 0.1 M. The reactions were carried out for 2 h at 30°C.

RESULTS

Purification of L and NS proteins. Purified VSV of both serotypes was first disrupted by Triton X-100 in the presence of 0.4 M NaCl. Under these conditions, essentially all of the viral G and M proteins were solubilized and could be removed by centrifugation. The pellets containing the N-RNA complex and L and NS proteins were further treated with 0.8 M NaCl to dissociate the latter proteins. Further purification of L and NS was achieved by phosphocellulose chromatography. The NS protein did not bind to the column and was recovered essentially free of L protein (Fig. 1, lane 1 [NS fraction]). A protein band migrating slightly faster than the NS protein was routinely seen in this preparation. By Western blot analysis with anti-N monoclonal antibody (12), the protein was shown to be N protein (data not shown). Presumably, a small fraction of N protein was released from the RNP by high salt treatment. The L protein that bound to the phosphocellulose column was eluted with 1 M NaCl (Fig. 1, lane 2 [L fraction]). A small fraction of the NS protein (ca. 5% of the total NS protein) contaminated the L protein fraction. In the case of L(IND), rechromatography of the L fraction in a phosphocellulose column virtually removed the NS protein (data not shown), whereas the corresponding purified L(NJ) protein retained a trace amount of NS protein which could be seen only by silver staining. The N-RNA complex was purified from both serotypes by the treatment of RNP with 0.8 M NaCl, followed by centrifugation on a Renografin cushion as described above. The purified N-RNA complex was transcriptionally inactive in vitro, and RNA synthesis occurred only after the addition of L and NS proteins (Fig. 2, lane A). The polyacrylamide gel electrophoresis pattern of in vitro-synthesized RNA in a typical reconstitution reaction is shown in Fig. 2. Addition of purified NS(IND) fraction to the N-RNA(IND) complex yielded virtually no RNA in vitro (Fig. 2, lane A). Similarly, addition of L(IND) fraction to the N-RNA(IND) complex yielded very little RNA (Fig. 2, lane B). Approximately a 30-fold stimulation of RNA synthesis occurred when saturating amounts of NS and L fractions, as determined by optimal RNA synthesis in vitro, were mixed (Fig. 2, lane C). These results indicated that NS protein alone was incapable of initiating RNA synthesis, but it significantly stimulated RNA synthesis when added together with the L fraction. When a similar reconstitution reaction was carried out with L(NJ) and NS(NJ) with N-RNA(NJ) template, a sixfold increase in

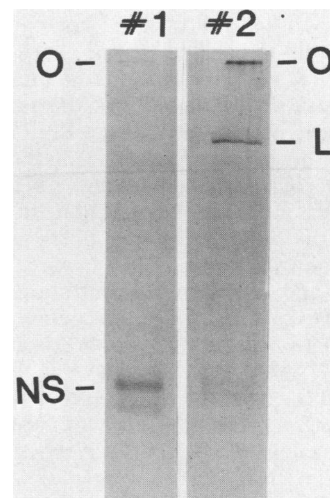

FIG. 1. Polyacrylamide gel electrophoresis of L and NS fractions of VSV(IND). The isolation and purification of L and NS proteins from purified virions are detailed in the text. Each fraction was analyzed by 10% polyacrylamide-sodium dodecyl sulfate gel electrophoresis (5). The proteins were fixed and stained with silver reagents (Bio-Rad Laboratories, Richmond, Calif.) derived by the method of Merrill et al. (24). Lane 1, Phosphocellulose unbound fraction; lane 2, 1 M NaCl eluate. Migration positions of L and NS are shown. O, origin.

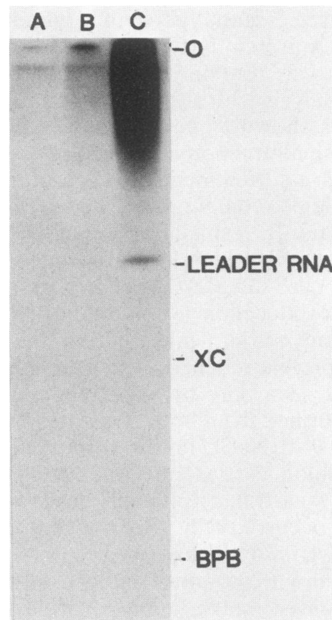

FIG. 2. Homologous reconstitution reaction with Indiana viral components. In vivo reconstitution reactions with N-RNA template and NS alone (A), L alone (B), and L + NS (C) were carried out as described in the text. The RNA products were analyzed by electrophoresis at 600 V for 15 h on a 20% polyacrylamide slab gel (30 by 14 by 0.15 cm) containing 8 M urea. RNA bands were located by autoradiography on Kodak XR-15 film. The migration positions of leader RNA, xylene cyanol (XC), and bromophenol blue (BPB) are shown.

RNA synthesis was observed. A small amount of RNA synthesis with L(NJ) alone was due to the presence of trace quantities of NS protein in purified L(NJ). It should be noted that the molar ratio of template and L and NS proteins in the reconstitution reactions was 1:0.1:1.2 compared to the ratio of 1:0.02:0.2 present in purified virions. Thus, the increased requirements for L and NS proteins in the reconstitution reaction demonstrates the inability of the system to mimic the structure of the native transcribing RNP complex.

Requirements for RNA synthesis from the N-RNA(IND) template in a heterologous reconstitution reaction. The RNA synthesizing ability of L and NS fractions of VSV(NJ) was first tested with the N-RNA(IND) complex as template. All heterologous reconstitution reactions were carried out at optimal protein and template concentrations (see above). The RNA synthesized in the heterologous reconstitution reactions was analyzed by electrophoresis in a 20% polyacrylamide gel. The results are shown in Fig. 3. To test whether purified L and NS fractions of VSV(NJ) were active, homologous reconstitution with N-RNA(NJ) was carried out. Efficient RNA synthesis occurred with the synthesis of leader RNA and mRNAs (radioactivity remaining at the top of the gel) (Fig. 3, lane A). However, purified L and NS fractions of VSV(NJ) failed to synthesize leader RNA and large RNA products when added to heterologous N-RNA(IND) complex (Fig. 3, lane B). A small amount of RNA was synthesized, the majority of which migrated with the bromophenol blue dye. Thus, the above results indicated that fractions L(NJ) and NS(NJ) failed to transcribe full-length leader RNA and mRNAs when mixed with the heterologous N-RNA(IND). However, an important observation was that when the NS(IND) was added to the L(NJ) and NS(NJ) fractions, efficient transcription ensued with the synthesis of leader RNA and mRNA species (Fig. 3, lane D). As expected, purified NS(IND) alone was inactive when added to N-RNA(IND) (Fig. 3, lane C). To further confirm that full-length mRNAs were indeed synthesized from the N-RNA(IND) complex reconstituted with L(NJ) and NS(NJ) fractions and NS(IND), the products were analyzed by velocity sedimentation. The RNA products sedimented with speed characteristic for VSV mRNAs only under the condition that NS(IND) was present in addition to the L(NJ) and NS(NJ) fractions to transcribe the N-RNA(IND) complex (Fig. 4). The above results clearly indicated that to transcribe the N-RNA(IND) complex there was a specific requirement for homologous NS protein, whereas the homologous L function could be replaced by the corresponding heterologous L fraction. It should be noted that the extent of RNA synthesis in Fig. 3, lane D was ca. 60% the amount synthesized when saturating amounts of homologous L and NS proteins and N-RNA(IND) template were used.

In the above studies, both L(NJ) and NS(NJ) fractions were added to the N-RNA(IND) complex. Thus, it was not clear whether there was also a specific requirement for the NS(NJ) fraction for heterologous transcription in vitro. The NS(NJ) fraction (Fig. 5, lane A) and the NS(IND) fraction (lane D) were inactive in transcription when added individually to the N-RNA(IND) complex. When L(NJ) alone was added to NS(IND) (Fig. 5, lane B), virtually no full-length leader RNA and mRNAs were synthesized. However, when NS(NJ) was subsequently added, RNA synthesis ensued as shown by the synthesis of both leader RNA and mRNAs (Fig. 5, lane C). These results clearly indicated that L(NJ),

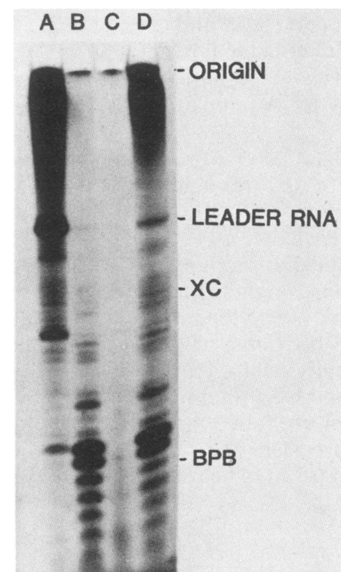

FIG. 3. Heterologous reconstitution reactions with N-RNA(IND) template. Reconstitution reactions with N-RNA(IND) template in vitro were carried out as detailed in the text. The RNA products were analyzed by polyacrylamide gel electrophoresis, followed by autoradiography as described in the legend to Fig. 2. Reconstitution with N-RNA(IND) template and L(NJ) + NS(NJ) (lane B), NS(IND) (lane C), or L(NJ) + NS(NJ) + NS(IND) (lane D); and reconstitution with N-RNA(NJ) + L(NJ) + NS(NJ) (lane A) are shown. XC, Xylene cyanol; BPB, bromophenol blue.

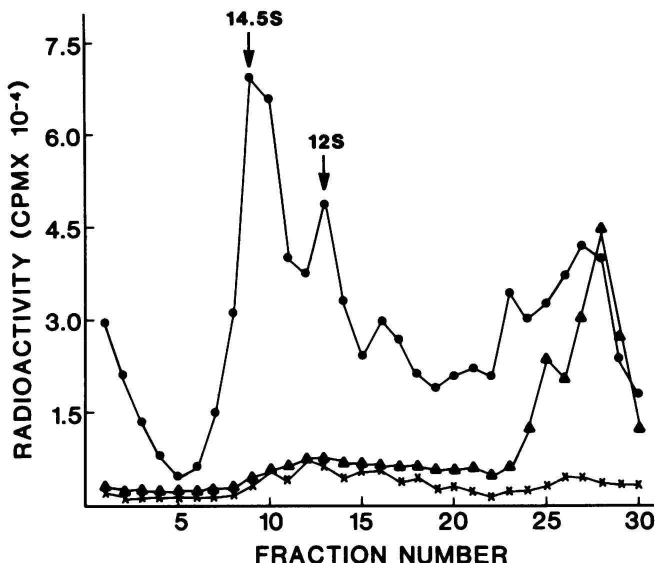

FIG. 4. Velocity sedimentation analysis of the in vitro RNA products synthesized in heterologous reconstitution reactions with N-RNA(IND) template. The RNA products synthesized in experiments described in the legend to Fig. 3 were analyzed by sedimentation in a 15 to 30% (wt/vol) sucrose gradient (30) in a Spinco SW40 rotor at 33,000 rpm at 23°C for 17 h. Fractions were collected from a hole pierced at the bottom of the tube, and acid-precipitable radioactivity in each fraction was determined. Symbols: X, RNA products synthesized by N-RNA(IND) template with NS(IND) along; ▲, those synthesized with L(NJ) + NS(NJ); ●, those synthesized with L(NJ) + NS(NJ) + NS(IND). The sedimentation coefficients of 14.5*s* (N-mRNA) and 12*s* (NS and M-mRNAs) are shown. Direction of sedimentation is from right to left.

NS(NJ), and NS(IND) were obligatory requirements for transcription of heterologous N-RNA(IND) template.

We have previously shown (8, 32) that the initiation of RNA synthesis in vitro by transcribing RNP can be studied by using a partial reaction containing ATP and [α-^{32}P]CTP as the only ribonucleoside triphosphate precursors. The synthesis of pppAC (and pppAACA) were indicative of the binding of enzyme with the template and occurrence of initiation on leader RNA and mRNA templates, respectively. Similar partial reactions were carried out in homologous and heterologous reconstitution reactions with various soluble fractions. L(NJ) bound and initiated oligonucleotides I (AC) and II (AAC + AACA) on the homologous N-RNA(NJ) template (Fig. 6, lane A). Similarly, L(IND) synthesized both oligonucleotides on the N-RNA(IND) template (Fig. 6, lane B), whereas NS(IND) alone virtually failed to initiate RNA chains on the homologous template (lane C). However, initiation of oligonucleotide synthesis occurred when L(NJ) was added to the N-RNA(IND) template (Fig. 6, lane D). The level of initiation was identical to that observed in a homologous reaction (Fig. 6, lane B), indicating that L(NJ) bound to heterologous N-RNA(IND) template and initiated RNA chains. Virtually no oligonucleotides were synthesized by purified L or NS preparations in the absence of the template (data not shown). Since addition of homologous NS protein was essential for a full-length mRNA synthesis (Fig. 2), it appears that the initiation function for transcription of the N-RNA(IND) template can be equally provided by L(NJ) and L(IND), whereas the elongation function resides specifically in the homologous NS protein.

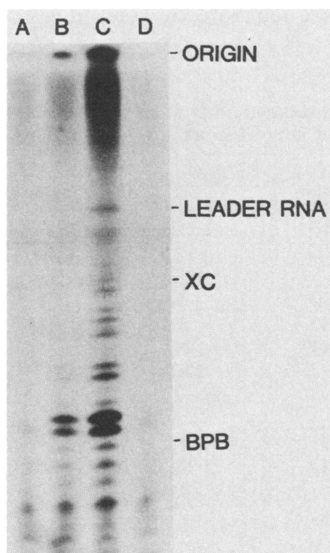

FIG. 5. Requirements for transcription of N-RNA(IND) template in heterologous reconstitution reactions. In vitro reconstitution reactions were carried out as described in legend to Fig. 3, and RNA products were analyzed by polyacrylamide gel electrophoresis. RNA products synthesized by N-RNA(IND) template with NS(IND) alone (lane A), L(NJ) + NS(IND) (lane B), L(NJ) + NS(NJ) + NS(IND) (lane C), and NS(NJ) alone (lane D) are shown. XC, Xylene cyanol; BPB, bromophenol blue.

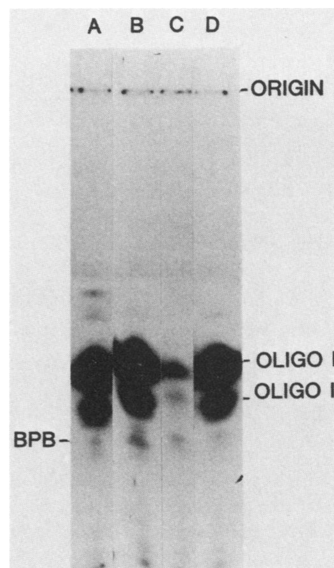

FIG. 6. Synthesis of oligonucleotides in the presence of ATP and [α-^{32}P]CTP in homologous and heterologous reconstitution reactions. Partial transcription reactions were carried out with N-RNA(NJ) + L(NJ) (lane A), N-RNA(IND) template + L(IND) (lane B), N-RNA(IND) + NS(IND) (lane C), and N-RNA(IND) + L(NJ) (lane D) in the presence of 1 mM ATP and 30 μM CTP containing 100 μCi of [α-^{32}P]CTP. After incubation for 2 h at 30°C, the oligonucleotides were isolated and treated with calf intestinal alkaline phosphatase (8), analyzed by electrophoresis in a 20% polyacrylamide gel, and autoradiographed. Oligonucleotide I (Oligo I) and oligonucleotide II (Oligo II) represent AC and a mixture of AAC and AACA, etc., respectively (8, 32). BPB, Bromophenol blue.

Requirements of RNA synthesis from the N-RNA(NJ) template in a heterologous reconstitution reaction. To study whether there was a similar specific requirement for NS(NJ) to transcribe N-RNA(NJ) in a heterologous reconstitution reaction, similar in vitro RNA synthesis was carried out as described above. In sharp contrast to the NS(IND) requirement for N-RNA(IND) template transcription, very little RNA was synthesized when a mixture of L(IND), NS(IND), and NS(NJ) was added to the N-RNA(NJ) template (Fig. 7, lane C). The product RNA was small and contained insufficient radioactivity for further analysis. In control experiments, RNA synthesis was normal in homologous reconstitution reactions for N-RNA(NJ) (Fig. 7, lane A) and N-RNA(IND) (lane B). These results indicated that, unlike L(NJ), L(IND) may lack specificity to bind NS(NJ) to synthesize RNA chains.

We next tested the L(NJ) requirement for transcription of the N-RNA(NJ) template. L(NJ) synthesized a small amount of RNA when added to the homologous N-RNA(NJ) template (Fig. 8, lane A). This residual RNA synthesis by the L fraction was due to the presence of contaminating NS protein. When NS(NJ) was added to L(NJ), as expected, a sixfold stimulation in RNA synthesis was observed (Fig. 8, lane B). Interestingly, when NS(NJ) was replaced by NS(IND), identical stimulation of RNA synthesis was observed (Fig. 8, lane C). These results indicated that the N-RNA(NJ) template, in contrast to the N-RNA(IND) template, specifically required homologous L protein, whereas NS protein could be interchanged. In separate experiments, using the N-RNA(IND) template and L(IND), NS(NJ) could not replace the function of NS(IND) in stimulating RNA synthesis (data not shown). From these results, we concluded that L(NJ) could interact with NS proteins of both serotypes, whereas L(IND) was specific for NS(IND). Thus, the N-RNA template for the two serotypes demonstrated contrasting requirements (and specificity) for transcription of their genome templates.

Table 1 summarizes the results of a series of reconstitution experiments. There were specific requirements for transcribing each N-RNA template in heterologous reconstitution

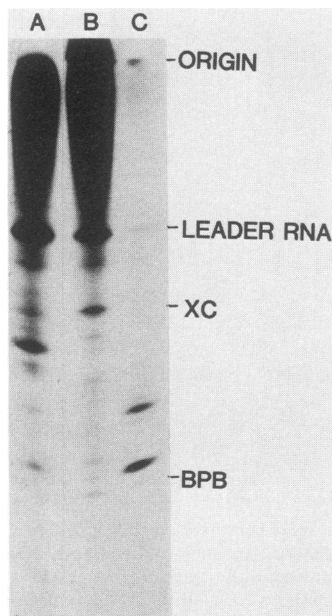

FIG. 7. Heterologous reconstitution with N-RNA(NJ) template. Reconstitution reactions in vitro were carried out with N-RNA(NJ) + L(NJ) + NS(NJ) (lane A), N-RNA(IND) + L(IND) + NS(IND) (lane B), and N-RNA(NJ) + L(IND) + NS(IND) + NS(NJ) (lane C) as described in the text. The RNA products were analyzed by polyacrylamide gel electrophoresis followed by autoradiography. XC, Xylene cyanol; BPB, bromophenol blue.

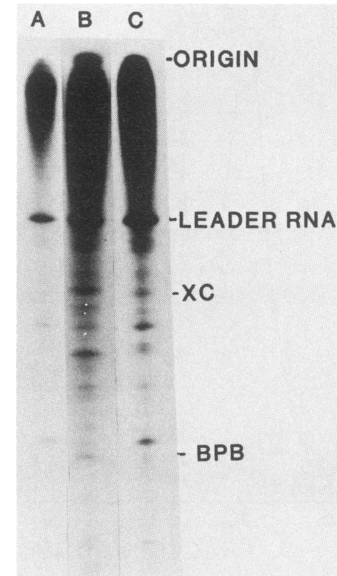

FIG. 8. Requirement for transcription of N-RNA(NJ) template in heterologous reconstitution reactions. In vitro reconstitution reactions were carried out as described in the legend to Fig. 3, and RNA products were analyzed by polyacrylamide gel electrophoresis. RNA products synthesized by N-RNA(NJ) template with L(NJ) alone (lane A), L(NJ) + NS(NJ) (lane B), and L(NJ) + NS(IND) (lane C) are shown. XC, Xylene cyanol; BPB, bromophenol blue.

TABLE 1. Requirements of L and NS fractions for transcription of heterologous N-RNA template

Template N-RNA	L fraction	NS fraction	RNA synthesis[a]	Initiation reaction[b]
IND	IND	IND	+	+
IND	IND		−	+
IND		IND	−	−
IND	IND	NJ	−	+
IND	NJ		−	+
IND	NJ	NJ	−	+
IND	NJ	IND	−	+
IND	NJ	NJ + IND	+	+
NJ	NJ		−[c]	+
NJ	NJ	NJ	+	+
NJ		NJ	−	−
NJ	NJ	IND	+	+
NJ	IND		−	+
NJ	IND	IND	−	+
NJ	IND	NJ	−	+
NJ	IND	IND + NJ	−	+

[a] +, RNA synthesis; −, denotes no RNA synthesis.

[b] +, Synthesis of initiated oligonucleotides in a partial reaction (Fig. 6 and 8); −, no initiation.

[c] RNA synthesis in the experiment was low yet reproducible (Fig. 8, lane A). This was due to the presence of contaminating NS in the L fraction (see text).

reactions. For the N-RNA(IND) and N-RNA(NJ) templates, specificities resided in the NS protein and L protein, respectively. Under all conditions, heterologous L proteins bound to the template and were capable of initiating oligonucleotides, whereas NS protein failed to initiate oligonucleotide chains.

DISCUSSION

Although significant advancement has been made in understanding the mode of transcription of VSV genome RNA in vitro by using the transcribing RNP, little is known regarding the precise roles played by the soluble transcriptases L and NS and the N-RNA template in the synthesis of mRNA in vitro. Reconstitution of RNA synthesizing activity by dissociated L and NS with purified N-RNA template (15) offers an excellent opportunity to study the nature of interactions of these proteins with the template. Moreover, availability of different serotypes of VSV allows design of suitable heterotypic reconstitution reactions to probe the specificity of interactions of each of the soluble proteins with the template N-RNAs.

In the present studies, we purified L and NS proteins of VSV(IND) and VSV(NJ) and showed that in a heterologous reconstitution reaction there was a specific obligatory requirement of L or NS functions for transcription of either serotype of VSV. For transcription of VSV(IND) genome, the L function could be replaced by the L and NS proteins of VSV(NJ). However, there was a specific requirement for NS(IND) to synthesize mRNAs (Fig. 2). It is important to note that L(NJ) alone was not able to replace the L(IND) function (Fig. 4); the presence of NS(NJ) was essential to impart transcriptional ability to the complex. Interestingly, L(NJ) alone initiated leader and mRNA-specific oligonucleotides on the N-RNA(IND) template (Fig. 6). Thus, the NS(NJ) requirement may possibly be necessary to stabilize the heterologous complex (23). Thus, it seems that there may be two distinct sites of interaction of NS(IND) and NS(NJ) on the transcribing N-RNA(IND) complex. NS(NJ) appears to interact with the L(NJ), and NS(IND) may interact with either L(NJ)-NS(NJ) complex or homologous N protein to synthesize RNA. In any event, it was apparent from the results that the function of L protein was to initiate RNA chains. The function of the homologous NS protein, which lacks RNA synthesizing activity alone, was to promote elongation of RNA chains. Thus, L and NS proteins of VSV are possibly subunits of the RNA polymerase complex having two specific functions for RNA synthesis. It should be noted that our results do not rule out the possibility that for initiation the L protein needs a high-affinity NS protein bound tightly at the initiation site which was not removed by high salt treatment of the template (33). Efficient RNA chain elongation, however, required additional excess of NS protein. It is interesting to note that the specificity of transcription of N-RNA(IND) template resides in the homologous NS protein. Thus, it seems that in addition to its interactions with heterologous L protein, there may be a specific interaction with homologous N-protein which imparts the observed specificity. In this respect, the NS(IND) resembles the sigma protein of bacterial DNA-dependent RNA polymerase, which lacks RNA synthesizing capability yet imparts specificity for promoter recognition by the core enzyme (7).

In contrast to the observed requirements for transcription of the N-RNA(IND) template, the N-RNA(NJ) template specifically needed the homologous L protein, whereas the NS protein could be totally replaced by heterologous NS protein (Fig. 7 and 9). Since L(IND) interacted with the N-RNA(NJ) template and initiated oligonucleotides (Table 1), the inability of NS(NJ) to yield RNA indicates that the protein may not interact with L(IND) to form a stable RNA polymerase complex. In contrast, NS(IND) can interact with L(NJ) to form an active RNA polymerase complex to transcribe the N-RNA(NJ) template (Fig. 8). The simplest interpretation for this specificity is that L(IND) lacks putative sites or domains at which NS(NJ) may interact, whereas L(NJ) possesses such domains for interactions with both NS(IND) and NS(NJ). Such putative sites or domains in the L protein may be located in the amino acid sequences or be generated by specific interactions with the N proteins and the RNA template. Complete nucleotide sequencing of the NS(NJ) gene (work currently in progress) and subsequent comparison with the published NS(IND) gene sequence (17) should help to locate areas of sequence homology, if any, in the two genes. Although the precise reasons for these specific requirements for transcription of heterologous templates are not presently understood, the above results clearly indicate that specific interactions between the L and NS proteins and also with the N protein-RNA complex determine the capability of the complex to transcribe the genome template. It is important to note that despite considerable sequence homology between the N genes of both serotypes (Banerjee et al., in press) a monoclonal antibody raised against N(IND) failed to cross-react with N(NJ) (12), indicating that there is an exposed epitope in the N-RNA(IND) complex which is highly specific to the Indiana serotype. Thus, it is possible that N protein may also play a role in this observed specificity in heterologous reactions. Further investigations along these lines would help delineate the nature and specificity of the protein-protein and protein-RNA interactions involved in the VSV transcription processes in vitro.

Finally, the above results have now given us insight into many of the previously unexplained phenomena in VSV replication processes. The heterotypic inhibition of VSV(NJ) by the defective interfering particles (DI-LT)(IND), which lack the L gene, can now be explained (26, 31). Wild-type L(NJ) can substitute for the L(IND) function during co-infection, resulting in efficient replication of DI-LT(IND) and the inhibition of VSV(NJ). Moreover, during coinfection, association of L and NS proteins of VSV(NJ) with replicating DI-LT particles has also been reported (9). This observation was expected since, as shown above, both L and NS proteins of VSV(NJ) are probably needed to transcribe DI-LT(IND) genome RNA. On the other hand, Adachi and Lazzarrini (1) reported that DI-LT(IND) can replicate well in the presence of wild-type VSV(NJ) Hazelhurst strain (and probably also Ogden strain) without inhibiting replication of VSV(NJ). Thus, it seems that heterotypic inhibition or noninhibition is dependent on the strain of VSV(NJ) used. Further studies should reveal the mechanism of interference of these DI-LTs in this heterologous coinfected system. In addition to the above results, partial complementation of temperature-sensitive mutants of group I of VSV(IND) by the wild-type VSV(NJ) at the nonpermissive temperature (29) is probably due to transcriptive performance of L function by wild-type L(NJ). Moreover, our results confirm earlier studies (4), demonstrating the inability of L and NS proteins of VSV(NJ) to transcribe the N-RNA(IND) template. We showed here that, in addition, NS(IND) was essential to transcribe its homologous template.

Despite these heterologous interchanges of proteins in vitro and in vivo by these two serotypes of VSV, no genetic complementation has been shown to occur between mutants

of different rhabdoviruses, including the Indiana and New Jersey serotypes (27). Although the precise reasons for this phenomenon are not known, the absence of complementation in vivo may be due to well-known heterotypic interference between VSV(IND) and VSV(NJ) (22). Clearly, this heterotypic interference is not absolute, since one-step simultaneous infection can be achieved under certain circumstances (28). The fact, as shown in our studies, that heterotypic exchange of proteins is clearly possible in vitro indicates that further studies are needed to better understand whether similar heterotypic interactions are also effective in vivo.

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