JOURNAL OF VIROLOGY, July 1982, p. 241–249
0022-538X/82/070241-09$02.00/0

Vol. 43, No. 1

RNA Polymerase-Associated Interactions Near Template Promoter Sequences of Defective Interfering Particles of Vesicular Stomatitis Virus

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Received 30 November 1981/Accepted 17 March 1982

Methylation protection studies suggested that the NS protein component of the RNA polymerase of vesicular stomatitis virus contacts the RNA templates of defective interfering (DI) particles at the sequence 3'...GUCUAUUUUUUAUUUUUGGUG...5', 17 to 37 nucleotides downstream from the site of initiation of in vitro transcription. The data indicated that vesicular stomatitis virus and DI particle RNAs contain different polymerase binding sequences and that NS may function as a transcription initiator protein for template recognition at both sequences. These results are thus compatible with the hypothesis that differences in the rate of defective and nondefective viral particle replication and autointerference are due to higher-affinity binding sites for polymerase at the 3' end of DI particle RNAs. In addition, a unique DI particle (DI-LT_2) RNA that contains a transcriptionally inactive vesicular stomatitis virus leader gene 72 to 118 nucleotides from its 3' end showed interactions with the viral polymerase similar to those reported previously for the 3'-terminal vesicular stomatitis virus leader gene (Keene et al., Proc. Natl. Acad. Sci. U.S.A. 78:6191–6195, 1981). The interaction of polymerase with the internal leader gene of DI-LT_2 RNA suggested that the lack of leader RNA and mRNA production by this particle is not due to the inability of polymerase to bind to internal sites along the template. Instead, the initiation of transcription is more likely influenced by the position of the polymerase binding site relative to the 3' end or by requisite interactions between the catalytic polymerase component (L) and the proposed initiator protein (NS).

Vesicular stomatitis virus (VSV), a member of the rhabdovirus group, produces several different types of defective interfering (DI) particles upon high-multiplicity passage. These DI particles replicate and often decrease the titer of infectious virus, a phenomenon known as autointerference (10). Most DI particles contain RNAs derived largely from the 5' half of the parental genome and have deleted the 3' half. A few DI particle RNAs, however, are derived from the 3' half of the VSV genome (4, 20, 35). Unlike VSV RNA, the majority of the DI particle RNAs have long complementary termini, and several models have been proposed to explain their origins (11, 13, 21). Recently, a unified model for the origin of DI particles that emphasizes several common properties of the VSV polymerase has been proposed (18).

One DI particle RNA derived from the 3' half of the VSV genome, DI-LT_2, has a unique RNA structure with both an internal deletion and complementary termini (9, 13, 27). An RNA sequence analysis of the 3' end of DI-LT_2 has revealed that beyond a 70-base region of terminal complementarity, the VSV leader gene and the genes for the N, NS, M, and G mRNAs are present. Interestingly, DI-LT_2 is transcriptionally inactive for the production of leader or mRNA, although it does synthesize the same small DI particle product that is synthesized from the 3' end of most other VSV DI particles (6, 31, 32). In contrast, nondefective VSV particles synthesize leader RNA and mRNAs in vitro in the presence of detergent and nucleoside triphosphates (3). Both VSV and its DI particles contain an associated RNA polymerase which has two components, the NS protein (molecular weight = 32,000) and the L protein (molecular weight = 190,000) (8).

DI particles require the presence of the nondefective helper virus to supply deleted functions for replication (10). Several studies have indicated that autointerference may result from the competition by DI particle templates for a helper virus function, probably replicase. Thus, the DI particle has a replicative advantage over the nondefective virus (26), and this advantage may be reflected in the affinity of replicase for the

template initiation sites. Sequence analyses of the RNAs of both the Indiana and New Jersey serotypes of VSV have revealed that the 3'-terminal sequences of the infectious and DI RNAs are largely homologous for about 20 nucleotides (14). Thus, a mechanism of autointerference based on higher-efficiency initiation by polymerase at the 3' terminus of the DI particle RNA is only plausible if sequences beyond the first 20 nucleotides are involved in recognition.

In view of the unusual transcriptional properties of DI particles and autointerference, it is important to determine the sites of polymerase interaction along the RNA template. Recently, we have presented evidence that there are two, possibly overlapping, sites on the VSV nondefective virus template that are involved in the initiation of transcription: a terminal initiation site where synthesis begins and an adjacent binding site (17). We have proposed that the NS protein, in binding to the template at the latter site, facilitates initiation by the L protein at the former site. In these studies, VSV nucleocapsids containing NS protein were examined by using methylation protection with dimethyl sulfate (DMS) (33). The region of NS interaction with the VSV RNA template was detected in the middle of the leader gene about 16 to 30 nucleotides from the 3' terminus.

In this study, we performed methylation protection analysis of RNA in nucleocapsids from several DI particles, including DI-LT_2. We found NS-associated interactions within the internally positioned leader gene of the DI-LT_2 and in a region approximately 17 to 37 nucleotides from the 3' termini of all DI particles examined. The base sequence in the latter region of the DI particle RNAs differed from that of the VSV leader RNA gene, although it is also A+U rich and is similarly positioned with respect to the 3' end. Thus, we conclude that base contact sites for polymerase at the 3' end occur largely in regions of nonhomology between the DI and VSV RNAs. We discuss these findings with respect to transcription, replication, and autointerference.

MATERIALS AND METHODS

Cells and viruses. DI particles of VSV Indiana were grown in BHK-21 monolayer culture as previously described (12). The virus particles were purified by two isopycnic bandings and rate velocity sedimentation in 10 to 40% sucrose gradients (16, 35).

Isolation of viral nucleocapsids. Purified DI particles were lysed in 1× HSS (0.72 M NaCl, 1.87% Triton X-100, 6×10^{-4} M dithiothreitol, 9.35% glycerol, 0.05 M Tris-hydrochloride [pH 7.4]) for 30 min either on ice or at 30°C (7). Nucleocapsids containing N and NS proteins were prepared by centrifugation of lysed DI particles for 15 h at 24,000 rpm in an SW27 rotor or at 27,000 rpm in an SW41 rotor through 15.2% Renografin in HSS and onto a shelf of 76% Renografin (8). The band at the 15.2%–76% Renografin interface was removed, diluted with 12 ml of 1× HSS, and pelleted onto a pad of glycerol for 2 h at 37 rpm in the SW41 rotor. The nucleocapsid pellets were suspended in 200 μl of cacodylate buffer and analyzed (methylation protection). The nucleocapsids were further purified by banding twice in 15 to 47% Renografin in a buffer of 100 mM NaCl and 10 mM Tris-hydrochloride (pH 7.4). Occasionally, two cycles of solubilization in HSS and centrifugation in Renografin step gradients were used before banding in linear Renografin gradients. The linear Renografin gradients were centrifuged for 16 h at 16,000 rpm in the SW41 rotor (17) or at 23,000 rpm in the SW27 rotor. Rate gradients of 15 to 35% glycerol were sometimes used to remove ribonucleoprotein aggregates. Centrifugation was for 2 h at 31,000 rpm.

Methylation protection. Isolated nucleocapsids or phenol-extracted RNAs were suspended in 200 μl of DMS buffer containing 50 mM sodium cacodylate (pH 7.0) and 10 mM $MgCl_2$ (25, 33). DMS (1 μl) was added to each sample, and the samples were then blended in a Vortex mixer and incubated at 30°C for 12 min. The DMS was inactivated by the addition of 50 μl of stop buffer (1.0 M mercaptoethanol, 1.5 M sodium acetate, 1 M Tris-acetate [pH 7.5], 0.1 mM EDTA). Methylated nucleocapsids were suspended in buffer containing 2 mM dithiothreitol, 100 mM NaCl, 10 mM Tris-hydrochloride (pH 7.4), 1.5 mM $MgCl_2$, and 10 mM KCl and were pelleted onto a pad of glycerol. The pelleted nucleocapsids were suspended in 300 μl of buffer (0.1 M NaCl, 0.05 M Tris-hydrochloride [pH 7.4], 0.01 M EDTA), adjusted to 0.5% sodium dodecyl sulfate, phenol extracted, and ethanol precipitated. Methylated RNA was labeled at the 3' end with cytidine 3',5'-bisphosphate as described previously (15, 16).

The terminally labeled RNA was either loaded directly onto sequencing gels or subjected to borohydride reduction and beta elimination with aniline (24). Before this treatment, in some cases, the DI-LT_2 RNA from nucleocapsids was purified on 10 to 30% sucrose-sodium dodecyl sulfate gradients to assure complete removal of VSV 42S RNA. All samples were analyzed on urea-acrylamide sequencing gels.

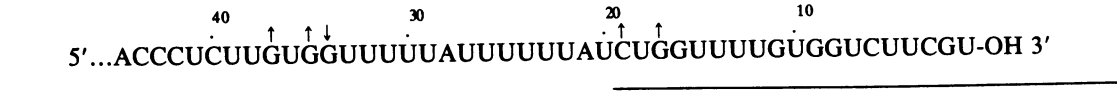
Sequence analysis. RNA sequences were determined by the chemical RNA sequence procedures of Peattie (24).

RESULTS

The active template for transcription in both VSV and its DI particles is a ribonucleoprotein complex consisting of the RNA and a tightly bound nucleocapsid protein, N (7). The presence of this protein on the RNA does not limit the accessibility of the bases to methylation by DMS (17). Thus, it is possible to analyze the template for specific contact points with other RNA-binding proteins. Alkylating agents have been used as probes to detect specific regions of protein-nucleic acid interaction (33) because the reactivity of the bases to alkylation is often altered when protein is bound. DMS reacts with RNA bases in the order G > C > A > U, and the

alkylation of phosphate groups in RNA has been reported (34).

Polymerase contacts at the 3′ end of DI particle RNAs. Nucleocapsids from purified DI particles were prepared by solubilization in HSS. This procedure solubilizes most of the viral proteins except the N protein, some NS protein, and possibly some L protein (7). The nucleocapsids were further purified by centrifugation through 15.2% Renografin onto a 76% Renografin shelf (8). After recovery of the nucleocapsids and treatment with DMS, the remaining proteins were removed by phenol extraction, and the RNA was labeled at the 3′ end with RNA ligase and cytidine 3′,5′-bisphosphate (16). Borohydride reduction and chain scission (aniline treatment) of the RNA produced fragments that were analyzed on RNA sequencing gels (15, 24). Figure 1 shows a comparison of RNA from DMS-treated DI-T nucleocapsids (lane B) and DMS-treated DI-T RNA (lane A). Lanes C and D show the U and C-U sequence markers, respectively, of DI particle RNA (15, 24). The methylation patterns to base 15 from the 3′ end show no significant differences between the RNA (lane A) and the nucleocapsids (lane B). However, beginning with position 17 and extending to about position 37, there are dramatic differences between deproteinized RNA and nucleocapsid RNA. The G residue in position 17 is enhanced, a new band appears at the C residue in position 19, and the G residues in positions 34 to 37 are altered. These alterations are indicated in Fig. 1 by arrows. Identical results were obtained with several DI particles, including DI-T, DI-011, DI-611, and DI-LT$_2$. The relative band intensities varied slightly from experiment to experiment, and some minor differences were occasionally noted at other positions, but the altered methylation patterns obtained when RNA and nucleocapsids in the region 17 to 37 nucleotides from the 3′ end were compared were exceptionally intense and highly reproducible. These sequence perturbations are denoted below as enhancements (↑) and protections (↓) of base methylation:

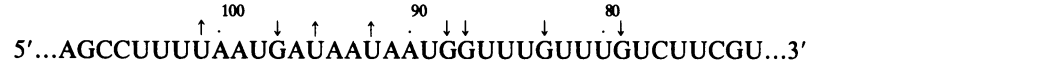


We conclude that a viral protein(s) interacts specifically with the DI particle RNA in the region 17 to 37 nucleotides from the 3′ end and alters the reactivity of base residues with DMS. As described below, the reduction of sequence perturbations in this region correlated with removal of the viral NS protein.

The DI particles used in this study have conserved at least 350 nucleotides from the 5′ end of VSV RNA. RNA sequencing has confirmed that the 3′ ends of the DI particle RNAs are complements of the 5′ end for 46 to 70 nucleotides (13, 30). In addition, DI-011 RNA has complementary termini for its full length of about 800 nucleotides (19). Thus, all of the DI particles examined have identical 3′ sequences up to position 46. DI-LT$_2$ is unique in that it contains the common DI particle 3′ sequence at its 3′ terminus as well as the VSV 3′ sequence (leader gene) at an internal position 71 nucleotides from its 3′ end. This unusual structure allows a comparison of polymerase interactions at this internal leader gene with those previously reported for the VSV terminal leader gene (17).

Polymerase contacts at the internal leader gene of DI-LT$_2$. Nucleocapsids were prepared from a purified stock of DI-LT$_2$ and were DMS treated as described above. After phenol extraction, the 31S DI RNA was isolated as described above. After 3′ labeling and chain scission (aniline treatment), the RNA fragments were analyzed on 12% polyacrylamide gels. Figure 2 shows the methylation pattern encompassing the leader gene of DI-LT$_2$ RNA (lane A) and DI-LT$_2$ RNA methylated in nucleocapsids (lane B). Lane C shows the A−G sequence of DI-LT$_2$ RNA as a marker (13). Lane D shows RNA fragments from the top of the 10 to 30% sucrose gradient used to purify the DI-LT$_2$ 31S RNA of lane B. Lane E shows the DMS-treated DI-LT$_2$ nucleocapsid RNA before chain scission at methylated bases. Lanes D and E were included because previous studies indicate that DMS may produce direct strand scissions at certain bases without aniline treatment (17). The numbers on the left side of Fig. 2 indicate the sequence position of each G residue in the DI-LT$_2$ RNA and the analogous positions of G residues in the VSV RNA leader gene (13). There was dramatic protection from methylation in the RNA from DI-LT$_2$ nucleocapsids between positions 73 and 107 (Fig. 2, lane B). This region has the same sequence as the proposed site of NS protein interaction found in the leader gene of VSV nucleocapsids (17), but appears to encompass a larger portion of the RNA. G residues flanking the leader region of DI-LT$_2$ (positions 73 and 118) were enhanced. As reported previously for the VSV leader gene, G47 (G118 in DI-LT$_2$) was enhanced in some experiments. In addition to the strongly protected region between positions 73 and 107, enhancements of methylation occurred at U residues 92, 95, and 101 in the RNA of DI-LT$_2$ nucleocapsids, and, as with VSV,

these fragments were generated without aniline treatment (lanes D and E) (17). DMS is normally least reactive with U residues in RNA, but the presence of the NS protein in this region apparently enhances their reactivity. Alternatively, DMS may alkylate the phosphate groups in these positions, resulting in direct chain scission through the formation of phosphotriesters. At the present time, however, their exact origin is unknown. Beyond position 107, the methylation patterns of DI-LT_2 naked RNA and DI-LT_2 RNA from nucleocapsids were very similar. Bands preceding position 73 (lane B) also showed differences in intensity compared with the RNA (lane A). Although these may represent additional sites of protein interaction, they were significantly less pronounced than those within the leader gene. Thus, we interpret the sequence perturbations detected in the leader gene of nucleocapsids of DI-LT_2 as noted below:



These data, together with previous studies of VSV nucleocapsids (17), suggest that polymerase contacts DI-LT_2 nucleocapsids in at least two regions: the region in the middle of the DI particle product coding sequence and the region in the middle of the internal leader gene. Interestingly, the former polymerase binding site is transcriptionally active for the synthesis of DI particle product, whereas the latter site is inactive for the synthesis of any detectable leader RNA or mRNA. Below, we show that the perturbations in the methylation patterns in both of these regions are diminished after NS protein is removed from nucleocapsids. These findings suggest that the NS protein contacts the DI-LT_2 RNA in these two regions.

Analysis of DI particle nucleocapsids after removal of NS protein. The DI particle nucleocapsids used in this study were prepared such that N and NS proteins, and occasionally small amounts of other VSV proteins, remained bound to the RNA. Most NS protein can be removed from nucleocapsids by two cycles of HSS solubilization at 30°C followed by banding in Renografin step gradients, sequential banding in linear 15 to 47% Renografin gradients, or both (17). These procedures leave N protein bound to the template, as is demonstrated by RNase resistance of the VSV and DI RNAs and by protein analysis on sodium dodecyl sulfate-acrylamide gels (17) (see Fig. 3). Similar results were obtained with DI particles (data not shown).

Nucleocapsids from DI-LT_2 were banded three times in Renografin gradients (see above) and treated with DMS. After end labeling and aniline treatment, the RNA fragments were analyzed on polyacrylamide sequencing gels. Figure 3 shows the methylation pattern of DI-LT_2 nucleocapsids after three successive Renografin bandings. Lanes A, B, and C contained identical samples but were electrophoresed for different lengths of time to allow resolution beyond the internal leader gene of DI-LT_2.

Lanes A and B of Fig. 3 show the methylation pattern starting from the 3' terminus, including the coding region for DI particle product. This pattern is very similar to the pattern obtained with deproteinized RNA (Fig. 1) (13). Microdensitometer scans of the gels and a computer analysis of the areas under the peaks were performed as described previously (17). From G2 to G16, the relative band intensities were the same as those of deproteinized RNA. Likewise, G17 was not enhanced, and the C cleavage in position 19 did not appear in the recycled nucleocapsids (Fig. 1, lane B). The methylation pattern at positions 34 to 37 did not precisely resemble that of either NS-containing nucleocapsids or deproteinized RNA. Since the last traces of NS protein are difficult to remove completely from the template, interactions persisting at positions 34 to 37 may be stronger than those seen elsewhere. Figure 3, lane C, shows the internal leader gene of DI-LT_2 in Renografin-purified nucleocapsids. The dramatic protection from methylation that was apparent in the presence of NS protein (Fig. 2, lane B) was significantly diminished after further purification in Renografin (Fig. 3, lane C). The G residues at positions 73 and 118 which flank the binding site remained slightly enhanced in the recycled nucleocapsids, as they were in NS-containing nucleocapsids. The U residues at positions 92, 95, and 101 also appeared in the recycled nucleocapsids but were much reduced in intensity. These bands often appeared lightly even in deproteinized RNA (Fig. 2, lane A) (17), but as yet we have no definite explanation for their origin. Again, it is possible that NS protein is very tightly associated with the RNA in this region, thus making its complete removal very difficult.

Overall, the methylation patterns of recycled DI particle nucleocapsids greatly resembled those of deproteinized RNA. Perturbations of the sequences shown in Fig. 1 and 2 were significantly reduced by the removal of NS protein. Thus, we conclude that perturbations of the RNA methylation patterns in DI particle nucleocapsids in the regions discussed above correlate with the presence of NS protein.

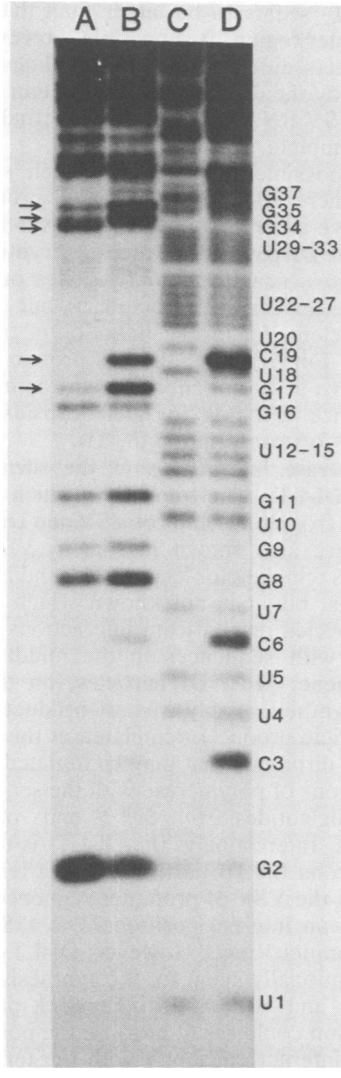


FIG. 1. Base methylation of deproteinized DI-T RNA (lane A) and DI-T nucleocapsids containing NS protein (lane B). After DMS treatment, RNA was extracted and labeled at the 3′ end. Strand scission at methylated bases was produced by borohydride reduction and beta elimination with aniline (lanes A and B). Lanes C and D show the U and C+U sequence markers, respectively, of DI-T RNA.

DISCUSSION

We used methylation protection to study the interactions of VSV RNA polymerase with the 3′ ends of several DI particle RNAs. By comparing methylation patterns of nucleocapsids containing only N protein with methylation patterns of nucleocapsids containing N and NS proteins, we observed altered reactivities of base residues in regions of the RNA sequence that correlate with the presence of the NS protein. We cannot totally rule out the influence of other template binding factors that are removed in conjunction with NS, nor can we rule out long-range conformational effects due to protein interactions at other sites.

The regions in which we detected NS-associated contacts with VSV and DI particle RNAs are shown in Fig. 4. Two points of comparison can be made. First, although the sites of interaction near the 3′ end of VSV RNA and DI RNAs have different sequences, the sequences are similar in that they are both A+U rich and are located in analogous positions with respect to the 3′ end. Second, the sizes of the regions in

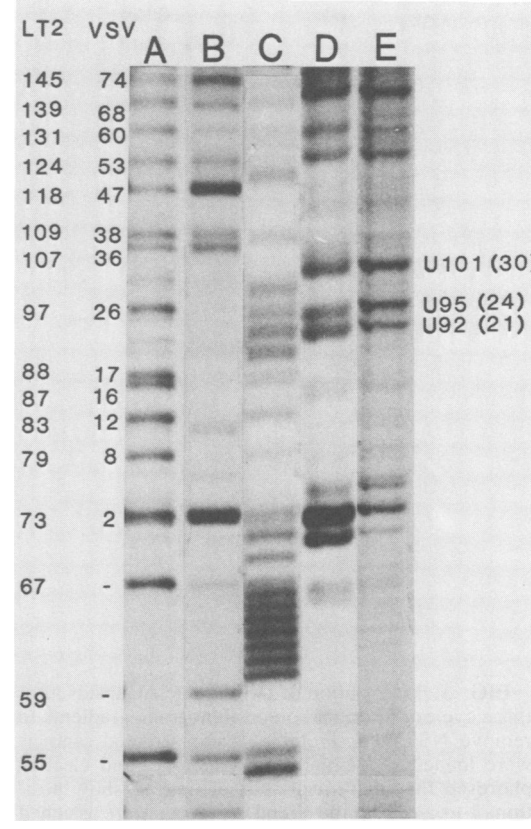


FIG. 2. Methylation of the leader gene of DI-LT_2 (lane A) and NS protein-containing DI-LT_2 nucleocapsids (lane B). Samples were treated identically to those in Fig. 1 except that the 31S DI-LT_2 RNAs were purified on 10 to 30% sucrose-sodium dodecyl sulfate gradients to assure complete removal of VSV 42S RNA. Lane C shows the A+G sequence marker of DI-LT_2 RNA. Lane D shows the RNA from the top of the 10 to 30% sucrose gradient of lane B and includes the chain scission products induced directly by DMS. Lane E shows the DMS-treated DI-LT_2 nucleocapsid RNA of lanes B and D before gradient separation and aniline treatment.

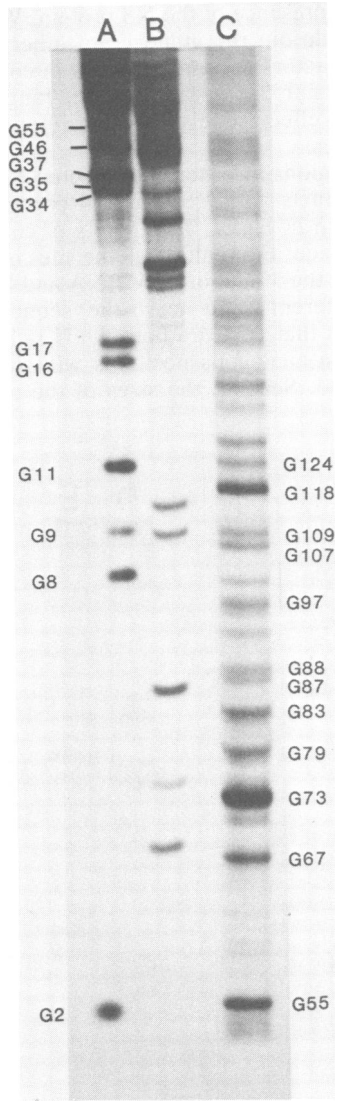


FIG. 3. Methylation of DI-LT_2 nucleocapsids after three cycles of purification on Renografin gradients to remove NS and L proteins. Aniline-treated samples were loaded sequentially on a 12% gel and electrophoresed for different periods of time to show positions 1 to 124 from the 3′ end. Positions were assigned by using chemical RNA sequencing ladders.

which interactions were observed are approximately the same for the DI particle RNAs, the VSV leader gene, and the leader gene of DI-LT_2. However, we cannot define the boundaries of protein binding sites by this method alone, since every base is not probed by DMS and in some cases the regions are defined by only a few bases. For example, the site of NS protein interaction detected in the internal leader region of DI-LT_2 seems to be larger than that of the VSV leader region. It is possible, however, that this reflects differences in NS binding that are affected by the disposition of N protein at the 3′ end of VSV RNA versus that at internal regions of the template.

It is possible that the A+U-rich sites described here interact with the NS protein and thus serve as promoters for RNA synthesis by VSV and DI particles. Other eucaryotic A+T-rich regions near the initiation sites of mRNA synthesis appear to be necessary, but probably not sufficient, for proper initiation of transcription (5). Furthermore, the NS protein of VSV may serve as an initiator protein for transcription, since it binds to nucleocapsids and is required for transcription (8, 23).

Polymerase interactions at the silent leader gene of DI-LT_2. The transcripts which are synthesized from the 3′ ends of VSV and DI particle RNAs are also shown in Fig. 4. VSV RNA serves as template for leader RNA and N mRNA synthesis, but it is not known which of these RNA species results from interactions of polymerase with sequences in the middle of the leader gene. Most DI particles, on the other hand, synthesize only a short product in vitro (DI particle product), templated at the extreme 3′ RNA terminus, that may be initiated through interactions of polymerase with the sequence 17 to 37 nucleotides from the 3′ ends of the DI particles. Interestingly, the DI-LT_2 is unique in that beyond the DI particle product template it contains the VSV 3′ promoter sequence (leader gene) at an internal position 72 to 118 nucleotides from its 3′ end. However, DI-LT_2 RNA is transcriptionally silent for the synthesis of leader RNA and mRNA (13). The lack of mRNA production could result because the polymerase fails to interact efficiently with the template at the internal leader gene. Our results, however, show that the RNA methylation pattern of DI-LT_2 nucleocapsids is altered in the internal leader gene in the presence of the polymerase. We conclude from this evidence that the failure to initiate transcription at the leader and N mRNA genes of DI-LT_2 is not due to an inability of polymerase to bind to the template.

Several studies have shown that VSV transcription proceeds first from the leader gene and is followed by the mRNA genes in the order that they occur along the RNA template (1, 2). Taken together, these findings indicate that the VSV polymerase requires a 3′-terminal initiation event to synthesize complete mRNA. Other factors, such as the topology of the template and proper interactions between the L and NS proteins, are probably also important for initiation. For example, these proteins may form an active polymerase complex only when the correct se-

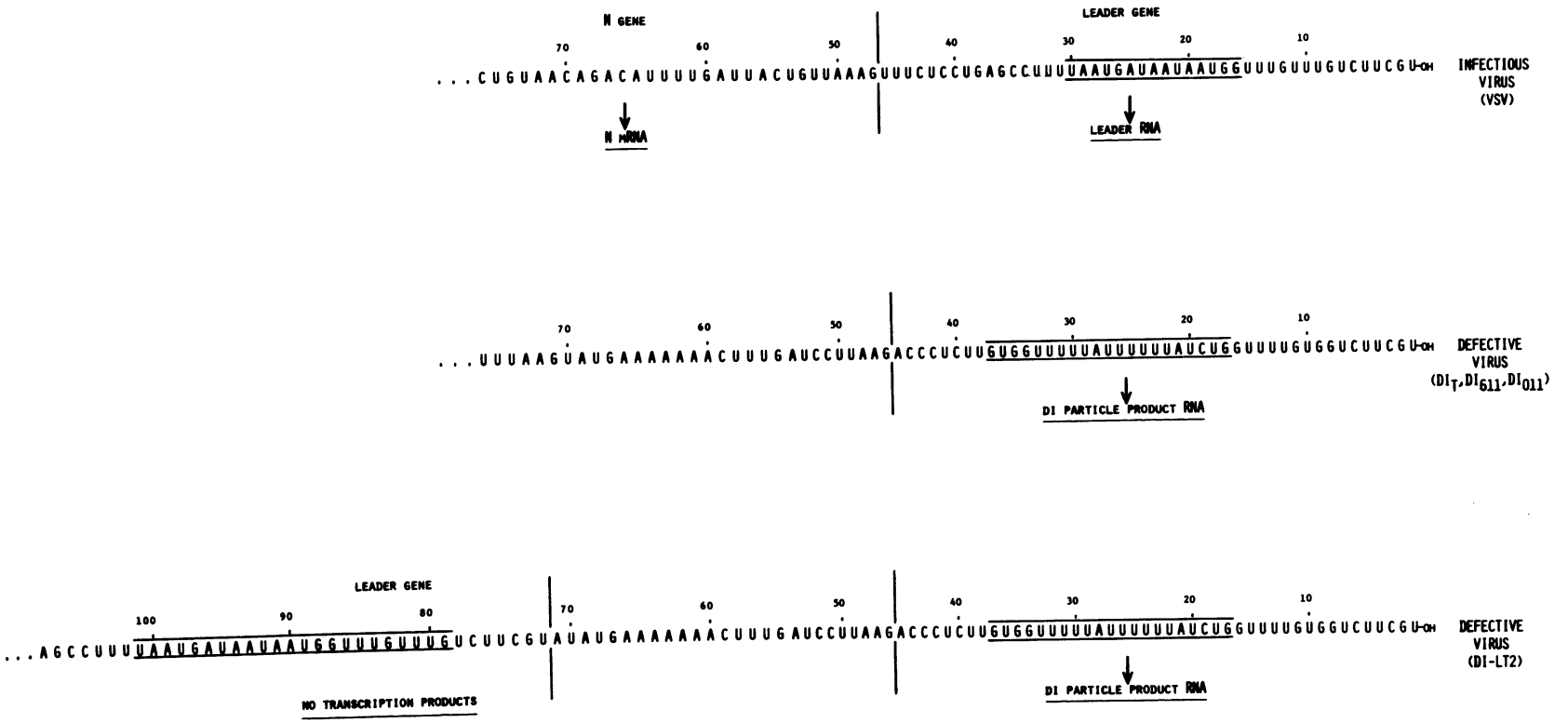


FIG. 4. Sequences at the 3′ ends of VSV and DI particle RNAs. Horizontal lines indicate areas of polymerase interaction detected by methylation protection. The arrows show the in vitro transcription products templated at the sequences between the vertical lines.

quence is at the 3′ end of the template. In the VSV genome, intergenic gaps vary in length from two to five nucleotides (15, 22, 28). Thus, the distance between the termination site of the DI particle transcription product of DI-LT_2 and the start of the internal leader gene (27 bases) may be too great for the active polymerase complex to span to continue synthesis.

The evidence we have presented here that NS protein binds to a specific internal site on the DI-LT_2 RNA template suggests that similar interactions may occur at other internal A+U-rich regions which are present 25 to 30 nucleotides before the start of each VSV messenger RNA (22, 28). These sequences may function as part of the transcription promoter sites for the VSV polymerase. Transcription of the genomic RNA may require the binding of NS protein at these sites in conjunction with a proper 3′-terminal entry for the polymerase complex.

Separation of polymerase binding and initiation sites. An examination of the 3′-terminal sequences of VSV and DI RNAs (Fig. 4) reveals only three base differences in the first 18 nucleotides from the end (16). Furthermore, studies of the New Jersey serotype of VSV (not shown) indicate complete homology between the 3′ ends of defective and nondefective viral RNAs for 21 nucleotides (14). Beyond this region of about 20 nucleotides, the VSV and DI particle RNA sequences diverge in both serotypes. Interestingly, it is in this region of divergency that we detected evidence of polymerase contact with the RNA. Thus, it appears that there are two important sites of template recognition by the VSV polymerase: the initiation site at the 3′ terminus and the A+U-rich NS binding site that starts about 16 or 17 nucleotides from the 3′ end. The degree of overlap between these two regions is uncertain at this time.

The proposed mechanism of autointerference maintains that the reduction in infectious virus titer by DI particles results from competition between the two particles for limiting amounts of replicase (11, 26). Several features of DI particles may play a role in this competition. For example, the length of terminal complementarity may be important, provided that the termini are free for base pairing in the nucleocapsid. On the other hand, the DI particle RNA may possess a higher-efficiency binding site for polymerase. Although the sequences at the 3′ termini of the defective and nondefective viral RNAs are almost identical, the regions in which we detected polymerase contacts with the RNA are not homologous. Thus, it is possible that the polymerase has different affinities for VSV and DI templates in the region of binding such that the DI particles compete more effectively than the infectious virus when the amount of polymerase is limiting. The finding of large amounts of NS protein in infected cells, however, makes it unlikely that NS alone is the limiting factor in autointerference (36). On the other hand, recent studies by Rubio et al. (29) suggest that NS may be limiting in the assembly of viral nucleocapsids, and it is possible that subforms of the NS protein serve different functions in the infected cell.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants R01 AI16099 and 1P01CA30246 and research training grant T32 CA09111. J.D.K. is the recipient of a Faculty Research Award from the American Cancer Society.

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