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Extent of terminal complementarity modulates the balance between transcription and replication of vesicular stomatitis virus RNA

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ABSTRACT **We compared the template properties of a subgenomic RNA that contained the authentic 5′ and 3′ ends of the vesicular stomatitis virus genome with those of RNAs in which the wild-type termini were engineered to extend their complementarity from 8 to 51 nucleotides as seen in defective interfering RNAs. The RNA with authentic 5′ and 3′ ends directed abundant transcription but low replication. In contrast, RNAs with complementary termini derived from either end of the genome replicated well but transcribed poorly or not at all. These results have implications for understanding the mechanisms of RNA replication and transcription; they explain the replicative dominance of defective interfering RNAs and demonstrate that the extent of terminal complementarity rather than its exact sequence is a major determinant of whether the template predominantly directs transcription or replication.**

During infection the negative-stranded RNA genome of vesicular stomatitis virus (VSV) directs two distinct RNA synthetic processes: transcription of five discrete capped and polyadenylylated mRNAs and replication of a perfect full-length copy of the genome. The same core RNA-dependent RNA polymerase is thought to execute both processes using as template a ribonucleoprotein complex of the viral genomic RNA. Transcription is the initial and predominant RNA synthetic event; synthesis of nucleocapsid (N) protein to encapsidate nascent genomic RNA is required for replication and is one trans-acting factor that modulates the balance between transcription and replication (1).

To examine the role of template sequences in the regulation of these two RNA synthetic processes, we established a system in which the entire replication cycle of a VSV defective interfering (DI) particle was reconstructed from cDNA clones (2). The DI RNA chosen for this work (DI-T) is a member of the copy-back family of DI RNAs in which the 5′ 2163 nt are derived from the wild-type (wt) VSV 5′ terminus, but the 3′ terminus is a 45-nt complement of the 5′ terminus (3–6). DI genomes of this type have a replicative advantage over wt VSV and interfere with its replication (4). Deletion of internal regions of the DI-T genomic RNA and replacement with heterologous sequences have shown that the inverted repeats of the 5′ end were sufficient to signal RNA replication (A. K. Pattnaik and G.W.W., unpublished data). Since the natural role of the 5′ terminal sequence is to act exclusively as an origin of replication during synthesis of full-length VSV negative strands, its presence at each end of the DI RNA has been proposed to account at least in part for the replicative advantage of this class of DI particle and hence for its ability to interfere with the replication of wt VSV (4).

In the work reported here, we took advantage of the ability to recover replicable RNAs from cDNA clones to compare the template properties of RNA analogues that contained the authentic VSV 5′ and 3′ termini with those of RNAs engineered to have increasing extents of terminal complementarity. The results showed that RNAs that had the 3′ end of the VSV genome were not only replicated by the VSV polymerase but also directed abundant transcription of capped and polyadenylylated mRNA analogues. Unexpectedly, however, a distinct replicative advantage was conferred by increased complementarity of the RNA termini, rather than by their sequence *per se*.

MATERIALS AND METHODS

Plasmid Construction, Mutagenesis, and Transfections. Plasmids that directed the transcription of analogues of VSV genomic RNA were constructed using the principles described previously in which VSV cDNA sequences were inserted between a promoter site for bacteriophage T7 RNA polymerase and a cDNA of the self-cleaving ribozyme from the antigenomic strand of hepatitis delta virus (HDV) (2). RNAs transcribed by T7 RNA polymerase from these plasmids contained two non-VSV nucleotides (GG) at their 5′ ends but cleaved themselves to yield 3′ ends that corresponded precisely to the 3′ end of the VSV genome (Fig. 1*A*). Within this general structure, individual plasmids differed from one another as follows: after the T7 promoter, plasmid 8 contained sequences derived from the two termini of the wt VSV genome, as shown in Fig. 1*A*. Plasmids 18, 22, and 28 were created by oligonucleotide-directed mutagenesis (8) to yield progressive replacement of the wt VSV 3′ leader sequence with the complement of the 5′ terminus, as in a DI RNA. The sequences of RNAs derived from these plasmids are shown in Fig. 1*C*. Plasmid 51 was made by using an *Afl* II site created at nt 48–53 to replace the 50 nt at the VSV 3′ end with a 49-nt inverted copy of the 5′ end. Conversely, plasmid 47(le) was made by replacing the 49 nt at the 5′ end with an inverted copy of the 50 nt at the 3′ end. The structures of all plasmids were checked by sequence determination. DNA transfections and virus infections were carried out in baby hamster kidney cells as described (2).

Analysis of RNA. At times between 15 and 24 hr posttransfection, transfected cells were exposed for 5 hr to [^{3}H]uridine (33 μCi/ml; 1 Ci = 37 GBq) and, where indicated, actinomycin D (10 μg/ml). Cells were harvested, and either total or N protein-encapsidated RNA was analyzed as described (2). RNAs were also analyzed by primer extension (2) using Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL) and a primer that annealed to positive-sense RNA in the N gene 83–66 nt from the 5′ end or with a primer that annealed to negative-sense RNA between positions 11,021 and 11,042 of the complete VSV genome sequence.

RESULTS

Effect of Terminal Complementarity on VSV RNA Replication. To test the ability of plasmid-derived RNA having the natural VSV RNA termini to replicate, and to examine the

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Abbreviations: wt, wild-type; VSV, vesicular stomatitis virus; DI, defective interfering; N, nucleocapsid protein; P, phosphoprotein; L, polymerase; HDV, hepatitis delta virus.

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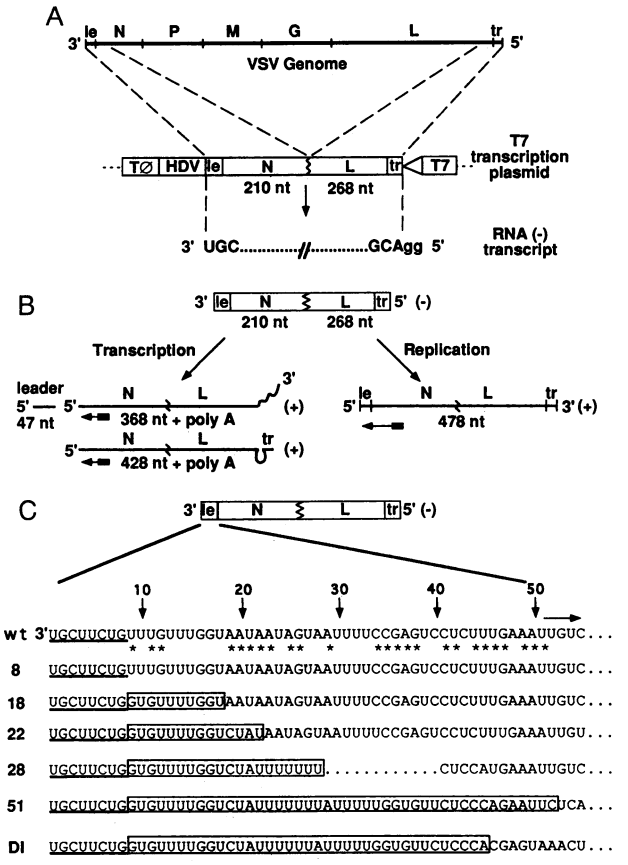

FIG. 1. (*A*) Diagram of VSV genomic analogues: A section of the T7 transcription plasmid showing regions of the VSV genome that were included, and the positions of the HDV ribozyme (HDV) and T7 terminator (Tϕ). The termini of the negative-sense genomic RNA analogue expressed from the T7 transcription plasmid after self-cleavage are shown. (*B*) VSV-specific RNA products expressed from the wt genomic analogue. The genome analogue should direct replication of a complete 478-nt complementary copy as well as transcription of the 47-nt, positive-strand leader RNA and an mRNA that initiated at the N mRNA start site, extended through the N/L gene fusion to terminate and polyadenylylate at the L gene signal prior to the trailer region. A second form of the mRNA also occurs that would not terminate after polyadenylylation but continues to copy the trailer (7). The primers and products of primer extension on positive-strand RNA products are illustrated. (*C*) Terminal exchanges: Sequence of 3′ termini of wt VSV negative-sense genome; the wt genomic analogue (8); the DI genomic analogue (51); and constructs having progressive exchanges of DI 3′ sequence (boxed) into the wt leader gene background (18, 22, and 28). Each construct is designated by the number of nucleotides at the 3′ terminus having uninterrupted complementarity to the 5′ terminus. The 8 nt underlined are complementary to the 5′ terminus in the wt and are the same in the DI 3′ terminus. Nucleotides in the 3′ wt terminus that lack complementarity with the 5′ terminus are indicated by an asterisk (*). The N mRNA transcriptional start site is at position 51.

importance of terminal complementarity in RNA template activity, we constructed a transcription plasmid that expressed the two ends of VSV RNA joined directly together as shown in Fig. 1*A*. For comparison, we constructed a plasmid that expressed an RNA of identical size and sequence to that described above, except that its 3′ terminal 51 nt were perfectly complementary to the 5′ end as in a DI RNA (Fig. 1*C*). In addition, we made a series of plasmids in which the VSV 3′ end was progressively changed to have increasing complementarity to the 5′ terminus and thereby to resemble the 3′ end of the DI RNA. This resulted in changing the terminal complementarity from 8 nt (the situation in wt VSV RNA) to 18, 22, 28, or 51 nt (Fig. 1*C*). All changes were engineered into the leader gene region of the wt subgenomic replicon and therefore all plasmids expressed negative-strand RNAs that contained the wt 5′ terminus of VSV RNA. Fig. 1*B* depicts the RNA synthetic events predicted for the wt subgenomic replicon (construct 8).

The ability of the RNAs expressed from these plasmids to be encapsidated with N protein and to serve as templates for the VSV RNA polymerase was examined by infecting cells with a recombinant vaccinia virus that expressed the T7 RNA polymerase (VTF7-3; ref. 9), and then transfecting simultaneously the plasmids that expressed the VSV genomic analogues and T7 transcription plasmids encoding the VSV N protein, phosphoprotein (P), and polymerase (L), to provide a source of the VSV proteins necessary to support encapsidation and replication. The RNAs synthesized were labeled with [^{3}H]uridine and examined by several direct biochemical assays chosen to discriminate among the different RNA species made. The products of the VSV polymerase could be labeled exclusively by [^{3}H]uridine incorporation in the presence of actinomycin D, which inhibited all DNA-dependent RNA synthesis. Omission of the plasmid that encoded the VSV polymerase protein, L, was used as a control to establish that particular RNAs were indeed products of the VSV polymerase (2). Encapsidation of RNAs with VSV N protein was assayed by immunoprecipitation of [^{3}H]RNA.

Fig. 2 shows total RNA synthesis (Fig. 2 *A* and *B*) as compared to actinomycin D-resistant RNA synthesis (Fig. 2 *C* and *D*) in cells transfected with the individual plasmids described above. RNAs of the correct size to represent the genome analogues were transcribed from all plasmids (Fig. 2*A*). The majority of RNA shown in Fig. 2*A* is due to transcription by the T7 RNA polymerase. The minor RNA species migrating slower than the major negative-sense transcript represents the small portion of primary transcript in which the HDV ribozyme has not cleaved (Fig. 2*A*, "uc"). The RNA species migrating faster than the genome analogue is a transcription product of the VSV polymerase as will be shown below. Approximately 5% of the primary transcripts expressed in the presence of the VSV N, P, and L proteins were immunoprecipitated, indicating that they had been encapsidated with N protein (Fig. 2*B*).

The ability of the encapsidated RNA genomic analogues to associate with the VSV polymerase and to direct VSV-specific RNA synthesis was measured by incorporation of [^{3}H]uridine in the presence of actinomycin D (Fig. 2 *C* and *D*). All of the constructs directed VSV-specific RNA synthesis. The nature of the products changed dramatically, however, as the sequence at the 3′ end of the RNA changed progressively from the wt VSV sequence (plasmid 8) to the DI-like sequence (plasmid 51). With plasmids 22, 28, and 51, RNA species that comigrated with the minus-strand RNA genomic analogue were readily labeled in the presence of actinomycin D and could be immunoprecipitated (Fig. 2*D*, lanes 20–22), showing that RNA from these constructs was encapsidated and replicated. In the case of construct 51, the replicated plus strand resolved from its complement in the low pH agarose/urea gels and can be seen as an RNA of slightly faster mobility (Fig. 2*D*, lane 22; ref. 2). However, in the case of plasmids 8 and 18, replicated RNA was barely detectable (Fig. 2*D*, lanes 18 and 19), suggesting that either the sequence of the VSV 3′ end or its limited complementarity with the 5′ end supported less efficient replication. In contrast, with RNA from constructs 8, 18, and 22, instead of carrying out high levels of RNA replication, the VSV polymerase catalyzed abundant synthesis of RNAs that migrated significantly faster than the genome analogue (Fig. 2*C*, lanes 13–15). The majority of these faster-migrating RNAs (>99%) were not immunoprecipitated (Fig. 2, panel *D*, lanes 18–21), indicating they were not encapsidated. Omission of the VSV L plasmid from the transfections abrogated all actinomycin D-resistant RNA

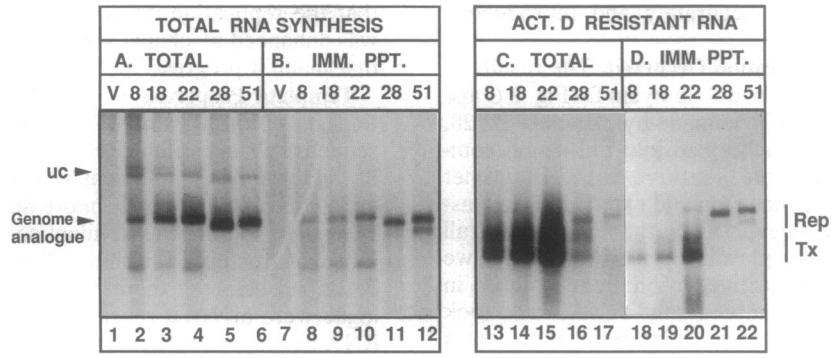

FIG. 2. Replication and transcription of RNAs expressed from the genome analogue constructs. Cells were infected with VTF7-3, transfected with cDNAs for the VSV genomic analogue construct 8, 18, 22, 28, or 51 and with the plasmids encoding the VSV N, P, and L proteins, and exposed to [^{3}H]uridine in the absence (lanes 1–12) or presence (lanes 13–22) of actinomycin D. Total RNA (*A* and *C*) or N protein encapsidated RNA (*B* and *D*) was analyzed. Total RNA samples in *A* represent 1/10th the amount of that in *B*; RNA in *C* and *D* represent equivalent amounts, but the fluorogram of *D* has been exposed 10 times longer. V, VTF7-3 infected only; uc, uncleaved T7 primary transcript; Rep, RNA replicated by VSV polymerase; Tx, RNA transcribed by VSV polymerase; IMM. PPT., immunoprecipitate.

synthesis, confirming that the RNAs were products of the VSV RNA polymerase (data not shown).

Analysis of VSV Transcription Products. The abundant VSV-specific RNAs that were made in cells transfected with plasmids 8, 18, and 22 were the appropriate size to be the products of transcription by the VSV polymerase. Such products should be of positive polarity, contain both N and L gene sequences, have polyadenylate tails, and have 5′ termini that map to the conserved transcription initiation site of the N mRNA. Each of these criteria was analyzed. Oligo(dT)-cellulose chromatography of the RNA from constructs 8, 22, and 51 showed that the two faster-migrating RNA species directed by RNA from plasmids 8 and 22 were polyadenylylated, whereas the genome-length RNAs were not (data not shown). The finding that the faster-migrating RNAs migrated as a doublet was most likely due to the fact that the VSV polymerase does not always terminate transcription following polyadenylylation of the L mRNA but may continue into the trailer yielding two forms of the mRNA (Fig. 1*B*; ref. 7). By Northern blotting (data not shown) and by primer extension analysis (see below), both polyadenylylated products were shown to contain positive-strand sequences of the expected N and L gene regions.

Primer Extension Analysis Identifies Products of Both Transcription and Replication. Analysis of the 5′ ends of the positive-strand RNAs by primer extension allowed determination of whether both a full-length replication product and an RNA with the correct 5′ end for an N/L transcript were made. An oligonucleotide primer that annealed to residues 83–66 from the 5′ end of positive-sense VSV RNA was used (see Fig. 1*B*). When extended by reverse transcriptase on a full-length, positive-strand RNA product of replication, this primer should yield a product of 83 nt, whereas on an RNA that started at the authentic transcriptional initiation site of N mRNA, it would yield a product of 33 nt. If the mRNA were capped, a primer extension product of 34 nt was predicted. In addition, products of 45–48 nt were expected as a result of primer extension on the T7 transcripts of the plasmid that expressed the N protein mRNA.

The data in Fig. 3, lanes 1, 3, 5, 7, and 8, show by the presence of the appropriately sized primer extension products that a full-length, positive-strand RNA product of replication was synthesized by the VSV polymerase using the negative-sense template RNA expressed from plasmids 8, 18, 22, 28, and 51. As expected, the products from the constructs varied slightly in size as predicted by their sequences (see Fig. 1*C*). In addition, a second major primer extension product, 34 nt in size, was observed by extension on RNA from constructs 8, 18, 22, and 28 (Fig. 3, lanes 1, 3, 7, and 8, respectively). This was the product expected from extension on a capped RNA that initiated at the authentic 5′ AACAG . . . 3′ initiation site for the N mRNA. No product of VSV transcription was detected from plasmid 51. When the VSV polymerase (L) plasmid was omitted from the transfections (Fig. 3, lanes 2, 4, and 6), neither the 83- nor the 34-nt product was detected, showing that both replication and transcription were catalyzed by the VSV polymerase. These experiments were repeated with a primer that annealed to the L gene portion of positive-strand RNA. The results confirmed the positions of the 5′ ends of both the replication and transcription products and showed that the mRNAs contained the expected L gene as well as N gene sequences, although the relative intensities of products differed (data not shown). This showed that the relative intensities of primer extension products in Fig. 3 cannot be interpreted quantitatively as the templates are minority species in a mixture of RNAs of both polarities (made both by the VSV and T7 polymerases), which can compete for primer or template. In contrast, Fig. 2*C* shows direct analyses of labeled RNA made by the VSV

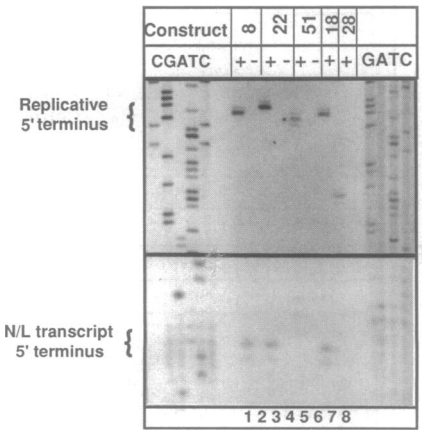

FIG. 3. Primer extension analysis of the 5′ termini of positive-stranded RNA replication and transcription products. RNAs isolated from cells transfected with plasmid construct 8, 18, 22, 28, or 51 and the plasmids for the VSV N, P, and L proteins (+ = Pol$^+$) or only the N and P proteins, omitting L (− = Pol$^-$), were analyzed by primer extension using a primer that annealed in the N gene to residues 83–66 from the 5′ terminus of the full-length, positive-strand RNA. The 34-nt N/L transcript product for construct 28 is visible only on longer exposures. The figure has been condensed by omitting the central portion of the gel, which contained only the products of primer extension on the N mRNA transcribed from the T7 N support plasmid. Dideoxy sequence ladders of plasmid constructs 8 and 51 using the same primer are shown for reference.

polymerase, and these data accurately and reproducibly reflect RNA abundances.

A Complete Cycle of Replication Occurred. The results of RNA labeling in the presence of actinomycin D (Fig. 2 *C* and *D*) showed clearly that the RNAs made by plasmids 22, 28, and 51 were encapsidated and copied into full-length complementary strands. The more sensitive assay of primer extension confirmed this conclusion and extended it to plasmids 8 and 18 (Fig. 3). However, since the levels of overall replication of the RNAs from plasmids 8 and 18 were low, we next examined whether the positive-strand progeny could, in turn, direct synthesis of negative-strand RNAs. We took advantage of the observation that during replication, extra nucleotides on the 5′ end of the initial VSV T7 transcripts were eliminated (2). Thus, by primer extension analysis, negative-strand products of VSV replication could be distinguished from those of T7 transcription and progeny negative-strand synthesis could be specifically assayed.

RNA transcribed from construct 8, 18, 22, 28, or 51 by T7 RNA polymerase in the absence of the VSV polymerase gave a predominant primer extension product that corresponded to the RNA template sequence: 5′ GGACG . . . (Fig. 4*A*, lanes 2, 4, and 6). An extension product 1 nt longer was also present and can be attributed to RNAs that were made by the T7 polymerase but then capped by the vaccinia virus guanylyltransferase (10). In contrast, RNA from cells where VSV RNA replication had occurred yielded additional primer extension products that were 2 nt shorter and corresponded to replicated RNA having the authentic 5′ end of the negative-strand VSV genome: 5′ ACG . . . (Fig. 4*A*, lanes 3, 5, 7, and 8). These data show that RNAs from plasmids 18, 22, 28, and 51 directed not only the synthesis of their positive-strand complements but also that these progeny RNAs were, in turn, competent templates for replication. The situation with plasmid 8 was less clear; only low levels of negative-strand replication products were detected (Fig. 4*A*, lane 1), and the level of overall replication was always less than that seen with the other plasmids. These results reinforced the conclusion

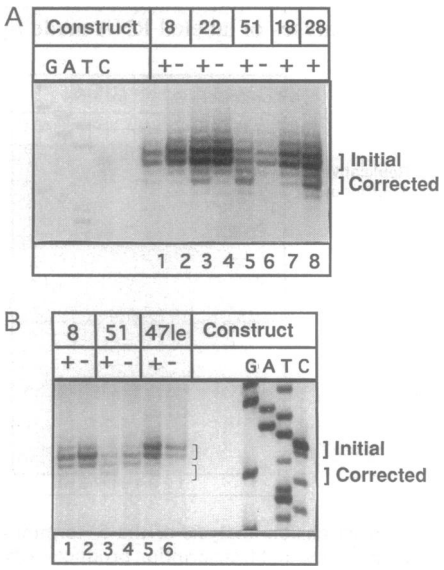

FIG. 4. Primer extension analysis of the 5′ termini of negative-stranded RNA products of replication. (*A*) RNAs isolated from cells transfected with plasmid construct 8, 18, 22, 28, or 51, as described in the legend to Fig. 3, were analyzed by primer extension using a primer that annealed to the negative-sense RNA 136–120 nt from its 5′ end. +, VSV polymerase gene transfected; −, polymerase gene omitted. A dideoxy sequence ladder of plasmid 8 with the same primer is shown for reference. (*B*) RNA isolated from cells transfected with plasmid construct 8, 51, or 47(le) was similarly analyzed; the reference sequence ladder is of plasmid 47(le).

that the replication efficiency of the negative-strand RNA was enhanced by changes in the sequence of the VSV 3′ end that made it progressively resemble the DI RNA 3′ end.

Template Properties of an RNA with Inverted Terminal Repeats of the 3′ End of the VSV Genome. Since these sequence changes at the RNA 3′ end also increased the length of the terminal complementarity, it was necessary to determine which factor was the more important: the sequence *per se* or the extent of the terminal complementarity. We therefore constructed a plasmid (47le) that was similar to those described before, except now the termini of the RNA analogue were inverted repeats of the 3′ end of VSV genomic RNA having perfect complementarity for 47 nt. Fig. 5 shows that replacement of the VSV 5′ end by a complement of the 3′ end profoundly changed the products of the wt VSV RNA synthesis. Instead of transcription, as seen with plasmid 8, the major RNA synthetic event changed to replication with plasmid 47(le) (Fig. 5, compare lanes 1 and 3). The level of replication was as high or higher than that seen with construct 51, but transcription was greatly reduced as compared to the wt construct 8. The difference in mobility of the RNA from constructs 51 and 47(le), which differ in size by only 1 nt, is due to the fact that RNA mobility in agarose/urea gels is a function of base composition as well as size (11).

Primer extension analysis of the positive-strand RNA products of construct 47(le) confirmed that a full-length replication product was made and also showed the presence of capped mRNA that initiated at the authentic start site of the N gene (data not shown). Primer extension analysis of the negative-strand RNAs showed that in the presence of the VSV polymerase (Fig. 4*B*, lane 5), negative-strand RNA that has a repaired, 2-nt shorter 5′ terminus was present. These data demonstrate that RNA from construct 47(le) carried out a complete cycle of replication. These results, together with those of the progressive 3′ end replacements described above, show that the efficiency of both transcription and replication of a VSV RNA template is profoundly influenced by the extent of its terminal complementarity. Furthermore, similar effects of increased terminal complementarity can be achieved either by changing the wt 3′ end to increasingly complement the 5′ terminus or by retaining the wt 3′ terminus and changing the 5′ end to complement the 3′ terminus.

DISCUSSION

In the work described above, we were able to distinguish the contrasting effects that mutations in the VSV leader gene have on transcription and replication by the use of direct

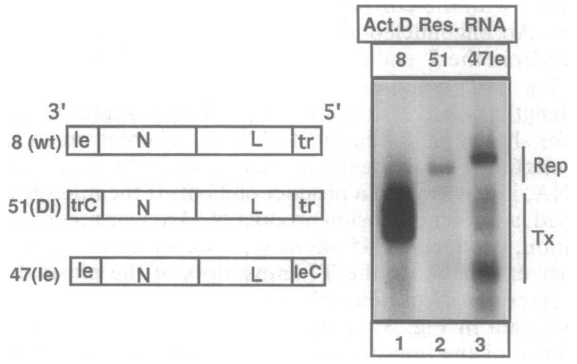

FIG. 5. Replication and transcription of RNAs expressed from genomic analogue constructs. Cells were infected with VTF7-3 and transfected with cDNAs for the genomic analogue construct 8, 51, or 47(le) and the plasmids encoding the N, P, and L proteins. Cells were exposed to [^{3}H]uridine in the presence of actinomycin D for 5 hr at 19 hr posttransfection and total cytoplasmic RNA was analyzed. trC, Trailer complement; leC, leader gene complement; Rep and Tx, as in Fig. 2.

biochemical assays for the corresponding RNA products. Such investigations have not been carried out previously because only recently has it become possible to alter negative-strand RNA virus genomes at the cDNA level and recover replicable RNA (2, 12–15). However, using an approach in which RNA is recovered from a cDNA clone by transcription in cells concomitantly synthesizing the proteins required for formation of functional nucleocapsids, we have been able to analyze directly both RNA transcription and replication from VSV RNA analogues recovered from cDNA clones. Consequently, the results have implications for understanding the mechanisms of VSV RNA replication, transcription, and DI particle interference.

RNA Replication. RNAs with inverted terminal repeats of the VSV 5′ end, which are characteristic features of the common copy-back (or panhandle) class of DI RNA, replicated efficiently when expressed from plasmids (construct 51, ref. 2). Conversely, RNA that had the 5′ and 3′ termini of wt VSV RNA (plasmid 8) replicated poorly. Primer extension analyses showed that although a positive strand replication product was made, it had low activity as a template for the synthesis of progeny negative strands. This result was surprising since the termini of wt VSV RNA support adequate RNA replication in the natural situation, although transcription is always the predominant RNA synthetic activity.

The low replicative activity of RNA that had the wt VSV genome 5′ and 3′ termini was greatly improved by progressive sequence changes to the 3′ end that increased its complementarity with the 5′ terminus. Alternatively, its replicative activity could be enhanced by changing the 5′ end so that it was an inverted complement of the 3′ end, as in the RNA expressed from plasmid 47(le). These results show that the possession of complementary termini, whose sequences can be derived from either end of VSV RNA, is a major factor in determining the efficiency of replication. It is difficult to escape the conclusion that the two ends of the RNA interact to form a structure that promotes RNA replication, perhaps by serving as a polymerase recognition site or by nucleating the encapsidation process for the nascent strand. The termini of wt VSV or DI RNAs in nucleocapsid form have not been shown to interact, although the complementary termini of deproteinized VSV DI RNAs base-pair (16). Nevertheless, despite the association with N protein, which renders encapsidated VSV RNA resistant to digestion by ribonucleases, the bases in the active RNP template are available for modification (17) and are also accessible to the polymerase during transcription and replication.

RNA Transcription. In contrast to its low activity as an origin of RNA replication, the 3′ end of wt VSV RNA in combination with the wt 5′ terminus was a strong transcriptional promoter that directed abundant synthesis of the predicted N/L chimeric mRNA. Interestingly, progressive replacement of the wt VSV 3′ sequence with the DI RNA 3′ sequence initially enhanced VSV transcription (plasmids 18 and particularly 22) and then suppressed it (plasmid 28). Although the situation was clearly complex, one of the factors affecting promoter strength was again the extent of terminal complementarity, since RNA from plasmid 47(le) directed only low levels of transcription, despite having the wt VSV 3′ terminal sequence at both ends and replicating well. This result showed that mismatches within the terminal region were necessary for full activity of the transcriptional promoter and suggested that the interrupted complementarity found at the ends of wt VSV RNA is the result of an evolutionary compromise to give the right balance between the requirements for transcription and replication. DI RNAs, on the other hand, which have no need to be transcribed, can acquire complementary termini that are optimized for replication.

Although many details of the mechanism of VSV transcription are not yet understood, most of the data point to a single polymerase entry site located at the extreme 3′ end of the VSV genome (18). Initiating here, the polymerase either makes leader RNA followed by the five mRNAs or it makes a full-length, encapsidated, positive-sense copy of the genome. The availability of N protein to encapsidate the nascent RNA is necessary for the latter process (1). However, the experiments presented here, all of which were carried out in the presence of N protein, show that the sequences at both termini of the template and, in particular, their extent of complementarity also affect the balance between transcription and replication. We are led to one of two conclusions: either encapsidation of the nascent strand can somehow be influenced by the secondary structure of the template or factors other than nascent strand encapsidation can affect the behavior of the polymerase at the leader/N junction, thereby altering the balance between transcription and replication.

DI RNA Interference. Finally, these results shed light on the mechanism of DI particle interference, since engineered RNAs that had longer regions of uninterrupted terminal complementarity had a clear replicative advantage over those with shorter regions. Among naturally occurring DI RNAs also, those with complementary termini are known to have the greatest competitive replication advantage, with complementarity varying from 45 to about 150 nt (4, 19).

In summary, the data presented show that increased terminal complementarity strongly promotes replication. The wt VSV genomic termini have evolved to possess a limited degree of terminal complementarity such that transcription is the predominant RNA synthetic event from the 3′ genomic terminus in order to generate sufficient mRNA to program protein synthesis to support the entire replicative life cycle of the virus. DI RNAs, on the other hand, replicate at the expense of the wt genomes and, not needing to transcribe mRNAs, have been selected because their terminal complementarity confers a powerful replicative advantage.

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