JOURNAL OF VIROLOGY, Dec. 1987, p. 3960–3967
0022-538X/87/123960-08$02.00/0

Vol. 61, No. 12

Translational Requirement for La Crosse Virus S-mRNA Synthesis: A Possible Mechanism

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Received 27 May 1987/Accepted 10 September 1987

Ongoing protein synthesis is required for La Crosse S-mRNA synthesis in vivo, and complete S-mRNA can be made in vitro only in the presence of an active rabbit reticulocyte lysate. Using in vitro systems based on the polymerase activity of purified virions, we further support the notion that it is translation of the nascent mRNA that is required for complete transcription. Since replacement of guanosine with inosine in the nascent mRNA substitutes for the translational requirement, it appears that translation is required to prevent interactions of the nascent chain from taking place, which, if not prevented, lead to premature termination. These interactions appear to be between the nascent mRNA chain and its nucleocapsid template. A model for the translational requirement for complete S-mRNA synthesis is presented.

La Crosse virus (LAC) is a member of the California encephalitis serogroup of the insect-transmitted bunyavirus family (15). The genome of these viruses consists of three segments of single-stranded RNA of negative polarity, each contained within a separate nucleocapsid (NC), labeled S, M, or L, each of which has helical symmetry and appears under the electron microscope as a circular, supercoiled structure (9). These NCs are the templates for mRNA synthesis, initiating transcription by a cap-snatching mechanism which is remarkably similar to that of influenza virus (6). However, unlike influenza virus, LAC transcription takes place in the cytoplasm (18) and uses a stable pool of mRNAs as substrates for primers. The LAC S-mRNA thus contains ca. 15 nontemplated nucleotides (nt) at its 5′ end, and its 3′ end, which is apparently not polyadenylated, has been mapped to ca. position 886 (12) (see Fig. 6).

Although bunyavirus mRNA synthesis in vivo is independent of host mRNA synthesis (10), it is unusually sensitive to drugs which disrupt protein synthesis. This finding was first reported by Abraham and Pattnaik for bunyamwera and akabane viruses (1, 13) and has since been confirmed for LAC and Germiston viruses (5, 16). Since a translational requirement for mRNA synthesis appeared to conflict with the transcriptional activity of purified LAC virions, the S-genome transcripts generated by purified virions were reexamined (2). We found that only incomplete transcripts were made under these conditions, the longest having extended 175 nt from the start of the template. However, when transcription was carried out in the presence of a rabbit reticulocyte lysate, complete S-mRNA was the most prominent product made and the incomplete transcripts were either undetectable or greatly reduced. Drugs which inhibit protein synthesis also abolished complete mRNA synthesis, and, in most cases, led to the reappearance of the incomplete transcripts. The incomplete S transcripts found in vitro could also be detected in virus-infected cells in which protein synthesis had been inhibited by drugs, but not in control cultures (17).

In the absence of translation, LAC mRNA synthesis therefore initiates normally, but terminates prematurely at well-defined sites. These premature termination sites are suppressed in the presence of ongoing protein synthesis, and complete S-mRNA synthesis thus appears to be coupled to translation. The direct coupling of transcription to translation is, of course, impossible for eucaryotic cell transcription, but no such limitation exists in the special case of a virus which makes its mRNA in the cytoplasm. In addition, direct coupling of transcription to translation is well known in bacteria in which the terms polarity and attenuation are used to describe mechanisms by which the concurrent translation of the nascent mRNA chain is used to modulate termination of RNA polymerase at premature termination sites (14, 19, 20). We have further investigated LAC premature transcription termination in vitro to study the mechanism by which mRNA synthesis is coupled to translation.

MATERIALS AND METHODS

Preparation of BHK cell extracts. Either uninfected or 8-h LAC-infected packed BHK cells (1 ml, ca. 10^8 cells) were washed with 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES [pH 7.5])–5 mM KCl–0.5 mM $MgCl_2$–0.5 mM dithiothreitol–0.2 M sucrose and then suspended in 1 ml of the same buffer without sucrose. After 30 min on ice, the cells were lysed by Dounce homogenization with a tight-fitting pestle (15 strokes) and then centrifuged for 5 min at 4,000 rpm in a Sorval HB4 rotor, followed by 10 min at 10,000 rpm. The supernatant was then diluted to 10% in glycerol, aliquoted, and stored at −70°C.

RNase mapping. Approximately 10^5 cpm of riboprobes were used with their complementary RNAs in a total of 9 μl of ET (1 mM EDTA, 10 mM tris chloride [pH 7.5]), heated for 1 min at 90°C, quick chilled, and then diluted to 0.3 M in NaCl in a total volume of 10 μl. The samples were annealed for 30 min at 70°C and then increased to a volume of 50 μl with 25 mM tris chloride (pH 7.4)–2.5 mM EDTA–0.375 M NaCl–0.1% sodium dodecyl sulfate–40 μg of RNase A per ml. After 60 min at 30°C, 600 μg of proteinase K per ml and an additional 0.1% sodium dodecyl sulfate was added. After a further 30 min at 30°C, the samples were phenol extracted and ethanol precipitated. The remaining RNAs were then dissolved in 80% formamide plus dyes, heated for 2 min at 70°C, and separated on a 6% sequencing gel.

RNase H treatment of RNA:DNA hybrids. Wheat germ extracts, either from Amersham Corp. or prepared according to the method of Morch et al. (8) were used as a source of RNAse H, since they were found to have stronger and more reproducible activity than commercial RNase H from

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several suppliers. To monitor hybrid formation, 7.5 μl of BHK cell extract was mixed with 6 μl of wheat germ extract and 100 pmoles of oligonucleotide in 1.5 μl of water. After 40 min at 30°C, the samples were phenol extracted twice, ethanol precipitated, and electrophoresed on a 1.5-mm-thick 4% polyacrylamide gel in 7 M urea. The gel was soaked in 10 mM Tris–5 mM sodium acetate–0.5 mM EDTA for 10 min and electroblotted onto Hybond-N membranes (Amersham) in the same buffer. Prehybridization, hybridization, and washing were done as previously described (2).

RESULTS

The available evidence suggests that the translational requirement for LAC mRNA synthesis is not for a translation product. For example, it does not seem reasonable that this requirement is for a viral protein, and if it were for a host protein, this protein would have to be extremely unstable, since cycloheximide treatment of infected cultures leads to an immediate cessation of new mRNA synthesis (16). The translational requirement would therefore appear to be for some other consequence of protein synthesis, such as ribosomal translocation, presumably on the nascent viral mRNA. However, our drug experiments did not rule out the possibility that it could also be for ribosome movement on host mRNAs. For example, it is possible that the translational requirement is for a stable host factor whose availability to the transcription complex is itself controlled by the level of protein synthesis. Furthermore, interpretations based on drug effects alone are always limited, since these drugs often have multiple effects, some of which are hard to explain (2, 17).

We therefore sought another way of preventing translation of the nascent mRNA which did not involve inhibitors of protein synthesis. One approach is to saturate the capacity of the lysate for protein synthesis with exogenous mRNAs so that ribosomes would be unavailable to translate the viral transcript. Alfalfa mosaic virus (AlMV) RNA 4 was therefore added in increasing amounts to a coupled virion polymerase reaction. Some reactions were labeled with [^{35}S]methionine to follow AlMV coat protein synthesis, whereas other reactions were used to estimate the amounts of incomplete and complete S-mRNAs made. These reaction products were first examined by RNase mapping (Fig. 1) with a riboprobe which includes positions 43 to 195. When no mRNA was added, most of the transcripts (87%) had extended past position 195 (the 152 band) and only a few had terminated at position 175 (the 132 band). However, as increasing amounts of mRNA were added, the 152 band steadily decreased in amount, whereas the 132 band increased and reached levels clearly in excess of the transcripts made without added mRNA. This finding reflects both increased termination at position 175 and increased initiation of the polymerase because of these high levels of mRNA. The same reaction products were also examined by Northern (RNA) analysis with a riboprobe containing positions 1 to 195 (Fig. 2). When no mRNA was added, this probe detected roughly equal amounts of complete mRNA and the premature transcript (175 nt). This difference between Northern analysis and RNase mapping occurred presumably because this probe preferentially detected the shorter of the two RNAs (cf. the relative amounts of incomplete mRNA made in the absence of the reticulocyte lysate with that of the complete mRNA made in its presence). When increasing amounts of mRNA were added, the complete mRNA was lost entirely, whereas the incomplete mRNA steadily increased, as in Fig. 1. These results demonstrate that premature termination at position 175 can be

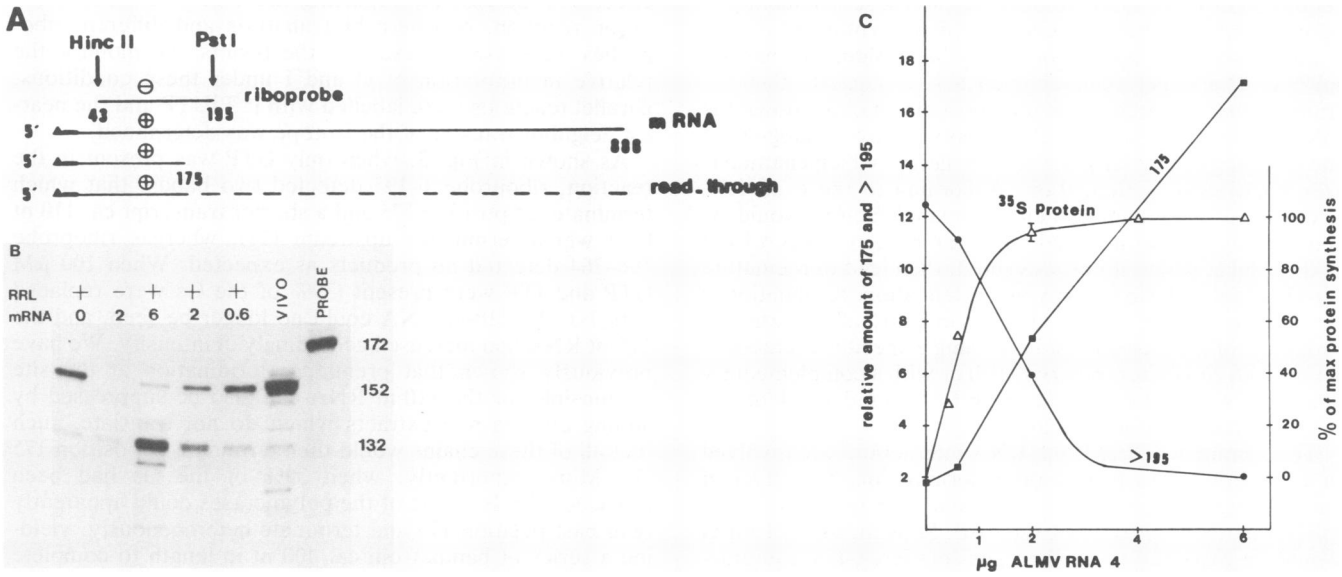

FIG. 1. Effect of exogenous mRNA on the in vitro-coupled LAC transcription reaction products by RNase mapping. LAC transcription reactions were carried out in the presence of a rabbit reticulocyte lysate (RRL) as previously described (2) to which increasing amounts of AlMV RNA 4 (μg) were added (top of panel B). After 45 min at 30°C, the reaction products were pelleted through a CsCl density gradient and annealed with an excess of minus-strand riboprobe representing nucleotides 43 to 195 (A and Fig. 6). The reaction products were then digested with RNase A before separation on a 12% sequencing gel (B). Lanes: PROBE, undigested riboprobe; VIVO, results of annealing cytoplasmic S-mRNA; +, presence; −, absence of the RRL. Numbers on the right indicate the length (in nucleotides) of the various RNAs. Results were quantitated by densitometry and are plotted (C). Parallel reactions were labeled with [^{35}S]methionine, and the amount of AlMV coat protein made in each reaction was determined by scanning the autoradiograms of the 175-nt RNA and >195-nt RNA products separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (not shown) and is also plotted (C).

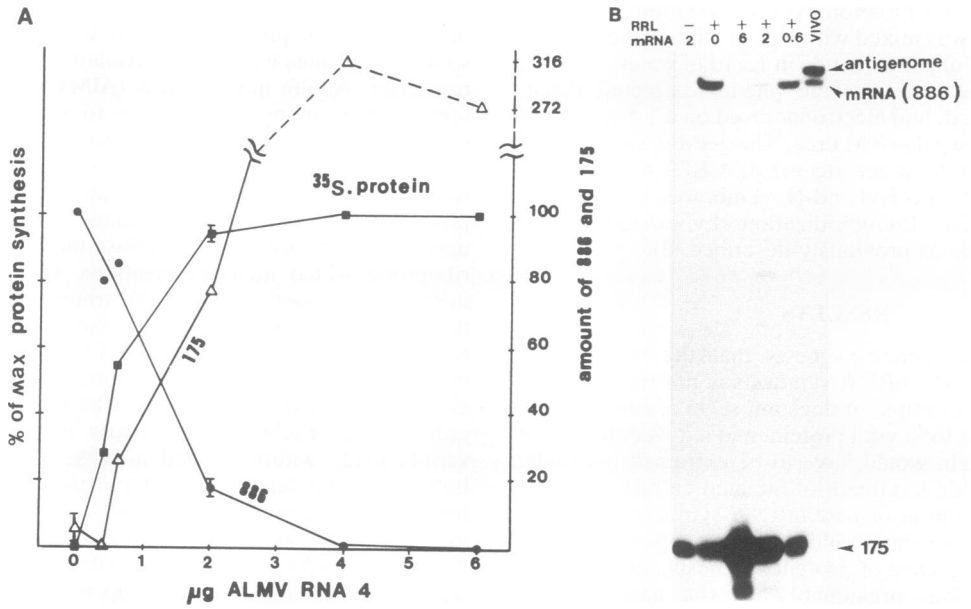

FIG. 2. Effect of exogenous mRNA on the in vitro-coupled LAC transcription reaction products by Northern analysis. The same reaction products shown in Fig. 1 were also separated on a 4% polyacrylamide gel and electroblotted onto zeta probe membrane. The membrane was then annealed with a minus-strand riboprobe representing nucleotides 1 to 195. The autoradiograms from two separate experiments were quantitated by densitometry. The autoradiogram from one of these experiments (B) is a longer exposure. Abbreviations for lanes are the same as in the legend to Fig. 1. The results are plotted (A) along with the amount of AlMV coat protein made in each reaction. The amounts of the 175-nt (△) and 886-nt (●) RNAs and the [^{35}S]methionine protein (■) are plotted here relative to themselves and not relative to each other (as in Fig. 1).

achieved in a coupled in vitro system without the use of drugs, presumably by using sufficient exogenous mRNA to outcompete the nascent mRNAs for ribosomes. This would also appear to eliminate the possibility that the translational requirement is for a stable host factor whose availability is controlled by the general level of protein synthesis.

Substitution of inosine for guanosine residues in nascent mRNA. The experiment detailed above suggests that the translational requirement is for ribosomes moving down the mRNA chain in concert with the polymerase, analogous to the coupling of transcription to translation, which characterizes polarity and attenuation. According to these models, active ribosomes and/or their associated factors would be required to prevent interactions of the nascent mRNA from taking place, which if not prevented, then lead to premature termination. In both polarity and attenuation, termination is thought to be the result, at least in part, of a particular sequence of the nascent chain forming a specific interaction, either with *rho* factor (polarity [19]) or with a complementary sequence within the nascent chain for *rho*-independent termination (attenuation [14, 20]).

To examine whether RNA-RNA interactions are involved in LAC premature transcription termination, the effect of substituting inosine (I) for guanosine (G) residues in the mRNA made in vitro was examined. Substitution of I for G would be expected to weaken possible RNA-RNA interactions, and this substitution has been shown to result in polymerase readthrough of the *trp* operon attenuator (7). However, when we completely replaced the GTP with ITP in an uncoupled virion polymerase reaction, we found that very little RNA was made. RNA synthesis was normal if only half the GTP was replaced, and under these conditions, iodine was efficiently incorporated (see below). Since identical results were obtained when synthesis only up to the first cytidine residue of the mRNA at position 10 was measured (not shown), it appeared that the viral polymerase could not initiate with ITP (GTP, although the penultimate coded nucleotide, is the first nucleotide added to the capped primer during mRNA synthesis [3, 12]). We therefore examined the effect of progressively replacing the GTP with ITP in the virion reaction. Northern blot analysis and different riboprobes were used to examine the results. To monitor the relative incorporation of G and I under these conditions, parallel reactions were labelled with [^{32}P]UTP and the nearest neighbor transfer of the isotope was determined.

As shown in Fig. 3, when only GTP was present in the reaction, riboprobe 1–195 detected two bands: that which terminates at position 175 and a shorter transcript ca. 110 nt long which terminates upstream (17), whereas riboprobe 196–764 detected no products as expected. When 100 µM GTP and ITP were present (39% of the Gs were replaced with Is), the 110-nt RNA could no longer be seen, and the 175-nt RNA had increased accordingly in intensity. We have previously shown that premature termination at the site responsible for the 110-nt RNA can also be suppressed by adding cytoplasmic extracts which do not translate, such that all of these chains would then terminate at position 175 (2). More importantly, when 39% of the Gs had been replaced with Is, some of the polymerases could apparently read past position 175 and terminate heterogenously, yielding a series of bands from ca. 400 nt in length to complete mRNA. When the ITP/GTP ratio was increased to 3/1 and 7/1, where 76 and 81%, respectively, of the Gs have been replaced with Is, these longer RNAs increased in amount only slightly, whereas the 175-nt RNA decreased sharply, such that the sum of the longer RNAs relative to the 175-nt RNA had risen significantly. The longest RNA which comigrated with complete mRNA also clearly increased with I substitution for G. This increase can best be seen with the 196–764 riboprobe which, for unknown reasons, detects the

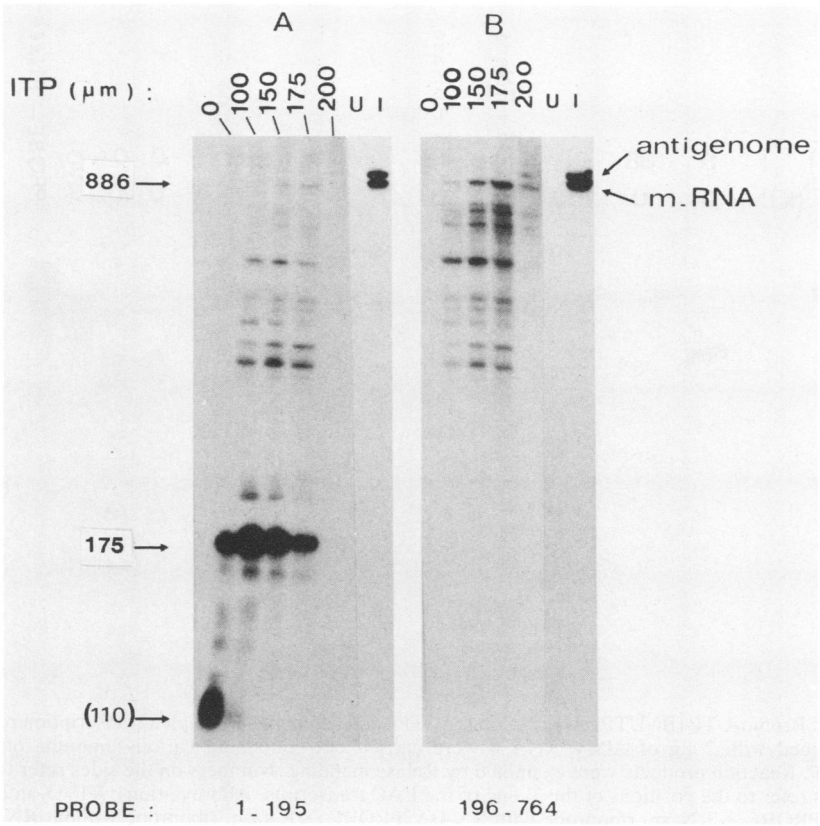

FIG. 3. Effect of ITP substitution for GTP in the uncoupled LAC transcription reaction products by Northern analysis using minus-strand riboprobes representing nucleotides 1 to 195 and 196 to 764 in individual blots. Virion transcription reactions were carried out without reticulocyte lysate but with 2 μg of AlMV RNA 4 which contained various amounts of ITP as indicated. The total amount of both ITP and GTP was always 200 μM. Lanes: U and I, 10 μg of total cytoplasmic RNA from uninfected and LAC-infected cells, respectively. Numbers 175 and 886 (on the left) refer to the positions of the 3′ ends of these RNAs; (110) refers to their estimated chain length.

longer transcripts preferentially compared with the 1–195 riboprobe (cf. the relative amounts of the longer RNAs detected by these two probes). When only ITP was present, the 1–195 riboprobe detected no products at this exposure, but the more powerful 196–764 riboprobe could still detect complete S-mRNA, which was then the most prominent product of the reaction, although the number of chains initiated was severely reduced.

The level of polymerase readthrough of position 175 was more accurately measured by RNase mapping, as in Fig. 1. When the RNA products of a polymerase reaction containing no ITP were used to protect this probe, only 5% of these transcripts had extended past position 195 (the 152 band), whereas the rest terminated at position 175 (left panel, Fig. 4). The termination site at ca. position 85 responsible for the 110-nt RNA was not detected in this instance because the resulting hybrid of ca. 42 base pairs was at the limit of stability under our RNase conditions. However, when ITP replaced 88% of the GTP in the reaction, then 80% of the RNAs made were extended past position 195 by this test. We also examined whether the effect on polymerase readthrough was due to the incorporation of I into the nascent chain, as opposed to a nonspecific effect such as the binding of a modified nucleotide to the polymerase and the subsequent alteration of its termination properties. We tested the effect of the total replacement of UTP with Bromo-UTP, which would be expected to increase base pairing. Bromo-UTP was found to be as efficiently incorporated by the LAC polymerase as UTP, but its product RNA did not extend past position 175 any more frequently than did the control (7% readthrough; right panel, Fig. 4). Similar results were also obtained with rTTP (not shown), which was also expected to strengthen base pairing. Furthermore, total replacement of the UTP with Bromo-UTP, in which ITP replaced 88% of the GTP in the reaction (left panel, Fig. 4), led to a limited but reproducible decrease (approximately half) in the fraction of the chains which extended past position 175. Since the incorporation of nucleoside analogs which increase base pairing did not allow increased polymerase readthrough but instead counteracted the effect induced by inosine incorporation, it seems reasonable that inosine incorporation into the nascent chains acted on polymerase readthrough by weakening RNA-RNA interactions.

Probable interaction of the nascent chain with its template causes premature termination. The experiments detailed above suggest that RNA-RNA interactions are important in LAC premature termination, and in this respect, the translational requirement resembles attenuation. However, when the first 175-nt region of the mRNA sequence was examined for complementarity, this region was predicted to be devoid of strong structure, and in particular, no G+C-rich stem similar to the terminator stem of the *trp* operon could be formed. Nevertheless, it is possible that a weaker or different structure could serve the same purpose. In the case of the *trp* operon, Fisher and Yanofsky (4) demonstrated that complementary oligonucleotides which specifically prevented formation of the terminator stem could by themselves cause the polymerase to read through the attenuator. We

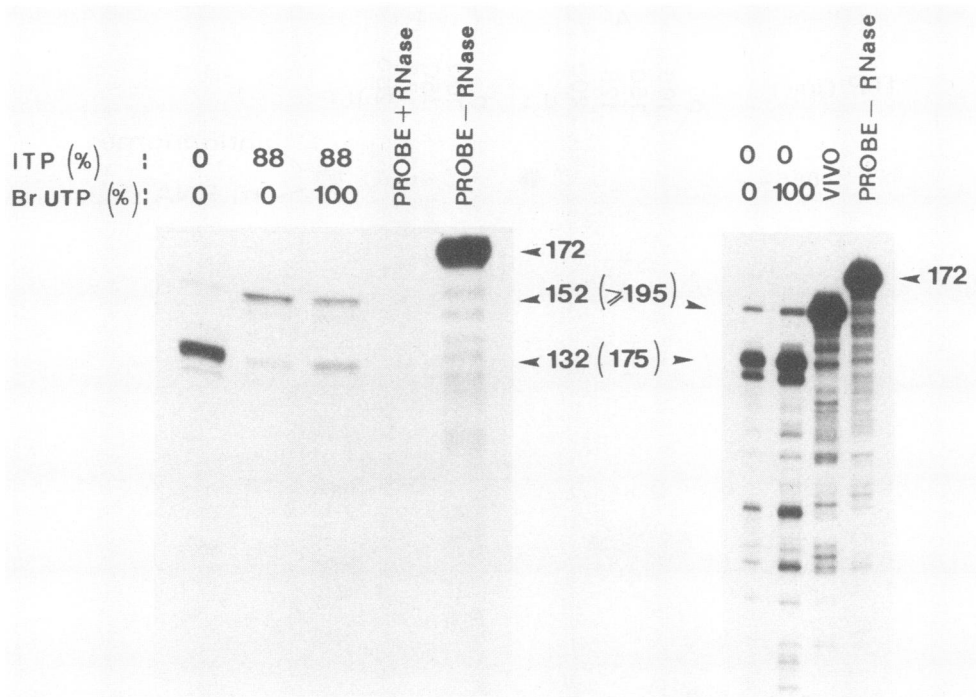

FIG. 4. Effect of ITP and Bromo-UTP (Br-UTP) substitution for GTP and UTP in the uncoupled transcription reaction by RNase mapping. Transcription reactions primed with 2 μg of AlMV RNA 4 were carried out containing various amounts of ITP relative to GTP and Bromo-UTP relative to UTP. Reaction products were examined by RNase mapping. Numbers on the sides refer to the lengths of the various RNAs; those in parentheses refer to the positions of the 3′ end of the LAC transcripts. Abbreviations: VIVO, infected-cell CsCl pellet RNA used to protect the probe; PROBE + RNase, riboprobe with RNAse; PROBE − RNase, riboprobe without RNase.

therefore argued that if LAC premature termination involves RNA-RNA interactions within the first 175 nt, these structures should be even more susceptible to interference by complementary oligonucleotides. To test this possibility, five oligonucleotides complementary to positions 82 to 179 were added either individually or in various combinations to the uncoupled virion polymerase reaction, and the resulting products were examined by RNase mapping for evidence of increased readthrough of position 175. However, even at concentrations of up to 13 μM, with variations in both the temperature and the nucleoside triphosphate concentrations to slow the progress of the polymerase, these oligonucleotides did not enhance polymerase readthrough, although all the oligonucleotides could be shown to anneal efficiently to purified S-mRNA (not shown). These results suggested that if base pairing of the nascent chain is involved in termination at position 175, these interactions are then more likely to be with the RNA genome template. In this case, the DNA oligonucleotides would have to compete with perfect RNA matches which are intramolecular as well, in that the template and nascent chain are joined at the polymerase.

We would thus like to examine whether such interactions between the nascent mRNA and its template (i.e., upstream of the polymerase and independent of those present at the growing 3′ end of the chain) take place during mRNA synthesis, but it is unclear how this examination can be approached experimentally. As a first step in this direction, however, we can examine whether sequences upstream of position 175 within genome NCs are free to anneal to complementary oligonucleotides under conditions where mRNA synthesis does not take place. To test for these possible interactions, a source of RNase H was also included so that the formation of RNA:DNA hybrids could be monitored by the specific cleavage of the NC RNA. Four plus-strand 20-mers complementary to positions 105 to 184 were individually incubated with Nonidet P-40-permeabilized virions and wheat germ extract (the source of RNase H), and the reactions were then examined by Northern blots. Unlike the reticulocyte lysate, the wheat germ extract by itself did not support LAC transcription, which remained dependent on the addition of mRNA (not shown). As shown in Fig. 5C, when either no oligonucleotide or a noncomplementary oligonucleotide (PC) was added, no evidence of any degradation of the minus-strand genome RNA could be detected. However, when the complementary oligonucleotides were added, specific cleavage of the genome RNA indicated that three of the oligonucleotides could anneal to the template, but to very different extents. When quantitated by densitometry, oligonucleotide 145–164 was found to have annealed the best, 27% of the template being specifically cleaved, whereas oligonucleotides 105–125 and 125–144 induced 13 and 4% cleavage, and only oligonucleotide 165–184 showed no evidence of any specific cleavage. As a control showing that all the oligonucleotides were equally competent to anneal to and cause RNase H to cleave unencapsidated minus-strand RNA, they were tested against a riboprobe containing positions 1 to 195. All of the oligonucleotides equally caused the specific cleavage of the minus-strand riboprobe (Fig. 5B).

We next examined whether genome NCs present in cytoplasmic extracts also contained regions that were differentially accessible to annealing with the same oligonucleotides. These extracts are transcriptionally active, but, again, only when supplemented with mRNA (2), which was withheld. As shown in Fig. 5A, essentially the same results were obtained as with virion NCs, except that there was a small

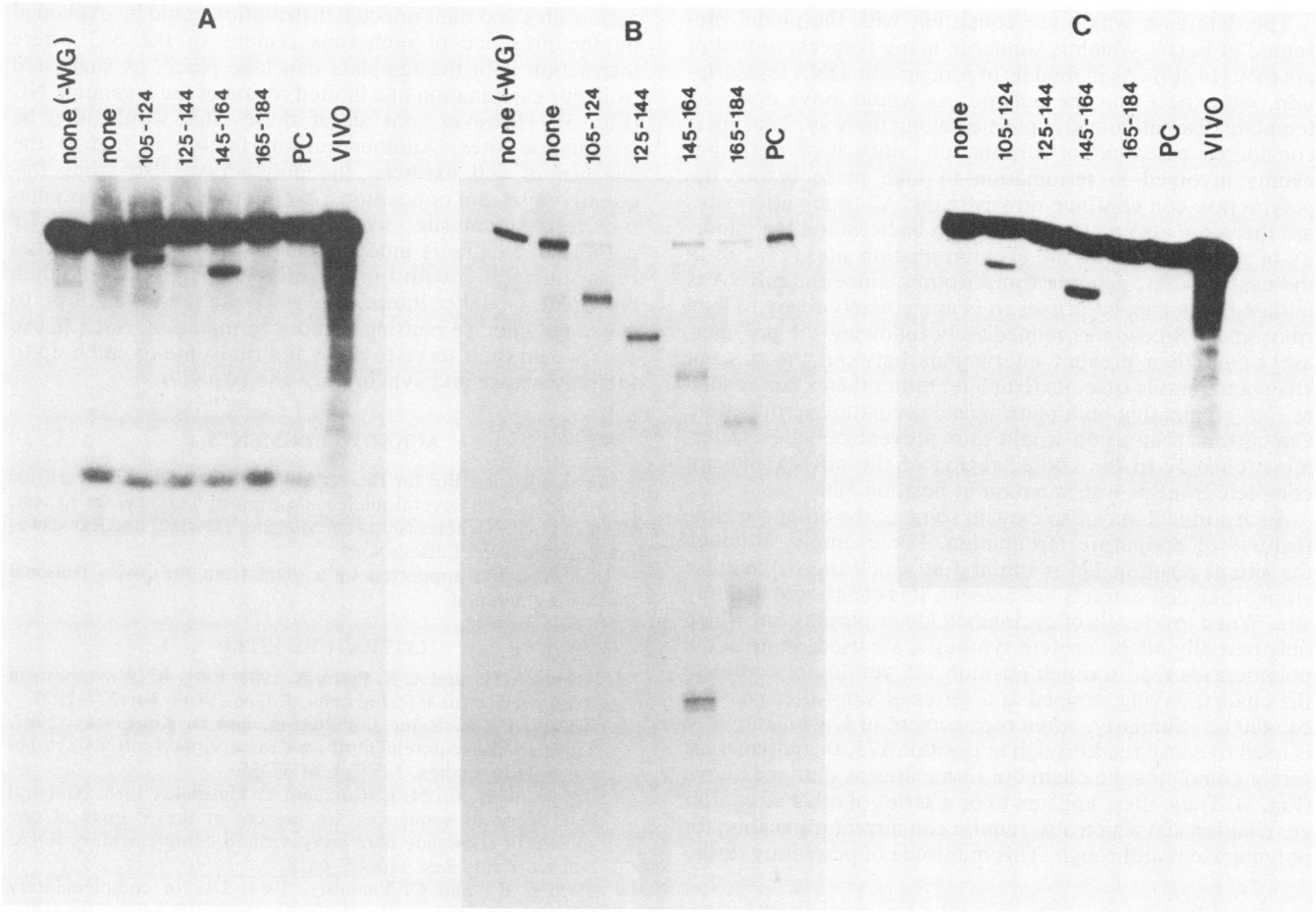

FIG. 5. Effects of oligonucleotides complementary to LAC S minus-strand genome RNA on the ability of RNase H to cleave NC RNA. LAC-infected BHK cell extracts (A) or purified virions containing 0.05% Nonidet P-40 (C) were incubated with the various oligonucleotides indicated plus wheat germ (WG) extract (see Materials and Methods). RNAs were then isolated and examined on Northern blots with complementary riboprobes containing nucleotides 196 to 764. (B) Radiolabeled minus-strand riboprobe representing nucleotides 1 to 195 was mixed with uninfected BHK cell extract and incubated with 10 pmol of the various oligonucleotides plus wheat germ extract as indicated, but for 5 min at 30°C and then separated on an 8% sequencing gel. Lanes: VIVO, total cytoplasmic RNA from infected cells used as a marker; PC, a Sendai virus-specific oligonucleotide. The weak band (A) just above the middle of the photo is apparently due to weak annealing of the probe to RNAs present in the wheat germ extract.

amount of nonspecific degradation due to the presence of the cell extract. The experiment detailed above demonstrates that some of the sequences within genome NCs are partially accessible to annealing with complementary oligonucleotides and that there is no difference between intracellular and virion NCs in this respect. Furthermore the availability of sequences within NCs for annealing would appear to be a property of the NC structure rather than of the RNA itself, since all the oligonucleotides could anneal efficiently to unencapsidated RNA.

In several experiments, the relative amounts of cleavage were found to be characteristic of each oligonucleotide, and in no case did maximum cleavage exceed 27%. Increasing the concentration of oligonucleotide above the level of 6.6 μM used in the experiments shown did not increase specific cleavage of the NC RNA, nor did the addition of commercial RNase H or doubling the amount of wheat germ extract increase specific cleavage, presumably because neither the oligonucleotide nor the RNase H was limiting. When the kinetics of NC RNA cleavage were examined, the specific cleavage products increased for the first 20 min and then remained constant for the next 20 min. It thus appears that the cytoplasmic and virion NCs examined represent a mixed population, constituting structures where some selected sequences are available for annealing and others where they are not.

DISCUSSION

The experiments presented here further support the notion that translation of the nascent mRNA is required to prevent the polymerase from terminating prematurely. In the absence of translation, the replacement of G with I residues in the nascent chain also suppresses premature termination. It is therefore likely that RNA-RNA interactions of the nascent mRNA are involved in premature termination. Attempts to demonstrate that these interactions were within the nascent chain alone were uniformly negative. We were, however, able to show that some of the sequences within genome NCs are available for annealing with complementary oligonucleotides in the absence of transcription. These template sequences might then also be available for interaction during mRNA synthesis and perhaps to a greater extent.

The data as a whole are consistent with the model presented in Fig. 6, which is similar in many respects to that of polarity (14, 19). As in models of procaryotic DNA transcription, we expect that the polymerase would move down its template discontinuously, pausing along the way. Pausing is considered essential for termination, since it allows other events involved in termination to take place before the polymerase can continue downstream. All termination sites are therefore also pause sites, but in bacteria, only a minority of these pause sites are also termination sites (19, 20). In the case of LAC genome transcription, since the mRNA is initiated on a capped primer, it is immediately ready to load ribosomes. Ribosomes immediately following the polymerase would then prevent interactions between the nascent chain and, in this case, its template, rather than a factor such as *rho*, eliminating both pausing and premature termination. Concurrent translation would thus prevent polymerase termination only in the coding region of the mRNA but not complete S-mRNA termination at position 886.

Such a model may also explain some of the other puzzling features of premature termination. For example, although the site at position 175 is the first at which the polymerase stops when cell extracts are present, it is clearly not the only site. When low levels of cycloheximide or puromycin, which only partially inhibit protein synthesis, are used, some of the polymerases read through position 175 yet do not complete the chains, having stopped at a series of sites after position ca. 400 (2). Similarly, when replacement of G with I in vitro is used to cause readthrough at position 175, the polymerase rarely completes the chain but terminates as outlined above (Fig. 3). Thus, there appears to be a series of other sites after ca. position 400 which also require concurrent translation for polymerase readthrough. This multitude of premature termination sites and their unequal distribution could be explained by the presence of numerous regions of the NC where interaction with the template can take place, as suggested from our examination of a limited region of the S genome NC (Fig. 5). However, not all of these sites would also be termination sites. Another curious feature is that in the absence of cell extracts, the polymerase also stops frequently upstream of position 175 (110-nt RNA). This premature termination site, which can be suppressed both by cytoplasmic extracts independent of translation and partial replacement of G with I in the mRNA (Fig. 3A), could then represent a weaker interaction whose function might be to cause polymerase pausing without termination. Such pause sites would then serve to allow the ribosome to catch up to the polymerase and synchronize the two (20).

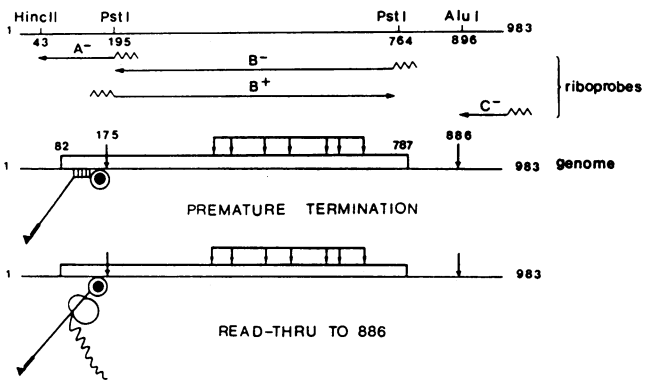

FIG. 6. Model for the translational requirement for complete LAC S-mRNA synthesis. A partial restriction map of the S genome segment and the various riboprobes used is shown. The genome template is shown below. The boxed area (positions 82 to 787) indicates the N protein-coding region. The arrow (position 175) indicates the first of the premature termination sites; another arrow (position 886) marks the termination site for complete S-mRNA. Downstream of position 175, a series of premature termination sites which similarly require translation for polymerase readthrough exist which are only detected when termination at position 175 is incomplete. Symbols: ◉, viral polymerase; ▬, 5′ end and capped primer of the nascent mRNA chain with the other end attached to the polymerase. In the absence of translation, nascent mRNA hybridizes to its template at certain sites causing the polymerase to stall and terminate at position 175. Upon translation, the movement of the ribosome behind the polymerase prevents these interactions and the polymerase reads through position 175 and all subsequent premature termination sites.

ACKNOWLEDGMENTS

We thank John Bol for the AlMV RNA 4 and David Ward for providing us with several nucleoside triphosphate analogs to test. We also thank Nicholas Acheson, Martinez Hewlett, and Ramaswamy Raju for helpful discussions.

This work was supported by a grant from the Swiss National Science Foundation.

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