Evidence for splicing of avian sarcoma virus 5'-terminal genomic sequences onto viral-specific RNA in infected cells

(gene splicing/retrovirus RNA/hybridization/RNase H hydrolysis)

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ABSTRACT The 5'-terminal nucleotide sequences of the avian sarcoma virus (ASV) genome are transcribed by the reverse transcriptase *in vitro* into a DNA transcript that represents the entire distance (~100 nucleotides) between the tRNA^{Trp} primer molecule and the 5' terminus. We have used these DNA₁₀₀ transcripts in hybridization reactions with ASV-specific RNA from infected avian cells and find nucleotide sequences complementary to these transcripts on all of the various size classes of viral mRNA identified. Similar hybridization results were obtained with a specific DNA transcript complementary to viral genomic nucleotide sequences between the tRNA^{Trp} primer molecule and up to, but not including, the terminal redundant sequences (DNA₇₀), indicating that the observed hybridization of DNA₁₀₀ to all size classes of viral RNA in infected cells did not reflect hybridization of DNA₁₀₀ to the terminal redundant sequences at the 3' end of the viral genome. *Escherichia coli* RNase H hydrolysis of RNA-DNA hybrids consisting of genomic 35S RNA obtained from virus and DNA₁₀₀ transcripts indicated that viral genomic sequences complementary to these DNA transcripts were not present at sites distal to the ends of the RNA genome and therefore not adjacent to the corresponding gene sequences representing the various species of viral mRNA from infected cells. These studies suggest that the 5'-terminal genomic nucleotide sequences, or a portion thereof, are somehow added or "spliced" onto each ASV-specific mRNA species in infected cells either during or after transcription of proviral DNA for some as yet undetermined purpose.

The 5' terminus of the avian sarcoma virus (ASV) genome appears to play an important role in the replication of the virus for several reasons. First, initiation of reverse transcription occurs on a tRNA^{Trp} primer molecule located near (~100 nucleotides) this end of the viral genome (refs. 1-10). Second, a portion of the 5' terminus (i.e., 21 nucleotides) is repeated at the 3' end of the viral genome and presumably is used to facilitate continued, uninterrupted transcription of the viral genome during proviral DNA synthesis (5, 11-14). Finally, the initiator codon, AUG, and nucleotide sequences implicated in the binding of eukaryotic ribosomes to mRNA are present within this region of the genome, suggesting a possible role in the initiation of translation of the viral genome (6).

In this communication we present a series of experiments indicating that the 5'-terminal genomic nucleotide sequences, or a portion thereof, are present on each size class of ASV-specific mRNA identified in infected cell cultures. Furthermore, we demonstrate that these nucleotide sequences are not present adjacent to the corresponding gene sequences in genomic RNA obtained from virus. These data suggest that the 5'-terminal nucleotide sequences of the ASV genome are somehow "spliced" onto the individual species of viral-specific mRNA present in infected cells to provide some as yet unknown

function. This phenomenon is similar to that reported recently in the adenovirus system in which a common 5'-terminal sequence of 150-200 nucleotides appears to be spliced onto the end of several mRNA species during maturation of the mRNAs from larger precursors (15, 16).

MATERIALS AND METHODS

Reagents and Virus. The sources and preparation of most of the materials used in this study have been reported (17-19). Avian myeloblastosis virus (AMV) (in plasma) and purified AMV RNA-directed DNA polymerase were from J. W. Beard of Life Sciences, Inc., St. Petersburg, FL. These materials were obtained through the Office of Resources and Logistics, Viral Cancer Program, National Cancer Institute. The B77 strain (subgroup C) and Schmidt-Ruppin (subgroup D) strains of Rous sarcoma virus (RSV) were propagated in avian embryo fibroblasts and purified as described (18).

Purification of Viral RNA. RNA was extracted from purified virus with Pronase/sodium dodecyl sulfate (NaDodSO₄)/phenol and subsequently fractionated into 70S RNA and free low molecular weight RNA by rate-zonal sedimentation (18). ³²P-Labeled RSV (B77 strain) was prepared and purified by described procedures (18).

Synthesis of Viral-Specific Complementary DNA Transcripts. Virus-specific DNA (cDNA), representing the majority of viral genomic nucleotide sequences, was synthesized with detergent-activated RSV as described by Stehelin *et al.* (20). The specific activity of the [³H]cDNA probe was approximately 2.4 × 10⁷ cpm/μg. The [³H]cDNA probe specific for the transforming gene sequences (cDNA_{src}) of ASV was prepared as described (20, 21).

The enzymatic synthesis of cDNA transcripts representing defined regions of the 5' terminus of the viral genome was performed in reconstructed reactions containing reconstituted 35S RNA-tRNA^{Trp} complexes (10 μg/ml) as template/primer and purified AMV DNA polymerase (30-60 units/ml) (19, 22). The reaction mixtures consisted of 0.1 M Tris-HCl (pH 8.1), 0.01 M MgCl₂, 1.4% (vol/vol) 2-mercaptoethanol and three unlabeled dNTPs at 60 μM and one labeled dNTP at 6 μM. Incubation was for 1 hr at 37°. Termination of enzymatic synthesis and subsequent purification of the DNA transcripts have been described (19).

Under these conditions of enzymatic synthesis, two predominant single-stranded species of initial DNA (iDNA) transcripts representing nucleotide lengths of 100, and 70, are

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Abbreviations: ASV, avian sarcoma virus; AMV, avian myeloblastosis virus; RSV, Rous sarcoma virus; NaDodSO₄, sodium dodecyl sulfate; iDNA, initial DNA.

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FIG. 1. Lack of detectable endonuclease activity associated with purified preparations of *E. coli* RNase H. ^{32}P -Labeled ASV 35S RNA (75,000 cpm, 15 ng) was incubated in the presence (broken line) or absence (solid line) of *E. coli* RNase H (0.04 unit) for 30 min at 37° in 0.1 M Tris-HCl, pH 8.1/0.01 M MgCl_2 /1.4% (vol/vol) 2-mercapto-ethanol. After the incubation, the reactions were adjusted to 20 mM EDTA and 1% NaDodSO₄ and subjected to electrophoresis in 98% formamide/3.5% polyacrylamide gels at 150 V for 2.5 hr as described (19).

synthesized at the 5' terminus of the viral genome (10, 19, 22). The individual iDNA species were further purified by formamide/polyacrylamide gel electrophoresis (17). iDNA transcripts 100 nucleotides long (iDNA₁₀₀) represent the genomic sequences from the 3'-OH of tRNA^{Trp} directly to the 5' end of the viral RNA (19). iDNA₇₀, on the other hand, represents genomic sequences that extend from the 3'-OH of tRNA^{Trp} primer up to but not including the terminal redundant sequences (12). iDNA₇₀ transcripts do not hybridize to the 3' end of the viral genome (10). Based upon nucleotide sequence analysis of viral RNA protected from RNase hydrolysis by these 5'-DNA transcripts, no contamination with cDNA representing other portions of the viral genome was detected (10, 12).

Oligo(dC)₁₂₋₁₈-initiated DNA transcripts were synthesized from 35S RNA-oligo(dC)₁₂₋₁₈ template/primer complexes with the purified AMV DNA polymerase. Oligo(dC)₁₂₋₁₈-initiated DNA transcripts have been characterized, and the bulk represent nucleotide sequences at the 5' end of the sarcoma gene approximately 2000–3000 nucleotides from the 3' end of the viral genome (M. S. Collet and A. J. Faras, unpublished data).

Purification of *Escherichia coli* RNase H. The purification of RNase H from *E. coli* was carried out by the procedure of Berkower *et al.* (23). These preparations were free of contaminating endoribonuclease and exoribonuclease activities because incubation with ^{32}P -labeled viral RNA at 37° under conditions used in our studies to hydrolyze RNA-DNA hybrid structures neither nicked the RNA genome internally (Fig. 1) nor released nucleotides from either termini (Fig. 4).

Preparation of Poly(A)-Containing RNA from Cytoplasm of RSV-Infected Chick Cells. RSV-infected cells used as a source of cytoplasmic RNA were harvested from roller bottles (2×10^8 cells) by scraping and washed three times with phosphate-buffered saline and once with RSB buffer (0.01 M Tris-HCl, pH 8.3/0.01 M NaCl/1.5 mM MgCl_2). Cytoplasmic RNA was extracted as described (21). Poly(A)-containing RNA was then isolated from cytoplasmic RNA by repeated affinity chromatography on poly(U)-Sephadex as described (8). Of the total RNA applied to the column, approximately 1–2% was bound to poly(U)-Sephadex and therefore contained poly(A) sequences.

Rate-Zonal Sedimentation and Hybridization Analysis of Poly(A)-Containing RNA. The conditions used for rate-zonal

centrifugation were essentially those described by McKnight and Schimke (24). Approximately 10 μg of poly(A)-containing RNA in 300 μl of TE buffer (0.02 M Tris-HCl, pH 7.4/0.01 M EDTA) containing 1% NaDodSO₄ was heat denatured by incubation at 65° for 10 min and then cooled rapidly to 0° . The RNA was layered directly onto a 15–30% (wt/vol) linear sucrose density gradient made in 0.01 M Tris-HCl, pH 7.4/5 mM EDTA/1% NaDodSO₄ and centrifuged in an SW 41 rotor at 25,000 rpm for 16 hr at 25° . The gradients were collected from the bottom, and the RNA in each fraction was precipitated in the presence of 0.2 M sodium acetate and 100 μg of yeast-tRNA with 2 volumes of ethanol. The RNA was collected by centrifugation and resuspended in 60 μl of TE buffer. A constant volume of each fraction, generally 3–10 μl , was dried in a vacuum desiccator, resuspended in 10 μl of 0.01 M Tris-HCl, pH 7.4/0.6 M NaCl/1 mM EDTA with single-strand ^3H - or ^{125}I -labeled cDNA (1500 cpm) and yeast tRNA (1.0 mg/ml), sealed in 30- μl capillary tubes, and incubated for 24–48 hr at 68° . Hybrid formation was detected by incubation with single-strand-specific S1 nuclease (*Aspergillus oryzae*) as described by Leong *et al.* (25).

RESULTS

Identification of Several Size Classes of ASV-Specific mRNA in Infected Permissive Cells. We (unpublished data), as well as others (26–28), have recently identified several major species of viral-specific mRNA in ASV-infected permissive cells. These species have been identified by isolating total poly(A)-containing cytoplasmic RNA from ASV-infected chick cells, fractionating the RNA by rate-zonal centrifugation, and hybridizing each fraction of the gradient with a constant amount of radiolabeled viral-specific cDNA probe to determine the number of size classes of viral-specific RNA present. The results of such an experiment are presented in Fig. 2A. Three major peaks of viral-specific RNA were observed in the gradient: one sedimenting with the 35S viral RNA genome, one at 28 S, and one slightly ahead of the 18S RNA marker, at 21 S. These viral-specific mRNA species represent molecular weight ranges on the order of $3.0\text{--}3.5 \times 10^6$ (29–31), $1.8\text{--}2.0 \times 10^6$, and $1.0\text{--}1.2 \times 10^6$, respectively, and are compatible with the size estimates for viral-specific RNA obtained recently by others (26–28). The RNA whose analysis is shown in Fig. 2 was selected by poly(U)-Sephadex chromatography and therefore contains poly(A); however, total cytoplasmic RNA exhibited an identical profile when analyzed in a similar manner.

A small amount of viral-specific RNA sedimenting between 12 and 15 S was also identified by these analyses. Although reconstructed experiments in which ^{32}P -labeled RNA was present throughout the RNA extraction procedure suggested that no breakdown of viral RNA occurred during extraction, it is conceivable that this population represents either transcription of the common (C) region (~ 800 nucleotides long) between the sarcoma (*src*) gene and the 3'-poly(A) tract (32) or the end-product of viral mRNA turnover in ASV-infected cells. That each of these viral-specific RNA species is indeed mRNA was demonstrated by their association with, and EDTA-mediated release from, polyribosomes (unpublished data). On the basis of hybridization analyses with cDNA probes representing specific gene sequences of the viral genome (e.g., cDNA_{src}, cDNA_{env}, cDNA_{gag/pol}) (refs. 26 and 27; Fig. 2A) and a uniform cDNA probe representing the entire viral genomic sequences (unpublished data), the 35S RNA species represents the entire viral genome, the 28S species represents the *env* and *src* genes, and the 21S represents only the *src* gene sequences.

FIG. 2. The presence of iDNA₁₀₀ and iDNA₇₀ complementary sequences in poly(A)-containing viral-specific mRNA from ASV-infected avian cells. Approximately 10 μ g of poly(A)-containing RNA was heat denatured (65°, 10 min) and centrifuged in a 15–30% (wt/vol) linear sucrose gradient, containing 0.01 M Tris-HCl (pH 7.4), 5 mM EDTA, and 1% NaDodSO₄, in a SW 41 rotor at 25,000 rpm for 16 hr at 25°. Labeled RSV 35S RNA and chick fibroblast 28S and 18S RNAs were included in a parallel gradient as sedimentation markers. The gradients were collected, and the RNA in each fraction was precipitated with ethanol, pelleted, and then resuspended in 60 μ l of TE buffer. A constant volume was removed from each fraction and hybridized with a radiolabeled cDNA probe at 68° for 24–48 hr. Hybridization was measured by the resistance of the cDNA probes to hydrolysis with S1 nuclease (25). (A) Hybridization with total [³H]-cDNA (○) and [³H]cDNA_{arc} (●) probes. (B) Hybridization with ¹²⁵I-labeled iDNA₁₀₀ (○) and ¹²⁵I-labeled iDNA₇₀ (●) transcripts.

Identification of Nucleotide Sequences Complementary to iDNA₁₀₀ Transcripts in All Size Classes of ASV mRNA from Virus-Infected Cells. Reverse transcription of the ASV genome is initiated on the tRNA^{Trp} primer molecule located approximately 100 nucleotides from the 5' end of the viral genome (1–10). We have recently characterized the DNA transcripts synthesized at, and therefore containing nucleotide sequences complementary to, the 5' end of the viral genome (10, 12). Of these iDNA₁₀₀ represents the entire distance between the tRNA^{Trp} primer and the 5' end of the viral genome; iDNA₇₀ represents genomic sequences from the tRNA^{Trp} primer molecule up to, but not including, the terminal redundant sequences (12).

We used iDNA transcripts in hybridization reactions with the fractionated viral-specific mRNA species from infected cells in an effort to determine which of the viral mRNA species contain nucleotide sequences complementary to these transcripts. When iDNA₁₀₀ transcripts were annealed in the presence of RNA from each of the gradient fractions, hybridization occurred not only to the 35S RNA species but also to the 28S and

21S mRNA species as well (Fig. 2B). To rule out the possibility that these results reflected hybridization of iDNA₁₀₀ transcripts to the terminal redundant nucleotide sequences present at the 3' end of the viral genome, we repeated this experiment with iDNA₇₀ transcripts which lack sequences complementary to the viral terminal redundancy (12) and do not hybridize to the 3' end of the viral genome (10, 12). The iDNA₇₀ transcripts also hybridized to all of the size classes of viral-specific mRNA present in ASV-infected cell cultures (Fig. 2B). Because no effort was made to drive the hybridization reaction to completion in these studies, the extent of hybridization of iDNA transcripts to the various size classes of viral-specific mRNA in infected cells was incomplete. However, when annealing was performed under conditions that facilitated maximal hybridization, approximately 70–80% of the iDNA₁₀₀ transcripts hybridized to all size classes of viral-specific mRNA. Because the iDNA₁₀₀/iDNA₇₀ transcripts hybridize to all of the identified size classes of poly(A)-containing viral mRNA, including those that were appreciably smaller than the ASV genome, these results indicate that nucleotide sequences present at the 5' terminus of the viral 35S RNA genome are also present on each of the size classes of mRNA detected in infected cells.

Analysis of ASV 35S Viral RNA for the Presence of Sequences Complementary to iDNA₁₀₀ Transcripts Distal to the 5' Terminus. The identification of nucleotide sequences complementary to iDNA₁₀₀ transcripts on every size class of viral mRNA provided the impetus to analyze 35S RNA obtained from virus for the presence of such sequences within the viral genome itself. In these studies ³²P-labeled viral 35S RNA was hybridized to iDNA₁₀₀ transcripts under conditions in which the DNA transcripts were in excess (20-fold) of the concentration required to saturate nucleotide sequences complementary to iDNA₁₀₀ transcripts on every molecule of viral RNA present in the hybridization reaction. The resultant viral RNA·iDNA₁₀₀ hybrids were then treated with *E. coli* RNase H, which lacked detectable contaminating endoribonuclease activity (Fig. 1), and the RNA was analyzed under denaturing conditions in 98% formamide/3.5% polyacrylamide gels (Fig. 3). No nicking of ³²P-labeled viral 35S RNA was detected by such an analysis after *E. coli* RNase H treatment of the hybrid structures, indicating that there were no genomic sites internal to the termini (i.e., >500 nucleotides) that contained sequences complementary to iDNA₁₀₀.

That *E. coli* RNase H was indeed active on viral 35S RNA·DNA hybrids under these conditions of hydrolysis was demonstrated by the following observations. First, 35S RNA·iDNA₁₀₀ hybrids lost the capacity to bind to oligo(dT)-cellulose subsequent to *E. coli* RNase H hydrolysis (data not shown). This result was attributable to the fact that iDNA₁₀₀ transcripts can bind to the terminally redundant nucleotide sequences adjacent to the poly(A) 3' terminus as well as the 5' terminus in order to provide a suitable substrate for *E. coli* RNase H at both ends of the viral genome. Loss of the poly(A) sequence from the viral RNA would therefore be expected to occur if *E. coli* RNase H were active on these hybrid molecules. Second, RNase H treatment of 35S RNA·iDNA₁₀₀ hybrid complexes released oligonucleotides of various sizes as demonstrated by polyacrylamide electrophoresis of these complexes on 20% slab gels (Fig. 4). In these studies the amount of radioactivity released (~1%) suggested that all of the 35S molecules were complexed to iDNA₁₀₀ transcripts and that most, if not all, of the viral RNA hydrogen-bonded to iDNA₁₀₀ transcripts were hydrolyzed by RNase H. Because the 5'-terminal capped nucleotide sequence is also released during this RNase H treatment (33), these results indicate that hydrolysis was occurring at the 5' end. Under

FIG. 3. Effect of *E. coli* RNase H hydrolysis on ^{32}P -labeled 35S viral RNA-cDNA hybrids containing DNA transcripts of defined sequences. (A) ^{32}P -Labeled 35S viral RNA (25,000 cpm, 5 ng) was reannealed with iDNA₁₀₀ (1 ng) in 2 μl of 0.02 M Tris-HCl, pH 7.4/0.6 M NaCl/0.01 M EDTA for 30 min at 68° and then added to RNase H hydrolysis buffer [0.01 M Tris-HCl, pH 8.1/10 mM MgCl₂/1.4% (vol/vol) 2-mercaptoethanol containing 500 μg of yeast tRNA per ml]. *E. coli* RNase H was added and the mixture was incubated for 30 min at 37°. Termination of the reaction was accomplished by adjusting the mixture to 1% NaDodSO₄ and 20 mM EDTA. Another sample of ^{32}P -labeled 35S RNA was treated in an identical fashion except that iDNA₁₀₀ was absent from the reannealing mixture. Both samples were subjected to electrophoresis in 98% formamide/3.5% polyacrylamide gels as in Fig. 1. Broken line, ^{32}P -labeled viral RNA-iDNA₁₀₀ hybrids incubated in the presence of *E. coli* RNase H; solid line, ^{32}P -labeled viral 35S RNA incubated in the presence of *E. coli* RNase H. (B) ^{32}P -Labeled viral RNA (75,000 cpm, 15 ng) was reannealed in the presence (broken line) or absence (solid line) of oligo(dC)₁₂₋₁₈-initiated DNA transcripts (1.5 ng), treated with *E. coli* RNase H, and subjected to electrophoresis on 98% formamide/3.5% polyacrylamide gels as in A.

similar conditions of RNase H treatment, viral 35S [^{32}P]RNA hybridized to the internal oligo(dC)₁₂₋₁₈-initiated DNA transcripts was completely degraded to small genomic fragments (Fig. 3B), indicating that the conditions were satisfactory for *E. coli* RNase H hydrolysis to occur on hybrid structures containing DNA transcripts distal to the termini.

Finally, the oligodeoxynucleotide (dC)₁₂₋₁₈ facilitated RNase H-mediated degradation of 35S [^{32}P]RNA after hybridization, suggesting that our conditions of RNase H hydrolysis were sufficient to detect even small regions of homology between the 5' terminal DNA transcripts and viral genomic sequences distal to the termini if they existed after hybridization (data not shown).

DISCUSSION

The data presented in this communication indicate that nucleotide sequences complementary to iDNA₁₀₀/iDNA₇₀ transcripts and representing the 5' end of the viral genome are found on all of the various size classes of viral-specific mRNA present in ASV-infected cells. These include: a genome-length 35S RNA species that contains the *gag*, *pol*, *env*, and *src* genes; 28S RNA which contains the *env* and *src* genes; and 21S RNA

FIG. 4. Release of nucleotides from ^{32}P -labeled 35S RNA-iDNA₁₀₀ hybrids by *E. coli* RNase H. ^{32}P -Labeled viral RNA was reannealed in the presence (lane A) or absence (lane B) of iDNA₁₀₀ transcripts at DNA:RNA ratios similar to those in Fig. 3 and treated with *E. coli* RNase H. The reaction was terminated by adjustment to 1% NaDodSO₄ and 20 mM EDTA, and the mixtures were layered onto a 3%/20% composite polyacrylamide slab gel after heating for 1 min at 100°. Electrophoresis was for 6.5 hr at 400 V at 10° in Tris-borate buffer, pH 8.3. The 3% polyacrylamide portion of the gel was removed, the [^{32}P]RNA was measured, and the remainder of the gel was subjected to autoradiography. The percentage of the radioactivity released due to RNase H hydrolysis of the ^{32}P -labeled viral RNA-iDNA₁₀₀ hybrids was determined by totaling the radioactivity between the arrows and comparing this value with the total radioactivity present in both the 3% and 20% portions of the gel. In this experiment, 0.93% of the total radioactivity was released from the ^{32}P -labeled 35S RNA by *E. coli* RNase H hydrolysis as oligonucleotides that migrated between the arrows. Because the theoretical maximal cpm that would be released from a region of the viral genome associated with DNA transcripts 100 nucleotides long would be 0.95% (e.g., 100 nucleotides $\div$ 9500 nucleotides), these data indicate that all of the 35S molecules present in the reannealing mixture were associated with iDNA₁₀₀ transcripts during hybridization.

which contains only the *src* genes (26-28). Initially, we interpreted the data to mean that sequences complementary to iDNA₁₀₀/iDNA₇₀ transcripts were present not only at the 5' terminus of the viral genome but also within the viral genome, adjacent to the *env* and *src* genes. However, when this presumption was tested directly by hybridizing iDNA₁₀₀ transcripts to radiolabeled viral 35S RNA, hydrolyzing the resultant hybrid complex with *E. coli* RNase H, and analyzing the viral RNA on polyacrylamide gels, the only regions of the viral genome complexed to the iDNA₁₀₀ transcripts were the 5' terminus from which these transcripts were originally derived and the 3' terminus adjacent to the poly(A) which contain the terminally redundant nucleotide sequences. Because, under our conditions of RNase H hydrolysis, even small regions (~20

nucleotides) of homology between the 5' DNA transcripts and viral RNA sequences distal to the termini would have been detected, it is apparent that the bulk (>80%) of the 5' genomic sequences are absent elsewhere on the viral genome.

These data indicate that, although the 5'-terminal nucleotide sequence of the ASV genome is present on all of the size classes of viral mRNA from infected cells, these sequences are not present adjacent to the corresponding gene sequences (e.g., *env* and *src*) within the viral genome. Because iDNA₇₀, which lacks nucleotide sequences complementary to the terminal redundancy (12) and does not hybridize to the 3' end of the viral genome (10), also hybridizes to all three species of RSV-specific mRNA from infected cells, these observations cannot be attributed to the redundant sequences adjacent to the poly(A) at the 3' end. These data are consistent with restriction endonuclease mapping studies of ASV proviral DNA which indicate that nucleotide sequences complementary to iDNA₁₀₀ transcripts are not present on restriction fragments of ASV-specific DNA that code for the 28S and 21S viral-specific mRNA species (34). Therefore, the nucleotide sequences complementary to iDNA₁₀₀ transcripts must be somehow attached or "spliced" onto the discrete classes of viral-specific mRNA identified in infected cells at some point during their synthesis or maturation.

A similar situation involving the splicing of leader sequences, present at one location on the viral genome, onto mRNAs representing another region of the genome has been recently described in both the adenovirus and simian virus 40 systems (15, 16). The mechanism for synthesis of mosaic mRNA molecules containing leader sequences from a specific region of the viral genome is unknown, as is the role of these leader sequences in viral gene expression. These leader sequences could be attached to a mRNA species either during or after transcription of particular mRNA species or during processing of heterogeneous nuclear RNA by some sort of ligation reaction. In the case of ASV, if the 5' genomic sequences present on viral-specific mRNA in infected cells are located at the 5' end of the various mRNA species, they could conceivably play an important role in the initiation of protein synthesis because they contain ribosome binding sequences (6). Irrespective of the function of these leader sequences in viral gene expression, their identification in yet another virus system suggests that this "splicing" phenomenon may be a common feature characteristic of the production and processing of all mRNA species in eukaryotic cells.

Note Added in Proof. While this paper was in review, Mellon and Duesberg (35) published data indicating that subgenomic, cellular RNA from Prague RSV-B-infected cells also contain sequences representing the 5' terminus of the viral genome. Since we submitted our manuscript, we have demonstrated that the 28S and 21S viral mRNAs contain the capped T₁ RNase-resistant oligonucleotide present at the 5' terminus of the viral genome. Furthermore, we have shown that the 5' viral genomic sequences present on the 28S and 21S mRNA species include the tRNA^{Trp} primer, binding site and at least some genomic nucleotide sequences distal to the 3' end of the primer binding site.

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