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Nucleotide sequence at the 5′ terminus of the avian sarcoma virus genome

(DNA sequence/RNA sequence/RNA-directed DNA synthesis/messenger RNA/ribosome binding)

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ABSTRACT **Transcription of DNA from the RNA genome of avian sarcoma virus by RNA-directed DNA polymerase *in vitro* initiates on a primer ($tRNA^{Trp}$) located near the 5′-terminus of the viral genome. One of the major products of transcription is a single-stranded DNA chain complementary to a sequence of 101 nucleotides immediately distal to the site of initiation of DNA synthesis. We have determined the complete nucleotide sequence of this transcribed chain for the Prague strain of avian sarcoma virus, a partial sequence of the transcribed chain for the Bratislava 77 strain of avian sarcoma virus, and the sequence of a DNA transcript that is shorter than the transcribed single-stranded chain. Our data define the location of $tRNA^{Trp}$ on the genome of avian sarcoma virus and provide the sequence of 119 nucleotides at the 5′-terminus of the genome. Portions of this sequence may be involved in the binding of RNA-directed DNA polymerase, the initiation of translation from viral messenger RNA, the extension of RNA-directed DNA synthesis from the 5′- to the 3′-terminus of viral RNA, and the integration of viral DNA into the host genome.**

Avian sarcoma viruses (ASV) are retroviruses (1) whose replication requires transcription of DNA from the viral genome by RNA-directed DNA polymerase (2). Transcription from the genome of ASV *in vitro* initiates on the primer $tRNA^{Trp}$ (3, 4) located near the 5′-terminus of the viral RNA (5, 6). One of the major products of transcription is a DNA chain (denoted $cDNA_{5'}$) complementary to 101 nucleotides of the viral RNA immediately distal to the site of initiation (7–9). We have previously purified and characterized $cDNA_{5'}$ for ASV and we have described the utility of this DNA for the analysis of viral structure and replication (10). We report here the complete sequence of the 101 nucleotides which constitute $cDNA_{5'}$ synthesized with the Prague subgroup C strain (Pr-C) of ASV and a partial sequence for $cDNA_{5'}$ of Bratislava 77 subgroup C strain (B77-C) of ASV.

Using the nucleotide sequence of $cDNA_{5'}$ and previous data from our laboratory, we have deduced the sequence of 119 nucleotides at the 5′-terminus of the Pr-C ASV genome. The details of this sequence and its secondary structure are of interest because the 5′-terminus of the viral genome is involved in the initiation of DNA synthesis (7, 11, 12), the binding of ribosomes for the initiation of viral protein synthesis (13, 14), the extension of RNA-directed DNA synthesis from the 5′-terminus to the 3′-terminus of the viral genome (7, 11, 12), and the integration of viral DNA into the host genome (7, 11, 12).

MATERIALS AND METHODS

Reagents. Carrier-free ^{32}P (orthophosphoric acid in H_2O) was obtained from New England Nuclear. [γ-^{32}P]ATP (approximately 1500 Ci/mmol) was prepared by the exchange reaction originally described by Glynn and Chappell (15) and modified by Maxam and Gilbert (16). Polynucleotide kinase [polynucleotide synthetase (ATP), poly(deoxyribonucleotide: poly(deoxyribonucleotide)ligase (AMP-forming), EC 6.5.1.1] from T_4-infected *E. coli* was a gift from N. Cozzarelli. Acrylamide and *N,N′*-methylene-bis-acrylamide were electrophoresis grade from Bio-Rad Laboratories. Urea (ultra-pure) was purchased from Schwarz/Mann.

Cells and Viruses. We have described previously the propagation and purification of ASV (17). B77-C ASV was obtained from R. Friis. Pr-C ASV was provided as concentrated suspensions by University Laboratories through the auspices of the Office of Program Resources and Logistics, National Cancer Institute. Both strains of virus have been extensively propagated and cannot be considered clonal stocks.

Isolation of $cDNA_{5'}$. The synthesis and purification of $cDNA_{5'}$ were carried out as described (10). The DNA was then fractionated by electrophoresis in a slab gel of 10% polyacrylamide; details are given for individual experiments.

Analysis of DNA Sequence. The entire procedure was as described by Maxam and Gilbert (16). DNA was labeled by phosphorylation with polynucleotide kinase, purified by electrophoresis in gels of 10% polyacrylamide, and then subjected to base-specific cleavages. The products of cleavage were separated by electrophoresis in a slab gel of 20% acrylamide and located on the gel by autoradiography.

Identification of 5′-Terminal Nucleotides in DNA. Single-stranded DNA was labeled at 5′-termini as described above, then exhaustively hydrolyzed with snake venom phosphodiesterase (phosphodiesterase I, oligonucleate 5′-nucleotidohydrolase, EC 3.1.4.1) (0.2 mg/ml, in 0.01 M $MgCl_2$/0.05 M Tris·HCl, pH 8.8; 2 hr at 37°). The mononucleotide products of hydrolysis were separated by electrophoresis for 1 hr at 3 kV in Whatman 3MM paper at pH 3.5, by using pyridine/acetate buffer as described previously (18). Radioactive mononucleotides were located by autoradiography.

RESULTS

$cDNA_{5'}$ was prepared as described previously (10) and labeled at 5′-termini by phosphorylation with polynucleotide kinase. After labeling, the DNA was run in a gel of 10% polyacrylamide to remove unincorporated radioisotope (Fig. 1*a*). The $cDNA_{5'}$ was eluted from the gel. A portion was reanalyzed by electrophoresis with single-stranded DNA markers to obtain a precise estimate of chain length (100 nucleotides) (Fig. 1*b*) and a final evaluation of purity. The remainder was used for analysis of nucleotide sequence. From the relative amounts of ^{32}P and ^{3}H recovered in $cDNA_{5'}$ we computed that 90% of the DNA chains were labeled at their 5′-termini.

Abbreviations: ASV, Avian sarcoma virus; Pr-C, Prague strain subgroup C; B77-C, Bratislava strain subgroup C; $cDNA_{5'}$, single-stranded DNA initiated on $tRNA^{Trp}$ and complementary to 101 nucleotides at the 5′-terminus of the ASV genome.

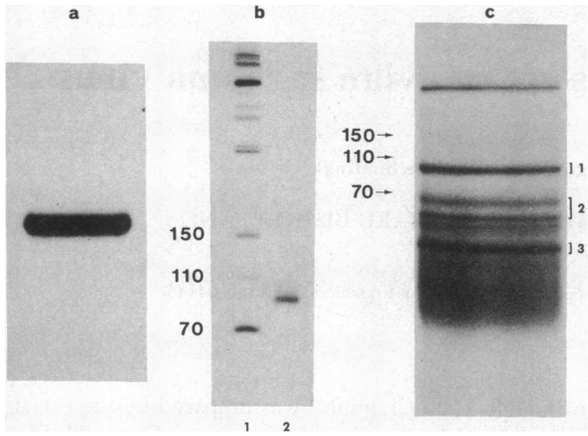

FIG. 1. Fractionation of DNA after labeling at 5′-termini. (*a*) $cDNA_{5'}$ was labeled at 5′-termini with [γ-^{32}P]ATP by using polynucleotide kinase, and then subjected to electrophoresis in a slab gel of 10% polyacrylamide. The ^{32}P-labeled DNA was located by autoradiography as illustrated, the DNA was recovered from the gel, and used for both further analysis (*b*) and determination of nucleotide sequence. (*b*) A portion of the DNA recovered from the gel in (*a*) was subjected to electrophoresis (200 V, 2.5 hr) in a slab gel (20 × 20 × 0.2 cm) of 8% polyacrylamide (lane 2). Lane 1 contained fragments of the single-stranded DNA of M13 bacteriophage obtained by cleavage with restriction nuclease *Hae* III and labeled at 5′-termini by phosphorylation with polynucleotide kinase. (*c*) DNA was synthesized with detergent-activated virions of B77-C ASV (using [^{3}H]TTP as the radioactive precursor). The extracted DNA was labeled with polynucleotide kinase and fractionated by electrophoresis in a slab gel of 10% polyacrylamide. The gel was then analyzed by autoradiography. The arrows indicate the positions of DNA fragments 70, 110, and 150 nucleotides in length. Bands 1 and 3 of the ^{32}P-labeled DNA from B77-C ASV were recovered for determination of nucleotide sequence.

A similar DNA preparation synthesized with B77-C ASV was labeled at 5′-termini prior to fractionation by electrophoresis (Fig. 1*c*). This preparation of DNA was synthesized in the presence of low concentrations of deoxynucleoside triphosphates (10 μM) and a significant percentage of the radioactivity was found in DNA species smaller than $cDNA_{5'}$ (10). Band 1 in Fig. 1*c* is $cDNA_{5'}$. The two major species of shorter DNAs (bands 2 and 3) correspond to DNA chains found previously when the genome of B77-C ASV was transcribed at a limiting concentration of dGTP (10); the slab gel used here resolved band 2 into a doublet not detected in the previous study. Bands 1 and 3 were eluted for analysis of nucleotide sequence.

The DNAs labeled at 5′-termini were sequenced with the procedure developed by Maxam and Gilbert (16). This method depends on modification and cleavage of the DNA at specific nucleotides, followed by separation of the DNA fragments by electrophoresis in a gel of 20% polyacrylamide containing 7 M urea. The sequence can than be read directly from the autoradiogram of the gel as illustrated for $cDNA_{5'}$ of Pr-C ASV in Fig. 2. The penultimate residue deoxyadenosine was run off the gel in this particular analysis; consequently, the illustrated sequence begins with thymidine. The first several residues at the 3′-terminus of the DNA are not well resolved in the illustrated gel (lane 1, Fig. 2). The identity of these nucleotides was verified by using longer periods of electrophoresis in separate analyses (data not illustrated).

The method of Maxam and Gilbert does not identify 5′-terminal nucleotides of DNA. Consequently, DNAs labeled at 5′-termini were hydrolyzed with snake venom phosphodiesterase and the mononucleotide products of hydrolysis were separated by electrophoresis as described in *Materials and Methods*. All of the radioactivity in mononucleotides from $cDNA_{5'}$ was identified as dAMP (data not illustrated).

The complete nucleotide sequence for $cDNA_{5'}$ of Pr-C ASV is illustrated in Fig. 3. Identical results were obtained with three separate preparations of DNA in our laboratory and in independent analyses by Haseltine, Maxam and Gilbert (19). A very similar sequence has been obtained for avian myeloblastosis virus (personal communication from E. Stoll, M. A. Billeter, and C. Weissman).

We also analyzed the nucleotide sequence of DNAs synthesized with B77-C ASV (Fig. 3). The amounts of $cDNA_{5'}$ available were insufficient to permit determination of the entire sequence, but conclusive results were obtained for the first 65 nucleotides; the sequence was found to differ from that of $cDNA_{5'}$ from Pr-C ASV at only two positions (residues 21 and 28). The nucleotide sequence of band 3 from the DNA synthesized with B77-C ASV (Fig. 1*c*) was identical to the sequence of $cDNA_{5'}$ over the first 40 residues and terminated in a staggered fashion at residues 41–43 (dG-dG-dG).

Using the nucleotide sequence of $cDNA_{5'}$ and previous data from our laboratory (20), we deduced the sequence of 119 nucleotides at the 5′-terminus of the genome of Pr-C ASV (Fig. 4*a*). Residue 1 is the inverted $m^7Gp(5')$ which is added in 5′- to 5′-linkage with the first encoded base of the viral genome (23, 24). Residues 2 through 102 are the complement of $cDNA_{5'}$; we believe that this sequence extends to the 5′-terminus of the viral RNA because residues 1 through 24 correspond to an oligonucleotide isolated from the 5′-terminus of ASV RNA by two laboratories (9, 25).

DNA synthesis begins opposite residue 102, and residue 103 lies opposite the 3′-terminal adenosine of the primer $tRNA^{Trp}$. Because our previous data indicated that the terminal adenosine of primer may not be paired with a complementary nucleotide (20), we were unable to deduce the identity of residue 103 from chemical data. However, when the viral RNA sequence is arranged into a base-paired structure (Fig. 4*a*), residue 103 could pair with either guanosine or uridine; we have arbitrarily designated residue 103 as adenosine. The sequence of nucleotides which base-pairs $tRNA^{Trp}$ to the ASV genome begins with the penultimate base (C) at the 3′-terminus of the tRNA and includes the next 15 contiguous nucleotides (20); this sequence was used to deduce residues 104 through 119 of the viral RNA.

The nucleotide sequence of the viral RNA can be arranged into three hairpin loops with base-paired duplex stems (Fig. 4*a*). In order to assess the relative stabilities of these loops, we computed the t_m (temperature at which strand separation is 50% complete) in 1 M NaCl for each loop according to Gralla and Crothers (26) with the following results: loop at positions 76–107, $t_m = 82°$; loop at positions 36–62, $t_m = 80°$; and loop at positions 16–28, $t_m = 71°$. The binding of $tRNA^{Trp}$ disrupts the base of the loop at positions 76–107 (Fig. 4*b*), and this reduces the t_m to 76°. Although these calculations are based on measurements made in 1 M NaCl (26), the results are believed to yield values comparable to those obtained in physiological buffers containing 10 mM magnesium (27).

DISCUSSION

$cDNA_{5'}$ is Complementary to the 5′-Terminus of the ASV Genome. Previous data indicated that the synthesis of $cDNA_{5'}$ by ASV is initiated on $tRNA^{Trp}$, followed by transcription from a sequence of nucleotides extending to the 5′-terminus of the viral genome (7–9, 11, 12). The nucleotide sequence of $cDNA_{5'}$ substantiates these conclusions. (*i*) The first eight nucleotides at the 5′-terminus of $cDNA_{5'}$ synthesized by either Pr-C or

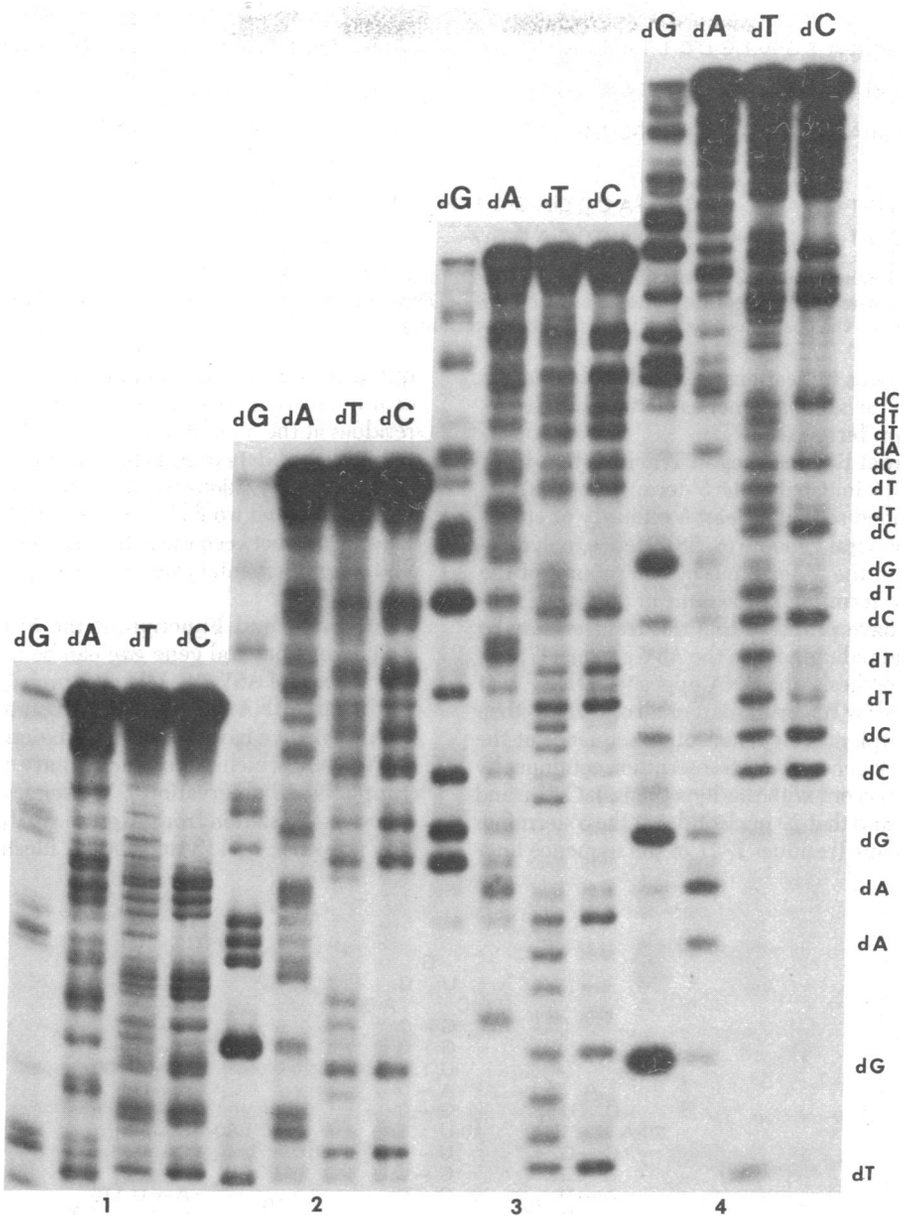

FIG. 2. Determination of nucleotide sequence by fragmentation of DNA labeled at 5′-termini. The $cDNA_{5'}$ of Pr-C ASV recovered from the gel shown in Fig. 1*a* was subjected to each of the four base-specific cleavages developed by Maxam and Gilbert (16). Samples from each of the reactions (lanes denoted dG, dA, dT, and dC) were fractionated by electrophoresis in a slab gel of 20% polyacrylamide containing 7 M urea. Sets of samples were loaded at 10-hr intervals in the order 1 through 4 as labeled on the figure. After electrophoresis for a total of 48 hr, the gel was removed for autoradiography. The DNA sequence was read from the autoradiogram by identifying which base was cleaved at each position in the DNA chain (16), as illustrated for 20 nucleotides at the 5′-end of $cDNA_{5'}$ (sample 4).

B77-C ASV are identical to the octanucleotide d(A-A-T-G-A-A-G-C) found previously at the 5′-terminus of DNA initiated on $tRNA^{Trp}$ (18). (*ii*) An oligonucleotide containing 24 residues and including the terminal structure $m^7G(5')ppp(5')Gm$ has been isolated from the 5′-terminus of the ASV genome (9, 24). The composition of this oligonucleotide is complementary to nucleotides at the 3′-terminus of $cDNA_{5'}$ (Fig. 3), although the order of nucleotides suggested by Beemon and Keith (25) does not conform to the sequence obtained by us (Fig. 4) (this discrepancy involves nucleotides well-resolved in Fig. 2 as well as residues definitively identified only in our unpublished data). We conclude that $cDNA_{5'}$ extends from the site of initiation of DNA synthesis to the 5′-terminus of the viral genome. Consequently, the length of $cDNA_{5'}$ (101 residues) defines the location of $tRNA^{Trp}$ on the genome of ASV (12).

The nucleotide sequences of $cDNA_{5'}$ synthesized with Pr-C ASV and B77-C ASV differ at only two positions (21 and 29) over the first 65 residues. These two differences account for previously reported differences between the oligopyrimidines of $cDNA_{5'}$ from Pr-C and B77-C ASV (10).

Termination of Transcription from the Template for $cDNA_{5'}$. Transcription of DNA from the genome of B77-C ASV at low concentrations of deoxynucleoside triphosphates generated discrete species of DNA shorter than $cDNA_{5'}$ (Fig. 1*c*). Two of these species (bands 2 and 3, Fig. 1*c*) had the same electrophoretic mobilities as DNA chains synthesized previously with limiting concentrations of dGTP (ref. 10, and unpublished results of the authors). The nucleotide sequence of DNA from band 3 was identical to that of the first 40 residues in $cDNA_{5'}$ and terminated in a staggered fashion at residues 41–43. These

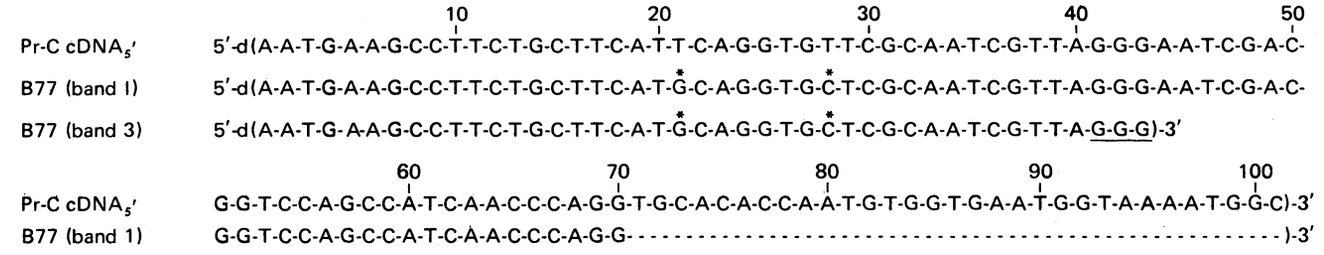

FIG. 3. Nucleotide sequence of DNA transcribed from the 5′-terminus of the ASV genome. Nucleotides marked with asterisks (bands 1 and 3) indicate positions where the nucleotide sequence of B77-C ASV cDNA$_{5'}$ differs from the sequence of Pr-C ASV cDNA$_{5'}$. The underlined sequence in band 3 denotes the positions at which the DNA chains terminate.

findings confirm previous indications (8, 10) that the shorter DNAs are initiated in the same manner as cDNA$_{5'}$ but terminate prematurely at preferred sites.

The Role of Terminal Redundancy in Transcription from the ASV Genome. DNA initiated at the 5′-terminus of the ASV genome can be extended so that at least a minority of chains represent much of the viral genome (8, 28). Hence, polymerization can move from the 5′-terminus of one subunit of the viral genome to the 3′-terminus of the same or a separate subunit. Other workers have proposed that this transfer may be facilitated by terminal redundancy in the ASV genome (7, 11). According to this hypothesis, the RNA opposite cDNA$_{5'}$ is at least partially removed by RNase H; part or all of cDNA$_{5'}$ then base-pairs with a complementary nucleotide sequence at the 3′-terminus of the viral genome and transcription continues by extending cDNA$_{5'}$. In accord with this hypothesis, J. Coffin and W. Haseltine have found that 20 nucleotides at the 5′-terminus of the Pr-B ASV genome (residues 2–21 of the sequence illustrated in Fig. 4) are reiterated at the 3′-terminus (personal communication), and we have found reiteration of at least 15 residues at the 5′- and 3′-termini of the genome of Pr-C ASV (unpublished data of A. Peter Czernilofsky). The duplex stem of the loop at positions 16–28 intrudes on the region of terminal redundancy and would have to be partially denatured to permit base pairing between the entire redundant sequence in cDNA$_{5'}$ and a complementary nucleotide sequence at the 3′-terminus of viral RNA.

Structure and Function at the 5′-Terminus of the ASV Genome. The viral gene *gag* can be translated directly from the genome of ASV (13, 14) and is probably located at the 5′-end of the viral RNA (29, 30). In collaboration with M. Kozak and A. Shatkin, we have found that ribosomes bind to a portion of ASV RNA which is complementary to 60–70% of cDNA$_{5'}$ (unpublished observations). We suggest that the site for initiation of translation from *gag* lies partly or completely within the sequence of 119 nucleotides illustrated in Fig. 4. Three

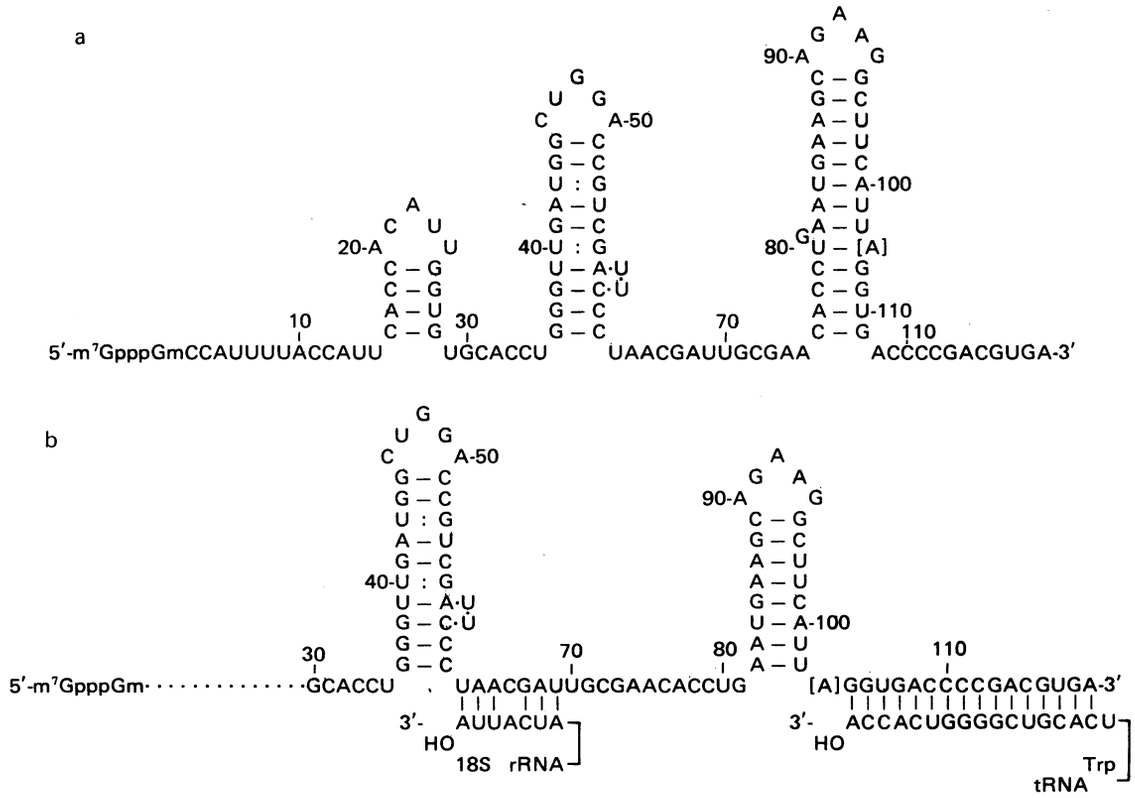

FIG. 4. Proposed nucleotide sequence at the 5′-terminus of the ASV genome. (*a*) Proposed structure of the 5′-terminal region of the Pr-C ASV genome in the absence of the tRNA^{Trp} primer. Residue 103 (in brackets) has been arbitrarily designated adenosine (see *text*), but the identity of this nucleotide is not known. (*b*) Proposed structure of the 5′-terminal region of the Pr- ASV genome when bound to tRNA^{Trp}. The 3′-terminus of tRNA^{Trp} is paired with viral RNA in accord with previously published data (20). Potential base pairing between viral RNA and the 3′-terminus of eukaryotic 18S ribosomal RNA (21, 22) is also illustrated.

features of the sequence conform to this suggestion. First, an initiator codon, AUG, occurs at residues 83–85, preceded immediately by the terminator codon UGA. [Another AUG occurs at residues 42–44, but is followed by the terminator codon UAA in phase at residues 63–65. The AUG at positions 83–85 in the genome of B77-C ASV is not preceded by a terminator codon (see Fig. 4)]. Second, six of the seven nucleotides at positions 63–69 are complementary to nucleotides located at the 3′-terminus of avian 18S ribosomal RNA (31); base-pairing of this sort has been implicated in the binding of eukaryotic ribosomes to messenger RNA (21, 22). Third, the initiator codon at positions 83–85 is bracketed by oligonucleotide sequences flanking initiator codons in messenger RNAs of reovirus (32).

A protein denoted pr76 is allegedly encoded at the 5′-end of the viral genome (13, 14, 29, 30), but the amino acid sequence of this protein has not been determined. Consequently, we cannot attempt to correlate the codons distal to the putative initiator codon with the structure of the gene product.

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1. Fenner, F. (1976) *Virology* **71,** 371–378.
2. Bishop, J. M. & Varmus, H. E. (1975) in *Cancer: A Comprehensive Treatise*, ed. Becker, F. F. (Plenum Press, New York), Vol. 2, pp. 3–48.
3. Dahlberg, J. E., Sawyer, R. C., Taylor, J. M., Faras, A. J., Levinson, W. E., Goodman, H. M. & Bishop, J. M. (1974) *J. Virol.* **13,** 1126–1133.
4. Harada, F., Sawyer, R. C. & Dahlberg, J. E. (1975) *J. Biol. Chem.* **250,** 3487–3497.
5. Taylor, J. M. & Illmensee, R. (1975) *J. Virol.* **16,** 553–558.
6. Staskus, K. A., Collett, M. S. & Faras, A. J. (1976) *Virology* **71,** 162–168.
7. Collett, M. S. & Faras, A. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1329–1332.
8. Haseltine, W. A., Kleid, D. G., Panet, A., Rothenberg, E. & Baltimore, D. (1976) *J. Mol. Biol.* **106,** 109–131.
9. Cashion, L. M., Joho, R. H., Planitz, M. A., Billeter, M. A. & Weissmann, C. (1976) *Nature* **262,** 186–190.
10. Friedrich, R., Kung, H.-J., Baker, B., Varmus, H. E., Goodman, H. M. & Bishop, J. M. (1977) *Virology*, in press.
11. Haseltine, W. A. & Baltimore, D. (1976) *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Baltimore, D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 175–214.
12. Taylor, J. M., Illmensee, R., Trusal, L. R. & Summers, J. (1976) *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Baltimore, D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 161–173.
13. von der Helm, K. & Duesberg, P. H. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 614–618.
14. Pawson, T., Martin, G. S. & Smith, A. E. (1976) *J. Virol.* **19,** 950–967.
15. Glynn, I. M. & Chappell, J. B. (1964) *Biochem. J.* **90,** 147–149.
16. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
17. Bishop, J. M., Levinson, W. E., Quintrell, N., Sullivan, D., Fanshier, L. & Jackson, J. (1970) *Virology* **42,** 182–195.
18. Taylor, J. M., Garfin, D. E., Levinson, W. E., Bishop, J. M. & Goodman, H. M. (1974) *Biochemistry* **13,** 3159–3163.
19. Haseltine, W. A., Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 989–993.
20. Cordell, B., Stavnezer, E., Friedrich, R., Bishop, J. M. & Goodman, H. M. (1976) *J. Virol.* **19,** 548–558.
21. Shine, J. & Dalgarno, L. (1974) *Biochem. J.* **141,** 609–615.
22. Dasgupta, R., Shih, D. S., Saris, C. & Kaesberg, P. (1975) *Nature* **256,** 624–628.
23. Furuichi, Y., Shatkin, A. J., Stavnezer, E. & Bishop, J. M. (1975) *Nature* **257,** 618–620.
24. Keith, J. & Fraenkel-Conrat, H. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3347–3350.
25. Beemon, K. L. & Keith, J. M. (1976) *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Baltimore, D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 97–105.
26. Gralla, J. & Crothers, D. M. (1973) *J. Mol. Biol.* **73,** 497–511.
27. Gralla, J., Steitz, J. A. & Crothers, D. M. (1974) *Nature* **248,** 204–207.
28. Collett, M. S. & Faras, A. J. (1975) *J. Virol.* **16,** 1220–1228.
29. Duesberg, P. H., Wang, L-H., Mellon, P., Mason, W. S. & Vogt, P. K. (1976) *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Baltimore, D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 107–125.
30. Joho, R. H., Stoll, E., Friis, R. R., Billeter, M. A. & Weissmann, C. (1976) *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Baltimore, D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 127–145.
31. Eladari, M-E. & Galibert, F. (1976) *Nucleic Acids Res.* **3,** 2749–2755.
32. Kozak, M. & Shatkin, A. J. (1977) *J. Mol. Biol.*, in press.