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Methylated Simian Virus 40-Specific RNA from Nuclei and Cytoplasm of Infected BSC-1 Cells

(transcription/mRNA/methylation)

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ABSTRACT **Host cell and virus-specific poly(A)-containing RNAs isolated from nuclei and cytoplasm of monkey kidney cells infected with simian virus 40 contain different methylated nucleotides. In the cytoplasmic simian virus 40-specific RNA, about 75% of the radioactivity derived from [*methyl*-^{3}H]methionine was in N^6-methyladenosine (N^6mA) after digestion with *Penicillium* nuclease and bacterial alkaline phosphatase. The remainder was in a negatively charged component with properties of 5′-terminal structures, i.e., digestion with nucleotide pyrophosphatase and bacterial alkaline phosphatase released 2′-*O*-methyladenosine (A^m), 2′-*O*-methylguanosine (G^m), and 7-methylguanosine (m^7G), consistent with a 5′-terminal structure of the type, m^7GpppN^m. The nuclear virus-specific RNA contained N^6mA, G^m, 2′-*O*-methyluridine (U^m), and a smaller proportion (10%) of nuclease-, phosphatase-resistant presumptive 5′ termini that also yielded A^m, G^m, and m^7G upon further hydrolysis. The infected cell nuclear and cytoplasmic RNAs that did not hybridize to DNA of simian virus 40 contained all four 2′-*O*-methylnucleosides. The possible role of methylation in the processing and translation of simian virus 40-specific mRNA is discussed.**

Methylation of mammalian cell mRNA is a recently recognized phenomenon (1, 2). It has been suggested that the maturation of cytoplasmic mRNAs, possibly via processing of heterogeneous nuclear RNA, may require RNA methylation (3). Although all animal cell mRNAs may not be derived by cleavage of larger precursors (4, 5), post-transcriptional methylation and processing of other types of cellular RNA, including ribosomal and transfer RNA, are well known (6).

In addition to a possible intracellular role in the processing of RNA, methylation is also involved in the synthesis and function of several viral mRNAs that apparently are monocistronic genome transcripts and not derived from larger molecules. They include the mRNAs of cytoplasmic polyhedrosis virus (7), reovirus (8), vaccinia virus (9, 10), and vesicular stomatitis virus (11). All are specifically methylated at the 5′ termini and contain structures of the type, $m^7G(5')ppp(5')N^m$. Furthermore, initiation of mRNA of cytoplasmic polyhedrosis virus *in vitro* by the virion-associated RNA polymerase requires the presence of the methyl donor, *S*-adenosylmethionine, and is coupled to methylation of the nascent RNA chains (12). Methylation of these viral mRNAs appears to be obligatory for their function, since translation of mRNAs of reovirus and vesicular stomatitis virus in cell-free extracts is completely dependent upon methylation of the mRNAs (13).

Because of its probable biological significance, it is of interest to study methylation of specific mRNAs in eukaryotic cells. Simian virus 40 (SV40)-specific mRNAs can be isolated from cells by DNA·RNA hybridization, and information is available on viral transcription in both lytically infected and virus-transformed cells (14–16). In the nuclei of infected BSC-1 monkey cells the SV40 genome is symmetrically copied, and the transcripts subsequently are selectively degraded (17). The resulting stable species of viral mRNA present in the cytoplasm are complementary to about 50% of one strand at early times and 50% of the other at late times in the lytic cycle (16). In nuclei of SV40-transformed (18) and productively infected cells (19, 20) there occur heterogeneous RNA molecules that are larger than the viral DNA and contain virus-specific sequences covalently linked to cell-specific sequences. In the lytic cycle those portions of the nuclear RNAs that are complementary to host cell DNA presumably are removed during processing to form the smaller, stable viral mRNAs in the cytoplasm. Symmetric transcription followed by RNA processing has also been observed in HeLa cell mitochondria (21) and adenovirus-infected HeLa cells (22). The mechanisms required for the extensive processing of SV40-specific and other mRNAs are unknown. As in the case of ribosomal RNA (6), information on the patterns of RNA methylation may help to elucidate the mechanism of mRNA maturation and/or function. We have, therefore, examined SV40-specific RNA for the presence of methylated nucleotides.

MATERIALS AND METHODS

Cells and Virus. A plaque-purified stock of SV40 (strain 777) was grown in the BSC-1 line of African green-monkey kidney cells (23). Confluent cultures of BSC-1 cells (4×10^6 cells per 100 mm dish) were infected with 1 ml of plaque-purified virus at a multiplicity of infection of 50–100 plaque-forming units per cell in Eagle's medium with 2% calf serum. After 2 hr at 37° for virus adsorption, 8 ml of fresh medium containing 2% calf serum were added to each culture. The infected cells were labeled with L-[*methyl*-^{3}H]methionine (150 μCi/3 ml of medium per culture; specific activity 11 Ci/mmol, from New England Nuclear) for 24–48 hr after infection.

RNA Extraction. At the end of the labeling period, the cultures were washed twice with phosphate-buffered saline (pH 7.4). To each plate were added 2 ml of phosphate/saline containing 0.2% Nonidet P-40. After 10 min on ice, the cytoplasmic lysate was removed and centrifuged at $500 \times g$ for 5 min at 4°; 300 μg/ml of polyvinyl sulfate and 0.5% sodium dodecyl sulfate ($NaDodSO_4$) (final concentrations) were added to the supernatant which was then extracted for cytoplasmic RNA. The cell nuclei that remain attached to the dish after Nonidet P-40 treatment were lysed by adding to each plate 2 ml of extraction buffer [7 M urea, 2% $NaDodSO_4$, 10 mM Tris·HCl (pH 8.0), 0.35 M NaCl, 1 mM EDTA].

Abbreviations: SV40, simian virus 40; $NaDodSO_4$, sodium dodecyl sulfate.

TABLE 1. *Selection of [³H]methyl-labeled SV40-specific RNA by hybridization to plaque-purified SV40 DNA*

RNA	Input (cpm)	DNA on filters: SV40 (10 μg)	DNA on filters: λ (6 μg)	DNA on filters: None
Cytoplasmic fraction	40,000	6.2*	0.04	0.05
Nuclear fraction	200,000	4.2	n.t.	0.03

Groups of 60–70 BSC-1 cell cultures (4×10^6 cells per plate) were infected at a multiplicity of 50–100 plaque-forming units per cell with plaque-purified SV40 and labeled with [*methyl*-³H]-methionine (50 μCi/ml) 24–48 hr after infection. RNA was extracted from the cytoplasmic and nuclear fractions as described in *Materials and Methods* and passed through a column of oligo-(dT)-cellulose. About 10% of the total [³H]methyl radioactivity in each fraction was bound. Aliquots of poly(A)-containing RNA were hybridized to DNA on filters in formamide and washed, and radioactivity was determined directly. n.t., not tested.

* Percent hybridized.

The lysates were extracted three times with a 1:1 mixture of phenol/chloroform containing 4% isoamyl alcohol (20) followed by four extractions with chloroform/4% isoamyl alcohol, all at room temperature. Two volumes of ethanol were added to the aqueous phase, and the solution was kept overnight at −20°. The precipitate was resuspended in $NaDodSO_4$ buffer (10 mM Tris·HCl, 1 mM EDTA, 0.1 M NaCl, 0.5% w/v $NaDodSO_4$) and re-extracted once with phenol and twice with chloroform/4% isoamyl alcohol. RNA was precipitated again from the aqueous phase by the addition of $LiCl_2$ to a final concentration of 2 M to remove DNA. The RNA precipitated after 18 hr at 4° was collected by centrifugation (Sorvall RC-2, 13,000 rpm, 30 min). The precipitate was resuspended in 10 mM Tris·HCl (pH 7.5) buffer containing 0.5 M NaCl and 1 mM EDTA and subjected to oligo(dT)-cellulose chromatography (24). The RNA that was bound to the column of oligo(dT)-cellulose was considered the poly(A)-containing RNA.

Selection of Virus-Specific RNA. The poly(A)-containing RNA isolated from 15 dishes was dissolved in 1 ml of hybridization buffer [0.75 M NaCl, 50 mM Tris·HCl (pH 7.4), 0.5% w/v $NaDodSO_4$, 55% v/v formamide] and hybridized with 10 μg of plaque-purified SV40 DNA component I immobilized on a nitrocellulose membrane filter at 37° for 18–22 hr (20). For recovery of the hybridized RNA, each filter was incubated for 2 hr at 37° in 1 ml of elution buffer (90 volumes of 100% formamide, 9 volumes of distilled water, and 1 volume of $NaDodSO_4$ buffer, final pH adjusted to 8.1). The eluted RNA was precipitated by the addition of 1/10 volume of 3 M NaCl and 2.5 volumes of ethanol.

Enzymatic Digestion. For *Penicillium* nuclease digestion, the RNA was dissolved in 0.1 ml of H_2O adjusted to 10 mM sodium acetate buffer (pH 6.0) and digested with 100 μg of enzyme (Yamasa Co., Tokyo, Japan) (8). RNA was treated with alkaline phosphatase (Worthington Biochemical Corp.) (20 units/ml) in 50 mM Tris·HCl buffer (pH 8.0). Nucleotide pyrophosphatase (Sigma Chemical Co.) was used at 0.05 unit/ml in 20 mM Tris·HCl buffer (pH 7.5), containing 1 mM Mg^{++}. All incubations were at 37° for 30 min. Samples were spotted on Whatman No. 3 MM paper and analyzed by electrophoresis at 2600 V for 40 min in pyridine/acetate buffer (pH 3.5). Authentic compounds were located under ultraviolet light, and the paper was dried and cut into 1 cm strips for counting in scintillation fluid. The counting efficiency was 10–15% as compared to eluted samples measured in Aquasol (NEN, Boston).

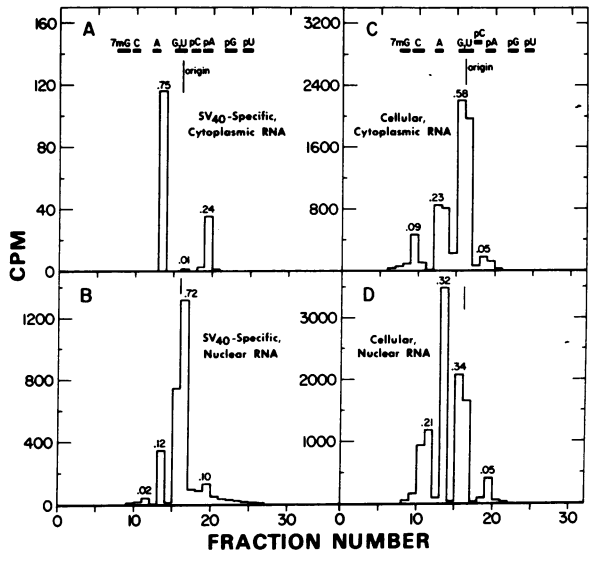

FIG. 1. Electrophoretic analyses of enzymatic digests of viral and cellular RNAs isolated from SV40-infected monkey cells. RNA was extracted from nuclear and cytoplasmic fractions, purified by binding to oligo(dT)-cellulose, and selected for virus-specific RNA by hybridization to plaque-purified SV40 DNA. The nonhybridized RNA, containing predominantly cellular mRNA, and SV40-specific mRNA were digested with *Penicillium* nuclease followed by alkaline phosphatase and analyzed by paper electrophoresis as described in *Materials and Methods*. Digested samples of SV40-specific cytoplasmic and nuclear RNAs and cellular cytoplasmic and nuclear RNAs contained 3000, 5000, 20,000, and 80,000 cpm, respectively, in panels A–D, as measured in Aquasol. Numbers above peaks indicate fraction of total cpm.

RESULTS

Selection of Poly(A)-Containing SV40-Specific RNA. BSC-1 cells infected with plaque-purified SV40 were labeled with [*methyl*-³H]methionine, and the nuclear and cytoplasmic RNAs were extracted as described in *Materials and Methods*. A total of 12×10^6 and 4.3×10^6 cpm were obtained in the nuclear and cytoplasmic RNAs, respectively. The RNAs were passed through oligo(dT)-cellulose, and the bound poly(A)-containing portion comprised 9–10% of the total [³H]methyl radioactivity in each preparation. The poly(A)-containing RNAs were eluted and hybridized to SV40 DNA. Four percent of the radioactivity in the oligo(dT)-bound nuclear RNA and 6% of the cytoplasmic poly(A)-containing ³H-labeled RNA hybridized to viral DNA (Table 1).

Methylated Constituents in SV40-Specific RNA. [³H]-Methyl-labeled RNA that hybridized to SV40 DNA was eluted and digested with *Penicillium* nuclease followed by alkaline phosphatase. *Penicillium* nuclease degrades polynucleotides, including sequences containing alkali-resistant, 2′-*O*-methylated residues, to 5′-mononucleotides (25, 26). RNA digests were analyzed by high-voltage paper electrophoresis (Fig. 1A and B). From the cytoplasmic RNA most of the radioactivity (75%) migrated in the position of adeno-

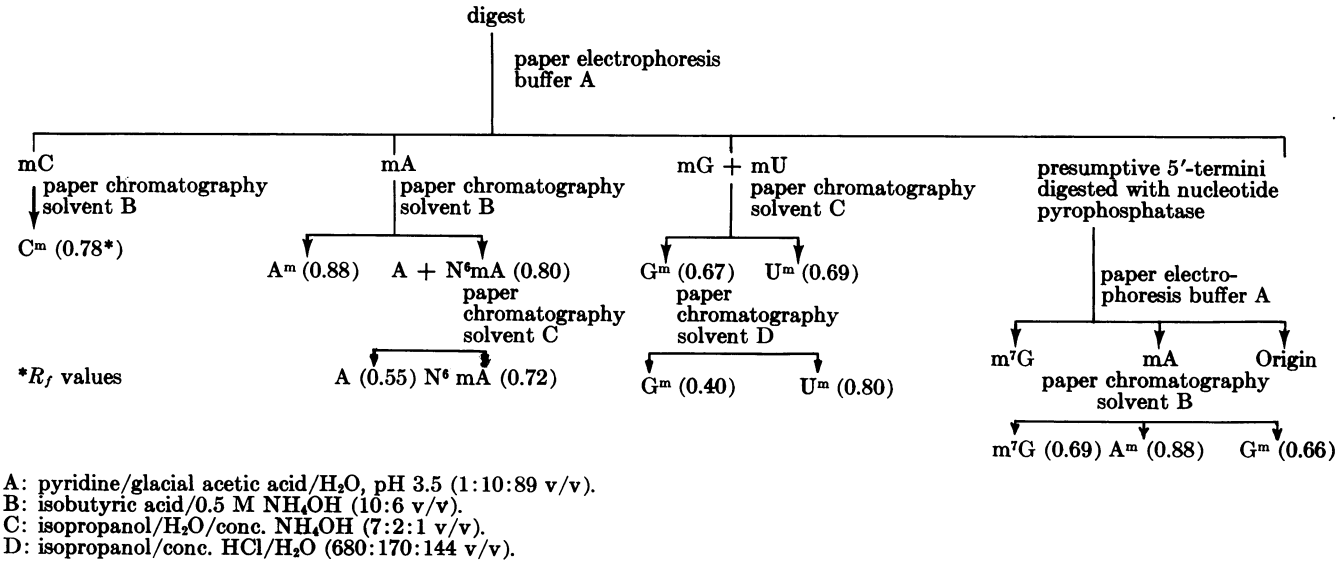

FIG. 2. Procedure for analysis of [^{3}H]methyl-labeled RNA after digestion with *Penicillium* nuclease + alkaline phosphatase.

sine; it was identified as N^6-methyladenosine by paper chromatography in solvents B and C, as described in Fig. 2 (Table 2). Radioactivity was absent from the guanosine region, indicating that [*methyl*-^{3}H]methionine was not utilized for incorporation of ^{3}H into ring positions during purine biosynthesis. The balance of the [^{3}H]methyl remained negatively charged after enzymatic digestion and migrated in the position of 5′-terminal structures, as described previously for several mRNAs (7–9, 11, 27) (Fig. 1A). The enzymatic digest of nuclear SV40-specific RNA also contained N^6-methyladenosine (12%), but most of the radioactivity in the nucleoside fraction migrated in the position of guanosine and uridine (Fig. 1B). By chromatography in solvents C and D (Fig. 2), this ^{3}H-labeled material was resolved into equal amounts of 2′-*O*-methylguanosine and 2′-*O*-methyluridine (Table 2). The radioactivity in the negatively charged, presumptive 5′ termini comprised only about 10% of the nuclear SV40-specific RNA (Fig. 1B).

The methylation patterns of poly(A)-containing infected cell RNA and SV40-specific RNA were different, indicating that the SV40-specific RNA was not contaminated by cellular RNA. After digestion of nuclear or cytoplasmic infected cell RNA with nuclease plus alkaline phosphatase and analysis by paper electrophoresis, [^{3}H]methyl radioactivity was present in the positions of all four ribonucleosides (Fig. 1C and D). They were identified further by chromatography (Fig. 2, Table 2). No 2′-*O*-methylated nucleosides were present in the nuclease plus phosphatase digests of SV40-specific cytoplasmic RNA (Table 2). Furthermore, virus-specific nuclear RNA digests contained no 2′-*O*-methylcytosine or 2′-*O*-methyladenosine (Table 2). In contrast to SV40-specific RNA, where the cytoplasmic fraction contained a 2.4-fold greater proportion of presumptive 5′ termini than the nuclear RNA (compare Fig. 1A and B), the proportion of [^{3}H]methyl radioactivity in phosphatase-resistant, presumptive 5′-terminal structures was the same (5%) in nuclear and cytoplasmic RNA that did not hybridize to SV40 DNA (Fig. 1C and D). The radioactive material in fractions 19 and 20 (Fig. 1D) was eluted from the paper and redigested with nuclease and alkaline phosphatase. Upon reanalysis by paper electrophoresis, the radioactivity migrated to the same position as the original presumptive 5′ termini.

TABLE 2. *Distribution of [^{3}H]methyl in nucleosides selected by digestion with Penicillium nuclease and bacterial alkaline phosphatase and analyzed by paper chromatography*

RNA	C^m	A^m	N^6mA	G^m	U^m
SV40-specific					
Cytoplasmic	—	—	99*	<1	—
Nuclear	—	<2	12	43	41
Infected cell					
Cytoplasmic	10	20	7	36	26
Nuclear	24	20	14	23	15

* % of cpm in nucleosides.

Analysis of Presumptive 5′-Terminal Structures. Viral mRNAs synthesized in the presence of the methyl donor, *S*-adenosylmethionine by the virion-associated RNA polymerase of cytoplasmic polyhedrosis virus (7), reovirus (8), vaccinia virus (9, 10), and vesicular stomatitis virus (11) contain blocked, methylated 5′-termini. The blocked 5′-terminal structures have been identified as m^{7}G(5′)ppp(5′)A^m for cytoplasmic polyhedrosis virus (7) and vesicular stomatitis virus (11), m^{7}G(5′)ppp(5′)G^m for reovirus (8), and a mixture of the two structures for vaccinia mRNA (9, 10). The same blocked 5′ structure is present in reovirus mRNA and in one strand of the double-stranded genome RNA isolated from infected cells (27). The identification of the 5′ termini of reovirus mRNA was facilitated by the use of *Penicillium* nuclease plus alkaline phosphatase, a combination which degrades RNA to the corresponding ribonucleosides and 5′-terminal structures (8). The 5′-terminal m^{7}GpppG^m from reovirus mRNA remained negatively charged after digestion and migrated toward the anode during high-voltage paper electrophoresis (8). The same results were obtained with enzymatic digests of poly(A)-containing [^{3}H]methyl-labeled RNA from SV40-infected BSC-1 cells (Fig. 1A and B). To characterize the negatively charged presumptive 5′ termini, the radioactive nucleotides obtained after hydrolysis with nuclease plus phosphatase (fractions 19 and 20, Fig. 1) were eluted, digested with nucleotide pyrophosphatase plus alkaline phosphatase, and again analyzed by paper electro-

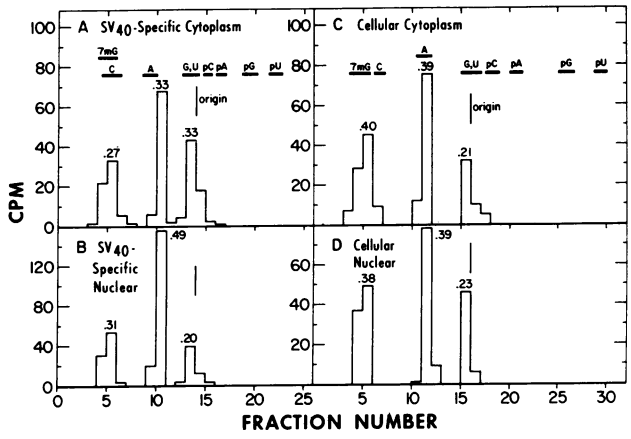

FIG. 3. Analysis of presumptive 5′-terminal structures of SV40-specific and cellular RNAs. SV40-specific and cellular RNAs from cytoplasmic and nuclear fractions were digested with *Penicillium* nuclease and alkaline phosphatase. After paper electrophoresis, the presumptive 5′ termini (corresponding to fractions 19 and 20 in Fig. 1) were eluted with H_2O, digested with nucleotide pyrophosphatase and phosphatase, and again analyzed by electrophoresis. The presumptive 5′ termini were obtained from digests of 10,000 cpm each of SV40-specific nuclear and cytoplasmic RNAs and 20,000 and 80,000 cpm of cellular cytoplasmic and nuclear RNAs, respectively.

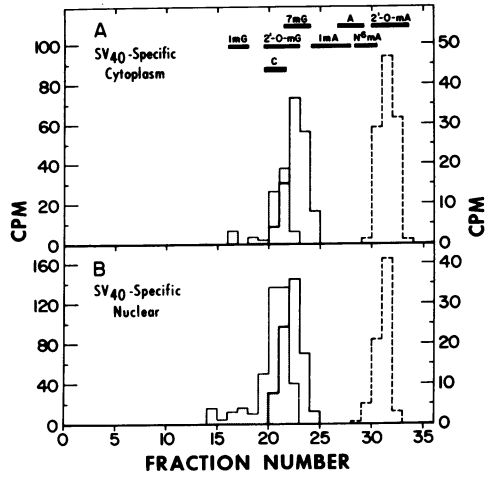

FIG. 4. Identification of methylated constituents in presumptive 5′ termini of SV40-specific RNAs. The radioactive components in Fig. 3A and B were eluted and again analyzed separately by descending paper chromatography in isobutyric acid/0.5 M NH_4OH (10:6 v/v). Material was eluted from the following fractions of Fig. 3: 10–11 (*broken curve*); 5–7 (*solid curve*); and 14–15 (*shaded area*). The left ordinate applies to the broken and solid curves; the right ordinate to the shaded area.

phoresis. All four samples of presumptive 5′ termini, i.e., nuclear and cytoplasmic SV40-specific and nonhybridizable RNA, yielded three [^{3}H]methyl-labeled components (Fig. 3). Two of the labeled components migrated in the positions of 7-methylguanosine and adenosine. The third had no net charge at pH 3.5 and remained at the origin in the position of guanosine and uridine. The nucleosides in the digests of the SV40-specific nuclear and cytoplasmic RNA were eluted and identified further by descending paper chromatography. As shown in Fig. 4, the ^{3}H-labeled constituents migrated with authentic 7-methylguanosine, 2′-*O*-methyladenosine, and 2′-*O*-methylguanosine. The presumptive 5′ termini of the nonhybridizable RNA, after nucleotide pyrophosphatase and alkaline phosphatase treatment, yielded m^7G, A^m, and a third radioactive component that was not identified but remained at the origin (the position of G^m and U^m) after paper electrophoresis.

In order to establish the number of phosphates in the linkage between m^7G and the 2′-*O*-methylated nucleotide, the net charge of presumptive 5′-terminal structures was measured by DEAE-cellulose chromatography. SV40-specific [^{3}H]methyl-labeled cytoplasmic RNA was digested with nuclease and alkaline phosphatase, mixed with oligonucleotide markers derived from tRNA by RNase digestion, and analyzed (Fig. 5). The elution profile included 75% of the radioactivity in the position of nucleosides, and this material was identified as N^6mA by paper chromatography. The remainder eluted with a net charge of between −2 and −3, consistent with the presence of three phosphates, one partially neutralized by m^7G, as observed previously for 5′-terminal m^7GpppG^m of reovirus mRNA (8).

The negatively charged material (fractions 23–31, Fig. 5) was pooled, desalted, and digested with nucleotide pyrophosphatase and alkaline phosphatase. Analysis by paper electrophoresis yielded three [^{3}H]methyl-labeled components: two migrating in the position of m^7G and A^m and the third remaining in the origin.

DISCUSSION

Methylated SV40-specific mRNA and cellular mRNA were obtained from lytically infected BSC-1 monkey cells incubated with [*methyl*-^{3}H]methionine. The patterns of RNA methylation were specific, as observed recently for a variety of eukaryotic mRNAs including those from Novikoff hepatoma (2), HeLa (Furuichi, Morgan, Darnell, Jelinek, Salditt-Georgieff, and Shatkin, unpublished results), and mouse L cells (ref. 1; Furuichi, LaFiandra, and Shatkin, unpublished results), and the viral-specific mRNAs of cytoplasmic polyhedrosis virus (7), reovirus (8), vaccinia virus (9, 10), vesicular stoma-

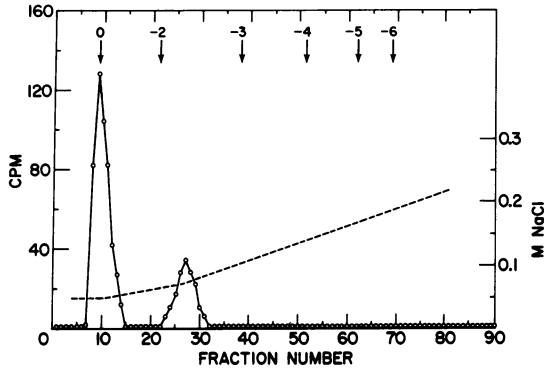

FIG. 5. DEAE-cellulose chromatography of SV40-specific cytoplasmic RNA digested with nuclease and alkaline phosphatase. Enzyme-digested, [^{3}H]methyl-labeled RNA was mixed with a yeast tRNA hydrolysate (1 mg of RNA, 100 μg of RNase A, 6 hr at 37°) and applied to a column (0.7 × 20 cm) of DEAE-cellulose equilibrated with 0.05 M Tris buffer (pH 8) containing 7 M urea and 0.05 M NaCl. The oligonucleotides were eluted with a salt gradient (50 ml each of 0.05 M and 0.3 M NaCl) in the same buffer. The *arrows* indicate the positions of the oligonucleotides, as measured by absorbance at 260 nm. Conductivity was used to determine the NaCl molarities. One-milliliter fractions were collected, and the radioactivity in 0.3 ml of each was measured in 0.4 ml of H_2O and 10 ml of Aquasol.

titis virus (11), and adenovirus (Furuichi, Darnell, and Shatkin, unpublished results). The methylated residues in poly(A)-containing infected cell RNA that did not hybridize to SV40 DNA yielded N^6mA and all four 2′-*O*-methylated nucleosides upon digestion with *Penicillium* nuclease and bacterial alkaline phosphatase. In addition, presumptive 5′-terminal structures of the type, $m^7G\text{-}pppN^m$, were obtained from both the nuclear and cytoplasmic cell RNAs. SV40-specific mRNA also contained N^6mA and presumptive 5′-terminal m^7GpppG^m and m^7GpppA^m, although the exact structures of the 5′ termini of the mRNAs remain to be established. However, in contrast to infected cell RNA, there were no other methylated nucleosides in the cytoplasmic SV40-specific mRNA. The nuclear poly(A)-containing SV40-specific RNA contained G^m and U^m in amounts that varied from 30 to 70% of the total methylated residues in several different RNA preparations. These 2′-*O*-methylated nucleosides presumably are in nonconserved regions of the RNA, in viral sequences and/or host sequences that are covalently associated with viral information in the nuclei of SV40-infected cells. The large nuclear RNAs may be cleaved, resulting in removal of G^m and U^m during maturation of the cytoplasmic 19S and 16S viral mRNAs (19, 20). The nuclei of SV40-transformed mouse 3T3 cells also contain large RNA molecules of mixed host and viral sequences (18), and an analysis of their methylation pattern will be of interest.

In addition to cleavage of host sequences from SV40-specific nuclear RNA, viral sequences are also apparently selectively degraded during maturation of cytoplasmic viral mRNA. The entire sequence of each strand of the viral genome is represented in the nuclear SV40-specific RNA of infected cells, but the cytoplasmic viral mRNA is a selected portion of the total transcripts (14–16). The stable cytoplasmic mRNA synthesized early in infection, i.e., before viral DNA replication, represents about 48% of one genome strand; late mRNA represents 52% of the other strand (16). The biochemical basis for sequence conservation in mRNAs is unknown. Stable cytoplasmic mRNA may be derived from larger nuclear RNA by endonucleolytic cleavage at specific, methylated sites followed by addition of m^7G to the resulting 5′ terminus by a "capping" reaction (3). Several preparations of cytoplasmic SV40-specific RNA contained the same relative amounts of N^6mA and 5′ termini, consistent with the presence of N^6mA near the 5′ end of stable viral RNA, possibly as part of a recognition sequence for mRNA maturation. Alternatively, m^7G may be added directly to 5′ termini of newly initiated SV40-specific mRNA chains, as in transcripts from virion-associated polymerases (8–12). Maturation would then involve conservation of the 5′ portions of the mRNA. The involvement of these mechanisms in mRNA formation remains to be demonstrated, but the presence in viral mRNA of 5′-terminal structures of the type, m^7GpppN^m, should permit the identification of sites in the viral genome that correspond to the 5′ sequences of stable mRNA. The presence of a blocked 5′ end may serve as an mRNA landmark, for example, as a ribosomal binding site, and also as protection against attack by 5′ exonucleases.

Although the present results do not establish a role for methylation in mRNA processing, they do indicate that SV40-specific mRNA is methylated and that the pattern of methylation is different in nuclear and cytoplasmic virus-specific RNAs. In vesicular stomatitis virus, reovirus, and hemoglobin mRNAs, the presence of 5′-terminal m^7G is required for translation of the RNAs in a cell-free protein-synthesizing extract prepared from wheat germ (ref. 13; Muthukrishnan, Furuichi, Both, and Shatkin, unpublished results). The same functional requirement for m^7G may apply to all eukaryotic mRNAs, including SV40-specific mRNA. Thus, although maturation of mRNA may involve both processing and methylation, the latter may be required ultimately for translation. It will be of interest to analyze the distribution of methylated nucleotides in mRNA from polysomes of uninfected and infected cells.

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