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# Further Characterization of mRNA's of Mouse Hepatitis Virus: Presence of Common 5'-End Nucleotides

MICHAEL M. C. LAI,* CHRIS D. PATTON, AND STEPHEN A. STOHLMAN

*Departments of Microbiology and Neurology, University of Southern California School of Medicine, Los Angeles, California 90033*

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The mouse hepatitis virus strain A59 codes for seven RNA species in the infected cells. These virus-specific RNAs were found to be polysome associated and therefore likely to represent mRNA's. All of them have common 3'-end sequences (Lai et al., J. Virol. 39:823–834, 1981). Their structure was further studied with respect to their 5'-end sequences. It was found that all of these mRNA's contained cap structures at their 5' ends. Furthermore, the cap-containing oligonucleotides which represent the sequences immediately adjacent to the 5' ends were found to be the same for most, if not all, of the seven virus-specific mRNA's. These sequences are also identical to the 5'-end sequences of the virion RNA genome. The 5'-end sequences were tentatively determined to be 5'-cap-N-UAAG. The presence of the common nucleotides in all of the virus-specific RNAs in mouse hepatitis virus strain A59 suggests several possible mechanisms of synthesis for these RNAs. The significance of these findings is discussed.

Mouse hepatitis virus (MHV) is a member of Coronaviridae and contains a positive single-stranded 60S RNA genome with a molecular weight of $5.4 \times 10^6$ (5). This RNA contains polyadenylic acid sequences at the 3' end (5, 16, 18) and a "cap" structure at the 5' end (6). It codes for at least three structural proteins, gp 90/180, pp 60, and gp 23 (12, 14), and probably three nonstructural proteins (1, 2, 10). The mode of synthesis of the viral RNA is still not clear.

Recently, several laboratories have shown that seven virus-specific RNAs could be detected in the cells infected with MHV (4, 6a, 11; H. Wege, S. Siddell, M. Sturm, and V. ter Meulen, *in* V. ter Meulen, S. Siddell, and H. Wege, ed., *Biochemistry and Biology of Coronaviruses*, in press). These RNAs include the genomic RNA and six subgenomic RNA species. All of them contain polyadenylic acid sequences, suggesting that they represent mRNA's. By oligonucleotide mapping of these RNAs, we have further shown that the sequences of each virus-specific RNA are included within the next-larger RNA species, and that the sequences of each RNA start from the 3' end of the genomic RNA (4). Each RNA contains polyadenylic acid sequences of comparable length, and all of them contain identical sequences in the region immediately adjacent to the polyadenylic acid (4). These results indicate that all of the subgenomic RNA species have identical 3'-end sequences of nearly 2 kilobases.

To understand the mechanism of synthesis of these subgenomic RNAs, it is important to determine their 5'-end sequences. Since all of the mRNA species overlap in the 3'-end sequences, it is expected that the 5'-half portions of each mRNA would have different sequences. But, the sequences at the very 5'-end of each mRNA could be identical or different, depending on the mechanism of their synthesis. For instance, if these subgenomic RNAs are derived by splicing of the genome-sized RNA, then all of them should contain identical sequences at the very 5' end. On the other hand, the lack of common 5' termini in these mRNA's would suggest a different mechanism of synthesis. In this study, we have shown that all of the mRNA's contain 5'-cap structures and that at least 4 nucleotides immediately adjacent to the cap are identical for most, if not all, of the virus-specific RNAs. This finding has implications in the possible mechanisms of synthesis of these RNAs.

## MATERIALS AND METHODS

**Cells and virus.** The MHV strain A59 (MHV-A59) used in these studies and the method of infection for the preparation of total intracellular viral RNA have been described previously (4).

For the preparation of polysomal RNA, L2 cells were grown in 15-cm plastic dishes in Dulbecco minimal essential medium supplemented with 5% fetal calf serum. The cells were infected with virus at subconfluency. At 10 to 12 h before infection with MHV-A59, the cell monolayers were washed twice with phosphate-free Dulbecco minimal essential medium and

refed with Dulbecco minimal essential medium which contained 1/10 the normal concentration of phosphate and twice the normal concentration of vitamins, amino acids, and glutamine, buffered with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.2) and supplemented with 0.5% fetal calf serum. After incubation for 10 to 12 h, the medium was decanted, and the cell monolayers were infected with MHV-A59 as previously described (4). After virus adsorption, the inoculum was replaced with 20 ml of phosphate-free Dulbecco minimal essential medium which contained 2× vitamins and amino acids, 3 μg of actinomycin D per ml, 1% dialyzed fetal calf serum, and 250 μCi of $^{32}P_i$ (ICN Pharmaceuticals) per ml. For preparation of polysome labeled with [$^3$H]uridine, the phosphate-free medium was replaced by regular medium. Otherwise, the same protocol was used.

**Polysome isolation.** The preparation of polysome was performed by a modification of the procedure of Neutschil and Kurth (7). At 8.75 h postinfection, cycloheximide dissolved in TKM buffer (10 mM Tris-hydrochloride [pH 7.2], 150 mM KCl, 10 mM $MgCl_2$) was added to the medium to a final concentration of 200 μg/ml. At 9.0 h postinfection, the medium was decanted, and the cell monolayers were placed in an ice bath. After this point, the cells and cell lysates were handled at ≤4°C. The monolayers were washed twice with ice-cold TKM which contained 200 μg of cycloheximide per ml and once with TKM which contained 500 μg of cycloheximide and 500 μg of dextran sulfate per ml (TKMCD). The cells were lysed by the addition of 2 ml of TKMCD which contained 0.5% Nonidet P-40. The monolayers were rocked for 5 to 10 min, and then the cells were scraped off with a rubber policeman. Nuclei and cell fragments were removed by centrifugation at 13,000 rpm for 10 min in a Sorval SS-34 rotor. The supernatant was layered onto a discontinuous sucrose gradient consisting of 6 ml each of 2.0 M, 1.0 M, and 0.5 M sucrose made in TKMCD and was centrifuged at 25,000 rpm for 2 h at 4°C in an SW27 rotor. Polysomes were collected from the 1.0 to 2.0 M sucrose interface and diluted 1:8 with TKMCD and was then layered onto a 9 to 49% continuous gradient of sucrose prepared in TKMCD and centrifuged at 26,000 rpm for 1.5 h at 4°C in an SW27 rotor. Fractions were collected by upward displacement using a density gradient fractionator and monitored by UV absorbence at 260 nm.

**Agarose gel electrophoresis of RNA.** The electrophoresis of RNA on 1% agarose gels was performed as described previously (4). Briefly, the RNA samples were dissolved in RE buffer (50 mM boric acid, 5 mM sodium borate, 1 mM EDTA, 10 mM sodium sulfate, pH 8.1), heat denatured by boiling at 100°C for 1 min, and electrophoresed in 1% agarose gel slabs made in RE buffer at 90 V for 5 h. After electrophoresis, the gels were wrapped with cellophane and exposed to Kodak XR films with an intensifying screen for various periods of time. The RNA was extracted from the gel by Dounce homogenization in distilled water and processed as described previously (4).

**Oligonucleotide fingerprinting and base sequence analysis.** The $^{32}$P-labeled RNA was digested with RNase $T_1$ and separated by two-dimensional polyacrylamide gel electrophoresis as previously described (4). After electrophoresis the oligonucleotides were excised from the gels, eluted with 0.5 ml of 0.5 M NaCl, and precipitated with 2.5 volumes of ethanol. The oligonucleotides were pelleted by sedimentation at 20,000 × *g* for 15 min, redissolved in 10 μl of a solution containing 0.04 M ammonium acetate (pH 4.4), 1 mM EDTA, 20 μg of RNase A per ml, 150 U of RNase $T_1$ per ml, and 5 U of RNase $T_2$ per ml at 37°C for 2 h. Alternatively, the oligonucleotides were redissolved in 10 μl of water containing RNase A at 0.1 mg/ml and incubated at 37°C for 30 min. After incubation, the reaction mixture was spotted on DEAE-cellulose paper, and electrophoresis was carried out at pH 3.5 (pyridine-acetate buffer) until a xylene cyanol FF blue dye had migrated 10 cm from the origin. The dried paper was then exposed to Kodak BB-5 film with an intensifying screen for appropriate periods of time. In some films, only the portion of electropherograms close to the origin was exposed with the intensifying screen.

## RESULTS

**Virus-specific RNAs associated with polysome.** The seven MHV-A59-specific RNAs in the MHV-infected L-2 cells contain polyadenylic acid sequences, suggesting that they are mRNA's (4). To provide more definitive proof of the messenger functions of these RNAs, we examined the RNA species associated with polysomes. The polysomes were isolated from the MHV-A59-infected L-2 cells 9 h postinfection, when the rate of RNA synthesis was at a maximum (4). As shown in Fig. 1A, the majority of the RNA was associated with polysome with sedimentation rate higher than 100S. All of these RNAs were released from the polysome when the latter was treated with 10 mM EDTA, indicating that all of the RNAs were polysomal RNAs. Furthermore, agarose gel electrophoresis showed that all of the virus-specific RNAs previously detected in MHV-infected L-2 cells (4) were present in the polysomes (Fig. 1B). The genomic RNA was associated with faster-sedimenting polysome fractions, whereas the smaller subgenomic RNAs were associated with slower-sedimenting polysome (Fig. 1B). Furthermore, the RNA released by EDTA from polysome contained all of the virus-specific RNAs previously reported (lane g in Fig. 1B). This result thus established that all of the MHV-A59-specific RNAs, including the genomic RNA, were mRNA's. As we have reported previously, the RNA no. 4 was usually not detectable under these conditions (4).

**Presence of the cap structure in all of the mRNA's.** We have shown that the 60S genomic RNA of MHV-A59 contains a cap structure (6). To determine whether the intracellular virus-specific mRNA's also contained a cap structure, the $^{32}$P-labeled mRNA's were isolated from agarose gels and digested with a mixture of RNases A, $T_1$, and $T_2$. The digests were then separated by electrophoresis on DEAE cellulose paper at pH 3.5. As shown in Fig. 2, most of the

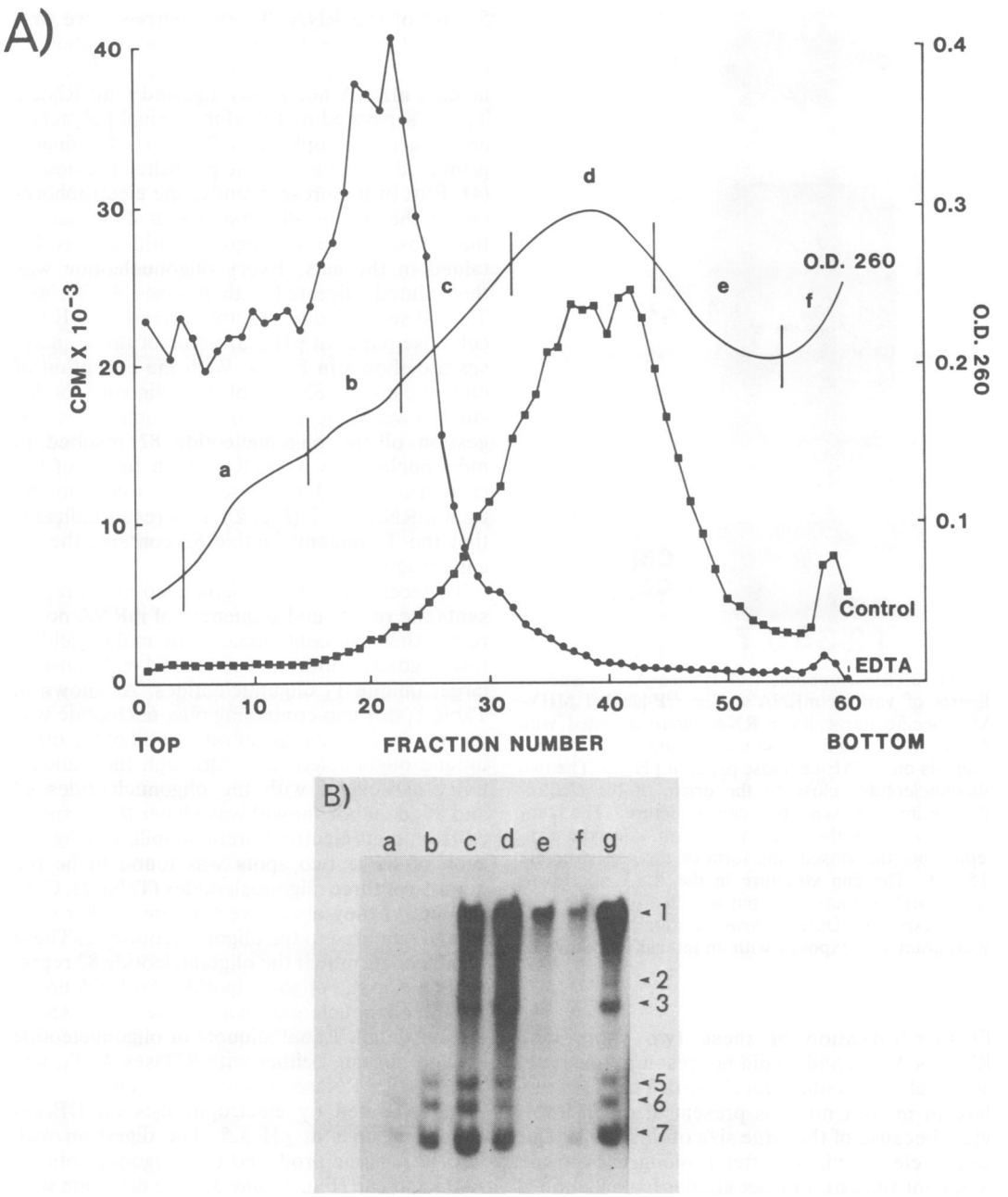


FIG. 1. Virus-specific RNAs associated with polysome. The $^{32}P$-labeled or [$^{3}H$]uridine-labeled polysome was isolated from the MHV-A59-infected cells at 9 h postinfection (A). The polysome preparations with or without EDTA (10 mM) treatment were sedimented through a 9 to 49% sucrose gradient at 26,000 rpm for 1.5 h at 4°C in an SW27 rotor. A sample of each fraction was precipitated with 10% trichloroacetic acid, and the radioactivity was counted in a liquid scintillation counter. The polysome RNAs of different size fractions were collected as indicated (a through f) and used for electrophoresis on 1% agarose gels (B). Lane g contained the RNA released from the polysome by EDTA treatment.

RNAs were digested into mononucleotides. However, all of the mRNA's also contained two nucleotides close to the origin of electrophoresis. These two nucleotides represent the cap structure. The fast-migrating spot ($cap_1$) probably represents the open ring form, whereas the slower-migrating spot ($cap_2$) represents the closed ring form of the cap structure (14, 19).

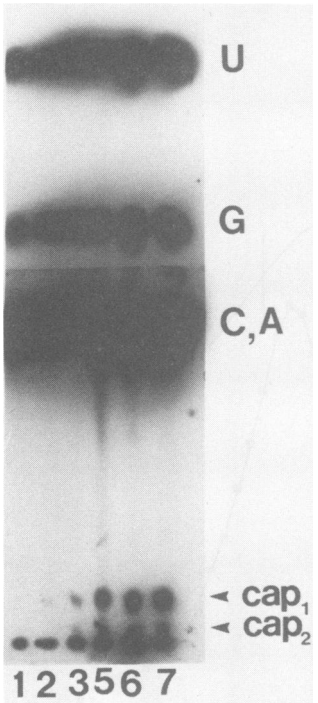


FIG. 2. Electrophoresis of RNase A, $T_1$, and $T_2$ digests of various mRNA's. The $^{32}P$-labeled MHV-A59-specific intracellular RNAs were digested with RNases A, $T_1$, and $T_2$ and then separated by electrophoresis on DEAE-cellulose paper at pH 3.5. The two oligonucleotides close to the origin of the electropherogram represent the cap structure. The $cap_1$ corresponds to the open ring form, and the $cap_2$ represents the closed ring form of the cap structure (15, 19). The cap structure in the RNA no. 1 was present in lower amounts, but was clearly visible after longer exposure. Only the lower portion of the electropherogram was exposed with an intensifying screen.

Further digestion of these two spots with RNases A, $T_1$, and $T_2$ did not result in release of any nucleotides (data not shown). The cap structure in mRNA no. 1 is present at much lower yield because of the large size of this RNA. This cap is clearly visible after prolonged exposure (data not shown). Further studies have identified a cap-containing oligonucleotide in mRNA no. 1 (see below). These results suggest that all of the intracellular MHV-A59-specific mRNA's contained a cap structure at their 5′ ends.

**Identification of the cap-containing $T_1$ oligonucleotides in viral mRNA's.** To understand the mechanism of synthesis of viral mRNA's, it is important to know their 5′-end sequences. Since we demonstrated that all of the mRNA's contained a 5′-cap structure, the cap-linked oligonucleotide provides a convenient marker for identifying the sequences immediately adjacent to the 5′ end of the RNA. To this purpose, we first searched for the $T_1$ oligonucleotide containing the cap structure in mRNA no. 7. The $^{32}P$-labeled mRNA no. 7 was digested with RNase $T_1$ and separated by two-dimensional polyacrylamide gel electrophoresis (Fig. 3). This fingerprint is identical to the one published previously (4). But, in the present study, the electrophoresis on the second dimension was shortened so that most of the $T_1$ oligonucleotides were retained in the gels. Every oligonucleotide was then eluted, digested with RNases A, $T_1$, and $T_2$, and separated by electrophoresis on DEAE-cellulose paper at pH 3.5. Some of these analyses are shown in Fig. 4. With the exception of oligonucleotide 82, all of the oligonucleotides studied were digested into mononucleotides. Digestion of the oligonucleotide 82 resulted in mononucleotides as well as both forms of the cap structure, identical to those found in the total mRNA no. 7 (Fig. 2). This result indicates that the $T_1$ oligonucleotide 82 contains the 5′-cap structure.

To ascertain that the oligonucleotide 82 represents the real 5′-end sequences of mRNA no. 7, rather than the contaminant, the molar yield of this oligonucleotide was compared with those of larger unique $T_1$ oligonucleotides. As shown in Table 1, this cap-containing oligonucleotide was present in the same molar ratio as all of the other unique oligonucleotides. Although the radioactivity associated with the oligonucleotides 83 and 84 (data not shown) was higher than expected from their electrophoretic mobilities (Fig. 3), each of these two spots was found to be the mixture of three oligonucleotides (Table 1). Consequently, they also have the same molar ratio when compared to the oligonucleotide 82. These results confirm that the oligonucleotide 82 represents the 5′-end oligonucleotide of mRNA no. 7.

This oligonucleotide was further analyzed in greater detail. Equal aliquots of oligonucleotide 82 were digested either with RNases A, $T_1$, and $T_2$, or with RNase A alone. The digests were then separated by electrophoresis on DEAE-cellulose paper at pH 3.5. The digestion with RNase A alone produced two oligonucleotides, AAG and cap (Fig. 5, lane 3). The digestion with RNases A, $T_1$, and $T_2$ produced U, G, 2A, and cap (lane 2) (in this case, the cap structure existed in two forms as explained above). The residue A was not well separated from the residue C under this electrophoretic condition. The identity of residue A was confirmed by further separation by electrophoresis on Whatman 3 MM paper at pH 3.5 (data not shown). From these results, the sequences of the oligonucleotide 82 were deduced to be cap-N-UAAG with N being A or other modified nucleotides.

**Presence of identical cap-containing oligonucle-**

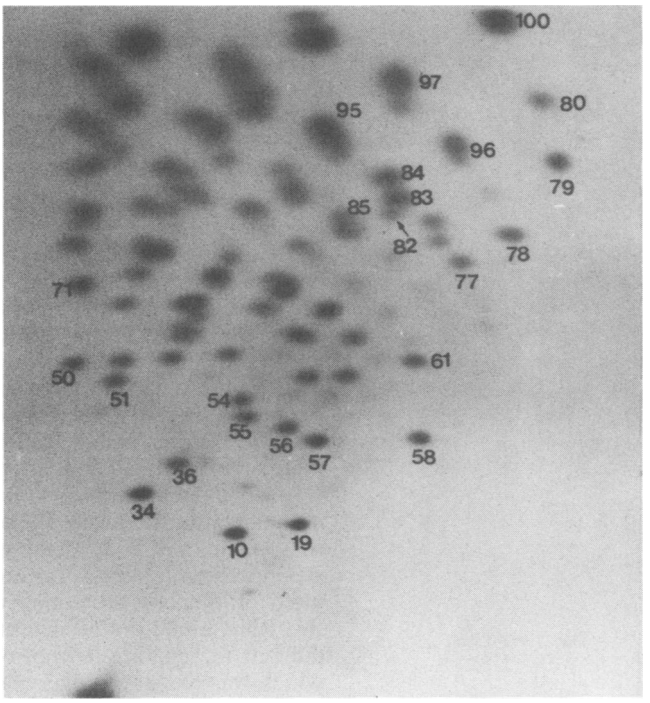


FIG. 3. Oligonucleotide fingerprint of RNA no. 7. The $^{32}$P-labeled mRNA no. 7 was extracted from the gel, digested with RNase $T_1$, and separated by two-dimensional polyacrylamide gel electrophoresis. The first-dimension separation was from left to right, and the second-dimension separation was from bottom to top. The numbering of spots was arbitrary and is consistent with the published system (4).

**otides in other mRNA's.** To determine whether all of the intracellular mRNA's contain the same or different 5'-end sequences, we examined the cap-linked $T_1$ oligonucleotides of the other mRNA's. Since RNAs no. 2 and 4 were present in lower amounts in the infected cells (4), only RNAs no. 1, 3, 5, and 6 were studied. These RNAs were first analyzed by $T_1$ oligonucleotide fingerprinting. As shown in Fig. 6, all of these RNAs, except RNA no. 1, contained an oligonucleotide clearly identical in position to oligonucleotide 82 from mRNA no. 7 (Fig. 3). To ascertain whether these oligonucleotides were the cap-containing oligonucleotides, the oligonucleotides identical to oligonucleotide 82 in these mRNA's were isolated from the gels, digested with RNase A, $T_1$, plus $T_2$ and separated by electrophoresis on DEAE-cellulose paper. Similar to the oligonucleotide 82 of RNA no. 7, the corresponding oligonucleotides of every mRNA also contained an RNase-resistant cap structure (data not shown). This result suggests that all of the mRNA's contained identical cap-containing oligonucleotides. This finding is surprising, since every intracellular MHV-A59 mRNA contains oligonucleotides representing the sequences in the 5' side of the RNA which are not present in the smaller mRNA species (4). It is possible that one of the unique oligonucleotides of each mRNA, which are not shared with the smaller mRNA's and thus are located at the 5' side of the RNA, might contain the real 5'-end cap-containing oligonucleotides. The oligonucle-

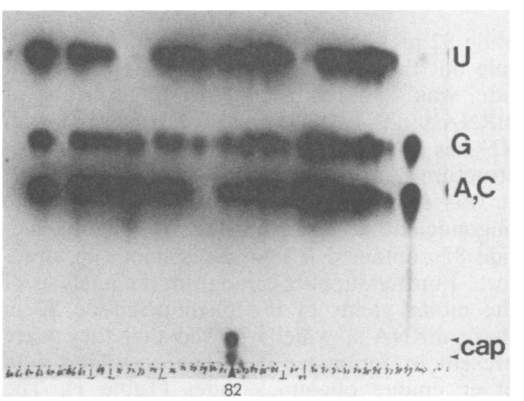


FIG. 4. Electrophoretic separation of RNase A, $T_1$, and $T_2$ digests of the oligonucleotides of mRNA no. 7. Every $T_1$ oligonucleotide of mRNA no. 7 was extracted from the gel as shown in Fig. 3 and then was digested with a mixture of RNases A, $T_1$, and $T_2$. The digests were separated by electrophoresis on DEAE-cellulose paper at pH 3.5. Only part of the electropherogram is shown. The oligonucleotide 82 is indicated whereas the rest of the oligonucleotides are not marked.

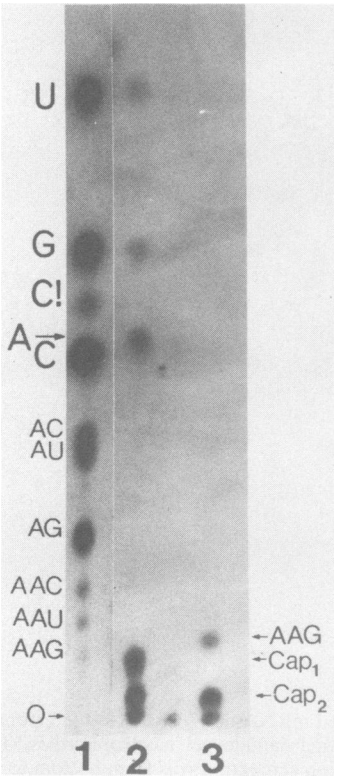


FIG. 5. Electrophoretic analysis of oligonucleotide 82 of mRNA no. 7. The $^{32}$P-labeled oligonucleotide 82 of RNA no. 7 was digested either with RNase A, $T_1$, and $T_2$ (lane 2) or with RNase A (lane 3) and then separated by electrophoresis on DEAE-cellulose paper at pH 3.5. The marker oligonucleotides are shown in lane 1.O, Origin.

otide 82 may then represent a contaminant. To rule out this possibility, every $T_1$ oligonucleotide was isolated from the fingerprints of mRNA's no. 1, 3, 5, and 6 and digested with RNases A, $T_1$, and $T_2$ for the detection of cap structure. Part of the analysis is shown in Fig. 7. It was found that in every mRNA, only the oligonucleotide corresponding to oligonucleotide 82 contained a RNase-resistant cap structure. Further support came from the analysis of the molar yields of the oligonucleotide 82 in these mRNA's, which showed that they were present in equimolar yields as compared with other unique oligonucleotides (Table 1). The only exceptions are oligonucleotides 83 and 84. As shown above, these two oligonucleotides are mixtures of many oligonucleotides and thus are not unique. The oligonucleotide 19a is present in low yield. The reason for its low yield is not clear. These results suggested that most, if not all, of the viral mRNA's in MHV-infected cells contain identical 5'-end sequences. This conclusion was supported by base composition analysis of the oligonucleotides corresponding to the oligonucleotide 82 in all of these mRNA's which showed that all of them contained cap-N-UAAG.

The mRNA no. 1 did not contain a distinct oligonucleotide corresponding to oligonucleotide 82 (Fig. 6, panel 1). Increased exposure of the autoradiograms did, however, suggest the possible presence of such an oligonucleotide. We therefore extracted the $^{32}$P-labeled material in the region corresponding to oligonucleotide 82, as well as all of the identifiable oligonucleotides in mRNA no. 1. These oligonucleotides were subjected to the same analysis for the detection of the cap structure. As shown in Fig. 7, only the region corresponding to the oligonucleotide 82 contained the cap structure similar to the results obtained for the other mRNA's analyzed. This result suggests that the mRNA no. 1 also contains the same 5'-end sequences. The reason for the lower molar yield of the 5'-cap structure in mRNA no. 1 is not clear. The molar yield of the cap-containing oligonucleotide in this RNA could not be determined since it was difficult to separate it from oligonucleotide 83, which was present in overwhelming excess.

Similar studies were done with the genomic RNA extracted from the virion (Fig. 6, panel V). Identical results were obtained (data not shown). Therefore, we conclude that most or all of mRNA's of the MHV-infected cells contained the same 5'-end sequences as that of the viral genomic RNA.

## DISCUSSION

We have previously shown that the seven intracellular virus-specific RNAs in MHV-A59-infected cells contained identical 3'-end sequences and that the shared sequences of each RNA species start from the 3' end of the genome (4). In this report we have further shown that all of these RNAs contain a cap structure followed by 5 nucleotides, N-UAAG. Therefore, all the RNAs appear to have identical sequences at both the 5' and 3' ends. At the present time, we do not know how many more nucleotides are shared at the 5' ends among these mRNA's. The presence of common 5'-terminal oligonucleotides in all of these RNAs is particularly interesting. This result suggests two possible mechanisms for the generation of these mRNA's. (i) The genome contains seven copies of a redundant sequence. These sequences serve as initiation sites for the synthesis of the subgenomic mRNA's. Such redundant sequences have so far not been detected in the viral genome. Examination of the oligonucleotide fingerprints of the viral genome (Fig. 6) did not reveal any large $T_1$ oligonucleotide that could be present in so many

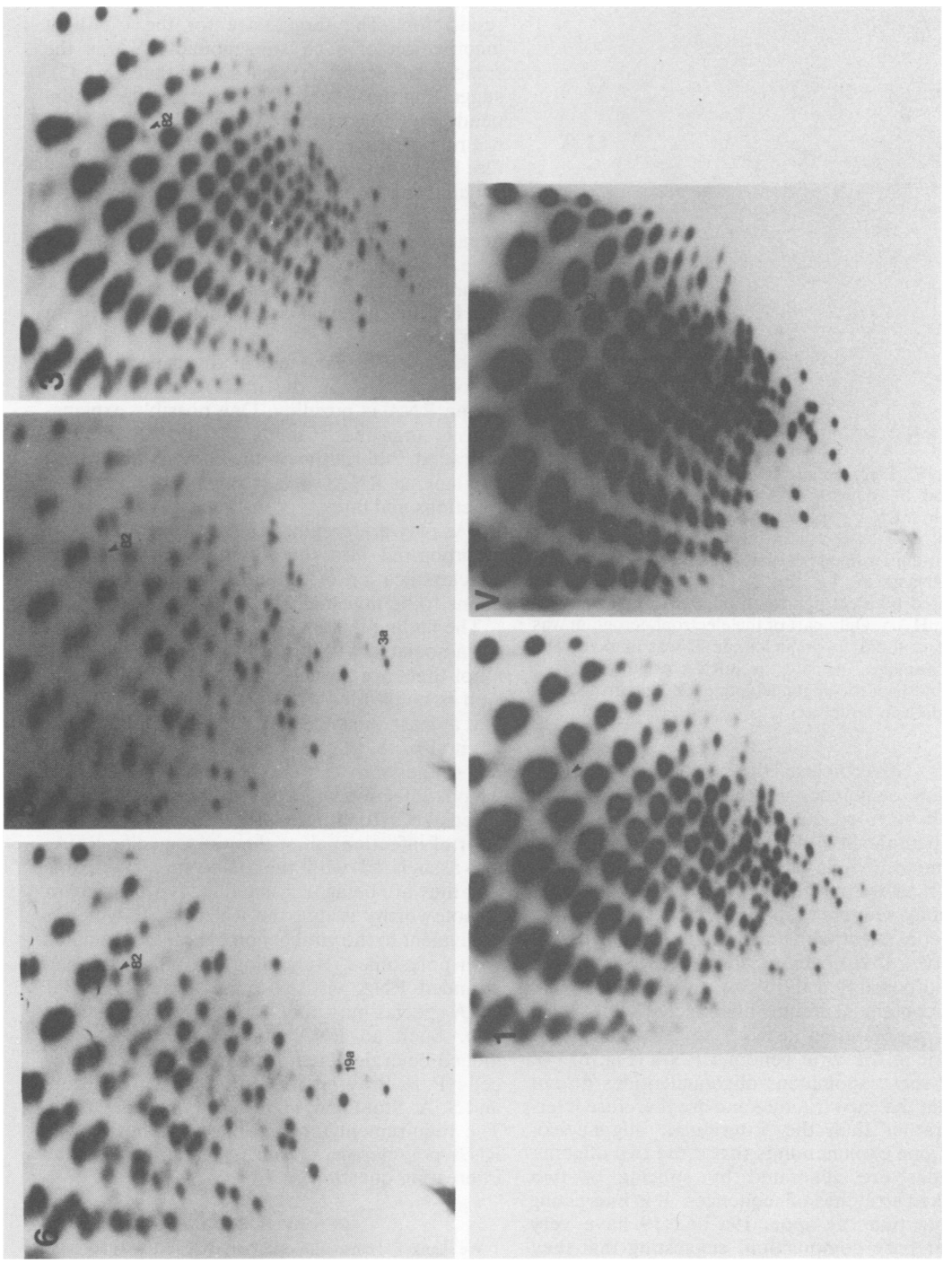


FIG. 6. Oligonucleotide fingerprints of the intracellular RNAs no. 1, 3, 5, and 6 and virion RNA. The $^{32}P$-labeled intracellular RNAs were extracted from the gels as shown in Fig. 1, and the virion RNA was extracted from the purified virus (5, 6). These RNAs were then analyzed by $T_1$ oligonucleotide fingerprinting. Oligonucleotide 82 was identified in all of the RNA. Oligonucleotides 19a and 3a are not present in the viral genome (4).

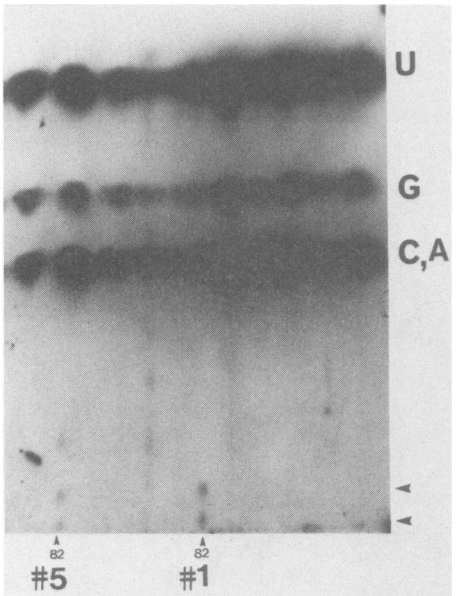


FIG. 7. Electrophoretic separation of RNase A, $T_1$, and $T_2$ digests of various oligonucleotides in different mRNA's. All of the oligonucleotides of different mRNA's as shown in Fig. 6 were extracted from the two-dimensional polyacrylamide gels and digested with RNases A, $T_1$, and $T_2$. The digests were then separated by electrophoresis on DEAE-cellulose paper at pH 3.5. Only part of the electrophoerogram was shown, and only oligonucleotide 82 was marked. The lower numbers indicate the mRNA origin (mRNA's no. 5 and 1) of the oligonucleotide 82. The arrowheads mark the cap structure.

copies. Nevertheless, the presence of such redundant sequences in the viral genome still cannot be ruled out since the copy number of such pentanucleotides would be very difficult to determine. (ii) An alternative possibility is that the RNAs are produced by splicing of the leader and body sequences in the RNAs. This possibility is consistent with an additional finding that at least two A59 subgenomic RNAs (i.e., no. 5 and 6), and possibly a third one, mRNA no. 4 (6a), each contain a unique oligonucleotide (19a in RNA no. 6 and 3a in RNA no. 5) which is not present in the viral genomic RNA (Fig. 6) (4). Since these anomalous oligonucleotides do not contain the cap structure and are therefore internal, rather than the 5'-terminal, oligonucleotides, one explanation is that these two oligonucleotides are generated by splicing of two unlinked stretches of sequences. It is interesting to note that the spots 19a and 19 have very similar base composition, suggesting that they might have very similar sequences, except for the residues close to the 3' end (next to G residues) (Table 1). Preliminary data suggest that the spot 3a is similarly related to 19a and 19. These data might suggest that these oligonucleotides represent junction sequences. However, two other pieces of data are contradictory to the possible involvement of RNA splicing in MHV replication. The target size for the UV light inactivation of each subgenomic RNA is the same as the actual physical size of that RNA (3), suggesting that each mRNA is transcribed independently. Furthermore, viral replication does not require the presence of the host cell nucleus (2a, 17); to date, it has not been shown that RNA splicing can take place in the cytoplasm. Therefore, the mechanism of RNA synthesis in mouse hepatitis viruses is still not clear at the present time. Determination of the size and sequences common to the 5' end of each subgenomic RNA will help resolve this issue. Such an experiment is in progress.

The lower molar yield of the cap-containing oligonucleotides in the mRNA no. 1 and genomic RNA is puzzling. One possible explanation is that the 5' ends of the RNAs were degraded. Alternatively, the cap-containing and cap-lacking RNA species might have different functions and fates. For instance, these different RNAs may preferentially be used as mRNA's or incorporated into the virion. Such functional subclassification of the virus-specific RNAs remains to be investigated.

The finding that all of the virus-specific RNAs are associated with the polysomes indicates that all of them are probably mRNA's. This conclusion is consistent with the recent observations that at least some of these RNAs can be translated in vitro (8, 9). The polysome preparations in our studies were isolated from the infected cells 9 h postinfection when the cells were undergoing extensive cytopathic change. Even at this late hour of infection, all of the virus-specific RNAs are associated with the polysome, suggesting that they are being translated in vivo. Particularly noteworthy is that the RNA no. 1, which is equivalent to the viral genome, is also associated with polysomes. By analogy to other positive-stranded RNA viruses, e.g., togaviruses, this RNA species may code for an RNA polymerase (13). Such an RNA polymerase activity has indeed been detected in the MHV-A59-infected cells (P. R. Brayton, M. M. C. Lai, C. D. Patton, and S. A. Stohlman, manuscript in preparation). The requirement for continued synthesis of an RNA polymerase so late in infection poses an interesting question.

ACKNOWLEDGMENTS

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TABLE 1. Quantitation of the $T_1$ oligonucleotides in MHV mRNA's

| Spot no.[a] | Base composition | No. of phosphate groups | Relative molar yield in mRNA[b] | | |
|---|---|---|---|---|---|
| | | | No. 7 | No. 6 | No. 5 |
| 82 | cap-N-UAAG | 8 | 1.0 | 1.0 | 1.0 |
| 10 | $U_6$ G $C_6$ (AC) ($A_2$C) ($A_4$N) | 23 | 1.0 | 0.8 | 0.8 |
| 19 | $U_7$ $C_2$ ($A_2$U) ($A_2$G) ($A_3$C) ($A_3$U) | 23 | 0.9 | –[c] | – |
| 34 | $U_2$ G $C_5$ (AC) ($A_2$C)$_3$ | 19 | 1.0 | 0.8 | 0.8 |
| 36 | $C_2$ (AC) (AG) ($A_2$U) ($A_3$U) ($A_4$N) | 18 | 0.8 | 0.7 | 0.8 |
| 57 | $U_5$ G $C_2$ (AC) ($A_4$N) | 15 | 1.1 | 0.9 | 0.9 |
| 56 | $U_4$ G $C_5$ (AC) (AU) | 14 | 1.0 | 1.0 | 1.1 |
| 55 | $U_2$ ($A_2$G) ($A_3$C) ($A_4$N) | 14 | 0.8 | 0.8 | 0.8 |
| 54 | $U_3$ $C_2$ (AC)$_2$ (AU) (AG) | 13 | 1.0 | 1.0 | ND[d] |
| 83[e] | $U_3$ $G_3$ (AU)$_4$ ($A_2$U) | 17 (mixture) | 1.0 | 1.5 | 1.8 |
| 84[e] | $U_6$ $G_2$ $C_2$ (AC) (AG) | 14 (mixture) | 1.1 | 1.6 | 2.1 |
| 51 | G $C_2$ (AC)$_3$ ($A_2$U) | 12 | 0.9 | 0.9 | ND |
| 19a | $U_5$ G $C_2$ (AU)$_2$ ($A_2$U) ($A_3$C) ($A_3$U) | 23 | – | 0.4 | – |
| 17 | $U_5$ $C_2$ ($A_2$C) ($A_2$U) ($A_2$G) ($A_3$C) | 20 | – | 0.8 | 0.9 |
| 37 | $U_2$ G C (AU) ($A_2$C) ($A_3$C)$_2$ | 17 | – | 0.8 | 0.9 |
| 39 | $U_2$ $C_2$ (AC)$_2$ (AU)$_2$ (AG) ($A_2$C) | 19 | – | 0.8 | 0.9 |

[a] Spot numbers are as described in Fig. 3 and 6.

[b] The $T_1$ oligonucleotides were cut out of the gels, and the radioactivity associated with each spot was determined by Cerenkov counting. The relative radioactivity of each spot was determined by dividing the radioactivity by the number of phosphate groups in each oligonucleotide. The relative molar yield was then deduced by comparing the relative radioactivity of each spot with that of the spot 82.

[c] –, Not present.

[d] ND, Not determined.

[e] The base compositions shown for the spots 83 and 84 are for the mRNA no. 7. Those for the mRNA's 6 and 7 were too complex to allow for accurate determination. These spots all represent mixtures of many oligonucleotides.

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