Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4170–4174, July 1981
Biochemistry

Sequences of mRNAs derived from genome RNA segment 7 of influenza virus: Colinear and interrupted mRNAs code for overlapping proteins

(mRNA structure/intervening sequences/myxovirus gene arrangement/nuclease S1 mapping/RNA genes in different reading frames)

ROBERT A. LAMB*, CHING-JUH LAI†, AND PURNELL W. CHOPPIN*

*The Rockefeller University, New York, New York 10021; and †Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Contributed by Purnell W. Choppin, March 31, 1981

ABSTRACT **RNA segment 7 of the influenza A virus genome codes for at least two proteins, M_1 and M_2, which are synthesized from separate mRNA species. Sequence analysis of the M_2 mRNA has shown that it contains an interrupted sequence of 689 nucleotides. The ≈51 virus-specific nucleotides comprising the 5′-end leader sequence of the M_2 mRNA are the same as those found at the 5′ end of the colinear M_1 mRNA. Following the leader sequence of the M_2 mRNA, where is a 271-nucleotide body region that is 3′ coterminal with the M_1 mRNA. Another small potential mRNA ($mRNA_3$) related to RNA segment 7 has been found. $mRNA_3$ has a leader sequence of ≈11 virus-specific nucleotides that are the same as the 5′ end of the M_1 and M_2 mRNAs, followed by an interrupted sequence of 729 nucleotides, and then a body region of ≈271 nucleotides that is the same as that of the M_2 mRNA. The nucleotide sequences found at the junctions of the interrupted sequences in M_2 mRNA and $mRNA_3$ are similar to those found at the splicing points of intervening sequences in eukaryotic mRNAs. In addition, both mRNAs contain 10–15 heterogeneous nonviral nucleotides at their 5′ ends that appear to be derived from cellular RNAs used for priming the transcription of viral RNAs. Because the 5′-end sequences of the M_1 mRNA and the M_2 mRNA are the same and share the 5′-proximal initiation codon for protein synthesis, the first nine amino acids would be the same in the M_1 and M_2 protein and then the sequences would diverge. The ≈271-nucleotide body region of the M_2 mRNA can be translated in the +1 reading frame, and the sequence indicates that M_1 and M_2 overlap by 14 amino acids. The coding potential of the $mRNA_3$ is for only nine amino acids, and these would be identical to the COOH-terminal region of the membrane protein (M_1).**

The genome of influenza virus consists of eight single-stranded RNA segments that are transcribed into mRNAs that code for the virus-specific polypeptides (for review, see ref. 1). Segment 8, the smallest RNA segment, codes for two proteins, NS_1 and NS_2, that are translated from separate mRNA species (2–4). Mapping and sequence analysis of the NS_1 and NS_2 mRNAs established that the first 56 nucleotides at the 5′ end of both mRNAs are identical and that this region contains the AUG codon for initiation of protein synthesis, so that NS_1 and NS_2 share nine amino acids at their NH_2-termini (5, 6). Following this shared sequence, there is an interrupted region of 473 nucleotides in the NS_2 mRNA, and then translation of NS_2 would continue in the +1 reading frame (5, 6). The coding regions of the NS_1 and NS_2 mRNAs that are in different reading frames overlap by 70 amino acids (6).

Recent analyses of the nucleotide sequences of RNA segment 7 from two strains of influenza A virus [PR/8/34 (H1N1) and Udorn/72 (H3N2)] have shown that, in addition to the reading frame coding for the membrane protein (M_1; 252 amino acids), there is a second open reading frame that is conserved and could code for 97 amino acids (7, 8, 9). We have identified a gene product for this second reading frame (M_2; apparent M_r ≈15,000) in infected cells and have shown by biochemical and genetic evidence that this protein is coded for by RNA segment 7 (10). Tryptic peptide maps of the M_2 protein synthesized by several strains of influenza virus could be correlated with the amino acid sequence deduced from the second open reading frame. In addition, we have identified a separate mRNA coding for M_2 protein (10).

We report here the results of experiments designed to determine the precise 5′-terminal nucleotides of the M_2 mRNA, to demonstrate directly that translation of the M_2 mRNA could occur in the second open frame, and to investigate whether the M_2 mRNA was analogous to the NS_2 mRNA in having an interrupted region.

MATERIALS AND METHODS

Virus and Cells. The influenza virus A/Udorn/72 (H3N2) strain was grown in embryonated eggs, and the HeLa (human) cell line was grown as described (2).

Preparation of Cloned DNA, Restriction Endonuclease Digestions, Nuclease S1 Mapping, and DNA Sequence Analysis. Clone PFV 45/M, constructed previously (11), was derived by hybridization of separate cDNA copies of RNA segment 7 and of its large mRNA (M DNA) and cloning in the *Pst* I site of pBR322. It was produced by transformation of *Escherichia coli* K-12, strain HB101. Purification of the M DNA after *Hpa* II digestion, preparation of restriction endonuclease digested fragments, nuclease S1 mapping, and 5′-terminal labeling were done as described (5, 6, 9, 11). DNA sequence analysis was done as described by Maxam and Gilbert (12, 13).

Primer Extension of the mRNAs to Obtain the Sequence of the 5′-Terminal Regions. The procedure was similar to that used previously (6).

RESULTS

Sizing the Body Sequences of M_2 mRNA. Fractionation of influenza virus-specific mRNAs on sucrose gradients and translation *in vitro* of the fractions indicated that protein M_2 was synthesized from a separate small polyadenylylated mRNA species (10). To investigate the size of the body segment of the M_2 mRNA, uniformly ^{32}P-labeled M DNA was hybridized to total influenza virus-specific mRNAs, digested with nuclease S_1, and analyzed on denaturing polyacrylamide gels. As shown in Fig. 1, a band of ≈1000 nucleotides, equivalent in size to protection of the M_1 mRNA, was observed. In addition, a band of ≈285

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

nucleotides, present in much less abundance and compatible in size with protection of the M_2 mRNA, was also seen.

Primer Extension to Analyze the 5′-Terminal Sequences of M_2 mRNA. The nucleotide sequence at the 5′ terminus of M_2 mRNA was obtained by reverse transcriptase extension to examine whether the M_2 mRNA had a leader sequence, to determine the possible initiation codon for translation of M_2, and to demonstrate directly that the M_2 protein was translated from a reading frame different from that for the M_1 protein. To obtain a primer for reverse transcriptase extension, a *Hin*fI-derived M DNA fragment, containing label at nucleotide 777, was hybridized to M_2 mRNA that had been isolated from infected cells and purified on sucrose gradients. The ^{32}P-labeled DNA primer was extended with reverse transcriptase, and the products were analyzed on denaturing acrylamide gels. Two major single-stranded DNA species, ≈61 and ≈108 nucleotides long, were obtained (Fig. 2) and both were sequenced. The larger species was thought the more likely candidate to represent the 5′-terminal sequences of the M_2 mRNA, as this size should include additional nucleotides beyond those expected for the second open reading frame coding for the M_2 protein. The sequence of the larger species (≈108 nucleotides) from nucleotide 763 and, on a longer gel run, its 5′-terminal end are shown in Fig. 3. The 5′ end of this mRNA species is heterogeneous for 10–15 nucleotides, which are followed by ≈51 nucleotides that correspond to the 5′-terminal portion of M_1 mRNA—i.e., complementary to the 3′-terminal portion of the virion RNA segment 7. This leader sequence is connected to the body of the mRNA beginning at nucleotides 740 and 741. The exact nucleotides at which the leader sequence and body sequence are joined cannot be determined because of the repetition of G-G at 740 and 741, but they are likely to be nucleotides 41 and 740 (see below). Thus, in this mRNA, there is an interrupted region of 689 nucleotides. This mRNA arrangement creates an open reading frame that begins with the AUG initiation codon at nucleotides 26–29 and continues to nucleotide 1004, with translation occurring in the same reading frame as that used for M_1 from nucleotides 20–51 (i.e., for nine amino acids); after nucleotide 740, translation switches to the +1 reading frame for 88 amino acids. The nucleotide sequence of the mRNA is compatible with the observed M_2 protein. The nucleotide sequence coding arrangement is shown in Fig. 4.

The sequence of the 5′-terminal nucleotides of the smaller primer-extended DNA species (61 nucleotides) is shown in Fig. 3. This mRNA species (designated $mRNA_3$ rather than M_3 RNA because a gene product has not yet been found) contains 10–15 heterogeneous nucleotides at its 5′ terminus, followed by ≈11 nucleotides that appear to be identical to the 5′ end of the M_1 and M_2 mRNAs. The leader sequence is then connected to the same body sequence as the M_2 mRNA at nucleotides 740 and 741 and therefore contains an interrupted region of 729 nucleotides. Examination of the coding potential of this mRNA (Fig. 4) indicates that the first AUG initiation codon is located 27–29 nucleotides from its discrete 5′ end (nucleotides 755–757) and, after codons for nine amino acids, there is a termination codon (782–784). These nine amino acids would be identical to the COOH-terminal region of the membrane protein (M_1).

To investigate the possibility that additional interrupted regions occur in the M_2 mRNA and the $mRNA_3$, a *Taq* I-derived M DNA fragment (871–943 on the minus strand) was labeled at its 5′ termini and cleaved with *Hae* III. The *Hae* III/*Taq* I (900–943) fragment was used as a primer for reverse transcriptase extension of the small mRNAs. The predicted sizes of the extended products, if there was no other interrupted region, were ≈265 nucleotides for the M_2 mRNA and ≈225 nucleotides for the $mRNA_3$. As shown in Fig. 5, the observed sizes, ≈269 and ≈222, respectively, were very close to those predicted. DNA sequence analysis of these extended DNA species confirmed that no other interrupted regions occurred in the M_2 mRNA and the $mRNA_3$ (Fig. 4). A schematic representation of the mRNAs and their coding regions is shown in Fig. 6.

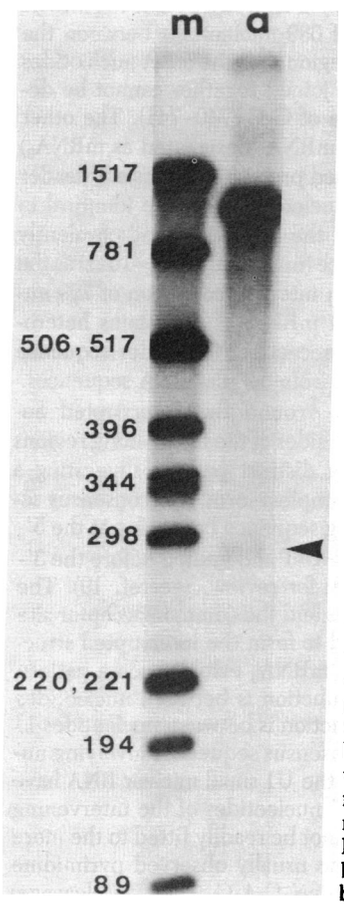

FIG. 1. Nuclease S1 analysis of mRNAs derived from RNA segment 7. Poly(A)-containing mRNAs from influenza virus-infected HeLa cells were hybridized with denatured ^{32}P-labeled M DNA in 80% formamide at 50°C for 2 hr, 48°C for 2 hr, and 46°C for 1 hr, consecutively. The hybridization mixtures were then treated with nuclease S1, and the digests were analyzed by electrophoresis on 4% polyacrylamide/9 M urea gels. Lane m: ^{32}P-labeled pFV M/45 plasmid DNA was digested with *Hin*fI, and the fragments were used as markers; numbers indicate polynucleotide length. Lane a: M DNA was hybridized to mRNAs, and the hybrid was treated with nuclease S1.

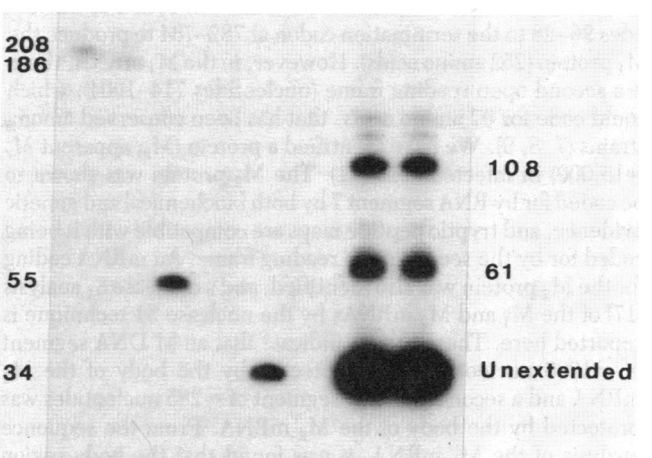

FIG. 2. 5′-terminal sequences obtained by primer extension of the small mRNAs derived from RNA segment 7. Poly(A)-containing mRNAs from infected cells were fractionated on sucrose gradients, and those fractions that translated *in vitro* to yield polypeptide M_2 were pooled. A^{32}P-labeled DNA fragment (nucleotides 741–777) was hybridized to the pooled mRNA fractions, and the primer was extended with reverse transcriptase. Markers of 208, 186, 55, and 34 nucleotides from M DNA cleaved with various restriction endonucleases were used. Samples were analyzed on 8% polyacrylamide/9 M urea gels. The two major extended products, ≈61 and ≈108 nucleotides, were heterogeneous in size due to the host cell-donated nucleotides at the 5′ ends of the mRNAs.

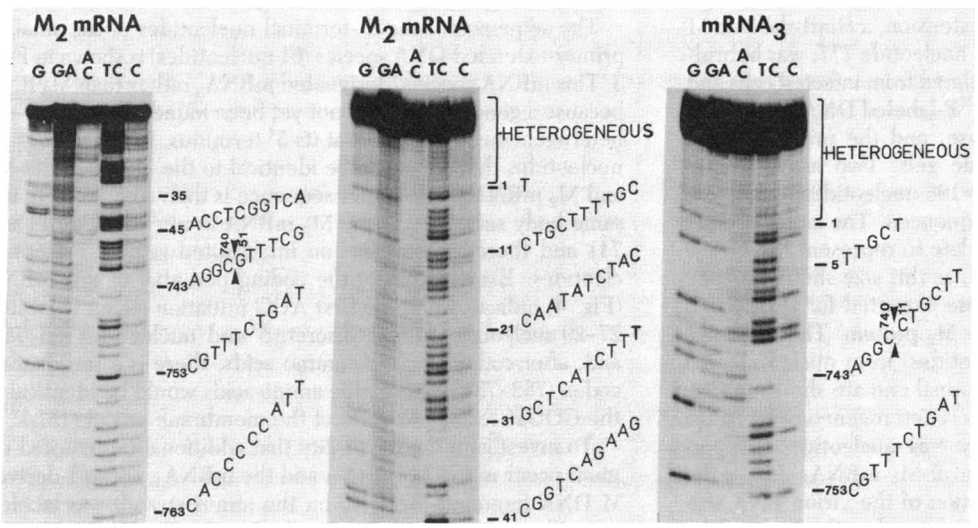

FIG. 3. Sequences of the cDNA copies of the 5′-terminal regions of the small mRNAs. The base-specific chemical cleavages are indicated over each lane. The sequence obtained from the gels is interpreted with the nucleotides numbered as shown in Fig. 4. The probable donor and acceptor nucleotides at which the interrupted sequence is excised are indicated by arrows. (*Left*) Twenty percent sequencing gel of the ≈108 nucleotide cDNA from the primer extension (Fig. 2) of M_2 mRNA, showing the junction of the leader and body sequences. (*Middle*) Eight percent sequencing gel of the same cDNA, showing the 5′-terminal region. (*Right*) Eight percent sequencing gel of the ≈61 nucleotide cDNA from the primer extension (Fig. 2) of the RNA segment 7-derived $mRNA_3$, showing the junction of the leader and body sequences and the 5′-terminal region. The presence of other bands in all lanes at position 740, which is predominantly a cytidine, is not readily explained unless the DNA was nicked at this position but has been observed in other junctions of leader and body sequences.

DISCUSSION

Structure of the mRNAs Derived from RNA Segment 7. The nucleotide sequences of influenza virus RNA segment 7 [1027 nucleotides] of strains PR/8/34 (H1N1) and Udorn/72 (H3N2) have been obtained (7, 8). The membrane protein (M_1) mRNA is a colinear, but incomplete, transcript of the virion strand (14, 15), and the polyadenylylation site has been shown to be an A-A-A-A-A-A region between nucleotides 1006-1011 (16). The M_1 mRNA is translated from the AUG initiation codon at nucleotides 26–28 to the termination codon at 782–784 to produce the M_1 protein (252 amino acids). However, in the M_1 mRNA, there is a second open reading frame (nucleotides 714–1004), which could code for 97 amino acids, that has been conserved among strains (7, 8, 9). We have identified a protein (M_2; apparent M_r ≈15,000) in infected cells (11). The M_2 protein was shown to be coded for by RNA segment 7 by both biochemical and genetic evidence, and tryptic peptide maps are compatible with it being coded for by the second open reading frame. An mRNA coding for the M_2 protein was also identified, and a nuclease S_1 analysis (17) of the M_1 and M_2 mRNAs by the nuclease S1 technique is reported here. These results indicate that an M DNA segment of ≈1000 nucleotides was protected by the body of the M_1 mRNA and a second M DNA segment of ≈285 nucleotides was protected by the body of the M_2 mRNA. From the sequence analysis of the M_2 mRNA, it was found that the body region consists of ≈271 nucleotides (depending on the exact poly(A) addition site), and the apparent small difference is probably due to incomplete denaturation in the urea gels used to analyse the nuclease S_1-digested products.

By using reverse transcriptase to extend DNA primers hybridized to the body region of the small mRNAs, we have demonstrated that there are two major species of small mRNAs derived from influenza virus RNA segment 7 in infected cells. Minor primer-extended species were also observed, but their significance is not clear and they have not been examined further. Sequence analysis has shown that the M_2 mRNA contains a 5′-leader sequence of ≈51 virus-specific nucleotides, which appears identical to the 5′ end of the M_1 mRNA, and this is covalently linked to the body sequence (nucleotides 740–1011). Preceeding the leader sequence are 10–15 nucleotides that are heterogeneous and appear to be derived from cellular mRNAs used for priming the transcription of viral RNAs. The M_2 mRNA has an interrupted sequence of 689 nucleotides between the leader sequence and the body region, but the exact nucleotides at which the two segments are joined together cannot be determined, due to the repetition of G-G (740–741). The other RNA segment 7-derived small mRNA (designated as $mRNA_3$) found as a major primer-extended product contains a 5′-leader sequence of 11 virus-specific nucleotides that are identical to the 5′ end of the M_1 mRNA or the M_2 mRNA and covalently linked to the same body sequence (nucleotides 740–1011) as the M_2 mRNA. This $mRNA_3$ has an interrupted region of 729 nucleotides. The observation that $mRNA_3$ also contains heterogeneous nonviral nucleotides preceeding the leader sequence suggests that these are derived from cellular RNA sequences.

Donor and Acceptor Sites Around the Interrupted Sequences. The sequences at both sides of the intervening regions in eukaryotic mRNAs follow a distinct pattern suggesting a "consensus" sequence. In its simplest form, the consensus sequence involves the intervening sequence beginning at the 5′-donor site with the dinucleotide G-T and ending before the 3′-acceptor site with A-G (ref. 18; for review, see ref. 19). The sequences at the two donor sites and the common acceptor site on the colinear M_1 mRNA used to form the interrupted structure of the M_2 mRNA and the $mRNA_3$ exhibit such a pattern (Fig. 4). For M_2 mRNA, the junction is between nucleotides 51 and 740; for $mRNA_3$, the junction is between nucleotides 11 and 740. More complicated consensus sequences involving nucleotides complementary with the U1 small nuclear RNA have been observed (20, 21). The 3′ nucleotides of the intervening sequence in the M_1 mRNA cannot be readily fitted to the more complicated model because the usually observed pyrimidine tract is not adjacent to the PyPyN-C-A-G.↓ (↓ = cleavage)

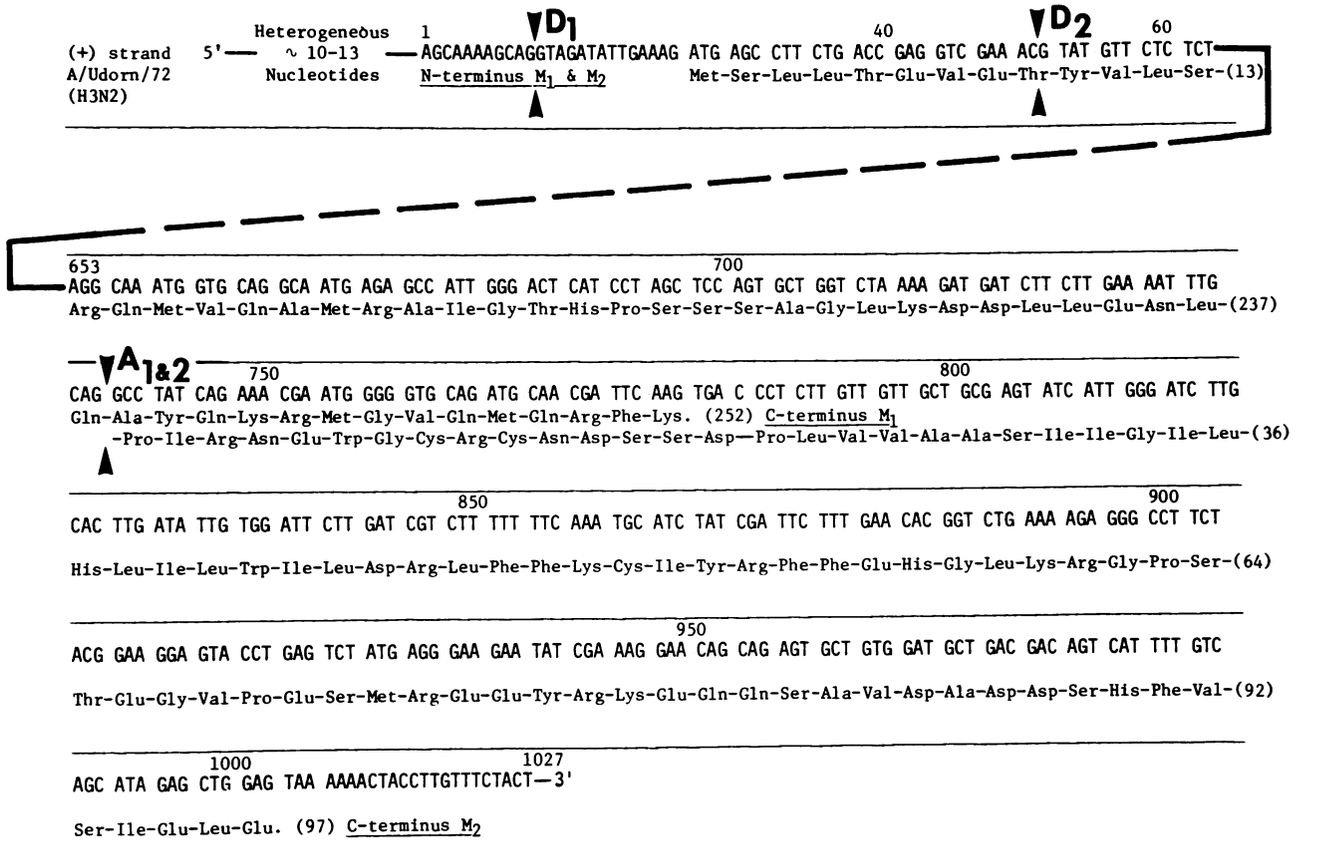

FIG. 4. Nucleotide sequence of M_2 mRNA and $mRNA_3$. The sequence shown here is an abbreviated version of the complete nucleotide sequence of the Udorn strain of influenza A virus RNA segment 7 and its M_1 mRNA (9). The heterogeneous 5'-terminal nucleotides donated from cellular RNAs are indicated as a group; the specific nucleotides found in this region on the cloned M_1 mRNA were shown earlier (9). The 3' terminus after the polyadenylylation site A-A-A-A-A-A (nucleotides 1006–1011) was obtained from the cDNA copy of the 5' end of the virion strand and would not be present on influenza virus mRNAs. D_1 and D_2, probable 5' junctions of the intervening sequences of the $mRNA_3$ and the M_2 mRNA, respectively; A_1 and A_2, probable 3' junctions of the intervening sequences, which are the same for M_2 mRNA and $mRNA_3$.

sequence. However, a seven-nucleotide pyrimidine tract is found five nucleotides to the left of the above sequence (Fig. 4).

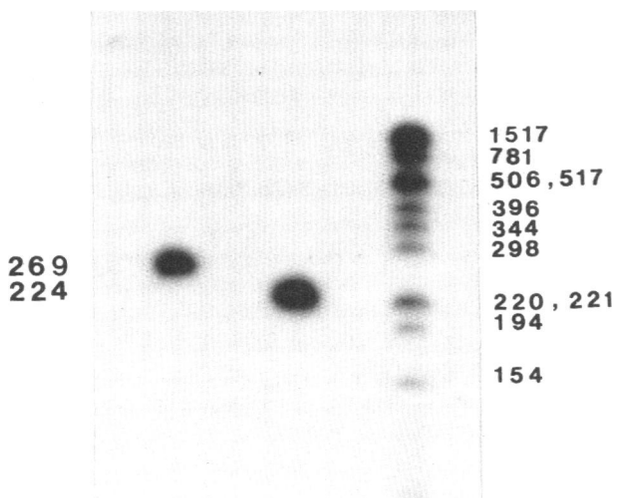

FIG. 5. Primer extension of M_2 mRNA and $mRNA_3$ to verify the absence of additional interrupted regions. A ^{32}P-labeled DNA fragment (nucleotides 900–943) was hybridized to the pooled mRNA fractions described in the legend to Fig. 2 and extended with reverse transcriptase. Markers from ^{32}P-labeled pFV 45/M cleaved with *Hin*fI were used. Samples were analyzed on 8% polyacrylamide/9 M urea gels, and then the bands were eluted and rerun separately on 6% polyacrylamide/9 M urea gels. The observed size of the primer-extended fragments correlates with the predicted size.

Mechanisms for the Synthesis of Interrupted Regions in Influenza Virus mRNAs. We have shown here that the M_2 mRNA and the $mRNA_3$ are analogous to the previously described NS_2 mRNA (6) in having junctions similar to those found in spliced eukaryotic mRNAs, and it seems likely that these mRNAs are derived by splicing from their colinear transcripts, M_1 and NS_1 mRNAs, respectively. The cell nucleus has been reported to be the site for primary transcription of the influenza virus genome

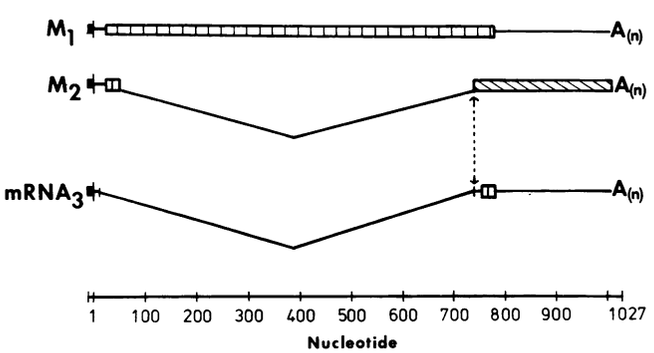

FIG. 6. Model for the arrangement of M_1 mRNA, M_2 mRNA, and $mRNA_3$ and their coding regions. Thin lines at the 5' and 3' termini of the mRNAs represent noncoding regions. Crosshatched areas represent the coding regions of the mRNAs. In the region 740–1004, M_2 mRNA is translated in a reading frame different from that used for M_1. No evidence has yet been obtained that $mRNA_3$ is translated, but its coding potential (which corresponds to the COOH-terminal region of the M_1 protein) is indicated. V-shaped lines represent interrupted regions; filled-in boxes represent heterogeneous nucleotides derived from cellular mRNAs that are covalently linked to the viral sequences.

(22, 23), and the processing of precursor colinear transcripts of mRNAs is thought to occur in the nucleus (24, 25). The small amounts of the processed mRNAs in influenza virus-infected cells suggest that the mechanism is controlled, as it has been found to be in the alternative splicing pathways to form different mRNAs from the colinear RNA transcripts of adenovirus and the papova viruses (for review, see ref. 26). However, with influenza virus, the situation is slightly different because the presumed precursor RNAs also act as mRNAs from which polypeptides are translated (M_1 and NS_1). In addition, processing of the full length (+) strands of template RNAs would have to be avoided. Less likely possibilities for the formation of influenza virus mRNAs involving the virion RNA strand and defective-interfering-particle formation have been discussed previously (6).

Host Cell RNA Sequences at the 5′ End of Influenza Virus mRNAs. Capped eukaryotic RNAs act as primers for the synthesis of influenza viral mRNAs synthesized both *in vitro* and *in vivo* (27, 29). *In vivo*, the host cell-donated sequences have been shown to be heterogeneous by sequence analysis of different cloned mRNAs and by primer extension sequencing on populations of a specific mRNA (6, 30, 31, 9); the size of these cellular sequences is ≈7–15 nucleotides. The mRNA sequences shown in Fig. 3 provide further evidence regarding the mechanism of this process. At the position opposite the 3′-terminal uracil residue of the virion strand, there is predominantly, but not exclusively, an adenine residue (in Fig. 3, this is shown as a thymine residue at nucleotide 1 because it is the cDNA of the mRNA). This finding is in contrast to the complete heterogeneity found by Caton and Robertson (31) but supports our earlier observations (6). The small amount of heterogeneity at this position in a population of specific mRNAs for one RNA segment is also represented in the nucleotides found at the position 1 in the cloned mRNAs (30, 31). This observation lends support to the theory that the virion transcriptase only elongates the primer starting at the second nucleotide at the 3′ end of the virion strand (29, 32). It can also be seen in Fig. 3 that adjacent to the preferred thymine residue in position 1, there is an indication of the predominance of a C-G dinucleotide among the heterogeneous nucleotides. This suggests that among the primer RNAs used, there is a selection process favoring those having the sequence G-C-A immediately before the site of cleavage and elongation to form influenza virus mRNAs.

Predicted Protein Sequences from the mRNA Species. The initiation AUG codon at nucleotides 26–28 in the M_1 mRNA is the only AUG in an open reading frame that could code for a polypeptide the size of M_1. Because translation of nearly all eukaryotic mRNAs begins with the 5′-proximal initiation codon (33) and the M_1 mRNA and M_2 mRNAs contain the identical 51 virus-specific nucleotides at their 5′ ends, the common proximal AUG codon at nucleotides 26–28 should be used for translation of the M_1 and M_1 polypeptides. After the interrupted region, translation of M_2 would continue in the +1 reading frame, so that M_1 and M_2 would share nine amino acids at their NH_2-termini, with the remaining sequences being different. Translation in the +1 reading frame continues to the termination codon TAA (1004–1007), which is adjacent to the polyadenylylation site of the mRNAs. The sequences indicate that M_1 and M_2 overlap by 14 amino acids, which are translated from different reading frames. Polypeptide M_2 has been estimated to have an apparent M_r ≈15,000 on polyacrylamide gels (10), but the sequence indicates it contains only 97 amino acids. Thus, the migration of the M_2 polypeptide on polyacrylamide gels, which varies among strains (10), is anomalous with respect to size and depends on amino acid composition.

Examination of the coding potential of $mRNA_3$ indicates that it would code for only nine amino acids, assuming that the 5′-proximal initiation AUG codon is used for translation. These amino acids would be identical to the COOH-terminal region of the M_1 protein.

We thank Mary Conners for excellent technical assistance. This work was supported by Research Grant AI-05600 from the National Institute of Allergy and Infectious Diseases. R.A.L. is an Irma T. Hirschl Career Scientist Awardee.

1. Schild, G. C. (1979) *Br. Med. Bull.* **35.**
2. Lamb, R. A., Etkind, P. R. & Choppin, P. W. (1978) *Virology* **91,** 60–78.
3. Lamb, R. A. & Choppin, P. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4908–4912.
4. Inglis, S. C., Barrett, T., Brown, C. M. & Almond, J. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3790–3794.
5. Lamb, R. A., Choppin, P., W., Chanock, R. M. & Lai, C.-J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1857–1861.
6. Lamb, R. A. & Lai, C.-J. (1980) *Cell* **21,** 475–485.
7. Winter, G. & Fields, S. (1980) *Nucleic Acids Res.* **8,** 1965–1974.
8. Allen, H., McCauley, J., Waterfield, M. & Gething, M.-J. (1980) *Virology* **107,** 548–551.
9. Lamb, R. A. & Lai, C.-J. (1981) *Virology* **112,** in press.
10. Lamb, R. A. & Choppin, P. W. (1981) *Virology* **112,** in press.
11. Lai, C.-J., Markoff, L. J., Zimmerman, S., Cohen, B., Berndt, J. A. & Chanock, R. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 210–214.
12. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
13. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
14. Hay, A. J., Lomniczi, B., Bellamy, A. R. & Skehel, J. J. (1977) *Virology* **83,** 337–355.
15. Plotch, S. J. & Krug, R. M. (1978) *J. Virol.* **25,** 579–586.
16. Robertson, J. S., Schubert, M. & Lazzarini, R. A. (1981) *J. Virol.* **38,** 157–163.
17. Berk, A. J. & Sharp, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1274–1278.
18. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4853–4857.
19. Lewin, B. (1980) *Cell* **22,** 324–326.
20. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L. & Steitz, J. A. (1980) *Nature (London)* **283,** 220–224.
21. Rogers, J. & Wall, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1877–1879.
22. Barrett, T., Wolstenholme, A. J. & Mahy, B. W. J. (1979) *Virology* **98,** 211–225.
23. Mark, G. E., Taylor, J. M., Broni, B. & Krug, R. M. (1979) *J. Virol.* **29,** 744–752.
24. Nevins, J. R. (1979) *J. Mol. Biol.* **130,** 493–506.
25. Piper, P., Wardale, J. & Crew, F. (1979) *Nature (London)* **282,** 686–691.
26. Ziff, E. B. (1980) *Nature (London)* **287,** 491–499.
27. Bouloy, M. B., Plotch, S. J. & Krug, R. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4886–4890.
28. Krug, R. M., Broni, B. A. & Bouloy, M. (1979) *Cell* **18,** 329–334.
29. Robertson, H. D., Dickson, E., Plotch, S. J. & Krug, R. M. (1980) *Nucleic Acids Res.* **8,** 925–942.
30. Dhar, R., Cahnock, R. M. & Lai, C.-J. (1980) *Cell* **21,** 495–500.
31. Caton, A. J. & Robertson, J. S. (1980) *Nucleic Acids Res.* **8,** 2591–2603.
32. Bouloy, M., Plotch, S. J. & Krug, R. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3952–3956.
33. Kozak, M. (1978) *Cell* **15,** 1109–1123.