JOURNAL OF VIROLOGY, Oct. 1996, p. 7236–7240 Vol. 70, No. 10
0022-538X/96/$04.00+0

The 3′ Untranslated Region of Coronavirus RNA Is Required for Subgenomic mRNA Transcription from a Defective Interfering RNA

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Received 1 February 1996/Accepted 17 June 1996

The 3′-end of mouse hepatitis virus (MHV) genomic RNA contains a recognition sequence (55 nucleotides [nt]) required for minus-strand RNA synthesis. To determine whether the 3′-end sequence is also involved in subgenomic mRNA transcription, we have constructed MHV defective interfering (DI) RNAs which contain a chloramphenicol acetyltransferase (CAT) gene placed behind an intergenic sequence and a 3′-end sequence with various degrees of internal deletions. The DI RNAs were transfected into MHV-infected cells, and CAT activities, which represent subgenomic mRNA transcription from the intergenic site, were determined. The results demonstrated that the deletions of sequence upstream of the 350 nt at the 3′-end, which include the 3′-untranslated region (3′-UTR), of MHV genomic RNA did not affect subgenomic mRNA transcription. However, deletions that reduced the 3′-end sequences to 270 nt or less completely abolished the mRNA transcription despite the fact that all of these clones synthesized minus-strand RNAs. These results indicated that mRNA transcription from an intergenic site in the MHV DI RNA requires most of the 3′-UTR as a *cis*-acting signal, which likely exerts its effects during plus-strand RNA synthesis. A substitution of the corresponding bovine coronavirus sequence for the MHV sequence within nt 270 to 305 from the 3′-end abrogated the CAT gene expression, suggesting a very rigid sequence requirement in this region. The deletion of a putative pseudoknot structure within the 3′-UTR also abolished the CAT gene expression. These findings suggest that the 3′-UTR may interact with the other RNA regulatory elements to regulate mRNA transcription.

Mouse hepatitis virus (MHV) is a prototype coronavirus, containing a linear, single-stranded, positive-sense RNA of approximately 31 kb (15, 17, 27). During MHV infection of susceptible cells, six to seven subgenomic mRNAs are transcribed, which are 3′-coterminal with the genomic RNA and have a nested-set structure (12). All of them contain a leader sequence, 72 to 77 nucleotides (nt) in length, at the 5′-end (12, 14, 32), which is derived from the 5′-end of the genomic RNA.

Coronavirus mRNA transcription has been shown to involve a discontinuous process, fusing the leader and the body sequences of mRNAs from two different RNA molecules (8, 18, 23). Several models have been proposed to describe the mechanism of discontinuous transcription (7, 12, 28). Among them, the minus-strand RNA jumping model (28) proposes that the discontinuous step occurs during negative-strand RNA synthesis, when the nascent minus-strand RNAs jump from an intergenic (IG) site to the leader region of the genomic RNA template to generate subgenomic minus-strand RNAs containing an antileader sequence. In contrast, another model, the leader-primed transcription model (12), proposes that the discontinuous step occurs during positive-strand RNA synthesis, when the positive-strand leader RNA dissociates from the template and reassociates with the IG site of the minus-strand RNA template (13). Both of these mechanisms require an interaction between the leader RNA and the IG sequence of positive or negative strand to effect subgenomic mRNA transcription. Transcription models combining the two mechanisms have also been proposed (7, 29).

The *cis*-acting sequence requirements for MHV genomic RNA replication and minus-strand RNA synthesis have previously been determined (9, 19, 20). The sequences required for genomic RNA replication include 470 to 859 nt from the 5′-end, an optional 135-nt internal sequence located at gene 1, and 436 nt from the 3′-end of the genome (9, 19). In contrast, minus-strand RNA synthesis requires only 55 nt plus poly(A) tail from the 3′-end (20). The apparent difference in the 3′-end sequence requirements between genomic RNA replication and minus-strand RNA synthesis suggests that the 3′-end sequence not only provides the signal for minus-strand synthesis, but also is involved in positive-strand RNA synthesis. These 3′-end sequences may interact with the 5′-end *cis*-acting sequences to regulate positive-strand RNA synthesis.

For subgenomic mRNA transcription, the major *cis*-acting signal is the IG sequence, since the insertion of an IG sequence into any site in a defective interfering (DI) RNA initiates subgenomic mRNA transcription (22). However, other RNA regions may also be required for, or can regulate, mRNA transcription. For example, the leader RNA has both *cis*- and *trans*-acting activities on the subgenomic mRNA transcription from a DI RNA (18, 38). The leader RNA derived in *cis* or in *trans* (i.e., from the same or a different RNA molecule) is incorporated into subgenomic mRNAs (8, 18, 38). The deletion of the leader sequence from an RNA template resulted in significant loss of transcription activity, even though the leader RNA for the subgenomic mRNA could have been supplied in *trans* from the helper viral RNA (18). These results suggest that MHV subgenomic mRNA transcription requires an IG

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sequence and is regulated by *cis*-acting sequences at the 5′-end and a *trans*-acting leader RNA.

In this communication, we examined the possible role of the 3′-end sequence on subgenomic mRNA transcription, in light of the finding that the 3′-end *cis*-acting signal for MHV RNA replication (9, 19) is longer than that for minus-strand RNA synthesis (20). By using a DI RNA construct containing a series of 3′-end deletions, we found that subgenomic mRNA transcription also required a 3′-end sequence that is longer than that for minus-strand RNA synthesis. These data suggest that the 3′-end sequence is involved in plus-strand RNA synthesis; thus, there is likely a direct or indirect interaction among 5′- and 3′-end sequences and the IG site of the viral RNA during subgenomic mRNA transcription.

For this study, we used a DI RNA vector containing a chloramphenicol acetyltransferase (CAT) gene behind an inserted IG 7 sequence (the intergenic sequence between genes 6 and 7) (18); the CAT activity could be expressed only when a subgenomic CAT mRNA was transcribed from the IG site in the presence of a helper virus (18). We used DIssF cDNA (24), instead of DIssE cDNA (18), to make DI vector RNA to take advantage of its longer 3′-end and convenient restriction enzyme sites for plasmid construction. The resulting clone, DF-CAT, was capable of RNA replication in the cells infected with MHV-A59 strain (data not shown). As shown previously (18), the CAT activity from this RNA system reflects subgenomic mRNA transcription and was detected only when this RNA was transfected into helper virus-infected cells but not mock-infected cells (data not shown).

Most of the 3′-UTR of the MHV genomic RNA is required for mRNA transcription from the DI RNA vector. A series of 3′-end deletion mutants derived from DF-CAT were constructed. These DI RNAs were transfected into A59-infected cells, and the cytoplasmic extracts were prepared at 7 to 8 h postinfection and assayed for their CAT activities. As shown in Fig. 1, DI RNA clones, DF2-4CAT, DF2-2CAT, DF3-4CAT, and DF-350CAT produced very high CAT activities (>100-fold) comparable to that of the wild-type DI RNA, while DF-305CAT had an approximately 15-fold increase over the background. In contrast, DF-270CAT, DF-240CAT, and DF6-1CAT yielded only background levels of CAT activities. Since all of the DI clones that contained a 3′-end sequence of no less than 305 nt produced significant CAT activities and those with no more than 270 nt did not, we concluded that at least 305 nt from the 3′-end of the genomic RNA were required for subgenomic mRNA transcription. It is interesting to note that 305 nt represents the complete 3′-untranslated region (3′-UTR) of MHV RNA (30). There were some variations in CAT activities between different clones; for example, DF3-4CAT had a slightly lower CAT activity than the others. The significance of this variation is not clear. However, DF-305CAT had a significantly lower CAT activity (only 15× background), suggesting that the nt 305 to 350 region from the 3′-end could enhance mRNA transcription.

In order to verify that the CAT activities detected reflect the synthesis of CAT-containing subgenomic mRNA, we used reverse transcription (RT)-PCR to detect the subgenomic CAT mRNA species. An oligonucleotide (3′CAT106) complementary to nt 87 to 106 of the CAT open reading frame was used to reverse transcribe the virus-specific RNA derived from A59-infected and DI-RNA-transfected cells. The cDNA was then amplified by PCR by using an additional primer, 5′L9, which corresponds to the 5′-end of the leader sequence (38). Since both the input DI RNA and the synthesized subgenomic mRNA contained CAT and leader sequence, both RNA species would be amplified by this RT-PCR. Consistent with the CAT activities described above, a PCR fragment expected from the subgenomic CAT mRNA was detected from cells transfected with DF-CAT, DF-350CAT, and DF-305CAT RNAs (Fig. 2A, lanes 2 to 4) but not from DF-270CAT, DF-240CAT, and DF6-1CAT RNAs (lanes 5 to 7). The PCR products corresponding to the full-length DI RNA were detected in all of the cells transfected with the DI RNAs. These results confirmed that the CAT activities detected in cells transfected with DF-305CAT and larger clones were derived from the subgenomic CAT mRNA. In contrast, the absence of CAT activity in cells transfected with DF-270CAT and smaller clones reflects their inability to synthesize a subgenomic CAT mRNA.

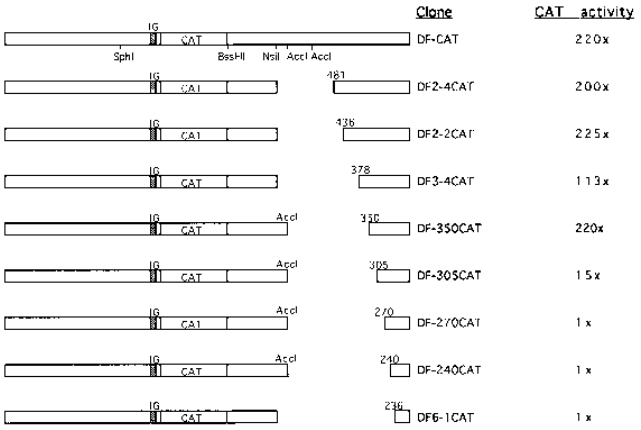

FIG. 1. Schematic diagram of the structure and CAT activities of DF-CAT and the 3′-end deletion clones. The restriction enzyme sites of DF-CAT used for plasmid construction are indicated. DF-CAT was derived from DF1-1 cDNA (19) by inserting the IG 7 (the IG sequence between genes 6 and 7) of MHV-A59 strain and the CAT open reading frame sequence into the *Spe*I site (19). DF2-4CAT, DF2-2CAT, DF3-4CAT, and DF6-1CAT were derived by inserting the *Sph*I-*Bss*HII fragment of DF-CAT into the corresponding sites of the previously constructed 3′-end deletion clones of DF1-1 (19). For other deletion clones, PCR fragments were generated by using a primer corresponding to the different nucleotides in the 3′-UTR region and another primer corresponding to the vector sequence immediately downstream of the DF1-1 viral sequence. The PCR fragments were inserted into the *Acc*I and *Xba*I (in the vector sequences) sites of DF-CAT, resulting in DF-350CAT, DF-305CAT, DF-270CAT, and DF-240CAT. These clones retain the *Acc*I site. Numbers of nucleotides left at the 3′-end of each clone are indicated on top of the junction site. All the constructs were confirmed by DNA sequencing. DI RNAs were transfected into A59-infected DBT cells, cell extracts were harvested at 8 h postinfection, and CAT activity was determined as previously described (19). CAT activity is summarized to the right of each clone; each value represents the average activity of more than five independent experiments. The CAT activity derived from A59-infected and mock-transfected cells was arbitrarily set as one fold (x).

All the 3′-UTR truncation mutants synthesized minus-strand RNAs. All of the DI RNA clones examined in Fig. 1 contain the 55 nt from the 3′-end plus poly(A) tail which are required for minus-strand RNA synthesis (20). Therefore, all the clones should be able to synthesize minus-strand RNA. However, the 305 nt in the 3′-end might contain sequences which modulate minus-strand RNA synthesis. To rule out this possibility, we performed a two-cycle RNase protection assay (20) to detect the minus-strand RNA in these cells by using a CAT-specific probe. As shown in Fig. 2B, all of the DI RNAs that did not yield a CAT activity, namely, DF-270CAT, DF-240CAT, and DF6-1CAT, synthesized minus-strand RNA, which was even more abundant than that of DF-350CAT, which yielded a high CAT activity. This result is consistent with the previous finding that mRNA transcription inhibits minus-strand RNA synthesis in *cis* (20). As a negative control, DE3′-0CAT, which does not have the 3′-UTR sequence at all (20),

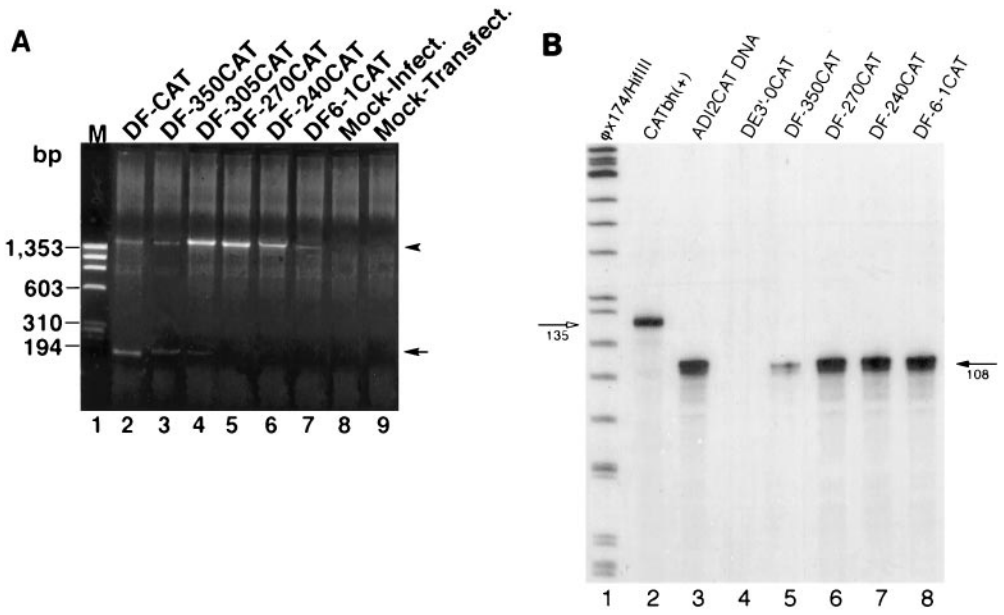

FIG. 2. Detection of RNA species made by the 3′-end deletion clones. (A) Positive-strand RNA species. RNA from the transfected cells was amplified by RT-PCR using a CAT-specific antisense primer, 3′CAT106, and a leader-specific sense primer, 5′L9 (38), and the PCR products were analyzed by agarose gel electrophoresis. Lanes 2 to 7, RT-PCR products of RNA samples from cells infected with MHV-A59 and transfected with various DI RNA as indicated at the top of each lane; lane 8, RNA from mock-infected and DF-CAT-transfected cells; lane 9, RNA from A59-infected and mock-transfected cells. The arrowhead and arrow indicate genomic and subgenomic RNAs, respectively. Lane 1, molecular size markers. (B) Minus-strand RNA species. RNA from the transfected cells was analyzed for the presence of minus-strand RNA by the two-cycle RNase protection assay (20), by using a ^{32}P-labeled CATbh(+) probe, which is complementary to the minus-strand CAT gene (20). The protected fragments were analyzed by agarose gel electrophoresis and detected by autoradiography. Lane 2, undigested CATbh(+) probe; lane 3, ADI2-CAT (19) DNA used for hybridization to ^{32}P-labeled CATbh(+) in RNase protection assay (20); lanes 4 to 8, cytoplasmic RNA from cells transfected with various RNAs. The protected fragment is indicated by a solid arrow, and the probe is indicated by an open arrow. Lane 1, *Hif*III-digested φx174 replicative form DNA fragments as size markers. The sizes of the probe and the protected bands are given in number of nucleotides.

did not synthesize any minus-strand RNA, in agreement with the previous finding (20). Therefore, we conclude that the failure of the 3′-end deletion clones to transcribe mRNAs is not due to their inability to synthesize minus-strand RNA. Thus, the 305 nt at the 3′-end of the genomic RNA must be required for mRNA transcription during plus-strand RNA synthesis.

Substitution of BCV for MHV sequence at nt 270 to 305 from the 3′-end abolished mRNA transcription. The results shown above suggested that the sequence at nt 270 to 305 from the 3′-end of MHV RNA contained at least part of the *cis*-acting signal for MHV subgenomic mRNA transcription. To determine the sequence specificity of this requirement, we substituted a bovine coronavirus (BCV) sequence which differs by 4 nt for the MHV sequence within this region. The resulting clone, 305BCV4CAT, did not express significant CAT activities. In contrast, the parental clone, DF-305CAT, expressed a CAT activity that is approximately 15-fold higher than the background (Fig. 3). Since these two sequences vary only by 4 nt, this result suggests that the sequence requirement in nt 270 to 305 from the 3′-end is very rigid.

Internal deletions within the 3′-UTR also reduced mRNA transcription. We further examined whether some other sequence within the 3′-UTR was also important for subgenomic mRNA transcription. For this purpose, the nt 122 to 270 region from the 3′-end (*Bst*EII-*Ppu*MI fragment of the cDNA) was deleted from DF-CAT. The resulting clone, DF-CATΔPK, expressed a relatively low level of CAT activity (fivefold over the background) (Fig. 3), whereas the parental clone, DF-CAT, expressed CAT activity of more than 200-fold over the background, suggesting that the nt 122 to 270 region is also required for efficient subgenomic mRNA transcription. Interestingly, this region includes a putative pseudoknot structure (from nt 182 to 240 from the 3′-end), which is important for coronavirus RNA replication (35). Whether the pseudoknot structure per se is required for mRNA transcription was not further studied.

These data combined suggested that the presence of most of the 3′-UTR sequence is required for subgenomic mRNA transcription. The partial deletion of the 3′-UTR inhibited subgenomic mRNA transcription but did not affect the ability of

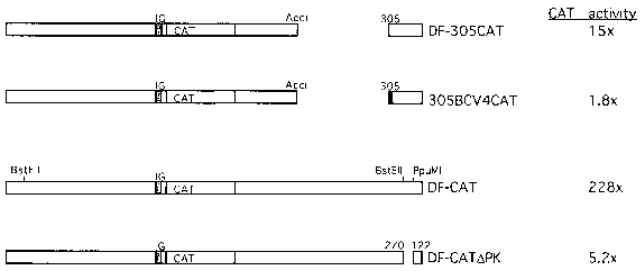

FIG. 3. Structure and CAT gene expression of DF-305CAT, 305BCV4CAT, DF-CAT, and DF-CATΔPK. The 305BCV4CAT plasmid was constructed by replacing the *Acc*I-*Xba*I fragment of DF-305CAT with a PCR fragment generated by using a primer containing the BCV-specific sequence in the nt 270 to 305 region from the 3′-end and another primer corresponding to the vector sequence (at the *Xba*I site) immediately following the viral sequences (19). DF-CATΔPK was generated by digesting DF-CAT with *Bst*EII (2 sites) and *Ppu*MI (1 site) and blunt-ended with T4 DNA polymerase. The two large fragments were isolated and ligated. The CAT activity is summarized to the right of each clone; each value represents the average activity of two to three experiments. The solid black box in 305BCV4CAT denotes BCV-specific sequences. The nucleotide numbers from the 3′-end are indicated.

these RNAs to synthesize minus-strand RNA (Fig. 2B), consistent with the previous finding that the initiation of minus-strand RNA synthesis requires only the 3′-terminal 55 nt and poly(A) (20). Thus, the remaining sequence of 3′-UTR most likely participates in regulating plus-strand mRNA synthesis. This result and previous findings (9, 19) combined show that the 3′-end sequence of genomic RNA may participate in the regulation of both RNA replication and transcription during positive-strand RNA synthesis. It is interesting that the 3′-end length requirements for replication and transcription are different (436 nt for RNA replication and 305 nt for mRNA transcription), suggesting that these two processes are separable.

Conceptually, the conclusion reached in this study seems unexpected, because positive-strand RNA synthesis must start from the 5′-end of the genome (or 3′-end of the negative-strand template); thus, it should be regulated by the 5′- and not the 3′-end sequence of genomic RNA. These studies therefore suggest a potential interaction between the 5′- and 3′-ends of RNA in regulating RNA replication and transcription. Such an interaction has been implicated in the regulation of RNA synthesis of an increasing number of RNA viruses. For example, the presence of the 3′-end sequence of turnip crinkle virus genomic RNA enhanced the viral positive-strand RNA synthesis in vitro (31). An RNA pseudoknot structure in the 3′-UTR of brome mosaic virus RNA also stimulated viral RNA replication (11). Furthermore, tobacco mosaic virus RNA has a pseudoknot structure in the 3′-UTR (33), which may interact with the 5′ leader sequence via protein factors, enhancing the efficiency of RNA replication (16). Several other viral RNAs, such as vesicular stomatitis virus DI RNAs (34), alphavirus genomic RNA (26), and influenza virus genomic RNAs (6), have complementary sequences between the 5′- and 3′-ends. The complementarity between the two ends has been shown to regulate the RNA synthesis of vesicular stomatitis virus and influenza virus (21, 34). Infectious bronchitis virus, an avian coronavirus, also has terminal complementary sequences (1), which potentially enable the genomic RNA to fold into a panhandle structure. However, MHV genomic terminal sequences do not have obvious complementarity (unpublished observation); therefore, any potential interaction between the 5′- and 3′-ends of MHV RNA would probably be mediated by protein-RNA and protein-protein interactions.

The results in this and other studies thus suggest that the positive-strand RNA synthesis of MHV is regulated by multiple RNA components, including an IG sequence, a *trans*-acting leader sequence, and the *cis*-acting sequences at both the 5′- and 3′-ends of genomic RNA (18, 38). These elements may interact with each other via protein-RNA and protein-protein interactions. Several cellular proteins have been shown to bind to the 5′- and 3′-ends and IG sites of MHV RNA (3, 36, 37). It remains to be seen if any of these proteins are involved in the putative interactions of various RNA regions.

A recent study of another coronavirus, porcine transmissible gastroenteritis virus, showed that when a negative-strand RNA containing only the IG and no other *cis*-acting sequence was transcribed from a DNA template by T7 RNA polymerase in situ (via a recombinant vaccinia virus), it could express CAT activity in cells infected with the transmissible gastroenteritis virus (5). This finding appears to contradict the requirement of *cis*-acting sequences, other than the IG sequence, for mRNA transcription. However, the RNA structural requirements for transcription as determined by using a vaccinia virus-T7 RNA polymerase system and those determined by RNA transfection often differ significantly. For example, transfection of a negative-strand RNA construct similar to that described above (5) into virus-infected cells did not result in CAT expression (unpublished observation). Since in RNA transfection studies, negative-strand RNA was synthesized from the RNA template by the coronaviral polymerase, this approach probably reflects more closely than the vaccinia virus-T7 polymerase system the natural requirement of viral RNA synthesis. It is possible that the IG sequence alone is sufficient to confer a basal level of transcription, which could be detected in the vaccinia virus-T7 polymerase expression system; the presence of other *cis*-acting sequences conceivably enhanced the transcriptional efficiency.

The results of this study contribute to the list of the functional roles of 3′-UTRs of RNAs. The 3′-UTR has previously been shown to affect the translation of various mRNAs (2, 4, 10, 16, 25). In this study, we found that the 3′-UTR also had a significant effect on the regulation of mRNA transcription. Such a functional role for the 3′-UTR of RNA may be common to many RNA viruses.

We thank Ching-Len Liao for his helpful discussions and critical comments. We also thank Daphne Shimoda for editorial assistance.

This work was supported by grant AI-19244 from the National Institutes of Health. M. M. C. L. is an investigator of the Howard Hughes Medical Institute.

REFERENCES

1. **Boursnell, M. E. G., T. D. K. Brown, I. J. Foulds, P. F. Green, F. M. Tomley, and M. M. Binns.** 1987. The complete nucleotide sequence of avian infectious bronchitis virus: analysis of the polymerase-coding region, p. 15–29. *In* M. M. C. Lai and S. A. Stohlman (ed.), Coronaviruses, vol. 218. Plenum Press, New York.
2. **Danthinne, X., J. Seurinck, F. Meulewaeter, M. van Montagu, and M. Cornelissen.** 1993. The 3′ untranslated region of satellite tobacco necrosis virus RNA stimulates translation in vitro. Mol. Cell. Biol. **13:**3340–3349.
3. **Furuya, T., and M. M. C. Lai.** 1993. Three different cellular proteins bind to the complementary sites on the 5′-end positive- and 3′-end negative-strands of mouse hepatitis virus RNA. J. Virol. **67:**7215–7222.
4. **Goodwin, E. B., P. G. Okkema, T. C. Evans, and J. Kimble.** 1993. Translational regulation of tra-2 by its 3′ untranslated region controls sexual identity in *C. elegans*. Cell **75:**329–339.
5. **Hiscox, J. A., K. L. Mawditt, D. Cavanagh, and P. Britton.** 1995. Investigation of the control of coronavirus subgenomic mRNA transcription by using T7-generated negative-sense RNA transcripts. J. Virol. **69:**6219–6227.
6. **Hsu, M.-T., J. D. Parvin, S. Gupta, M. Krystal, and P. Palese.** 1987. Genomic RNAs of influenza viruses are held in a circular conformation in virions and in infected cells by a terminal panhandle. Proc. Natl. Acad. Sci. USA **84:** 8140–8144.
7. **Jeong, Y. S., and S. Makino.** 1992. Mechanism of coronavirus transcription: duration of primary transcription initiation activity and effects of subgenomic RNA transcription on RNA replication. J. Virol. **66:**3339–3346.
8. **Jeong, Y. S., and S. Makino.** 1994. Evidence for coronavirus discontinuous transcription. J. Virol. **68:**2615–2623.
9. **Kim, Y.-N., Y. S. Jeong, and S. Makino.** 1993. Analysis of cis-acting sequences essential for coronavirus defective interfering RNA replication. Virology **197:**53–63.
10. **Kwon, Y. K., and N. B. Hecht.** 1993. Binding of a phosphoprotein to the 3′ untranslated region of the mouse protamine 2 mRNA temporally represses its translation. Mol. Cell. Biol. **13:**6547–6557.
11. **Lahser, F. C., L. E. Marsh, and T. C. Hall.** 1993. Contributions of the brome mosaic virus RNA-3 3′-nontranslated region to replication and translation. J. Virol. **67:**3295–3303.
12. **Lai, M. M. C.** 1990. Coronavirus: organization, replication and expression of genome. Annu. Rev. Microbiol. **44:**303–333.
13. **Lai, M. M. C., C.-L. Liao, Y.-J. Lin, and X. Zhang.** 1994. Coronavirus: how a large RNA viral genome is replicated and transcribed. Infect. Agents Dis. **3:**98–105.
14. **Lai, M. M. C., C. D. Patton, R. S. Baric, and S. A. Stohlman.** 1983. The presence of leader sequences in the mRNA of mouse hepatitis virus. J. Virol. **46:**1027–1033.
15. **Lai, M. M. C., and S. A. Stohlman.** 1978. The RNA of mouse hepatitis virus. J. Virol. **26:**236–242.
16. **Leathers, V., R. Tanguay, M. Kobayashi, and D. R. Gallie.** 1993. A phylogenetically conserved sequence within viral 3′ untranslated RNA pseudoknots regulates translation. Mol. Cell. Biol. **13:**5331–5347.
17. **Lee, H.-J., C.-K. Shieh, A. E. Gorbalenya, E. V. Koonin, N. La Monica, J. Tuler, A. Bagdzyahdzhyan, and M. M.-C. Lai.** 1991. The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. Virology **180:**567–582.

18. **Liao, C.-L., and M. M. C. Lai.** 1994. Requirement of the 5′-end genomic sequence as an upstream cis-acting element for coronavirus subgenomic mRNA transcription. J. Virol. **68:**4727–4737.
19. **Lin, Y.-J., and M. M. C. Lai.** 1993. Deletion mapping of a mouse hepatitis virus defective-interfering RNA reveals the requirement of an internal and discontiguous sequence for replication. J. Virol. **67:**6110–6118.
20. **Lin, Y.-J., C. L. Liao, and M. M. C. Lai.** 1994. Identification of the *cis*-acting signal for minus-strand RNA synthesis of a murine coronavirus: implications for the role of minus-strand RNA in RNA replication and transcription. J. Virol. **68:**8131–8140.
21. **Luo, G., W. Luytjes, M. Enami, and P. Palese.** 1991. The polyadenylation signal of influenza virus RNA involves a stretch of uridines followed by the RNA duplex of the panhandle structure. J. Virol. **65:**2861–2867.
22. **Makino, S., M. Joo, and J. K. Makino.** 1991. A system for study of coronavirus mRNA synthesis: a regulated, expressed subgenomic defective-interfering RNA results from intergenic site insertion. J. Virol. **65:**6031–6041.
23. **Makino, S., S. A. Stohlman, and M. M. C. Lai.** 1986. Leader sequences of murine coronavirus mRNAs can be freely reassorted: evidence for the role of free leader RNA in transcription. Proc. Natl. Acad. Sci. USA **83:**4204–4208.
24. **Makino, S., K. Yokomori, and M. M. C. Lai.** 1990. Analysis of efficiently packaged defective interfering RNAs of murine coronavirus: localization of a possible RNA-packaging signal. J. Virol. **64:**6045–6053.
25. **Ostareck-Lederer, A., D. H. Ostareck, N. Standart, and B. J. Thiele.** 1994. Translation of 15-lipoxygenase mRNA is inhibited by a protein that binds to a repeated sequence in the 3′ untranslated region. EMBO J. **13:**1467–1481.
26. **Ou, J.-H., E. G. Strauss, and J. H. Strauss.** 1983. The 5′ terminal sequences of the genomic RNAs of several alphaviruses. J. Mol. Biol. **168:**1–15.
27. **Pachuk, C. J., P. J. Bredenbeek, P. W. Zoltick, W. J. M. Spaan, and S. R. Weiss.** 1989. Molecular cloning of the gene encoding the putative polymerase of mouse hepatitis coronavirus strain A59. Virology **171:**141–148.
28. **Sawicki, S. G., and D. L. Sawicki.** 1990. Coronavirus transcription: subgenomic mouse hepatitis virus replicative intermediates function in RNA synthesis. Proc. Natl. Acad. Sci. USA **64:**1050–1056.
29. **Sethna, P. B., S. L. Hung, and D. A. Brian.** 1989. Coronavirus subgenomic minus-strand RNAs and the potential for mRNA replicons. Proc. Natl. Acad. Sci. USA **86:**5626–5630.
30. **Skinner, M. A., and S. G. Siddell.** 1983. Coronavirus JHM: nucleotide sequence of the mRNA that encodes nucleocapsid protein. Nucleic Acids Res. **11:**5045–5054.
31. **Song, C., and A. E. Simon.** 1994. RNA-dependent RNA polymerase from plants infected with turnip crinkle virus can transcribe (+)- and (−)-strands of virus-associated RNAs. Proc. Natl. Acad. Sci. USA **91:**8792–8796.
32. **Spaan, W. J. M., H. Delius, M. Skinner, J. Armstrong, P. Rottier, S. Smeekens, B. A. M. van der Zeijst, and S. G. Siddell.** 1983. Coronavirus mRNA synthesis involves fusion of non-contiguous sequences. EMBO J. **2:**1839–1844.
33. **van Belkum, A., J. P. Abrahams, C. W. A. Pleij, and L. Bosch.** 1985. Five pseudoknots are present at the 204-nucleotides long 3′ noncoding region of tobacco mosaic virus RNA. Nucleic Acids Res. **13:**7673–7686.
34. **Wertz, G. W., S. Whelan, A. LeGrone, and L. A. Ball.** 1994. Extent of terminal complementarity modulates the balance between transcription and replication of vesicular stomatitis virus RNA. Proc. Natl. Acad. Sci. USA **91:**8587–8591.
35. **Williams, G. D., R.-Y. Chang, and D. A. Brian.** 1995. Evidence for a pseudoknot in the 3′-untranslated region of the bovine coronavirus genome, p. 511–514. *In* P. J. Talbot and G. A. Levy (ed.), Corona and related viruses, vol. 380. Plenum Press, New York.
36. **Yu, X., W. Bi, S. R. Weiss, and J. L. Leibowitz.** 1994. Mouse hepatitis virus gene 5b protein is a new virion envelope protein. Virology **202:**1018–1023.
37. **Zhang, X., and M. M. C. Lai.** 1995. Interactions between the cytoplasmic proteins and the intergenic (promoter) sequence of mouse hepatitis virus RNA: correlation with the amounts of subgenomic mRNA transcribed. J. Virol. **69:**1637–1644.
38. **Zhang, X., C.-L. Liao, and M. M. C. Lai.** 1994. Coronavirus leader RNA regulates and initiates subgenomic mRNA transcription, both in *trans* and in *cis*. J. Virol. **68:**4738–4746.