IN VIVO AND IN VITRO TRANSCRIPTION OF SMALL mRNAS CONTAINING A LEADER SEQUENCE FROM MOUSE HEPATITIS VIRUS STRAIN JHM

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Mouse hepatitis virus (MHV) genomes are divided into at least seven coding regions. Recently, it has been shown that one or two additional small mRNAs (mRNA8 and 9) are synthesized in DBT cells infected with MHV strains A59, -1 and -S[1]. It is suggested that the transcription may occur via a leader-priming mechanism whereby a trans-acting leader RNA binds to highly conserved intergenic sequences, UC(U/C)AAAC, on the full-length negative-stranded template to prime transcription of subgenomic mRNAs[2,3]. mRNA8 is initiated from a perfectly conserved intergenic sequence, UCCAAAC, at 828 nt of the nucleocapsid (N) protein gene of MHV-A59. mRNA9 is initiated from a nearly perfect sequence, UCUAAAU, at 982 nt. However, whether mRNA8 and 9 are synthesized in MHV-infected mice and whether the products from mRNA8 and 9 play a biological role in infected cells remain unknown. In this experiment we studied the course of synthesis of these two small mRNAs in DBT cells and in mouse organs.

Total RNA was extracted from DBT cells infected with MHV-JHM at a multiplication of infection (m. o. i.) of 2.0 as described previously[4] and cDNA was synthesized using reverse transcriptase (RT) and primer-R (5′ TGCCGACATAGGATTCATTCTCT 3′) corresponding to 1368-1390 nt of the N protein gene of JHM. The synthesized cDNA was amplified by polymerase chain reaction (PCR) using primer-F1 (5′ TATAAGAGTGATTGGCGTCCG 3′) and -F2 (5′CTCTAAAACTCTTGTAGTTT 3′) corresponding to 1-21 nt and 37-56 nt in the leader RNA, respectively, as a forward pimer. Primer-R was used as a reverse primer. The amplified products were analyzed by Southern blot hybridization using cDNA of mRNA7[5], which contains sequences of mRNA 8 and 9, as a probe as described previously[4].

Cellular RNA was extracted from DBT cells infected with JHM and A59 strains of MHV at 37°C at 6 h p.i. The amplified products derived from mRNA7 and 2 small mRNAs were observed in both strains using primer-F1 and -R (Fig. 1a). Sequence data of the PCR

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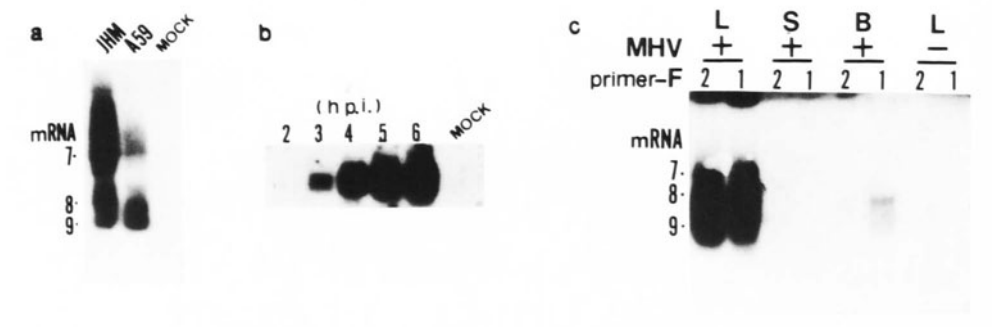

Figure 1. Small mRNAs in DBT cells and the mouse tissues infected with JHM-MHV. The cellular RNA was extracted from DBT cells infected with MHV-JHM and -A59 at 6 h p. i. (a), and from cells infected with MHV-JHM at 2, 3, 4, 5 and 6 h p. i. (b). RNA was extracted from the liver (L), spleen (S) and brain (B) of the infected mouse at 5 days p. i. and amplified by RT-PCR using primer-F1 or -F2 and -R (c). + and - represent the infected and uninfected mouse, respectively. The RT-PCR products were analyzed by Southern blot hybridization using cDNA of mRNA7 as a probe.

products showed that these small mRNAs corresponded to the mRNA8 and 9 reported by Schaad & Baric[1] (data not shown). These small mRNAs from JHM-MHV were detected at 3 h p. i. by analysis using RT-PCR (Fig. 1b). Since ORF1a gene products of MHV that contain RNA-dependent RNA polymerases are translated from genomic RNA at 1.5-3 h p. i.[6], mRNA8 and 9 were synthesized in the DBT cells infected with JHM-MHV at the early stage of the infection. This result suggests that the products from mRNA8 and 9 of MHV may play a role in the early stage of the viral replication cycle.

When a C57BL/6 mouse was inoculated i.p. with $3X10^5$ PFU MHV-JHM. The mRNA8 and 9 were observed in the liver and brain of the infected mouse at 5 days p.i. by RT-PCR amplifications of the RNAs (Fig. 1c). This result showed that mRNA8 and 9 were synthesized in the liver and brain, which are target tissues of MHV-JHM. Therefore, the products of mRNA8 and 9 may play a role in MHV infection in vivo. The mRNA8 and 9 synthesized in vivo contained the leader sequence (data not shown). Although mRNA8 is synthesized from a perfectly conserved sequence, mRNA9 is from an imperfect sequence[1]. In the infected cells, it is suggested that mRNAs of MHV are synthesized by an imprecise trans-acting leader-primed transcription mechnism[2,7,8]. This study suggested that the imperfect intergenic sequence also can serve as an initiation site for the leader-primed mRNA synthesis of MHV in vivo.

REFERENCES

1. Schaad, M. C., Baric, R. S. Evidence for new transcriptional units encoded at the 3′-end of the mouse hepatitis virus genome. Virol. 1993; 196: 190-198.
2. Baric, R. S., Stohlman, S. A., Lai, M. M. C. Characterization of replicative intermediate RNA of mouse hepatitis virus: Presence of leader RNA sequences on nascent chains. J. Virol. 1983; 48: 633-604.
3. Makino, S., Joo, M., Makino, J. K. A system for study of coronavirus mRNA synthesis: a regulated, expressed subgenomic defective interfering RNA results from intergenic insertions. J. Virol. 1991; 65: 6031-6041.
4. Mizutani, T., Hayashi, M., Maeda, A., Yamashita, T., Isogai, H., Namioka S. Inhibition of mouse hepatitis virus multiplication by antisense oligonucleotide complementary to the leader RNA. J. Vet. Med. Sci. 1992; 54: 465-472.
5. Skinner, M.A., Siddell, S.G. Coronavirus JHM; nucleotide sequence of the mRNA that encodes nucleocapsid protein. Nucleic Acids Res. 1983; 11: 5045-5054.

6 Denınson, M R , Zoltıck, P W , Hughes, S A , Gıangreco, B , Olson, A L , Perlman, S , Leıbowıtz, J L , Weıss, S R Intracellular processıng of the N-terminal ORF1a proteins of the coronavırus MHV-A59 requıres multıple proteolytıc events Vırol 1992, 189 274-284
7 Barıc, R S , Stohlman, S A Razavı, M K , Laı, M M C Characterızatıon of leader-related small RNAs ın coronavırus ınfected cells Further evıdence for leader-prımed mechanısm of transcrıptıon Vırus Res 1985, 3 19-33
8 Makıno, S , Laı, M M C Evolutıon of the 5′ end of genomıc RNA of murıne coronavıruses durıng passages ın vıtro Vırol 1989, 169 227-232