Both Antisense and Sense RNAs against the Nucleocapsid Protein Gene Inhibit the Multiplication of Mouse Hepatitis Virus

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ABSTRACT. DBT cells and several transfected cell lines which express antisense or sense RNA against the nucleocapsid protein gene of mouse hepatitis virus (MHV) were examined for the yields of MHV. The transfected cells showed 95 and 99% reduction of virus yield at 9 and 12 hr postinfection (p.i.) as compared with untransfected DBT cells. A remarkable decrease in MHV-specific RNA synthesis was observed in both transfected cell lines at 3.5 hr p.i. The result suggested that both antisense and sense RNAs inhibited viral replication at the initial stage of infection.—KEY WORDS: antisense RNA, mouse hepatitis virus, nucleocapsid protein, sense RNA.

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Mouse hepatitis virus (MHV) is a member of the Coronaviridae family, which causes a variety of diseases, including hepatitis and encephalomyelitis in laboratory mice [12, 33]. MHV is an enveloped virus containing a helical nucleocapsid structure composed of a single-stranded, positive-polarity RNA of approximately 31 kilobases (kb) in length [25]. In the replication cycle of MHV, RNA-dependent RNA polymerase translated from the 5'-portion of MHV genomic RNA [3, 17] transcribes the virion RNA into a full length negative-stranded RNA and subgenomic negative-stranded RNA [15, 26]. Genomic RNA (mRNA1) and six species of subgenomic mRNAs (mRNA2~7) form a 3'-coterminal nested set extending for different lengths in the 5'-direction [18], ending with an identical leader sequence of approximately 70 nucleotides [1, 16]. The free leader RNA species initially synthesized dissociates from negative-stranded RNA and then rebinds to the negative-stranded RNA at the initiation sites of six subgenomic mRNAs, taking part in leader-primed transcription [1].

An antisense RNA has been reported to inhibit the expression of the target gene [6], resulting from the hybrid formation. Inhibition of viral replication and multiplication by antisense RNA has been demonstrated [10, 14], while the effects of antisense RNA on the multiplication of positive-stranded RNA viruses in infected cells have not been reported. In our previous paper, an antisense oligonucleotide against leader RNA was shown to inhibit MHV-multiplication *in vitro* [22]. The inhibition percentage of viral multiplication by antisense oligonucleotide was 75% at a concentration of 10 μM at 6 hr postinfection (p.i.). It has been shown that a specific interaction occurs between the nucleocapsid (N) protein which is encoded by mRNA7 and the sequence of the leader RNA in the transcriptional complex [31]. The N protein also plays an important role in viral replication [2, 7]. Since neutralizing monoclonal antibodies to the N protein protect mice against a lethal challenge of MHV, the N protein might be important in the pathogenesis of MHV [24]. In the present study, we examined the effect of sense and antisense RNAs against the N protein on MHV multiplication.

MATERIALS AND METHODS

Chemicals: DNA polymerase I Klenow fragment and G418 were purchased from Nippon Gene Co. and Gibco Laboratories, respectively. SP6 RNA polymerase was from Takara Shuzo Co. RNase inhibitor and T7 RNA polymerase were from Boehringer Mannheim GmbH. ^{32}P-dCTP and ^{32}P-UTP (110 TBq/mmol) were from ICN Radiochemical.

Plasmids: Plasmids which expressed antisense and sense RNA were constructed as shown in Fig. 1. A cDNA of MHV-mRNA7 which was kindly provided by Dr. Siddell [30] was inserted into the *Pst* I site (pGEM-MHV) of

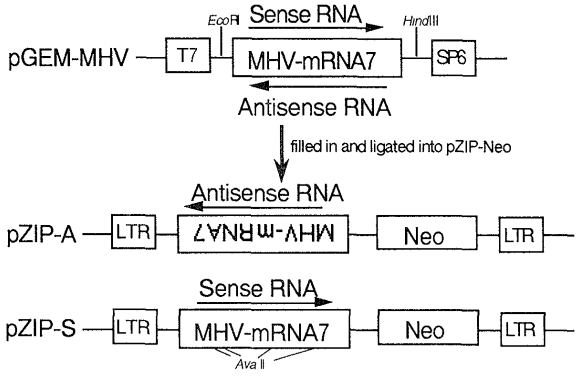

Fig. 1. Construction of recombinant plasmid pZIP-A and -S. The cDNA of MHV-mRNA7 was inserted into plasmid pGEM-1 at the *Pst*I site (pGEM-MHV). The 1.8 kbp cDNA fragment of mRNA7 was inserted into the *Bam*HI site of pZIP-Neo in both sense and antisense orientations, which were designated pZIP-S and pZIP-A, respectively, as described in Materials and Methods. T7 and SP6: bacteriophage T7 and SP6 RNA polymerase promoter; LTR: Moloney murine leukemia virus-LTR; neo: neomycine resistance gene.

plasmid pGEM-1. After digestion of the pGEM-MHV with *Hind*III and *Eco*RI, a 1.8 kbp cDNA fragment of MHV-mRNA7 was isolated and the sticky ends of this fragment were filled in with the Klenow fragment of DNA polymerase I. Plasmid pZIP-Neo SV(X) 1 (pZIP-Neo) [5] was cleaved with *Bam*HI and filled in. The cDNA fragment of MHV-mRNA7 was inserted into the pZIP-Neo vector. The clones which expressed the mRNA7 in sense and antisense orientations, designated pZIP-S and pZIP-A, respectively, were selected based on their *Ava*II digestion patterns.

Virus, Cell and Transfection: The JHM strain of MHV [19] was used in the present study. DBT cells [13] grown in Eagle's minimum essential medium (MEM) supplemented with 5% calf serum (CS) at 37°C in a humidified 5% CO_2 atmosphere, were transfected with the constructed plasmids using the standard calcium phosphate precipitation procedure [9]. Briefly, 5×10^5 cells in 6-cm dishes were treated with 10 μg of pZIP-A or -S DNA, and they were replated on the following day at a density of 1×10^5 cells per dish in selection medium containing 1 mg G418/m*l*. G418-resistant clones were established two months later.

Viral infection and Plaque assay: The parent and transfected DBT cells were infected with MHV-JHM at a multiplicity of infection (m.o.i.) of 0.1 for 1 hr at 37°C. After washing twice with CS-free MEM and adding MEM with CS, the culture supernatants were titrated for infectious progeny by plaque assay at each p.i. time.

Northern blot analysis: Cellular RNA samples were prepared from the parent or transfected cells infected with MHV according to the method of Silver *et al.* [29], and electrophoresed in 1.0 or 1.2% agarose gels containing formaldehyde, and blotted onto nitrocellulose membranes [20]. A probe cDNA of MHV-mRNA7 was ^{32}P-labeled by nick-translation, hybridization, washing and autoradiography were performed as described previously [22]. Using a template (Fig. 1), sense and antisense RNA probes were synthesized by reaction for 1 hr at 37°C in a mixture containing 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 10 mM ATP, CTP and GTP, 1.85 MBq of ^{32}P-UTP, 1 μg of linearlized pGEM-MHV, 1.5 U/μl RNasin and 30 U of T7 polymerase for sense RNA probe or SP6 polymerase for antisense RNA probe. Then, after adding 80 μl of 1% SDS to the mixture, synthesized RNA was extracted with phenol:chloroform (1:1), purified from unincorporated NTPs by Sephadex G50 chromatography and subjected to Northern blot hybridization analysis as described previously [22].

RESULTS

Antisense and sense RNA expression vectors: Two recombinant plasmids were constructed to see for antisense and sense RNA to inhibit the multiplication of MHV. A cDNA of MHV-mRNA7 (1.8 kb) was inserted into the pZIP-Neo vector in the sense or antisense orientation, referred to as pZIP-S and pZIP-A, respectively (Fig. 1). The pZIP-A or pZIP-S DNA was transfected into DBT cells and several transfected cell lines expressing antisense or sense RNA were selected by G418. No difference in cellular viability and growth rate was observed between the transfected and parent cells.

Northern blot analysis of RNA extracts from the four transfected cell lines using a probe cDNA of mRNA7, showed that RNAs 5 to 7 kb in size containing the N protein gene sequence were synthesized (Fig. 2). The RNA also hybridized with Neo and MLV-LTR sequences (data not shown). The N protein of MHV was undetectable in the transfected cell lines expressing the sense RNA by immunoprecipitation analysis using monoclonal and polyclonal antibodies.

Inhibition of the MHV multiplication by antisense and sense RNAs: After infection of the pZIP-Neo introduced DBT cells with MHV-JHM at 0.1 m.o.i., no inhibition of MHV multiplication was observed (data not shown), indicating that the pZIP-Neo vector sequence did not affect the MHV multiplication. Also the presence of G418 in the medium at a concentration of 1 mg/m*l* did not affect the multiplication of MHV.

The effects of antisense and sense RNAs against mRNA7 on the viral multiplication were investigated in two transfected cell lines which expressed either antisense

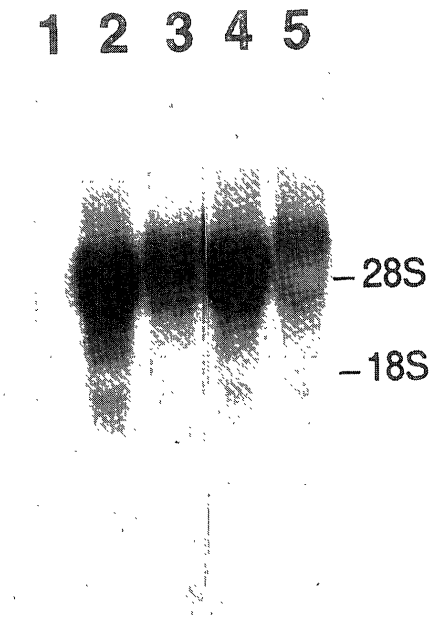

Fig. 2. Northern blot analysis of transfected cells. The cellular RNA samples (10 μg) prepared from the parent DBT cells and the transfected cells were analyzed on a 1.2% denatured agarose gel and hybridized with cDNA of MHV-mRNA7 as a probe. lane 1: parent DBT cells; lane 2: S1 cells; lane 3: S2 cells; lane 4: A1 cells; lane 5: A2 cells.

Table 1. Inhibition of MHV multiplication by antisense and sense RNAs

Cell line	9 hr p.i. PFU/ml[a)]	9 hr p.i. Inhibition (%)[b)]	12 hr p.i. PFU/ml	12 hr p.i. Inhibition (%)
parent DBT	2.47×10^5		1.47×10^6	
A1	8.10×10^3	96.7	9.52×10^4	93.5
A2	4.28×10^4	82.7	7.20×10^5	51.0
S1	1.13×10^4	95.4	1.90×10^4	98.7
S2	3.35×10^5	—	1.60×10^6	—

a) Cells were infected with MHV-JHM at 0.1 m.o.i.

b) Percentage of inhibition = $[1-(\frac{\text{PFU obtained from transfected cells}}{\text{PFU obtained from parent DBT cells}})] \times 100$ (%)

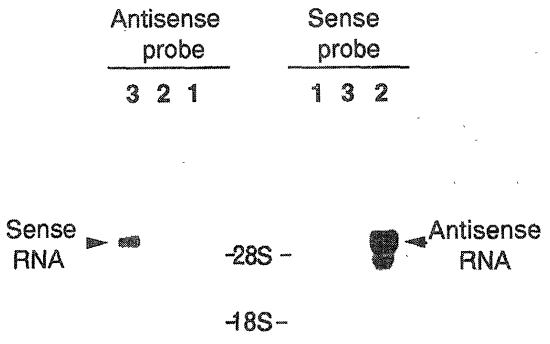

Fig. 3. Northern blot analysis using single-stranded RNA probes. The RNA samples (10 μg) prepared from the transfected A1 and S1 cells analyzed on a 1.0% denatured agarose gel. Sense and antisense RNA probes were synthesized from the pGEM-MHV using bacteriophage T7 and SP6 RNA polymerase, respectively (Fig. 1). lane 1: parent DBT cells; lane 2: A1 cells; lane 3: S1 cells.

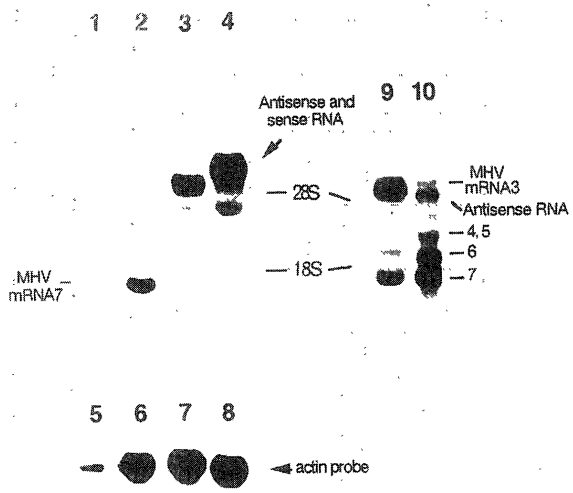

Fig. 4. The effect of antisense and sense RNA on viral mRNA synthesis. The A1, S1 and parent DBT cells were infected with MHV-JHM. At 3.5 and 7 hr p.i., the cellular RNAs were prepared and analyzed on 1% denatured agarose gel, and hybridized with cDNA of MHV-mRNA7 as a probe. lane 1: mock-infected parent DBT cells; lane 2: infected parent DBT cells; lanes 3 and 10: infected A1 cells at 3.5 and 7 hr.p.i.; lanes 4 and 9: infected S1 cells at 3.5 and 7 hr.p.i.; lanes 5 to 8 were rehybridized with β-actin DNA as a probe after dehybridization of the filter.

RNAs, A1 and A2, or sense RNAs, S1 and S2, at several intervals after infection with MHV at 0.1 m.o.i. for 1 hr at 37°C. As shown in Table 1, the yield of infectious particles from the both transfected cells expressing antisense or sense RNA was remarkably reduced as compared with that from the parent DBT cells. The A1 cells exhibited inhibition rates of 96.7 and 93.5% at 9 and 12 hr p.i., respectively, whereas the A2 cells 82.7% at 9 hr p.i. The inhibitory effect was rapidly reduced with incubation time showing 51.0% at 12 hr p.i. The S1 cells with sense RNA expression strongly inhibited the MHV multiplication at rates of 95 and 99% at 9 and 12 hr p.i., respectively. On the other hand, no inhibitory effect was observed in S2 cells, which expressed the sense RNA with lower copy numbers than S1 cells. The S1 and A1 cells were shown to express high levels of sense and antisense RNA, respectively (Fig. 3).

Viral transcription in the transfected cell lines: Syncytia formation first appeared in the infected cell monolayers at 6 to 7 hr p.i. and involving 80% cells at 12 hr p.i. To investigate the inhibitory effects of antisense and sense RNAs against MHV-mRNA7 on the MHV-specific mRNAs synthesis, RNAs from MHV-infected A1 and S1 cells at 3.5 and 7 hr p.i. were analyzed by Northern blot hybridization using a probe cDNA of MHV-mRNA7 (Fig. 4). Since genomic RNA and all subgenomic mRNAs contained a sequence complementary to mRNA7, all

mRNA species were hybridized with cDNA of mRNA7. At 7 hr p.i., MHV-mRNA3, 4, 5, 6 and 7 were detected in both infected A1 and S1 cells. In the parent DBT cells infected with MHV, only MHV-mRNA7, a most abundant mRNA species in infected cells, was detected at 3.5 hr p.i. The mRNA7 was not observed in either S1 or A1 cells.

DISCUSSION

In the present study, both antisense and sense RNAs against MHV-mRNA7 were shown to inhibit MHV multiplication. In MHV-infected cells, the positive-stranded virion RNA is initially transcribed into a negative-stranded RNA being of full-length and subgenomic, which is transcribed into subgenomic mRNA as a template. Both positive- and negative-stranded RNAs might be eligible for the targets of sense and antisense RNAs. The N protein coded by mRNA7 may play a regulatory role in the transcription and replication of MHV [2, 7, 31] and all negative- and positive-stranded MHV RNAs contain the related sequence of mRNA7. Thus mRNA7 might be an adequate target of antisense and sense RNAs inhibiting MHV multiplication. Skinner and Siddell [30] reported that the sequence of mRNA7 of the MHV-JHM is similar to that of strain A59, showing a 94% overall homology. Therefore, the transfected cell lines which express the antisense or sense RNA complementary to the N protein sequence of the MHV-JHM, may inhibit the multiplication of other MHV strains. After infection of A1 and S1 cells with MHV-A59 at 0.1 m.o.i., 96 and 91% inhibition at 9 hr p.i. were obtained.

In S2 cells expressing the sense RNA at a lower level than S1 cells, no inhibitory effect was observed. The A2 cells, which expressed a lower level of antisense RNA, showed lower inhibitory effect than that of A1 cells, and the inhibitory effect decreased with incubation time. The inhibitory effects might depend upon the level of sense or antisense RNA expression.

We previously showed that 10 μM of an antisense oligonucleotide against MHV-leader RNA reduced the viral multiplication at 6 hr p.i. as well as the synthesis of virus-specific mRNAs at 4.5 hr p.i. [22]. Also with higher concentration of sense oligonucleotide, some inhibitory effect was observed. Although the negative-stranded RNAs was reported to appear at 3 hr p.i. [8] and mRNA7 was produced at 3.5 hr p.i. in MHV-infected cells (Fig. 4, lane 2), no viral mRNA was detectable at 3.5 hr p.i. in the A1 and S1 cells. On the contrary, viral-specific mRNAs were produced at the same level at 7 hr p.i. in both A1 and S1 cells, while the inhibition of MHV growth was observed at 9 and 12 hr p.i., suggesting that the presence of antisense and sense RNAs in the early steps of viral replication might be important for inhibiting viral multiplication.

The mechanism by which the antisense and sense RNAs inhibited viral transcription and multiplication remains unclear. It has been proposed that the antisense RNAs

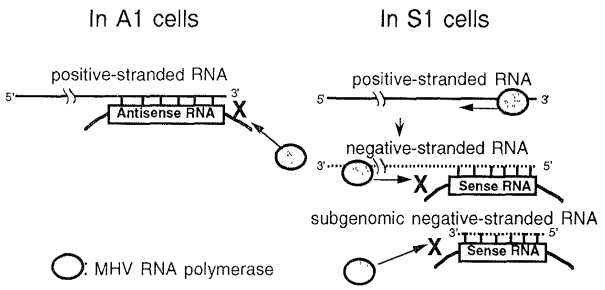

Fig. 5. Possible model for inhibition of MHV multiplication in the S1 and A1 cells. (A) and (B) indicate models of A1 cells and S1 cells, respectively.

can interfere with the translation of mRNA [11, 21], though the sense RNA cannot. The antisense RNA may also inhibit the transcription of the negative-stranded RNA from virion RNA. During the initial stages (3.5 hr p.i.) of infection in A1 cells, antisense RNA is thought to be hybridized with positive-stranded RNA (Fig. 5). On the other hand, the sense RNA expressed in S1 cells might be hybridized with negative-stranded RNAs being of full-length and/or subgenomic. The inhibition may be conformational in the activity of viral RNA polymerase at/before 3.5 hr p.i. Several hours after infection, when the MHV-RNAs dissociate from antisense or sense RNA, the viral transcription might restart.

In the transfected cells expressing the sense RNA, the N protein could not be detected in the present study. Neither antisense nor sense RNA was spliced out although pZIP-Neo contained the splicing signal (Fig. 3). The unspliced form of the sense RNA or RNA with the LTR and Neo gene sequence might not be translated into the N protein. Therefore, the inhibitory effect of sense RNA was not due to interference by the N protein synthesized from the sense RNA in transfected cells.

A sense RNA has been shown to block replication of a plant RNA virus genome by competitive inhibition of viral replicase [23]. It has been reported that the cells expressing the sense RNA of the tat mRNA leader sequence and the 5'-leader/gag region inhibit HIV-1 replication [14, 27, 32]. Since RNA-RNA and RNA-protein interactions are crucial for HIV-1 replication and transcription [4], the sense RNA could compete with HIV-1 mRNAs for binding of RNA and/or protein resulting in inhibition of HIV-1 multiplication. It has been reported that interaction between the N protein and MHV mRNA and that between the free leader RNA and the negative-stranded RNA, are crucial for MHV replication [2, 15, 31]. The sense RNA may act as a competitive inhibitor for the binding of N protein resulting in the inhibition of MHV multiplication.

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REFERENCES

1. Baric, R. S., Stohlman, S. A., and Lai, M. M. C. 1983. Characterization of replicative intermediate RNA of mouse hepatitis virus: presence of leader RNA sequences on nascent chains. *J. Virol.* 48: 633–640.
2. Baric, R. S., Nelson, G. W., Fleming, J. O., Deans, R. J., Keck, J. G., Casteel, N., and Stohlman, S. A. 1988. Interactions between coronavirus nucleocapsid protein and viral RNAs: implications for viral transcription. *J. Virol.* 62: 4280–4287.
3. Brayton, P. R., Lai, M. M. C., Patton, C. D., and Stohlman, S. A. 1982. Characterization of two RNA polymerase activities induced by mouse hepatitis virus. *J. Virol.* 42: 847–853.
4. Cann, A. J. and Karn, J. 1989. Molecular biology of HIV: new insights into the virus lifecycle. *AIDS* 3: S19–S34.
5. Cepko, C. L., Roberts, B. E., and Mulligan, R. C. 1984. Construction and applications of a highly transmissible murine retrovirus shuttle vector. *Cell* 37: 1053–1062.
6. Colman, A. 1990. Antisense strategies in cell and developmental biology. *J. Cell. Sci.* 97: 399–409.
7. Compton, S. R., Rogers, D. B., Holmes, K. V., Fertsch, D., Remenick, J., and McGowan, J. J. 1987. *In vitro* replication of mouse hepatitis virus strain A59. *J. Virol.* 61: 1814–1820.
8. Denison, M. R., Zoltick, P. W., Hughes, S. A., Giangreco, B., Olson, A. L., Perlman, S., Leibowitz, J. L., and Weiss, S. 1992. Intracellular processing of the N-terminal ORF 1a proteins of the coronavirus MHV-A59 requires multiple proteolytic events. *Virology* 189: 274–284.
9. Graham, F. W. and Van Der EB, A. J. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52: 456–467.
10. Han, L., Yun, J. S., and Wagner, T. E. 1991. Inhibition of Moloney murine leukemia virus-induced leukemia in transgenic mice expressing antisense RNA complementary to the retroviral packaging sequences. *Proc. Natl. Acad. Sci. U.S.A.* 88: 4313–4317.
11. Harland, R. and Weintraub, H. 1985. Translation of mRNA injected into *Xenopus* oocytes is specifically inhibited by antisense RNA. *J. Cell. Biol.* 101: 1094–1099.
12. Henderson, R. M., Griffin, D. E., McCormic, U., and Weiner, L. P. 1975. Mouse hepatitis virus-induced recurrent demyelination. *Arch. Neurol.* 32: 32–35.
13. Hirano, N., Fujiwara, K., Hino, S., and Matumoto, M. 1974. Replication and plaque formation of mouse hepatitis virus (MHV-2) in mouse cell line DBT culture. *Arch. Gesamte. Virusforsch.* 44: 298–302.
14. Joshi, S., Van Brunschot, A., Asad, S., Van Der Elst, I., Read, S. E., and Bernstein, A. 1991. Inhibition of human immunodecifiency virus type 1 multiplication by antisense and sense RNA expression. *J. Virol.* 65: 5524–5530.
15. Lai, M. M. C., Patton, C. D., and Stohlman, S. A. 1982. Replication of mouse hepatitis virus: negative-stranded RNA and replicative form RNA are of genome length. *J. Virol.* 44: 487–492.
16. Lai, M. M. C., Patton, C. D., Baric, R. S., and Stohlman, S. A. 1983. Presence of leader sequences in the mRNA of mouse hepatitis virus. *J. Virol.* 46: 1027–1033.
17. Lee, H.-J., Shieh, C.-K., Gorbalenya, A. E., Koonin, E. V., LaMonica, N., Tuler, J., Bagdzhadhzyan, A., and Lai, M. M. C. 1991. The complete sequence (22 kilobase) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. *Virology* 180: 567–582.
18. Leibowitz, J. L., Wilhelmsen, K. C., and Bond, C. W. 1981. The virus-specific intracellular RNA species of two murine coronaviruses: MHV-A59 and MHV-JHM. *Virology* 114: 39–51.
19. Makino, S., Taguchi, F., Hayami, M., and Fujiwara, K. 1983. Characterization of small plaque mutants of mouse hepatitis virus, JHM strain. *Microbiol. Immunol.* 27: 445–454.
20. Maniatis, T., Frisch, E. F., and Sambrook, J. 1982. Electrophoresis of RNA through gels containing formaldehyde. pp. 202–203. *In*: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, New York.
21. Melton, D. 1985. Injected antisense RNAs specifically block messenger translation *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 82: 144–148.
22. Mizutani, T., Hayashi, M., Maeda, A., Yamashita, T., Isogai, H., and Namioka, S. 1992. Inhibition of mouse hepatitis virus multiplication by an oligonucleotide complementary to the leader RNA. *J. Vet. Med. Sci.* 54: 465–472.
23. Morch, M. D., Joshi, R. L., Denial, T. M., and Haenni, A. L. 1987. A new "sense" RNA approach to block viral RNA replication *in vitro*. *Nucleic Acids Res.* 15: 4123–4130.
24. Nakanaga, K., Yamanouchi, K., and Fujiwara, K. 1986. Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. *J. Virol.* 59: 168–171.
25. Pachuk, C. K., Bredenbeak, P. J., Zoltick, P. W., Spaan, W. J. M., and Weiss, S. R. 1989. Molecular cloning of the gene encoding the putative polymerase of mouse hepatitis coronavirus, strain A59. *Virology* 171: 141–148.
26. Sawicki, S. G. and Sawicki, D. L. 1990. Coronavirus transcription: subgenomic mouse hepatitis virus replicative intermediates function in RNA synthesis. *J. Virol.* 64: 1050–1056.
27. Sczakiel, G., Pawlita, M., and Kleinheinz, A. 1990. Specific inhibition of human immunodeficiency virus type 1 replication by RNA transcribed in sense and antisense orientation from the 5'-leader/gag region. *Biochem. Biophys. Res. Commun.* 169: 643–651.
28. Siddell, S. G., Wege, H., and ter Meulen, V. 1983. The biology of coronaviruses. *J. Gen. Virol.* 64: 761–776.
29. Silver, S., Smith, M., and Nonoyama, M. 1979. Transcription of the Marek's disease virus genome in virus-induced tumors. *J. Virol.* 30: 84–89.
30. Skinner, M. A. and Siddell, S. G. 1983. Coronavirus JHM: nucleotide sequence of the mRNA that encodes nucleocapsid protein. *Nucleic Acids Res.* 11: 5045–5054.
31. Stohlman, S. A., Baric, R. S., Nelson, G. N., Soe, L. H., Welter, L. M., and Deans, R. J. 1988. Specific interaction between coronavirus leader RNA and nucleocapsid protein. *J. Virol.* 62: 4288–4295.
32. Sullenger, B. A., Gallardo, H. F., Ungers, G. E., and Gilboa, E. 1990. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. *Cell* 63: 601–608.
33. Weiner, L. P. 1973. Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). *Arch. Neurol.* 28: 298–303.