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THE BIOLOGICAL FUNCTION OF SMALL mRNAs FROM MOUSE
HEPATITIS VIRUS STRAIN JHM IN VITRO AND IN VIVO

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Mouse hepatitis virus (MHV), a member of the Coronaviridae, causes variable diseases in laboratory mice, including encephalomyelitis, hepatitis and gastroenteritis. MHV is an enveloped virus containing a helical nucleocapsid structure composed of a plus-sensed, single-stranded RNA. Although the mechanism of MHV transcription is still under study, it is suggested that virion RNA is initially transcribed into a full-length negative-stranded RNA. In turn, genomic and subgenomic mRNAs (mRNAs1-7) are synthesized from the negative-stranded RNA. Recently, it has been reported that one or two additional small mRNAs (mRNA8 and 9) are synthesized in DBT cells infected with MHV strains A59, -1, and -S. However, the biological roles of the products from mRNA8 and 9 remain unknown.

The present experiment showed that two additional small mRNAs were observed at 3 hours post-infection in DBT cells infected with the JHM strain of MHV. Therefore, the products from mRNA8 and 9 may play a role in the early stage of the viral replication cycle in infected DBT cells. Since mRNA8 and 9 were found in the liver and brain of an infected mouse, it was suggested that the products from these mRNAs might play a role *in vivo* infection by MHV and in the immune response in mice.

Intramuscular injection (i.m.) of DNA expression vectors into mice has been demonstrated to result in the uptake of DNA by the muscle cells and expression of the protein encoded by the DNA. Endogenous expression of a virus-specific antigen generates cytotoxic T lymphocytes (CTLs). Therefore, i.m. of vector DNA expressing viral protein may generate viral-specific CTLs. In the present study, vectors expressing the nucleocapsid (N) protein of JHM-MHV, which is coded by mRNA7 of MHV, directed by Rous Sarcoma virus (RSV) LTR and human elongation factor 1 α (EF1 α) promoter, named pRSV-mRNA7 and pEF-mRNA7, respectively, were constructed. Intramuscular injection of the vector resulted in the generation of CTLs specific for the N protein of JHM-MHV. The injection of pRSV-mRNA7 resulted in high cytolytic activity (>10%), which is thought to be effective to protect mice from MHV infection. These results suggest that intramuscular injection of the expression vector coding the N protein of MHV generates CTLs. Thus, use of the expression vector as an immunogen offers effective immunity without the need for self-replication agents or adjuvants.