ANTIVIRAL EFFECT OF OLIGONUCLEOTIDE AND RNA COMPLEMENTARY TO MOUSE HEPATITIS VIRUS Nc PROTEIN mRNA

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Mouse hepatitis virus (MHV), a member of the Coronaviridae, is associated with respiratory illness, gastroenteritis and neurological disease in the laboratory mouse. The virus contains a single-stranded, positive-sense RNA genome with a size of 18kb. The virion RNA is transcribed into a genomic-sized negative-stranded RNA, and the negative-stranded RNA serves as a template for the transcription of the genomic RNA and subgenomic mRNAs. The subgenomic RNAs share a 5' leader sequence of about 70 nucleotides. It is suggested that the learder RNA serves as a primer for the transcription of viral mRNAs. The nucleocapsid (Nc) protein encoded by mRNA7, combines with the virion genomic and the negative-stranded RNA. It was suggested that Nc protein might play an important role in the regulation of viral transcription.

In order to investigate the role of the Nc protein during viral multiplication, antisense oligonucleoties and antisense RNA against mRNA of MHV Nc protein were introduced into MHV-permissive DBT cells. A 14–mer anti-leader oligonucleotide contained a sequence complementary to the consensus sequece, UCUAA, of MHV leader RNA. An antisense mRNA7 oligonucleotide was complementary to 15 nucleotides from initiation codon AUG and an antisense junction oligonucleotide contained a sequence complementrary to the sequence between UCUAA and AUG. These three oligonucleotides complementary to the 5' side of Nc protein mRNA, inhibited the synthesis of MHV Nc proteins and exerted an antiviral activity against MHV.

The expression vector of the antisense mRNA was constructed, from pZIPneo and cDNA of MHV mRNA7, which included the leader sequence and an mRNA body. Several trasfected cell lines which expressed the antisense mRNA constitutively were established from DBT cells and MHV-semipermissive LM cells. In these transfected cells, the multiplication of MHV was markedly inhibited.

These results suggested that the synthesis of Nc protein was essential for the multiplication of MHV. The present study is the first report demonstrating that antisense RNA exerted an antiviral activity against a single-stranded positive-sense RNA virus.