Specific Cleavage of 28S Ribosomal RNA in Murine Coronavirus-Infected Cells

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1. INTRODUCTION

Mouse hepatitis virus (MHV), a prototypic coronavirus, causes gastrointestinal and upper respiratory tract illnesses in animals and man. The 32 kb-long MHV genome is a positive-sense, single-stranded RNA (Lai *et al* 1981, Lee *et al* 1991) that encodes 11 open reading frames, expressed through the production of a genomic-size and six to eight species of subgenomic mRNAs (Lai *et al* 1981). Genomic-size mRNA encodes the 5'-most 22 kb-long gene 1, which encodes the RNA polymerase function (Lee *et al* 1991). MHV contains three envelope proteins, S, M and E proteins. S protein binds to the coronavirus receptor (Dveskler *et al* 1991). M and E proteins are important for the formation of the MHV envelope (Kim *et al* 1997).

Extensive morphological and physiological changes occur in coronavirus-infected cells. Some of these changes contribute to damage of cells and tissues. The specific basis, for these deleterious effects on host cells, is not well understood. MHV-infection causes host protein translation inhibition (Hilton *et al* 1986, Tahara *et al* 1994). Inhibition of host protein synthesis accompanies an increase in MHV protein synthesis (Siddell *et al* 1981). Some host mRNAs are degraded in MHV-infected cells, while others are transcriptionally upregulated (Kyuwa *et al* 1994). The mechanism of selective MHV-specific protein synthesis in infected cells, concomitant with

host protein inhibition, is poorly characterised, although it has been suggested that binding of N protein to MHV leader sequences may act as a strong translation initiation signal (Tahara *et al* 1994, Tahara *et al* 1998).

In the present study, we described a hitherto unknown phenomenon in MHV-infected cells: the specific cleavage of 28S ribosomal RNA (rRNA). This phenomenon was not restricted to any particular MHV strain or host cell type. Our data also suggested that the observed cleavage was probably not regulated by the apoptotic pathway. Hence we demonstrated that MHV-induced 28 rRNA cleavage was different from other known ribosomal RNA cleavage events.

2. MATERIALS AND METHODS

2.1 Viruses and cells

The plaque-cloned A59 strain of MHV (Lai *et al* 1981), MHV-JHM and MHV-2 were used. Mouse DBT cells were used for MHV growth.

2.2 Northern (RNA) blotting

Northern blot analysis was performed using [³²P]-γATP-labeled oligonucleotide probes as previously described (Makino *et al* 1991). Probe 1 binding to nucleotides 1532-1551 from the 5'-end of mouse 28S rRNA, was used to detect 28S rRNA and its cleavage products. Probe 2 and probe 3, binding to nucleotides 921-940 and nucleotides 1846-1870 from the 5'-end of mouse 18S rRNA, respectively, were used to detect 18S rRNA. All hybridisation were performed at 60°C.

3. RESULTS

Agarose gel electrophoresis of intracellular RNA from MHV-A59-infected DBT cells showed a major band that migrated between the 28S and 18S rRNAs. No MHV-specific probe hybridised with this RNA band and its size differed from all MHV subgenomic mRNAs (data not shown). Northern blot analysis of intracellular RNA from MHV-infected cells, using a 28S rRNA-specific probe 1 (Fig. 1A) revealed a major 28S rRNA cleavage product, 28S-CL1 and four minor 28S rRNA bands, CL2-CL5, in addition to the intact 28S rRNA. 28S-CL1 appeared as early as 4 h p.i. (data not shown)

and it remained a major cleavage product until 12 h p.i. 28S-CL-3 and 28S-CL4 accumulated late in infection. The amount of intact 28S rRNA decreased significantly with increasing time of infection. In contrast, 18S rRNA did not undergo cleavage in MHV-infected DBT cells (Fig. 1B). The amount of 18S rRNA in MHV-A59-infected cells remained similar to that in uninfected cells, until 16 h p.i., when it decreased slightly.

Figure 1. Specific degradation of 28S rRNA in MHV-infected DBT cells. Cytoplasmic RNA was extracted from MHV-infected cells at indicated times and Northern blot analysis was performed using oligonucleotide probes against mouse 28S rRNA (A) and 18S rRNA (B).

Infecting DBT cells with UV-inactivated MHV inoculum did not cause any 28S rRNA cleavage (Fig. 2A), demonstrating that binding of MHV to MHV receptors alone or other unidentified substances, did not induce 28S rRNA cleavage. Induction of 28S rRNA cleavage required MHV replication. Comparing the kinetics of 28S rRNA cleavage with other cytopathic effects in MHV-infected cells, we found 28S rRNA cleavage started earlier than the onset of MHV-A59-induced cell fusion. Infection with a nonfusogenic MHV strain, MHV-2, also caused 28S rRNA cleavage, in DBT cells (data not shown). These data demonstrated that MHV-induced 28S rRNA cleavage was not directly related to cell fusion. A substantial increase in the amount of 28S-CL1, in MHV-infected cells, preceded the peak of MHV RNA synthesis and the cleavage continued at a time when MHV RNA synthesis declined (Sawicki and Sawicki 1986).

We examined the relationship between 28S rRNA cleavage and host protein synthesis in MHV-infected cells. Consistent with previous studies (Siddell *et al* 1981, Tahara *et al* 1994), host protein synthesis inhibition in MHV-infected cells was evident by 6 h p.i. (data not shown); 28S-CL1 appeared as a major cleavage product starting 5 h p.i. Hence, the 28S-CL1 cleavage product needed to accumulate before host protein synthesis inhibition could occur. Thereafter, both 28S rRNA cleavage and host protein synthesis inhibition continued as infection proceeded.

Figure 2. MHV replication is required for mature cytoplasmic 28S rRNA cleavage. (A) Cytoplasmic RNA from mock-infected (M) or UV-irradiated MHV-A59-infected (I) DBT cells were extracted at 8 h p.i. and analysed by Northern blot. (B) Ribosomal precursors were labelled with ^3H -uridine and chased for 13 hr. Then, cells were mock-infected (M) or infected with MHV-A59 (I). 8 h p.i. cytoplasmic RNA was extracted. U= Uninfected.

To determine whether mature cytoplasmic rRNA is cleaved in MHV-infected cells, precursor rRNA molecules, in DBT cells, were labelled with ^3H -uridine and allowed to be processed and transported to the cytoplasm. Cells were then infected with MHV-A59 and labelled, mature cytoplasmic RNA were analysed by electrophoresis (Fig. 2B). Mock-infected cells showed intact 28S rRNAs while MHV-infected cells showed a reduced amount of intact 28S rRNA and the presence of 28S-CL1. We concluded that MHV infection induced cleavage of mature 28S rRNA, that was processed and transported to the cytoplasm, prior to MHV infection.

28S rRNA cleavage was not confined to any particular cell type or MHV strain. MHV-A59-infection to DBT and 17Cl-1 cells produced all 28S rRNA cleavage products; the kinetics of appearance of the cleavage products and reduction of intact 28S rRNA in MHV-A59-infected DBT and 17CL-1 cells were similar (data not shown). MHV-JHM-infection to DBT and 17Cl-1 cells also induced similar 28S rRNA cleavage products but progress of MHV-JHM-induced 28S rRNA cleavage was slow (data not shown); A lower level of MHV-JHM replication efficiency in infected cells may be related to less efficient 28S rRNA cleavage.

There have been some reports of rRNA degradation occurring in apoptotic cells. MHV-infected 17Cl-1 cells undergo apoptosis, but not MHV-infected DBT cells (An *et al* 1999). Nevertheless, 28S rRNA cleavage occurred in both DBT and 17Cl-1 cells (Fig. 1, and data not shown). If apoptosis regulates MHV-induced 28S rRNA cleavage then blocking apoptosis should block 28S rRNA cleavage. Accordingly we treated MHV-infected 17Cl-1 cells with an irreversible caspase-3 inhibitor, Z-DEVD-fmk, that effectively blocks apoptosis. We found that blocking apoptosis in MHV-infected 17Cl-1 cells did not block or delay 28S rRNA cleavage (Fig. 3). Thus, MHV-induced 28S rRNA cleavage was upstream of caspase-

activation, or apoptosis induction and 28S rRNA cleavage occurred via two independent pathways.

Figure 3. Blocking apoptosis does not block MHV-induced 28S rRNA cleavage. 17Cl-1 cells were mock-treated or treated with 80 μ M Z-DEVD-fmk and mock-infected or infected with MHV-A59. 8 h p.i. cytoplasmic RNA was extracted and analyzed by Northern blot.

4. DISCUSSION

We report a novel MHV-induced 28S rRNA cleavage in the present study. The MHV-induced rRNA cleavage occurred only in 28S rRNA, and not in 18S rRNA. Specific cleavage of 28S rRNA required ongoing MHV replication since UV-inactivated MHV failed to induce 28S rRNA cleavage. Fully processed, mature cytoplasmic 28S rRNA underwent cleavage. Currently, we do not know which step of the viral life cycle or specific viral factor(s) causes this 28S rRNA cleavage. MHV infection to all susceptible cell lines, using several different MHV strains, induced 28S rRNA cleavage. MHV-induced 28S rRNA cleavage was independent of virus-induced CPE, and it differed from apoptosis-related rRNA degradation, in that blocking apoptosis had no effect on the cleavage of 28S rRNA.

MHV-induced 28S rRNA cleavage could occur via the activation of a cellular or viral RNase. That same RNase may degrade host mRNAs, which maybe partly responsible for the host protein shut-off in MHV-infected cells (Hilton *et al* 1986). Alternatively, binding of MHV factors or host factors may alter the structure of 60S ribosome, allowing a cellular or viral RNase to access specific regions of 28S rRNA and cleave it.

28S rRNA being an integral component of the 60S ribosomal subunit, MHV-induced 28S rRNA cleavage may affect protein synthesis by rendering polysomes, containing cleaved 28S rRNA, translationally inactive. Polysomes with intact 28S rRNA maybe used for MHV-specific protein synthesis, resulting in host mRNA translation inhibition. Alternatively, ribosomes, containing 28S cleavage products, maybe structurally altered to better translate increasing amounts of MHV-specific mRNAs. Thus, MHV-induced 28S rRNA cleavage may alter the cellular environment and

translation machinery to slow down host protein synthesis and allow efficient expression of viral proteins.

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