EFFECTS OF MOUSE HEPATITIS VIRUS INFECTION ON HOST CELL METABOLISM

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ABSTRACT

A time dependent decrease in cell surface expression of major histocompatibility complex (MHC) class 1 proteins was found during JHMV infection of the mouse macrophage J774.1 cells line by radioimmunoassay. MHC class I, actin and CSF-1 receptor mRNA levels were also found to decrease during infection. Surprisingly, not all host cell mRNA were similarly affected, suggesting that the apparent MHV-induced translational shut off of host cell protein synthesis during infection was specific for only some host cell mRNAs. Interestingly, two mRNAs found to be refractory to JHMV infection encode monokines, suggesting a role in pathogenesis. To understand the mechanism(s) of this preferential mRNA stability and the apparent shut off of host cell mRNA, translation lysates were prepared from infected and uninfected cells. Translation of host mRNAs in these extracts showed no apparent loss of translational ability in the infected cells vs. the uninfected cells; however, viral mRNAs were preferentially translated in the lysates from the infected cells. Chimeric mRNAs containing the MHV leader upstream of a globin reporter gene showed that preferential translation was a property of the MHV leader RNA. Deletional analysis showed that the sequences responsible for this cis translational augmentation are in a 12 nucleotide (nt) tract at the 3' end of the leader. The previously reported interaction of the nucleocapsid protein with these nts suggest that it may play a role in translational augmentation of MHV mRNAs.

INTRODUCTION

Many cytolytic viruses divert host cell macromolecular synthesis for use by the virus. Indeed, the preferential shut off of host cell translation is a property common to many cytolytic viruses[1]. The mechanisms used by viruses include alterations in host

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translation factors, increased turnover of host mRNAs, and competition due to vastly increased viral mRNA levels. Analysis of protein synthesis during mouse hepatitis virus (MHV) infection of susceptible cells shows a conversion to viral protein synthesis at the expense of host protein synthesis[2]. MHV has been suggested to actively suppress host cell translation through a combination of effects manifested by a loss of polysomes and degradation of host mRNAs[3].

METHODS

Virus and cell lines: The DL isolate of JHMV and the A59 strain of mouse hepatitis virus (MHV) were propagated and plaque assayed as previously described[4]. Northern blot analyses were carried out using the J774.1 Balb/c monocytic cell line[5]. Translation lysates were prepared from either the J774.1 or DBT murine astrocytoma cell lines as previously described[6]. Lysates were prepared from uninfected DBT cells grown in suspension using Joklik's modified MEM while the lysates from infected cells were prepared from monolayers infected with MHV-A59 an m.o.i. = 5 to 10 at 4.5 - 5.0 hr post infection.

Northern Analysis: Infected and uninfected J774.1 cells were lysed in guanidine isothiocyanate and the RNA purified by centrifugation through CsCl. RNA (30μgm) was analyzed by electrophoresis through 1 to 1.2% agarose gels containing 0.66mM formaldehyde and transferred to High Bond N (Amersham) membranes. The membranes were hybridizided with probes specific for actin, Il-1a, Ilb, TNFa, tubulin, fms (the CSF receptor) 28S ribosomal RNA, interferon (IF)-a and MHC class 1 and class 2. Probes were labeled by random priming and hybriziation was carried out for 24-96 hrs at 42 C. RNA levels were determined by densitometry.

Plasmids: Chimeric plasmids containing the MHV mRNA #6 leader sequence 5' of the human globin sequence were cloned by PCR and inserted using standard techniques. All constructions were confirmed by sequence analysis.

In vitro translation: Cell-free extracts were treated with 125 U/ml micrococcal nuclease at 21 C for 0-12 min. In vitro translations were performed in a 15μl volume containing 9μl of nuclease treated extract and 10 μCi ^{35} S-methionine (Amersham, Arlington Heights, IL) 60 min at 37 C. Synthetic, capped mRNAs were added at a concentration of 16.7 μg/ml. Reactions were terminated by addition of 1 ml of cold acetone. Precipitates were collected, resuspended in Laemmli sample buffer and analyzed on 15% SDS-polyacrylamide gels (SDS-PAGE). Bands were quantitated by densitometery.

RESULTS

The mRNA levels in J774.1 cells infected with JHMV were determined by northern blot to insure the quality of the RNA and quantitated by densitometry and compared to the levels in uninfected cells. Figure 1a shows that there is little variation in the quantity of 28S ribosomal RNA. In contrast, decreased levels of actin mRNA were found consistent with previous data[3]. In addition, the levels of MHC class 1 mRNAs were also decreased, consistent with decreased cell surface expression as determined by radioimmunoassay[7]. Figure 1a also shows that the CSF-1 receptor mRNA decreased during infection. Three additional mRNAs were analyzed, MHC class 2, IL-Iα and IF-α. No mRNA for these two putative macrophage mRNAs could be detected, although MHC class 2 mRNA was detected after IFN-γ induction of uninfected cells (data not shown).

Not all host cell mRNAs decreased following JHMV infection. Figure 1b shows the analysis of tubulin, IL-1β and TNF mRNAs. All three of these mRNAs increased following infection. Neither the TNF nor IL-1β mRNAs were at significant levels in the uninfected cells. Treatment of the cells with 1 μg/ml actinomycin D prevented the accumulation of these mRNAs, suggesting that infection activated transcription and/or a

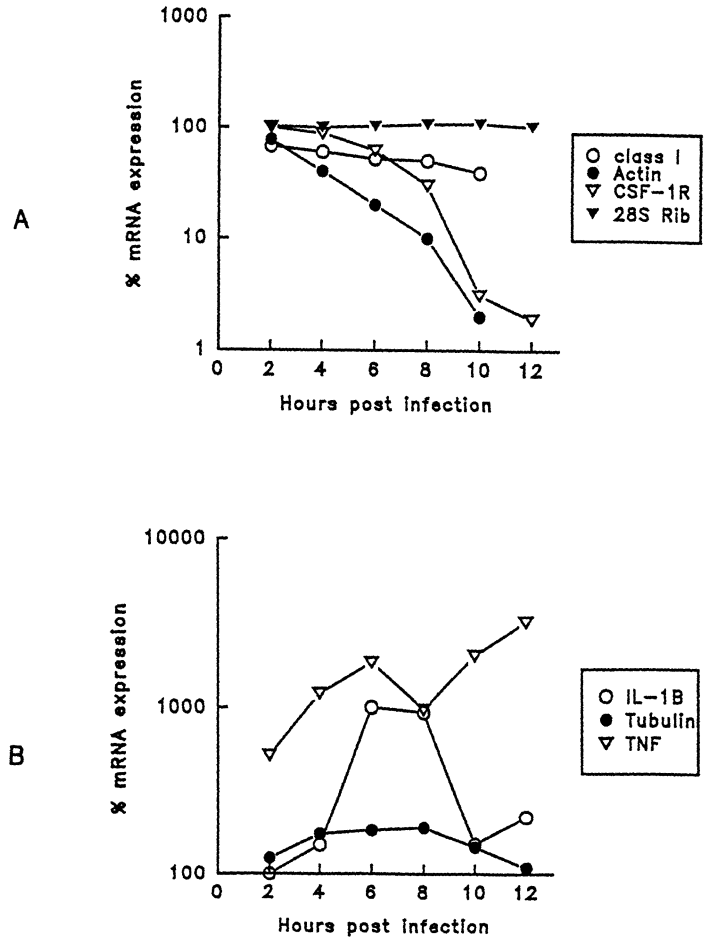

Figure 1. Relative mRNA levels determined from northern blots. Panel A: mRNA levels that decrease during infection. Panel B: mRNA levels that increased during infection. Levels are relative to those found in uninfected cells.

post transcription process. The most rapid and dramatic increase was seen in TNF mRNA which increased throughout infection. The induction and stability of these mRNAs suggested that the proteins were synthesized throughout the acute infection. Preliminary experiments indicate that TNF is indeed released from infected cells.

These data suggested that the conversion of protein synthesis from host to viral with time during infection may be abnormal in the J774.1 cells infected with JHMV. Analysis of protein synthesis in these cells infected with JHMV showed decreased host cell protein synthesis by 6 hr post infection accompanied by an increase in JHMV proteins. Later time points show only viral proteins being synthesized suggesting the possibility of translational regulation of protein synthesis in infected cells.

To determine if MHV altered host cell translation, lysates were prepared from either uninfected and JHMV-infected J774.1 cells or uninfected and A59-infected DBT cells for in vitro translation. Initial experiment examined the efficiency of translation of globin and CAT as model host genes and poly A selected MHV RNA from infected DBT cells. The globin mRNA was translated equally well in all lysates indicating that the apparent decrease in translation of host cell mRNAs is not due to diminished translation, and further indicating that there is no loss in host cell translation factors. By contrast, the poly A-selected viral mRNAs were translated more efficiently in the lysates of infected cells.

These data suggested that the viral mRNAs may contain a cis translational augmentor. To determine if the 5' leader RNA sequence, which is common to all MHV mRNAs, could function as the translational augmentor, chimeric genes were constructed containing the leader sequence in both the plus (+) and (-) negative orientations 5' of a human globin reporter sequence.

Synthetic 5' capped mRNA from these genes were tested in lysates from uninfected and infected DBT cells. Translation products were separated by SDS-PAGE and the globin quantitated by densitometry. No difference in the translation of native globin or synthetic CAT mRNAs was found in lysates from infected vs. uninfected DBT or J774.1 cells, therefore the quantity of globin translated was used for comparison to control for lysate differences. Translation of these mRNAs in the lysates from infected and uninfected cells showed that translation in the infected lysates was enhanced only by MHV leader in the (+) orientation. In addition, deletion of the 3' MHV leader sequence which is the N protein binding site 8, abolishes the enhanced translation (Fig. 4). Table 1 shows the relative levels of protein synthesis in the two lysates.

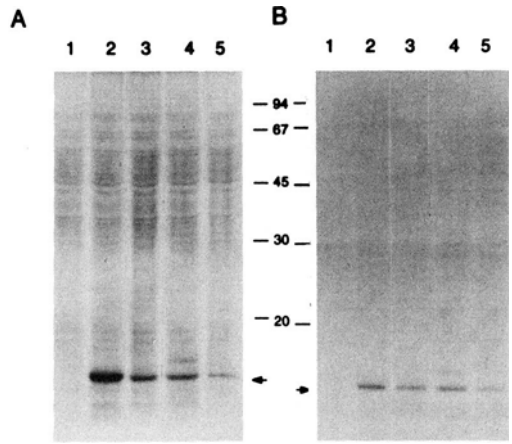

Figure 2. Translation of the MHV-leader globin chimeric mRNAs in lysates from infected (Panel A) and uninfected (Panel B) DBT cells using 250 ng of RNA per reaction. Lane 1: No mRNA added; Lane 2: haGL1 (Leader +); Lane 3: haGL12 (Leader -); Lane 4: haGL 62-74 (Leader deletion); Lane 4: haG (globin).

Table 1. Relative activity of MHV-leader globin chimeric RNAs.

mRNA	Uninfected Cells[1]	Infected Cells
haG	1.00	1.00
haGL1	2.70 ± 0.60[2]	10.31 ± 1.70
haGL12	2.38 ± 0.65	2.94 ± 0.68
haGL62-74	1.63 ± 0.35	3.39 ± 0.37

[1]Lysates of DBT cells. [2]Mean ≤ standard deviation

DISCUSSION

The data in this report contain a number of novel concepts relative to the biology and immunobiology of MHV infection. The analysis of host cell mRNA stability during infection indicated that not all host mRNAs are degraded, even though viral protein translation becomes predominant. These data suggest three fundamentally important concepts in the biology of MHV infection. First, in contrast to other cytolytic viral infections[1] MHV infection does not prevent the translation of host mRNAs. Secondly, the differential stability of the host mRNAs indicates that the degradation of host cell mRNA must be regulated since virus is able to persist without cytopathology, both in vitro and in vivo[8,9]. Third, the ability of the macrophage to upregulate mRNA levels for two cytokines following infection, one involved in enhancing antigen presentation (IL-1), and the other with antiviral activity (TNF) suggest that these cells are able to at least partially circumvent the deleterious effects of infection. TNF is cytolytic for oligodendroglial cells[11], suggesting that a vigorous immune response to infection within the CNS, particularly the release of TNF following infection of newly recruited macrophages, may contribute to JHMV-induced CNS demyelination.

Analysis of translational regulation in MHV-infected cells showed that there is no loss of the cells ability to translated host mRNAs. This is consistent with the 5' capped, poly adenylated nature of the MHV mRNAs and the ability of virus to persist in vitro and in vivo[2,8,9]. However, observations in a number of cell lines has shown that during infection there is decreased host protein translation with concommitant increases in viral translation[2]. Analysis of chimeric mRNAs clearly demonstrated that the 5' MHV leader RNA sequence functions as a cis augmentor and is at least partially responsible for the apparent increase in viral over host cell translation. The presence of this cis effect only in the lysates from the infected cells further suggested the presence of a transacting factor. Since this putative augmentor would bind leader RNA and such an interaction has been described[11], the N protein binding sequence (nts 62-74) was deleted. This deletion abolished the cis augmentation effect. Preliminary experiments replacing the 3' leader nts that comprise the N protein binding site within the 3' end of the native globin leader sequence show enhanced translation in infected vs. uninfected lysates. Although there is no direct proof that the N protein functions as the transacting protein, the kinetics of N protein synthesis and its subcellular distribution are both consistent with a major regulatory role in MHV infection, possibly controlling both transcription and translation as well as fulfilling its structural role in forming the helical nucleocapsid.

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