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Mengovirus Leader Is Involved in the Inhibition of Host Cell Protein Synthesis

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The presence of a leader peptide in picornaviruses is restricted to the *Cardiovirus* and *Aphthovirus* genera. However, the leader peptides of these two genera are structurally and functionally unrelated. The aphthovirus leader is a protease involved in viral polyprotein processing and host cell translation shutoff. The function of the cardiovirus leader peptide is still unknown. To gain an insight into the function of the cardiovirus leader peptide, a mengovirus leader peptide deletion mutant was constructed. The deletion mutant was able to grow at a reduced rate in baby hamster kidney cells (BHK-21). Mutant virus production in mouse fibroblasts (L929 cells), however, could be demonstrated only after inoculation of BHK-21 cells with the transfected L929 cells. Analysis of cellular and viral protein synthesis in mutant virus-infected cells showed a delayed inhibition of host cell protein synthesis and a reduced production of viral proteins. In a single-cycle infection, mutant virus produced only 1% of wild-type virus yield at 8 h postinfection. Host cell translation shutoff in L929 cells infected with mutant virus was restored by the addition of the kinase inhibitor 2-aminopurine. Mutant virus production in 2-aminopurine-treated L929 cells was increased to 60% of wild-type virus yield at 8 h postinfection. Our results suggest that the cardiovirus leader peptide is involved in the inhibition of host cell protein synthesis.

The picornavirus family can be subdivided into five different genera, the enteroviruses, rhinoviruses, cardioviruses, aphthoviruses, and hepatoviruses. They all possess a single-stranded RNA genome of approximately 7.5 kb with positive polarity which contains an unusual long 5′ nontranslated region (5′ NTR), a 3′ NTR at their 3′ end, and a genetically encoded poly(A) tract. The RNA genome is functionally equivalent to cellular mRNA and encodes a single polyprotein of approximately 220 kDa, which is subsequently processed by virus-encoded proteases into the structural and nonstructural viral proteins (12). The polyprotein comprises the P1 region containing the structural proteins that form the viral capsid and the P2 and P3 regions that deliver the nonstructural proteins involved in virus translation and replication. Poliovirus is the best studied of the picornaviruses, but the specific protein functions seem to be similar in all genera within this family with the notable exception of the 2A proteins and the leader peptides. The enteroviruses and rhinoviruses contain a 2A protein of 16 kDa which possesses proteolytic activity (8). In contrast, the aphthovirus 2A peptide consists of 19 amino acid residues that are involved in the nonenzymatic cleavage of the virus polyprotein at the 2A-2B junction (13). Aphthoviruses contain a leader peptide located upstream of the P1 region, while the enteroviruses and rhinoviruses lack the leader peptide. Cardioviruses, on the other hand, contain both leader and 2A proteins.

The 2A protease ($2A^{pro}$) of the enteroviruses and rhinoviruses and the leader protease (L^{pro}) of aphthoviruses exhibit similar activities. $2A^{pro}$ cleaves at its N terminus at the VP1-2A junction while L^{pro} cleaves at its C terminus at the L-VP4 junction. The enterovirus and rhinovirus $2A^{pro}$ is a zinc-binding protein which also causes the proteolytic degradation of the 220-kDa subunit of the eukaryotic translation initiation factor 4 (eIF-4G) (8, 17). The leader protease (L^{pro}) of aphthoviruses exhibits similar activity (5). As a consequence of eIF-4G degradation, translational initiation of cellular, 5′-capped mRNAs will be inhibited whereas initiation of viral translation is mediated by an internal ribosome entry site within the 5′ NTR, independent of a 5′-cap structure (2). $2A^{pro}$ and L^{pro} are also probably involved in the interaction between one or several cellular factors and the 5′ NTR of the viral RNA to enhance viral translation initiation (3, 22). In contrast to the other picornaviruses, no degradation of eIF-4G occurs during cardiovirus infection. Recently, Chen et al. demonstrated that, like enterovirus and rhinovirus $2A^{pro}$, the Theiler's encephalomyelitis virus (TMEV) L peptide contains a zinc-binding motif (1, 17), the presence of which might indicate that the L peptide possesses nucleic acid binding properties. However, the biological function of the cardiovirus leader is still unknown. Kong et al. recently constructed a series of TMEV DA strain deletion mutants lacking parts of the leader peptide-encoding region and showed that, whereas the leader peptide is dispensable for TMEV growth in baby hamster kidney (BHK-21) cells, growth in mouse fibroblasts (L929 cells) was dependent on a functional L peptide, suggesting that host cell restriction of virus growth was due to the production of interferon by L929 cells (6).

In this paper, we describe a deletion mutant of mengovirus, which lacks leader peptide activity. Cellular and viral protein syntheses were studied in both BHK-21 and L929 cells in the presence or absence of fibroblast interferon and the kinase inhibitor 2-aminopurine (18, 23). We found that deletion of the leader peptide resulted in host cell-restricted virus growth. Host cell protein synthesis shutoff was delayed in cells infected with mutant virus. The presence of 2-aminopurine in mutant virus-infected L929 cells resulted in restored shutoff of host cell protein synthesis whereas production of mutant virus was increased. The results reported here suggest that the cardiovi-

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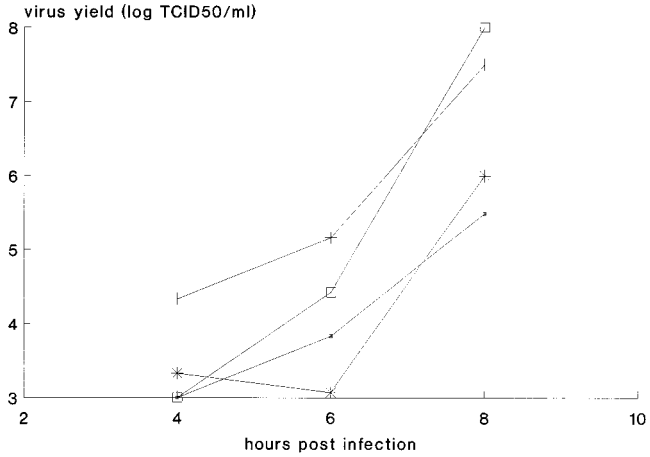

FIG. 1. Single-cycle growth curves in BHK-21 cells of vM16.1 (□) and vM16.1ΔL(12-52) (∗) and in L929 cells of vM16.1 (+) and vM16.1ΔL(12-52) (⊡). Cells were infected with virus at an MOI of 1. Cultures were incubated at 36°C and harvested at 4, 6, and 8 h postinfection. Virus titers were determined by titration on BHK-21 cells at 36°C.

rus leader protein is involved in the inhibition of host cell protein synthesis.

MATERIALS AND METHODS

Cells and viruses. BHK-21 cells and L929 cells were used for RNA transfections, virus propagation, and plaque assays. Production of virus stocks and virus titrations were performed in BHK-21 cells. Virus titers were determined with eight replicates by titrating decimal dilutions in 96-well microtiter plates (16). Fifty percent tissue culture infective doses ($TCID_{50}$) were calculated according to the method of Reed and Muench (11).

Construction of the L deletion mutant. The infectious mengovirus cDNA clone pM16.1 (2) was kindly provided by A. Palmenberg (University of Wisconsin). Site-directed mutagenesis was performed with the Altered Sites in vitro mutagenesis system according to the instructions of the manufacturer (Promega). To achieve mutagenesis, the *Eco*RV-*Sma*I fragment (nucleotides 45 to 3847) from pM16.1 was first cloned into the *Sma*I site of pALTER-1, which resulted in the clone pALTER-ML. Then, unique *Xho*I cleavage sites were introduced into the L-encoding region at positions 749 and 870 with the mutagenesis oligonucleotides 5′-GCA TTC TTC AAA GGT CAT GGC TCG AGC ACA AAT CTC TTG TTC-3′ and 5′-CAA ATC AGG ATC GAA CAC TCG AGC TTC ACC ATC AGT CAA CGA-3′ (Isogen, Amsterdam, The Netherlands).

The *Bln*I-*Bgl*II fragment (nucleotides 296 to 3814) of the original pM16.1 cDNA clone was replaced by the *Bln*I-*Bgl*II fragment of the mutated pALTER-ML constructs. The deletion mutant was created by *Xho*I digestion and subsequent self-ligation. The construct was verified by sequence analysis using the Ampli Cycle sequencing kit according to the manufacturer's instructions (Perkin-Elmer). The mutant mengovirus cDNA clone pM16.1ΔL(12-52), which lacks the region which encodes amino acids 12 to 52 of the leader peptide, was obtained with this procedure.

In vitro transcription and transfection. BHK-21 and L929 cells were transfected with 2 to 4 μg of in vitro-transcribed RNA by the DEAE-dextran method (19) with some modifications. Briefly, copy RNA in a volume of 20 μl was mixed with 150 μl of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-buffered saline solution (containing 20 mM HEPES [pH 7.05], 137 mM NaCl, 5 mM KCl, 0.7 mM NaH_2PO_4, and 5.5 mM glucose) and 150 μl of 1-mg/ml DEAE-dextran in HEPES-buffered saline solution and incubated on ice for 30 min. BHK-21 or L929 cells grown in 25-cm^2 flasks were washed three times with phosphate-buffered saline (PBS) (pH 7.4) and incubated with the RNA–DEAE-dextran mixture at room temperature for 30 min. Monolayers were washed three times with PBS and subsequently incubated at 36°C in the appropriate cell culture medium. Transfected cells were maintained for up to 5 days or until complete cytopathic effect due to virus growth was observed and subjected to three cycles of freezing and thawing. Lysates of cells transfected with mutant RNA were used to inoculate fresh cells when described. Aliquots of virus stocks were stored at −80°C.

Plaque assay. Plaque assays of both wild-type and deletion mutant virus were performed with 100% confluent monolayers of BHK-21 and L929 cells on 10-cm^2 dishes. Cells were infected with various virus dilutions and incubated at room temperature for 30 min. After removal of the inoculum, L929 cells were overlaid with 5 ml of medium containing 1% plaque agarose (Servo) and neutral red and grown for 3 to 5 days. Because of the irregular structure of BHK-21 monolayers, a modified procedure for the plaque assay on these cells was used. Briefly, BHK-21 cells were overlaid with 5 ml of medium containing 1% methylcellulose. To visualize the plaques generated on BHK-21 cells, the methylcellulose overlay was removed 3 days after infection and the cells were washed three times with PBS. The cells were fixed with 100% methanol and stained by Giemsa. The plaque assays were performed at 33, 36, and 39°C.

Single-cycle growth curves. Monolayers (25 cm^2) of BHK-21 and L929 cells were inoculated with vM16.1 and vM16.1ΔL(12-52) at a multiplicity of infection (MOI) of 1 $TCID_{50}$ per cell. Virus suspensions were replaced by fresh culture medium after 30 min of absorption at 36°C. At specific times postinfection as indicated in the text, cells were disrupted by three cycles of freezing and thawing. Virus titers were determined by titration of the supernatants on BHK-21 cells.

Analysis of protein synthesis in vivo. Monolayers of BHK-21 and L929 cells were inoculated with vM16.1 and vM16.1ΔL(12-52) at an MOI of 50 $TCID_{50}$ per cell. Virus suspensions were replaced by fresh medium after 30 min of absorption at 36°C. At various times postinfection, monolayers were washed three times with PBS (pH 7.2). Cells were incubated for 1 h with methionine-free medium containing 10 μCi of [^{35}S]Met per ml (ICN). Cellular extracts were prepared by lysis with a buffer containing 0.5 M Tris-HCl (pH 8.0), 0.15 M sodium chloride, 1% Nonidet P-40, 0.05% sodium dodecyl sulfate (SDS), and 0.1 mM phenylmethylsulfonyl fluoride. Protein synthesis was further analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

Treatment of L929 cells with interferon and 2-aminopurine. Monolayers of L929 cells were treated 2 h preinfection with 100 U of mouse fibroblast interferon per ml (Sigma). 2-Aminopurine (Sigma) was dissolved to a final concentration of 150 mM in PBS containing 0.5% acetic acid. Aliquots of this stock solution were stored at −80°C. Cells were treated with 2-aminopurine at a concentration of 10 mM 2 h preinfection.

RESULTS

Construction of the leader peptide deletion mutant. At first instance, we deleted the complete L peptide-encoding region from the infectious cDNA clone of mengovirus pM16.1 (2). However, in vitro translation of the mutant copy RNA revealed that viral protein synthesis was markedly reduced (data not shown). It has indeed been reported that translation initiation efficiency of encephalomyocarditis virus depends on the nucleotide sequence just downstream from the start codon (4). Therefore, to avoid problems in translation initiation not related to L activity, unique *Xho*I sites were introduced at positions 749 and 870 on pM16.1. Digestion with *Xho*I and subsequent self-ligation of the mutated cDNA resulted in the construct pM16.1ΔL(12-52), in which amino acids 12 to 52 of the L peptide were deleted.

Characterization of the leader peptide deletion mutant. In vitro-transcribed RNA of both pM16.1 wild-type and mutated cDNA was used for transfection of BHK-21 cells and L929 cells. Transfection of both these cell lines with wild-type copy RNA resulted in complete cell lysis within 24 h. Transfection of BHK-21 cells with pM16.1ΔL(12-52) copy RNA resulted in a cytopathic effect within 3 days. No cytopathic effect was observed after transfection of L929 cells with pM16.1ΔL(12-52) copy RNA. Inoculation of BHK-21 with the lysate of transfected L929 cells, however, resulted in a cytopathic effect within 3 days. Subsequent virus characterizations were performed with mutant virus vM16.1ΔL(12-52) stocks produced in BHK-21 cells. Titration of mutant virus with L929 cells revealed that a cytopathic effect could be observed only after infection at an MOI of at least 10. On the other hand, infection of BHK-21 cells with mutant virus at an MOI of 10^{-4} resulted in complete cytopathic effect within 3 days. Viral growth kinetics were measured by single-cycle growth curves (Fig. 1). Mutant virus yields in both cell types at 8 h after infection were approximately 1% of those of the wild-type virus. Plaque assays were performed in BHK-21 cells and L929 cells. Plaques of 2 to 3 mm were obtained with wild-type virus in L929 cells at 5 days postinfection. However, no plaques were observed when vM16.1ΔL(12-52) was inoculated on L929 cells (Fig. 2). Because of the irregular structure of the cellular monolayer, BHK-21 cells cannot be maintained for more than 3 days after

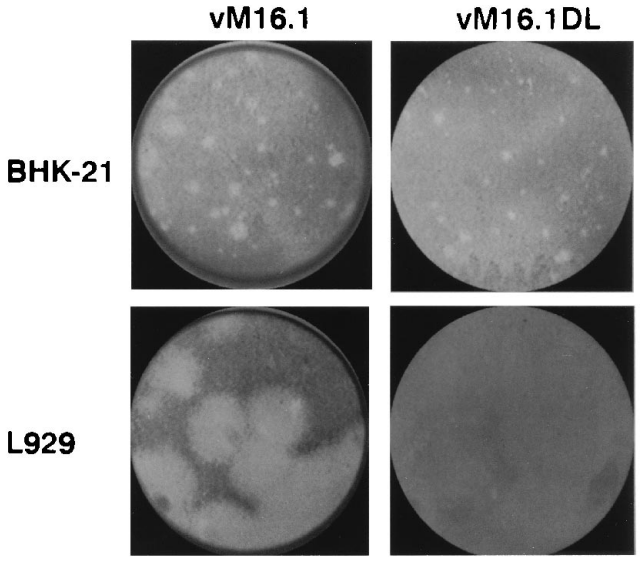

FIG. 2. Plaque phenotype of vM16.1 and vM16.1ΔL(12-52). Assays were performed in BHK-21 and L929 cells.

infection. Therefore, inoculation of BHK-21 monolayers with vM16.1 resulted in 1- to 1.5-mm plaques. Small plaques of 0.2 to 0.4 mm were obtained with vM16.1ΔL(12-52) inoculated on BHK-21 cells (Fig. 2).

Analysis of viral protein synthesis in vivo. Viral protein synthesis was studied by pulse-labeling of infected BHK-21 and L929 cells with [^{35}S]methionine. Infection of BHK-21 cells with wild-type virus resulted in a decrease of host protein synthesis until 5 h after infection, after which viral protein synthesis increased and continued until at least 8 h after infection (Fig. 3A). Infection of L929 cells with wild-type virus resulted in complete inhibition of host cell protein synthesis within 5 h after infection (Fig. 3B). Viral protein synthesis continued until 7 to 8 h after infection. However, when BHK-21 and L929 cells were infected with vM16.1ΔL(12-52), host cell protein synthesis still continued in both cell types until at least 7 to 8 h after infection, whereas only minor amounts of viral products were produced (Fig. 3).

Influence of fibroblast interferon on virus growth. Kong et al. suggested that the restricted mutant virus growth in L929 cells is caused by the interferon production by infected L929 cells (6). In order to determine further the effect of interferon on virus growth, L929 cells were treated with mouse fibroblast interferon prior to infection. Figure 4 illustrates that infection with wild-type virus of L929 cells treated with interferon resulted in a decreased virus titer which is similar to that obtained with mutant virus infection of untreated L929 cells. When pretreated L929 cells were infected with mutant virus, virus yields were not further affected.

Influence of 2-aminopurine on mutant virus growth. In order to prevent the antiviral action of interferon produced by infected L929 cells, virus growth was measured in L929 cells pretreated with the kinase inhibitor 2-aminopurine. The kinase inhibitor interferes with the interferon-induced signal transduction pathway. Infection with vM16.1ΔL(12-52) of L929 cells treated with 2-aminopurine resulted in mutant virus production of 60% of wild-type virus yield (Fig. 4). Protein synthesis of infected L929 cells after treatment with 2-aminopurine was determined by pulse-labeling at 2 and 7 h after infection (Fig. 5). Infection with vM16.1ΔL(12-52) of L929 cells treated with 2-aminopurine resulted in a complete shutoff of host cell protein synthesis at 7 h after infection, while viral protein synthesis was clearly visible.

DISCUSSION

In order to gain an insight into the function of the cardiovirus L peptide, we characterized a mutant in which the major part of the L-encoding region of mengovirus was deleted. Production of L deletion cardiovirus mutant was dependent on the host cells used for infection. Virus growth in BHK-21 cells infected with mutant virus was reduced but finally resulted in complete cell lysis. Production of mutant virus in L929 cells appeared to be hampered. Kong et al. recently described a similar host cell-restricted necessity for the leader peptide in TMEV (6). Any deletion within the TMEV L-encoding region abolished virus growth in L929 cells, while wild-type growth characteristics were found in BHK-21 cells (6). It is unclear

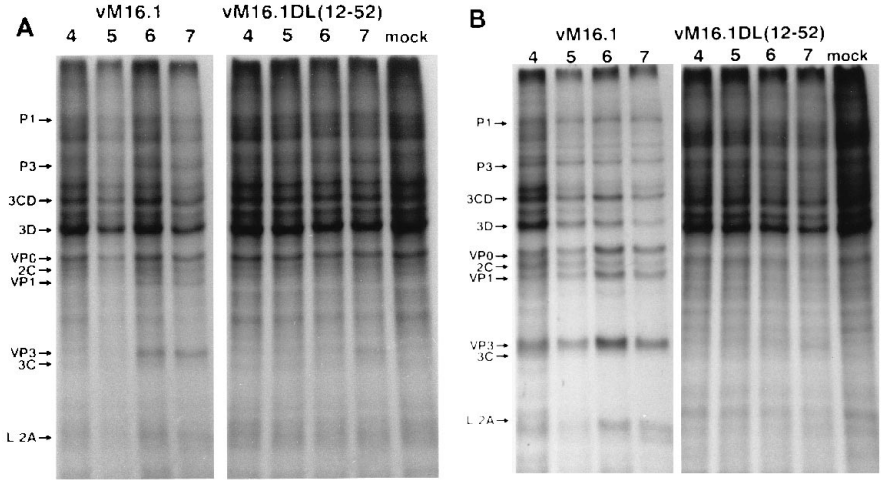

FIG. 3. Protein synthesis in vM16.1- and vM16.1ΔL(12-52)-infected BHK-21 (A) and L929 (B) cells. Cells were infected at an MOI of 50 and grown at 36°C. At the times (in hours) indicated above the lanes, culture medium was replaced by methionine-free and serum-free medium supplemented with [^{35}S]methionine as described in Materials and Methods. Labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis.

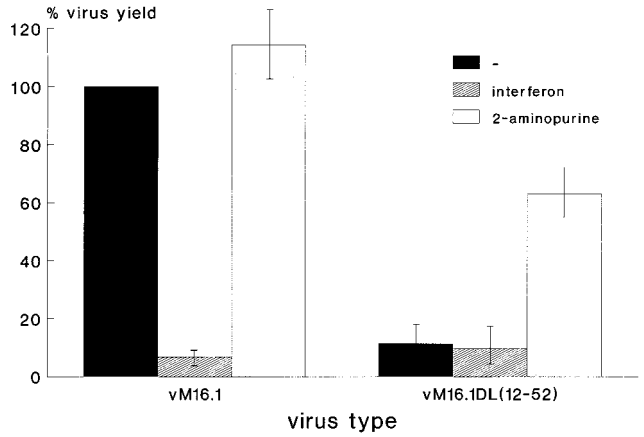

FIG. 4. Effect of fibroblast interferon and 2-aminopurine on vM16.1 and vM16.1ΔL(12-52) production. L929 cells were treated with either 100 U of mouse fibroblast interferon per ml or 10 mM 2-aminopurine at 2 h preinfection. Untreated and treated cells were infected at an MOI of 1 and grown at 36°C. Cells were harvested at 8 h postinfection. Virus titers were determined by titration on BHK-21 cells. Virus production is expressed as the percentage of vM16.1 production in untreated cells.

what causes this contradiction between the TMEV L deletion mutants and our mengovirus L deletion mutant in growth characteristics in BHK-21 cells. Kong et al. suggested that the observed host cell-restricted growth characteristics of the TMEV mutants were caused by the production of interferon in infected L929 cells (6). Indeed, analysis of L929 cells treated with interferon and infected with wild-type mengovirus shows that virus production was abrogated. We found that wild-type virus production in interferon-treated cells was comparable with mutant virus production in untreated cells. The influence of interferon on cardiovirus infections in L929 cells has been described previously by Muñoz and Carrasco (9), who showed that L929 cells additionally treated with fibroblast interferon could survive wild-type mengovirus infection. Our results with L929 cells infected with the wild-type virus in the presence of interferon are consistent with those reported by Muñoz and Carrasco (9).

L929 cells infected with the L deletion mutant displayed a delay of host cell protein synthesis shutoff. Total protein synthesis decreases from 5 h postinfection, although host cell protein expression continues for at least 8 h postinfection. This is consistent with the finding that, although virus production is abrogated, the host cell protein synthesis continues in infected cells pretreated with interferon (9). Because host cell protein synthesis is not completely shut off after infection with the L deletion mutant, L929 cells will still produce interferon. A minority of cells will be infected after infection at a low MOI, and the interferon produced by the cells infected with mutant virus may therefore protect the adjacent uninfected cells in the monolayer. It was indeed observed in L929 cells after infection with mutant virus at an MOI of less than 1 $TCID_{50}$ per cell. In parts of the monolayer, lysed cells were visible 24 h after infection with the L deletion mutant. These regions were, however, overgrown by L929 cells, and the cellular monolayer was completely restored 3 days postinfection. This phenomenon confirmed the observation by Kong et al. (6) that TMEV L deletion mutant-infected L929 cells resulted in a cytopathic effect for a limited number of cells. However, the cellular monolayer was restored completely within a few days (6).

The interaction between interferon and cells results in an intracellular signal transduction which is regulated by a cascade of phosphorylation and dephosphorylation reactions and will finally result in the transcription of specific genes (14). The process of signal transduction and subsequent transcription triggered by interferon can be influenced by kinase inhibitors. The kinase inhibitor 2-aminopurine has been described as a selective inhibitor of gene expression induced by interferons and double-stranded RNA (18). However, it was also suggested that 2-aminopurine may interfere more generally with host cell transcription or translation (23). Hence, 2-aminopurine may be able to complement the mutant virus with respect to the inhibition of host cell protein synthesis. Treatment of L929 cells with 2-aminopurine and subsequent infection with the mengovirus L deletion mutant indeed resulted in mutant virus production increasing from 1 to approximately 60% of wild-type yield. Analysis of protein synthesis showed that, after treatment of L929 cells with 2-aminopurine, host cell translation was completely inhibited after mutant virus infection whereas viral translation was not affected. In a study of the function of poliovirus 2A, a viable mutant which did not cleave eIF-4G was constructed (10). Similar to the mengovirus L deletion mutant, this 2A mutant was incapable of inhibiting host cell translation completely and viral protein synthesis was reduced. As for the effect of 2-aminopurine in L deletion mutant mengovirus-infected L929 cells, treatment of cells with 2-aminopurine before infection with the poliovirus 2A mutant resulted in the rescue of poliovirus protein synthesis and in the complete host cell translation shutoff (10).

Recently, Chen et al. reported that the cardiovirus leader peptide contains a zinc binding motif (1). Although the significance of this motif is unclear, it was suggested that the leader peptide possesses RNA binding capacities. Remarkably, human rhinovirus 2A, which is equivalent to enterovirus 2A, is a zinc-containing enzyme as well, and 2A activity fully depends on zinc binding (17). Enhancement of viral translation by poliovirus 2A and aphthovirus L has also been described elsewhere (3, 22). Recently, Ziegler et al. demonstrated that enhancement of viral translation by enterovirus and rhinovirus $2A^{pro}$ depends on the $2A^{pro}$-mediated cleavage of cellular proteins like eIF-4G (21). In conjunction with the result of the work of O'Neill and Racaniello (10) described above, it seems that enhancement of viral translation and inhibition of host cell protein synthesis are two features of the same process. Al-

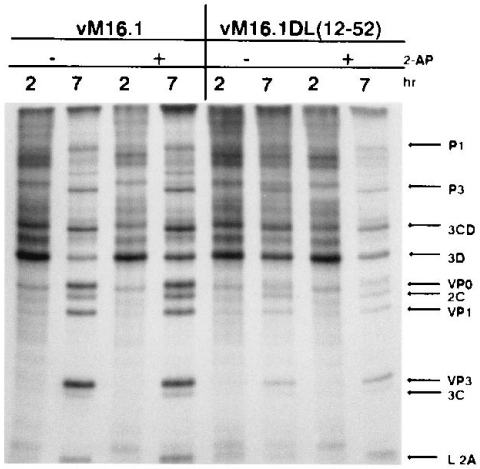

FIG. 5. Protein synthesis in 2-aminopurine (2-AP)-treated cells. L929 cells were treated with 10 mM 2-aminopurine at 2 h preinfection. Cells were infected at an MOI of 50 and grown at 36°C. At 2 and 7 h postinfection, [^{35}S]methionine labeling was performed as described for Fig. 3.

though the mechanism of enhancement of translation is still unknown, binding of the 2A protein to the viral RNA may be essential in this process. A number of translation initiation factors and other cellular proteins, like the polypyrimidine-binding protein and the La autoantigen, are involved in the translation initiation of the picornaviral RNA (7, 20). Viral peptides may be necessary to recruit these cellular factors or mediate binding of the cellular proteins to the viral RNA. The function of the cardiovirus leader peptide may be similar to the function of the enterovirus 2A and the aphthovirus L proteins. Binding of the cardiovirus leader peptide to the viral RNA may be necessary for the recruitment of one or more cellular factors involved in viral translation. The affinity of cellular translation initiation factors for the cardiovirus internal ribosome entry site may be enhanced by the leader peptide. This enhancement may be essential especially when a restricted host factor is involved. Scheper et al. indeed reported that inhibition of host cell translation in cardiovirus-infected cells depends partly on competition between viral RNA and cellular mRNA for restricted host factors, like eIF-2B (15). Early in infection, only minor amounts of viral RNA are present. So, leader peptide-mediated binding may be essential if host cell translation is to be shut off. The cardiovirus leader peptide seems to be involved in this inhibition of host cell protein synthesis, but the way in which the protein acts still remains to be elucidated.

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REFERENCES

1. **Chen, H. H., W. P. Kong, and R. P. Roos.** 1995. The leader peptide of Theiler's murine encephalomyelitis virus is a zinc-binding protein. J. Virol. **69:**8076–8078.
2. **Duke, G. M., and A. C. Palmenberg.** 1989. Cloning and synthesis of infectious cardiovirus RNAs containing short, discrete poly(C) tracts. J. Virol. **63:**1822–1826.
3. **Hambidge, S. J., and P. Sarnow.** 1992. Translational enhancement of the poliovirus 5′ noncoding region mediated by virus-encoded polypeptide-2A. Proc. Natl. Acad. Sci. USA **89:**10272–10276.
4. **Kaminski, A., G. J. Belsham, and R. J. Jackson.** 1994. Translation of encephalomyocarditis virus RNA: parameters influencing the selection of the internal initiation site. EMBO J. **13:**1673–1681.
5. **Kirchweger, R., E. Ziegler, B. J. Lamphear, D. Waters, H. D. Liebig, W. Sommergruber, F. Sobrino, C. Hohenadl, D. Blaas, R. E. Rhoads, and T. Skern.** 1994. Foot-and-mouth disease virus leader proteinase: purification of the Lb form and determination of its cleavage site on eIF-4γ. J. Virol. **68:**5677–5684.
6. **Kong, W. P., G. D. Ghadge, and R. P. Roos.** 1994. Involvement of cardiovirus leader in host cell-restricted virus expression. Proc. Natl. Acad. Sci. USA **91:**1796–1800.
7. **Meerovitch, K., Y. V. Svitkin, H. S. Lee, F. Leibkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg.** 1993. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. J. Virol. **67:**3798–3807.
8. **Merrick, W. C.** 1992. Mechanism and regulation of eukaryotic protein synthesis. Microbiol. Rev. **56:**291–315.
9. **Muñoz, A., and L. Carrasco.** 1981. Protein synthesis and membrane integrity in interferon-treated HeLa cells infected with encephalomyocarditis virus. J. Gen. Virol. **56:**153–162.
10. **O'Neill, R. E., and V. R. Racaniello.** 1989. Inhibition of translation in cells infected with a poliovirus $2A^{pro}$ mutant correlates with phosphorylation of the alpha subunit of eucaryotic initiation factor 2. J. Virol. **63:**5069–5075.
11. **Reed, L. J., and H. A. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
12. **Rueckert, R. R.** 1990. Picornaviridae and their replication, p. 507–547. *In* B. N. Fields (ed.), Virology. Raven Press, New York.
13. **Ryan, M. D., A. M. Q. King, and G. P. Thomas.** 1991. Cleavage of foot-and-mouth disease virus polyprotein is mediated by residues located within a 19 amino acid sequence. J. Gen. Virol. **72:**2727–2732.
14. **Samuel, C. E.** 1991. Antiviral actions of interferon; interferon-regulated cellular proteins and their surprisingly selective antiviral activities. Virology **183:**1–11.
15. **Scheper, G. C., A. A. M. Thomas, and H. O. Voorma.** 1991. The 5′ untranslated region of encephalomyocarditis virus contains a sequence for very efficient binding of eukaryotic initiation factor eIF-2/2B. Biochim. Biophys. Acta **1089:**220–226.
16. **Schmidt, N. J.** 1979. Cell culture techniques for diagnostic virology, p. 65–139. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Washington, D.C.
17. **Sommergruber, W., G. Casari, F. Fessl, J. Seipelt, and T. Skern.** 1994. The 2A proteinase of human rhinovirus is a zinc containing enzyme. Virology **204:**815–818.
18. **Tiwari, R. K., J. Kusari, R. Kumar, and G. C. Sen.** 1988. Gene induction by interferons and double-stranded RNA: selective inhibition by 2-aminopurine. Mol. Cell. Biol. **8:**4289–4294.
19. **Van der Werf, S., J. Bradley, E. Wimmer, F. W. Studier, and J. J. Dunn.** 1986. Synthesis of infectious poliovirus RNA by purified T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **83:**2330–2334.
20. **Witherell, G. W., A. Gil, and E. Wimmer.** 1993. Interaction of polypyrimidine tract binding protein with the encephalomyocarditis virus mRNA internal ribosomal entry site. Biochemistry **32:**8268–8275.
21. **Ziegler, E., A. M. Borman, F. G. Deliat, H. D. Liebig, D. Jugovic, K. M. Kean, T. Skern, and E. Kuechler.** 1995. Picornavirus 2A proteinase mediated stimulation of internal initiation of translation is dependent on enzymatic activity and the cleavage of cellular products. Virology **213:**549–557.
22. **Ziegler, E., A. M. Borman, R. Kirchweger, T. Skern, and K. M. Kean.** 1995. Foot-and-mouth disease virus Lb proteinase can stimulate rhinovirus and enterovirus IRES-driven translation and cleave several proteins of cellular and viral origin. J. Virol. **69:**3465–3474.
23. **Zinn, K., A. Keller, L. Whittemore, and T. Maniatis.** 1988. 2-Aminopurine selectively inhibits the induction of β-interferon, c-fos, and c-myc gene expression. Science **240:**210–213.