Fate of mRNA of L-Cells Infected with Mengovirus

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Mengovirus infection of L-cells results in an inhibition of host protein synthesis which is detectable *in vivo* by a decreased rate of incorporation of radioactive amino acids into acid-insoluble material and by a concomitant reduction in polysome content. The inhibition of host protein synthesis occurs early in the infection cycle, at a time when there is little synthesis of viral proteins. In this paper the stability of polyadenylic acid [poly(A)]-containing mRNA of uninfected L-cells and cells infected with mengovirus is compared. Our results suggest that there is no increase in the rate of degradation of cellular mRNA upon virus infection. The continued integrity of host mRNA throughout infection was verified by acrylamide gel electrophoresis.

Most lytic animal viruses inhibit cellular protein synthesis. These include vaccinia (27), herpes simplex (31), the arboviruses (18) and most, if not all, of the picornaviruses, such as poliovirus (24, 25) and mengovirus (10). In the case of vaccinia (21), the protein synthesis inhibitor appears to be closely associated with the virion and is brought into the cell during infection. On the other hand, host protein synthesis shut off by picornaviruses, requires the synthesis of a viral gene product, since inhibitors that block the translation of the viral genome also prevent the shutoff (10, 25).

Coincident with the diminished rate of amino acid incorporation into cellular proteins in picornavirus-infected cells there is a decrease in polysomes (5, 14, 32). Host ribosomes do not appear to be inactivated, since they form polysomes with viral RNA and manufacture viral proteins throughout the growth cycle of the virus (30). Using cycloheximide to decrease the rate of chain elongation, Leibowitz and Penman have shown that the reassembly of some host polysomes can occur (14). This suggests that the inhibition of host protein synthesis is not simply a result of nucleolytic activities which degrade host mRNA.

Host RNA synthesis is also reduced during picornavirus infection (9, 10). The rapid decrease in host protein synthesis early in infection, however, cannot be a result of the inhibition of host mRNA synthesis. Mammalian mRNA is very stable: its half-life in growing L-cells has been estimated to be 10 to 20 h (11; this publication) and that of HeLa cells has been estimated to be 2 to 3 days (22). We would

thus expect cellular message to continue to participate in protein synthesis throughout the 8-h growth cycle of a picornavirus. The decrease in protein-synthesizing capacity of infected cells must reflect, therefore, either a loss of preexisting host cell mRNA, or a regulation of translation of that RNA.

Recently, it has been shown that most of the cellular mRNA molecules contain 3' terminal polyadenylic acid [poly(A)]-rich sequences (7, 8, 13, 15). A simple assay based on the ability of these poly(A)-rich mRNA molecules to anneal to polyuridylic acid [poly(U)]-impregnated filters has been developed to measure cellular mRNA (28). Making use of these developments, we have investigated the fate of preexisting L-cell mRNA after infection by mengovirus.

MATERIALS AND METHODS

Virus and cell culture. Mouse L-929 cells were grown in suspension culture in minimal essential medium (MEM) for suspension culture, supplemented with 10% fetal calf serum (FCS). Mengovirus, small plaque strain, was propagated in suspension cultures of L-929 cells. For stock preparations of virus, cultures of L-929 cells were pelleted by centrifugation at $1,000 \times g$ for 10 min, washed once with serum-free MEM, and resuspended at 2×10^7 cells/ml in fresh MEM. The virus was adsorbed at a multiplicity of infection (MOI) of 10 PFU/cell for 30 min at 37°C. The cells were then diluted to 1×10^6 cells/ml with MEM for suspension culture containing 10% FCS (complete medium) and incubated at 37°C for 20 h. The resulting lysate was stored at -20°C for further use.

Infectious center assay. The detection of individual infected cells was achieved by plating infected cells on confluent monolayers of L-cells under plaque assay conditions. Suspension culture cells were in-

fecting under conditions just described. After the adsorption period, they were diluted into complete medium and incubated for 1 h at 37 C. The cells were then centrifuged at $1,000 \times g$ for 10 min and resuspended in MEM containing anti-encephalomyocarditis (EMC) antibody, which had previously been shown to inactivate mengovirus. The cells were incubated for 30 min at 37 C and then washed three times by centrifugation and resuspension in complete medium. They were ultimately resuspended in a small volume of complete medium and counted. Appropriate dilutions were made in complete medium. Samples of 1.5 ml were added to washed confluent monolayer cultures, and the cultures were incubated for 2 h at 37 C to permit attachment of infected cells. The medium was gently aspirated, and an agarose overlay consisting of 0.7% agarose in MEM containing 5% FCS was added. After 2 to 3 days at 37 C the cultures were stained for 1 h with a solution of neutral red (1:10,000) in phosphate-buffered saline (PBS), and the plaques were scored.

Incorporation of radioactive amino acids into whole cells. Protein synthesis *in vivo* was measured by the incorporation of [^3H]amino acids into acid-insoluble material. Cells were cultured and infected as above, except that in all experiments the MOI was 25 PFU/cell. After the adsorption period, the cells were diluted in fresh complete medium. At various times, 1.5-ml samples were removed and pulsed with 25 μCi of [^3H]amino acid mixture for 15 min at 37 C. Duplicate 0.5-ml samples were then removed and diluted with 3 ml of cold PBS. The cells were pelleted by centrifugation and resuspended in 0.5 ml of PBS. Protein was precipitated with 5% trichloroacetic acid, and the samples were heated for 10 min at 90 C, filtered on membrane filters (Millipore Corp.), and washed with 5% trichloroacetic acid. Filters were dried and counted in Omnifluor-toluene.

Preparation and detection of poly(A)-containing host mRNA from infected and uninfected cells. L-cells were treated with actinomycin D at 90 ng/ml for 30 min and then pulsed with 0.5 μCi /ml of [^3H]uridine for 1.5 h. The cells were pelleted by centrifugation, washed twice with MEM, resuspended at 5×10^7 cells/ml, and divided into two portions. One was infected with mengovirus at a multiplicity of 25 PFU/cell and the other was sham infected. Actinomycin D at 90 ng/ml and cordycepin at 25 μg /ml were present in the medium from the start of virus adsorption to the preparation of the lysates. After virus adsorption at 37 C for 30 min, the cells were diluted to 1×10^6 /ml in complete medium and further incubated at 37 C.

Cell extracts were prepared from 100-ml portions of the above cultures as described by J. Kates (personal communication). Cells were centrifuged at $1,000 \times g$, washed once with PBS, and lysed at a concentration of 5×10^7 /ml in 0.5% Triton X-100, 150 mM NaCl, 50 mM Tris-hydrochloride (pH 8.5), and 1.5 mM magnesium acetate. The lysate was centrifuged for 10 min at $12,000 \times g$ at 4 C. EDTA and sodium dodecyl sulfate (SDS) were added to the supernatant fraction to a final concentration of 10 mM and 0.5%, respectively.

The lysate was extracted twice with an equal volume of a 1:1 mixture of phenol and chloroform saturated with 10 mM sodium acetate (pH 6.0), 0.1 M NaCl, and 1 mM EDTA. The water phase was adjusted to 0.5 M in NaCl, and the nucleic acids were precipitated by the addition of 2 volumes of 95% ethanol. The samples were stored for at least 2 h at -20°C . The precipitates were collected by centrifugation at $12,000 \times g$ for 20 min and resuspended in 10 mM Tris-hydrochloride, pH 7.5, and 120 mM NaCl. The preparation of poly(U) glass fiber filters and the annealing procedure were carried out as previously described (28).

[^{14}C]rRNA. [^{14}C]rRNA markers were prepared from L-cells grown for 24 h in complete medium supplemented with [^{14}C]uridine, 0.5 μCi /ml. Cells (7.5×10^7) were lysed and centrifuged at $12,000 \times g$ as described above. Ribosomes, pelleted by centrifugation for 3 h at $85,000 \times g$, were resuspended in 50 mM Tris-hydrochloride (pH 7.5), 12.5 mM magnesium acetate, 880 mM KCl, and 1 mM dithiothreitol and incubated for 5 min at 37 C. The incubation mixture was layered over 2 ml of 20% sucrose in the above buffer and the ribosomes were pelleted by centrifugation at $80,000 \times g$ for 19 h. RNA was extracted as described above starting with the EDTA and SDS step.

[^3H]mengovirus RNA. Cells were grown to confluency in a roller bottle (approximate cell number per roller bottle 2×10^8) in MEM containing 10% FCS. The medium was removed, and the cell layer was washed with PBS. The cells were infected with an MOI of 10 PFU/cell in 15 ml of MEM. After 30 min at 37 C, 30 ml of MEM containing 5% FCS was added. At 5 h, 0.3 μCi of [^3H]uridine was added and the infection continued until 20 h postinfection. The cells were frozen and thawed in an acetone-dry ice bath to release the intracellular virus. The virus was purified as described by Kerr and Martin (12). The viral RNA was extracted from the purified virus by the same method used for the preparation of cellular poly(A)-containing RNA, starting with the addition of EDTA and SDS.

Sucrose gradient analysis of polysomes. Cell extracts were prepared as described above for the isolation of poly(A)-containing RNA. The extracts were centrifuged at $12,000 \times g$ for 10 min and 0.75-ml portions of the supernatant fraction were analyzed on 11-ml 15 to 45% sucrose gradients containing 20 mM Tris-hydrochloride (pH 7.4), 50 mM KCl, and 2 mM magnesium acetate. The gradients were collected using an ISCO gradient fractionator and UV analyzer and a Heathkit recorder. Fractions containing either monosomes and ribosomal subunits or polysomes were pooled. The pooled fractions were adjusted to 100 mM NaCl, 1 mM EDTA, and 0.5% SDS and precipitated with 2 volumes of 95% ethanol. After 2 h at -20°C , the precipitates were collected by centrifugation at $20,000 \times g$ for 20 min. The pellets were dissolved in 100 mM NaCl, 1 mM EDTA, 0.5% SDS, and 10 mM Tris-hydrochloride (pH 7.4), and extracted as described above.

When larger quantities of polysomal poly(A) RNA were required, 4-ml portions of cell extract were

analyzed on 28-ml 15 to 30% sucrose gradients prepared in the above buffer.

Purification of poly(A)-containing RNA and its analysis by gel electrophoresis. Poly(A)-containing RNA was annealed to and eluted from poly(U) glass fiber filters as described (28, 29). Carrier RNA (1 mg of *Escherichia coli* tRNA) was added to the eluate and nucleic acids were precipitated with 2 volumes of 95% ethanol. The precipitate was collected as described above and resuspended in 10 mM Tris-hydrochloride (pH 7.5), 120 mM NaCl, and analyzed on 2.5% acrylamide gels as described by Loening (16, 17). The electrophoresis buffer (pH 7.8) consisted of 36 mM Tris-hydrochloride, 30 mM NaH_2PO_4 , 1 mM EDTA, and 0.2% SDS. The gels were cut into 1-mm sections, and each section was dissolved at 50 C in 0.1 ml of 30% H_2O_2 and counted in 4 ml of PCS.

Analysis of in vivo labeled proteins by gel electrophoresis. Uninfected or infected cells (10^7) were harvested by centrifugation at $1,000 \times g$ for 10 min at room temperature. They were resuspended at 4×10^6 /ml in amino acid-free medium to which was added [^3H]amino acid mixture (NET 250) at 10 $\mu\text{Ci}/\text{ml}$ and unlabeled methionine, tryptophan, and cysteine in the same molar quantities as the radioactive amino acids. The cells were incubated in the radioactive medium at 37 C for 7 min. Complete medium was then added to dilute the cells to 10^6 /ml, and the incubation was continued for 45 min. The cells were pelleted by centrifugation at 4 C, washed in cold PBS, repelleted, and resuspended in 0.5 ml of 0.01 M phosphate buffer (pH 7.2) and 0.1% mercaptoethanol. The suspension was made 1% in SDS and heated at 90 C for 2 min. The dissolved proteins were analyzed on urea-SDS polyacrylamide gels by the method of Maizel (19).

Chemicals and radioisotopes. Cordycepin (3' deoxyadenosine) was purchased from Sigma Chemical Co., St. Louis, Mo.; actinomycin D was from General Biochemicals, Chagrin Falls, Ohio; and poly(U) was from Miles Laboratories, Kankakee, Ill. [^3H]uridine, 20 Ci/mmol, [^{14}C]uridine, 54 mCi/mmol, and PCS were obtained from Amersham-Searle Corp., Arlington Heights, Ill. L-Amino acid mixture- ^3H (G), NET 250, was purchased from New England Nuclear, Boston, Mass. Anti-EMC antibody was the generous gift of Charles Buckler, National Institutes of Allergy and Infectious Diseases. Whatman #GF/C glass fiber filters were used.

RESULTS

Use of cordycepin to terminate radioactive labeling of poly(A)-containing RNA. In order to study the fate of host cell mRNA in mengovirus-infected L-cells, conditions were established under which it was possible to examine the decay of poly(A)-containing RNA. This required a method by which poly(A)-containing RNA could be labeled with a radioactive substrate during a defined period and further incorporation of radioactivity after that period could be prevented. Host cell mRNA was la-

beled with [^3H]uridine for 1.5 h in the presence of low levels of actinomycin D to suppress the synthesis of rRNA (6). Labeling of the cytoplasmic poly(A)-containing RNA was stopped by transferring the cells to medium lacking [^3H]uridine and containing 25 μg of cordycepin per ml. Cordycepin was used to terminate the labeling of cytoplasmic poly(A)-containing RNA because simple dilution of the radioactive uridine with a large excess of unlabeled uridine at the end of the labeling period is ineffective in preventing the continued incorporation of radioactivity into RNA (6, 22). It has been shown that cordycepin treatment of HeLa cells results in an inhibition of the appearance of new mRNA in the cytoplasm (23) and a reduction in the length of poly(A) sequence found on the small amount of mRNA which does accumulate (1).

Figure 1 shows that cordycepin treatment of

FIG. 1. Inhibition of accumulation of cytoplasmic poly(A)-containing RNA by cordycepin. Cells were labeled with [^3H]uridine as described in Materials and Methods. After the labeling period, the cells were placed in nonradioactive medium and cordycepin, 25 $\mu\text{g}/\text{ml}$, was added. At the times indicated, portions were removed and the cytoplasmic RNA was measured. For each RNA fraction, the amount of trichloroacetic acid-precipitable radioactivity (O) and radioactivity which could be bound to poly(U) glass fiber filters (Δ) were determined as described in Materials and Methods.

[^3H]uridine-labeled L-cells prevents further accumulation of [^3H]poly(A)-containing RNA in the cytoplasm. As anticipated, the total acid-insoluble radioactivity of the cytoplasm increases after the removal of the radioactive uridine because of the continued labeling and transport of non-poly(A)-containing RNA such as rRNA and tRNA.

Making use of these pulse-chase conditions, we have measured the half-life of poly(A)-containing RNA in uninfected L-cells (Fig. 2). A half-life of approximately 20 h was observed.

Effect of cordycepin on viral and host functions. In order to make use of the above method to study the fate of host cell mRNA during mengovirus infection, it was necessary to determine the effect of cordycepin on viral growth and on the virus-mediated inhibition of cellular protein synthesis. Figure 3 shows that 25 μg of cordycepin per ml has no effect on either the length of the growth cycle or on the ultimate yield of virus. The average yield in three experiments was 1,150 PFU/cell.

The effect of cordycepin on the mengovirus-mediated inhibition of amino acid incorporation was then examined. Figures 4A and B depict the rates of incorporation of amino acids into proteins of uninfected and infected cells in the absence or presence of cordycepin during a 15-min pulse period. As can be seen from the two lower curves in Fig. 4A and B, virus infection decreases the rate of amino acid incorporation. In both cases the percentage of inhibition at 4 h after infection compared to their respective zero time points is approximately the same (50%). As also shown in Fig. 4A and B, if

FIG. 2. Half-life of poly(A)-containing RNA of uninfected cells. Cells were labeled and RNA was extracted and analyzed as for Fig. 1 at 0, 8, and 20 h after the addition of cordycepin.

FIG. 3. Growth curve of mengovirus in L-cells in the presence and absence of cordycepin. Cells were infected as described in Materials and Methods. When used, cordycepin, 25 $\mu\text{g/ml}$, was added after adsorption of the virus. Portions of the infected cells were harvested and frozen at the times indicated. The titer of virus in each portion was determined as described in Materials and Methods. Symbols: O, no cordycepin; Δ , 25 μg of cordycepin per ml.

FIG. 4. Host cell protein synthesis inhibition by mengovirus in the presence and absence of cordycepin. The *in vivo* incorporation of [^3H]amino acids at various times postinfection was measured under pulse-labeling conditions as described in Materials and Methods.

one focuses on the zero time points, incorporation into protein of both infected and uninfected cultures is decreased approximately 25 to 35% in the presence of cordycepin. The effect of cordycepin on the rate of incorporation in both infected and uninfected cultures may result

from a decreased aminoacylation of transfer RNA. Cordycepin is probably phosphorylated in the cell and may then act as an analogue of ATP, thus competing with ATP for binding to aminoacyl-tRNA synthetases in the charging of tRNA.

The effect of cordycepin on the polysome content of infected and uninfected cells was also compared. Polysomes were isolated from cytoplasmic extracts from infected and uninfected cells by sucrose gradient centrifugation as described in Materials and Methods. The polysome content of each extract was estimated by pooling those fractions of the sucrose gradient containing polysomes and measuring its absorbance at 260 nm. Planimetric measurements of the recorded polysome profiles were also employed to quantitate the amount of polysomes under the studied conditions. These agreed fairly well with the absorbance measurements at 260 nm.

The results (Fig. 5) were qualitatively the same as those shown in Fig. 4. Three hours after virus infection there is a large decrease (approximately 50%) in the polysome content of host cells (compare Fig. 5A to Fig. 5B and D). As in Fig. 4, cordycepin itself causes a depression in polysome content in both uninfected (compare Fig. 5A and C) and infected (Fig. 5B and D) cells. Here again, this may arise from a decreased level of transfer RNA charging. In each case the reduction in polysome content is accompanied by an increase in single ribosomes.

To examine whether cordycepin might have any selective inhibitory effect on particular classes of proteins, the proteins of uninfected cells and cells infected for 5 h were labeled with a mixture of [^3H]amino acids as described in Materials and Methods and then analyzed by acrylamide gel electrophoresis. The results (Fig. 6A and B) show that cordycepin inhibits the synthesis of both host proteins and viral pro-

FIG. 5. Sucrose gradient analysis of cytoplasmic extracts of infected and uninfected cells. Cells were harvested 3 h after mock adsorption, virus adsorption, and/or cordycepin treatment at $25 \mu\text{g/ml}$. Extracts were prepared and sucrose gradient analysis was carried out as described in Materials and Methods. Gradients were centrifuged for 100 min at $160,000 \times g$ in an International SB 283 rotor.

FIG. 6. Effect of cordycepin on the synthesis of proteins in infected and uninfected cells. The proteins of infected and uninfected cells with or without cordycepin treatment at the time of infection were labeled with [^3H]amino acids at 5 h after adsorption or mock adsorption and prepared and analyzed on polyacrylamide gels as described in Materials and Methods. Electrophoresis was carried out at 5 mA per gel for 10 h.

teins to approximately the same extent. This is to be expected if cordycepin is affecting a reaction such as the aminoacylation of tRNA, which is required for the synthesis of both host and viral proteins. The viral protein peaks in Fig. 6B are lettered according to the terminology of Butterworth et al. (3) for EMC virus, since the *in vivo* synthesized mengovirus proteins are nearly identical to those made by

EMC virus in terms of molecular weight, size, and precursor relationships (J. Lucas-Lenard, manuscript in preparation).

The observation that cordycepin has no effect on either the length of the growth cycle or on the ultimate yield of virus probably reflects the high noninfectious particle to infectious particle ratio (approximately 100:1) (26) in the infected cell. Sufficient proteins and RNA are probably synthesized to maintain the same yield of infectious units per cell in spite of the inhibitory effect of cordycepin on protein synthesis.

These results show that although cordycepin inhibits protein synthesis in both uninfected and infected cells, the effect appears to be nonselective, depressing the synthesis of all proteins equally and resulting in a breakdown of host polysomes. Since the viral-mediated shut-off of host protein synthesis is readily observable in the presence of cordycepin, it was decided that this system could be used to study the fate of host mRNA during mengovirus-mediated inhibition of host protein synthesis.

Efficiency of infection. Since the shut-off of host protein synthesis after mengovirus infection is not total, it was essential to determine whether all or only a fraction of the cells are infected. The efficiency of infection was determined using an infectious center assay as described in Materials and Methods.

As shown in Table 1, at multiplicities of infection of either 10 or 25, the number of plaques found slightly exceeds the number expected if all the cells plated served as infectious centers. This discrepancy may indicate the range of error in counting the cells prior to dilution.

Analysis by acrylamide gel electrophoresis of the proteins made at various times after infection also suggests that all or most of the cells are infected. As shown in Fig. 7, by 3 h after infection there is a large reduction in the labeling with radioactive amino acids of all

TABLE 1. Infectious center assay^a

Dilution series	MOI	No. of cells plated	No. of plaques found
1	10	100	123
			120
2	10	106	140
			109
1	25	98	100
			109
2	25	104	110
			159

^aThe conditions are described in Materials and Methods.

FIG. 7. Acrylamide gel analysis of proteins synthesized in uninfected and infected cells. Infected and uninfected cells were labeled with [^3H]amino acids at the times indicated. The labeled cells were dissolved in SDS-mercaptoethanol and analyzed on SDS-urea gels as described in Materials and Methods. Electrophoresis was carried out at 5 mA per gel for 10 h.

species of proteins. By 4 h the change-over to viral protein synthesis is evident, and by 5 h most of the proteins made are viral. The progression from host to viral synthesis would not be this pronounced if a fraction of cells were uninfected and continued to synthesize host proteins at a normal rate.

Stability of host poly(A)-containing RNA.

The relative stability of host mRNA after infection was determined by comparing the amount of poly(A)-containing cytoplasmic RNA of infected and uninfected cells at 15 min, 1.5 h, 3 h, and 5 h after adsorption or mock adsorption. The data (Table 2) indicate that the ratio of poly(A)-containing RNA from infected cells to that of uninfected cells does not vary significantly for the first 3 h of infection. Thus, the loss of protein-synthesizing capacity of the host cell, which is detected in the first hour after infection and reaches a 40% inhibition by 3 h, is not paralleled by a corresponding loss of poly(A)-containing RNA molecules.

At 5 h after infection there is an increase in the ratio of poly(A)-containing RNA in infected cells to that in uninfected cells over and above

TABLE 2. Relative stability of poly(A)-containing RNA of infected (I) and uninfected (U) cells^a

Time after adsorption	I/U ^b (counts/min bound)
15 min	1.19
1.5 h	1.17
3 h	1.16
5 h	1.36

^a The host poly(A)-containing RNA was labeled with [^3H]uridine. At the time periods indicated, portions were removed and the labeled RNA was isolated and measured as described in Materials and Methods.

^b These ratios represent averages of two to four experiments.

that seen at the earlier time periods. As will be shown later, this results from the synthesis of viral RNA in infected cells.

Gel analysis of poly(A)-containing RNA present after infection. Although the data shown in Table 2 suggests that the poly(A)-containing mRNA of the host cell is stable throughout 5 h of infection, it was necessary to deter-

mine whether the radioactivity bound to poly(U) filters after infection is entirely host mRNA or a mixture of host and newly synthesized and labeled viral RNA which could arise from the existing [^3H]uridine pools. Studies in this laboratory (unpublished results) and other laboratories (20) have indicated that mengovirus, like poliovirus (2, 33), contains a 3' terminal poly(A) sequence. The synthesis of this poly(A) terminus in mengovirus is apparently

insensitive to cordycepin, as in the case of poliovirus (23).

The cytoplasmic poly(A)-containing RNA of both infected and uninfected cells, harvested at 3 h after adsorption, was prepared by annealing the RNA to poly(U)-containing filters and eluting the bound RNA with formamide. The resulting RNA fractions were analyzed on 2.5% polyacrylamide gels (Fig. 8). In both cases the eluted RNA is characteristic of uninfected cell

FIG. 8. Polyacrylamide gel electrophoresis of poly(A)-containing RNA isolated at 3 h after infection. Radioactive poly(A)-containing RNA of infected and uninfected cells was isolated at 3 h after virus adsorption or mock adsorption as described in Materials and Methods. The preparation of [^3H]mengovirus virion RNA and [^{14}C]RNA markers is as described also in Materials and Methods. Electrophoresis was carried out for 3.5 h at 5 mA per gel.

mRNA. No viral RNA, which would migrate more slowly than the 28S rRNA marker, is detectable at this time. These data and those of Table 2 indicate that the host poly(A)-containing RNA is not extensively degraded during the first 3 h of infection. The data, however, do not exclude the possibility that a few nucleotides are removed from the 5' end of the RNA by virus-induced exonucleases present in the extracts of infected cells.

Similar preparations of poly(A)-containing RNA were purified from uninfected cells and cells infected for 5 h (Fig. 9A and B). At this time after infection there is a distinct increase in the radioactivity located in the high-molecu-

FIG. 9. Polyacrylamide gel electrophoresis of poly(A)-containing RNA isolated at 5 h after infection. Poly(A)-containing RNA from infected and uninfected cells at 5 h after adsorption was prepared and analyzed as in Fig. 8. The rRNA markers were analyzed on a separate gel run at the same time.

lar-weight region of the gel (note slices 5 to 13). This probably represents newly synthesized mengovirus virion RNA (see Fig. 8c for the behavior of mengovirus RNA). Since the intact mengovirus RNA migrates in a region of the gel in which few host RNA molecules appear, an estimate of the amount of viral RNA occurring in poly(A)-containing RNA preparations 5 h postinfection can be made by plotting the difference in counts in the gels presented in Fig. 9. The data from two such determinations are presented in Table 3. Mengovirus RNA accounts for 10 to 20% of the poly(A)-containing RNA detected in cells infected for 5 h. The nonviral RNA [host poly (A)-containing RNA] in the infected cell preparation is estimated by subtracting the virus-specific counts from the total counts in the gel (Table 3). The ratio of infected cell host poly(A)-containing RNA estimated by this method to that of uninfected cells was 1.25 and 1.02 in these two determinations. Thus, there is little decay of host poly(A)-containing RNA even as late as 5 h after infection.

Amount of poly(A)-containing RNA associated with polysomes in infected and uninfected cells in the presence of cordycepin. In order to determine if infection results in the displacement of poly(A)-containing RNA from cellular polysomes, the distribution of such RNA in polysomes was investigated. Sucrose gradients of extracts from uninfected and infected cells were prepared. The region corresponding to polysomes was pooled and the poly(A)-containing RNA was extracted as described in Materials and Methods. Table 4 presents data from the analysis of polysomes prepared at 3 and 5 h after infection. In each case, the polysomes of infected cells were compared with those of uninfected cells treated with cordycepin for the same number of hours (Fig. 5C and D). The data of Table 4 suggest that there is little reduction in the poly(A)-containing RNA associated with polysomes at 3 h after infection. This is to be compared with a 40% reduction in amino acid incorporation (Fig. 4B)

TABLE 3. Estimate of the contribution of viral RNA to the poly(A)-containing RNA at 5 h after infection^a

Expt	Counts/min poly(A)-RNA			Infected cell counts/min which are viral (C/A × 100) (%)	Counts/min infected cell host RNA (A-C)	Ratio I host/U host (A-C/B)
	Infected cell (A)	Uninfected cell (B)	Viral RNA ^b (C)			
1	92,244	58,429	18,767	20.3	73,477	1.25
2	141,216	122,973	15,914	11.3	125,302	1.02

^a Poly(A)-containing RNA of infected and uninfected cells was prepared and analyzed on polyacrylamide gels as described in Materials and Methods.

^b The number of counts migrating in the viral RNA region was estimated from difference plots of uninfected and infected cells.

TABLE 4. *Distribution of poly(A)-containing RNA in sucrose gradients^a*

Expt	Time after adsorption (h)	Poly(A) counts/min associated with polysomes (%)	
		Uninfected cells	infected cells
1	3	60	58
2	3	52	56
1	5	50	38
2	5	55	38

^a The conditions are described in Materials and Methods.

and a 20 to 30% reduction in polysomes (Fig. 5C and D) at this time. The interpretation that all of the host mRNA remains in the polysome fraction at this time is not firm. It is possible that our methodology might not have enabled us to detect a small displacement of mRNA from polysomes. Also, the separation of monosomes and disomes was poor in our sucrose gradients. A 20% contamination by monosomes containing mRNA could have obscured the expected 20 to 30% displacement of mRNA out of the polysomes.

At 5 h after infection (Table 4) there is a noticeable reduction in the percentage of poly(A)-containing RNA associated with polysomes. As seen in Table 3, 10 to 20% of the total poly(A)-containing RNA of the infected cells is viral RNA. Thus, in order to determine the percentage of host mRNA still associated with polysomes, it was necessary to locate the viral RNA in the gradients.

RNA prepared from the polysomal and monosomal regions of sucrose gradients similar to those in Fig. 5C and D was annealed to poly(U)-containing filters, eluted, and analyzed on polyacrylamide gels as above. As shown in Fig. 10C and D, all of the viral RNA is found in the polysome fraction of infected cells (gel slices 2 to 10). This permits a further calculation of the reduction of host mRNA in polysomes after infection. Five hours after infection approximately 40% of the total poly(A)-containing RNA is associated with polysomes (Table 4). From our previous calculation, 10 to 20% of this RNA is viral RNA. When the viral RNA is subtracted from the total poly(A)-containing RNA in polysomes, only 20 to 30% of the host mRNA remains in polysomes. This indicates a reduction of 40 to 60% of that found in association with the polysomes of uninfected cells.

The difference plot of infected and uninfected polysomal poly(A)-containing RNA (Fig. 10D) shows the presence of RNA peaks which are

FIG. 10. Polyacrylamide gel electrophoresis of poly(A)-containing RNA from polysomes and monosomes. The poly(A)-containing RNA was labeled, cells were infected or mock infected, and polysomes were analyzed on sucrose gradients as described in Materials and Methods. Fractions containing either monosomes and ribosomal subunits or polysomes were pooled, and the poly(A)-containing RNA was isolated. The RNA fractions were analyzed on polyacrylamide gels as described for Fig. 8. The difference between the infected and uninfected cell polysomal RNAs was determined by subtracting the counts of each slice of the uninfected cell gel from those of the corresponding slice of the infected cell gel.

smaller than mengovirus RNA (Fig. 8C). It is not clear whether these are fragmented viral RNAs or host RNAs which are synthesized in

response to infection (4). However, similar peaks were detected in a difference plot of infected and uninfected cell poly(A)-containing RNA at 3 h after infection, at which time no detectable amounts of viral RNA has been synthesized (data not shown). As shown in Table 2, infected cells seemed to contain more poly(A)-containing RNA than uninfected cells at all times after infection. The peaks in Fig. 10D may represent the "excess" poly(A)-containing RNA detected in infected cells.

DISCUSSION

The virus-mediated inhibition of host protein synthesis begins early in infection at a time when few viral proteins are being synthesized. This permits an examination of the mechanism of inhibition of translation at a time when there is little competition between the host and viral messages for initiation factors and ribosomes.

Our data indicate that preexisting host cell poly(A)-containing RNA is as stable in infected cells through 5 h of infection as it is in uninfected cells. It thus appears that an increased rate of degradation of the host poly(A)-containing RNA is neither the cause nor the result of the virus-mediated inhibition of host protein synthesis. This conclusion, however, must be taken cautiously, since our experiments do not rule out the possibility that a few nucleotides are removed from the 5' terminus of host mRNA in infected cells by a virus-induced exonuclease, thereby rendering the mRNA inactive in protein synthesis. Also, the use of cordycepin to terminate the labeling of cytoplasmic poly(A)-containing RNA has added a complication to the interpretation of our results, since cordycepin itself inhibits protein synthesis and causes a considerable decrease in host polysome content (see Fig. 4, 5, and 6).

The virus-mediated decrease in the polysome content at 3 h after infection does not appear to be accompanied by a decrease in the polysomal poly(A)-containing RNA at that time. One possible explanation for this result is that there is a lowered rate of initiation relative to elongation of protein chains in infected cells. However, a lack of sensitivity of the methodology or contamination by monosomes cannot be eliminated as possible alternative explanations. Leibowitz and Penman (14) have come to the conclusion that the primary effect of poliovirus infection on HeLa cell protein synthesis may be an inhibition of the rate of initiation.

At 5 h after infection there is a significant displacement of host poly(A)-containing RNA out of the polysomes of infected cells. Concomi-

tantly, there is considerable entry of viral RNA into polysomes. Because a large amount of mengovirus RNA is being translated at this time, there may be a competition at the initiation step of protein synthesis between host and viral mRNA. This may account for the displacement of host RNA from polysomes at late times in infection.

More poly(A)-containing RNA was found in infected cells than in uninfected cells at all time periods after infection. Late in infection part of this difference results from the synthesis of mengovirus RNA. Early in infection, however, no recognizable viral RNA is synthesized.

Contreras et al. (4) have presented data which suggest that the synthesis of several new message-like RNAs is induced immediately after infection of HeLa cells with poliovirus. The RNAs were synthesized between 15 and 40 min after infection and were detected in the cytoplasm with no delay. An estimate of the molecular weights of these molecules was not made; however, they are smaller than any of the known viral RNAs. The poly(A) content of these RNAs was not determined. It is possible that the "excess" poly(A)-containing RNAs of infected cells detected in our experiments from 15 min to 3 h after adsorption and those of Contreras et al. result from the same viral function. Work is in progress to further characterize this RNA.

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