Translation of Capped Virus mRNA in Encephalomyocarditis Virus-infected Cells

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SUMMARY

The pattern of protein synthesis in HeLa cells simultaneously infected with encephalomyocarditis virus (EMCV) and Semliki Forest virus (SFV) has been analysed throughout the time course of the infection. The ratio of the picornavirus protein γ versus the togavirus late protein C increased when the m.o.i. of EMCV was raised, and the ratio of C/γ increased with higher multiplicities of SFV. Under some conditions, the co-infected cells simultaneously synthesized the picornavirus and togavirus proteins, and the cells exclusively translated the capped 26S mRNA from SFV at the end of the co-infection experiment. The influence of the time of addition of the second virus on the relative translation of the capped and uncapped mRNAs was also studied. When HeLa cells were co-infected with 5 p.f.u./cell of EMCV and 200 p.f.u./cell of SFV, only the synthesis of SFV proteins was apparent, whereas if SFV was added 1 to 3 h later during the course of EMCV infection the cells synthesized picornavirus and togavirus proteins. If the cells were superinfected with SFV 1 h after EMCV addition, host protein synthesis was drastically inhibited after 3 h of EMCV infection. By 5 h post-infection both kinds of virus proteins were synthesized and at 7 h post-infection the cells preferentially translated the capped 26S mRNA from SFV. If cells were first infected with SFV (10 p.f.u./cell) and co-infected or superinfected at 1 h with EMCV (50 p.f.u./cell), the shut-off of host protein synthesis occurred 3 h after infection and the cells synthesized both kinds of virus proteins. However, 9 h after infection the cells synthesized SFV proteins only. When double-infected HeLa cells were placed in a hypotonic medium, they mainly synthesized the togavirus late proteins, whereas under hypertonic conditions, they translated the picornavirus RNA exclusively. These results suggest that the two kinds of mRNAs (SFV 26S mRNA and EMCV 35S mRNA) are present in the infected cells and that the relative translation of each of them depends on the external ionic conditions.

INTRODUCTION

The infection of mammalian cells with a picornavirus results in a specific inhibition of host protein synthesis (Darnell & Levintow, 1960; Leibowitz & Penman, 1971; Lawrence & Thach, 1974; Egberts et al., 1977) but the mechanism of inhibition still remains obscure. A correlation has been observed in cardiovirus (Carrasco & Smith, 1976) and Sindbis virus-infected cells (Garry et al., 1979) between the inhibition of host protein synthesis and the increase in membrane permeability to ions and low molecular weight compounds. Such a correlation is not apparent in poliovirus-infected cells (Nair et al., 1979). There is still
controversy as to whether poliovirus and EMCV inhibit host protein synthesis by similar mechanisms (Jen et al., 1980; Detjen et al., 1981).

It has been suggested that poliovirus infection leads to the specific inactivation of an initiation factor required for the translation of capped mRNAs (Helentjaris & Ehrenfeld, 1978; Rose et al., 1978; Trachsel et al., 1980). In agreement with this suggestion, poliovirus infection of HeLa cells interferes with the development of herpesvirus and leads to the inhibition of vesicular stomatitis virus (VSV) protein synthesis under some conditions (Doyle & Holland, 1972; Saxton & Stevens, 1972; Ehrenfeld & Lund, 1977). However, poliovirus infection of monkey cells does not shut off protein synthesis of simian virus 5 (SV5) under conditions where the synthesis of cellular proteins is inhibited (Choppin & Holmes, 1967). Moreover, infection of cells with mengovirus leads to interference or non-interference with vaccinia virus development and with VSV protein synthesis, depending on the conditions of infection used (Freda & Buck, 1971; Otto & Lucas-Lenard, 1980).

In the present work we have analysed the interference by EMCV with the translation of a capped mRNA, the SFV 26S mRNA, in infected cells.

METHODS

Cells and viruses. HeLa cells were propagated in Falcon culture flasks containing 6 ml Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum (Gibco) and incubated at 37 °C in a 5% CO2 atmosphere. EMCV was grown on L-929 cells in a mixture of Eagle's medium and phosphate-buffered saline supplemented with 1% newborn calf serum. SFV was grown on BHK cells in DMEM supplemented with 1% calf serum. The fraction obtained after removal of cell debris by low-speed centrifugation was used as the source of the corresponding virus.

Conditions of infection and protein labelling. Hela cells are grown in 3.5 cm diam. Petri dishes containing 2 ml DMEM supplemented with 10% newborn calf serum. When the cell monolayer was confluent, the medium was removed and the cells infected with EMCV and/or SFV in the presence of 10 μg/ml actinomycin D. The inoculum was then removed and replaced by 1 ml DMEM supplemented with 1% newborn calf serum containing 10 μg/ml actinomycin D. At the times indicated, the medium was removed and replaced by 0.5 ml methionine-free DMEM supplemented with 1% newborn calf serum containing 10 μg/ml actinomycin D. Labelling of newly synthesized proteins was carried out by incubating the cell cultures with 6 μCi [35S]methionine (Amersham International; 717 Ci/mmol; 9 mCi/ml) for 1 h at 37 °C.

Polyacrylamide gel electrophoresis (PAGE). After incubation of cells in the presence of [35S]methionine, the medium was removed and the cell monolayer washed with 2 ml phosphate buffer; the cells were dissolved in 0.1 ml 0.2 M-NaOH containing 0.1% SDS, plus 0.2 ml sample buffer (62.5 mM-Tris pH 6.8, 2% SDS, 0.1 M-dithiothreitol and 17% glycerol). Each sample was sonicated to reduce viscosity and heated at 90 °C for 2 min. Five-μl samples were analysed by PAGE on 15% polyacrylamide gels at 30 V overnight. The gels were stained and destained, and then fluorography was carried out with 2,5-diphenyloxazole/dimethyl sulfoxide (20%, w/w). The dried gels were exposed using Kodak XS-5 X-ray films. Densitometric profiles of the films were made in a microdensitometer Optronics P1700.

RESULTS

The infection of an animal cell with two different viruses leads, in many instances, to interference in the normal development of one of the viruses (Choppin & Holmes, 1967; Marcus & Carver, 1967; Freda & Buck, 1971; Doyle & Holland, 1972; Saxton & Stevens, 1972; Ehrenfeld & Lund, 1977; Otto & Lucas-Lenard, 1980). We have studied interference
Fig. 1. Time course of protein synthesis in HeLa cells co-infected with a low m.o.i. of SFV and different m.o.i.s of EMCV. Cell cultures were simultaneously doubly infected with SFV from –1 h to zero time at an m.o.i. of 5 and at the indicated m.o.i.s of EMCV (0, 10, 30, 50 and 100). Protein synthesis was estimated at the indicated times after infection (0, 2, 4, 6 and 8 h) as described in the text. The positions of SFV capsid protein C and EMCV proteins, D, E, ε, γ, H and I, are indicated on the autoradiograph of the gel. The graph shows γ/C ratios with the different m.o.i.s of EMCV used, calculated from the densitometric scans of the autoradiographs corresponding to the proteins synthesized 4 to 5 h post-infection.
Fig. 2. Time course of protein synthesis in HeLa cells co-infected with a low m.o.i. of EMCV and different m.o.i.s of SFV. Cell cultures were simultaneously doubly infected with EMCV at an m.o.i. of 5 and at the indicated m.o.i.s of SFV (0, 5, 30, 100 and 200). Protein synthesis was estimated at the indicated times after infection (0, 2, 4, 6 and 8 h) as described in the text. The positions of SFV proteins, p62, E\textsubscript{1} and C, and the EMCV proteins, D, E, \(\varepsilon\), \(\alpha\), \(\gamma\), H and I, are indicated on the autoradiograph. The graph shows \(C/\gamma\) ratios with the different m.o.i.s of SFV used, calculated from the densitometric scans of the autoradiographs corresponding to the proteins synthesized 4 to 5 h post-infection.
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at the level of protein synthesis in human HeLa cells doubly infected with a picornavirus (EMCV) and a togavirus (SFV). This system is of interest because we can follow the interference of the translation of a single species of a capped mRNA, the 26S mRNA from SFV, and the translation of a single species of a naturally uncapped mRNA, the EMCV mRNA.

Fig. 1 shows the time course of protein synthesis in HeLa cells simultaneously infected with a low m.o.i. of SFV and increasing m.o.i. of EMCV. The pattern of proteins synthesized by the infected cells depended to a considerable extent on the ratio of the different m.o.i. of the two viruses used. The ratio of the picornavirus protein γ versus the togavirus protein C was calculated from the areas obtained in the densitometric scans of the autoradiographs using a microcomputer programme. A fivefold increase in this ratio occurred when the m.o.i. of EMCV was raised to 100. Fig. 2 shows the time course of protein synthesis in cells infected with a low m.o.i. of EMCV and increasing m.o.i. of SFV. From the ratio of C/γ, it is also apparent that the synthesis of picornavirus proteins decreased when the m.o.i. of SFV was raised. Under some conditions, the infected cells simultaneously synthesized the picornavirus and togavirus proteins. It is interesting to note that under those conditions the cells translated mainly the capped 26S mRNA from SFV at later times after infection.

The influence of the time of addition of the second virus challenge on the relative translation of the capped and uncapped mRNA was analysed. Fig. 3 shows the time course of protein synthesis in HeLa cells infected with EMCV at a low m.o.i. of 5 and SFV at an m.o.i. of 200 at different times. When cells were simultaneously infected under such conditions of multiplicity with these two viruses, only the synthesis of SFV proteins was apparent, whereas if the EMCV-infected cells were superinfected with SFV at 1 or 3 h during the course of EMCV infection, those cells synthesized both kinds of proteins. When the cells were superinfected with SFV at 1 h after EMCV infection, host protein synthesis was drastically inhibited at 3 h after infection and at that time only picornavirus proteins were detected. Five h after infection the cells were synthesizing substantial amounts of EMCV proteins, and also translating the subgenomic SFV 26S mRNA. At 7 and 9 h after infection, the cells were mainly translating the SFV capped mRNA.

In the experiment in Fig. 4, the cells were infected with SFV at an m.o.i. of 10 and superinfected at different times with EMCV at an m.o.i. of 50. Under these conditions of multiplicity the shut-off of host synthesis induced by EMCV occurred 3 h after infection. At this time, the cells co-infected with EMCV and SFV preferentially translated the capped 26S mRNA from SFV. Five h after infection, both kinds of mRNA were translated and, interestingly, by 9 h the cells exclusively translated the capped virus mRNA. Addition of EMCV at later times after SFV infection reduced the translation of the picornavirus mRNA considerably. The general conclusion from Fig. 3 and 4 is that the time of addition of each virus is also a very important factor influencing the kind of virus proteins synthesized by the infected cell.

Infection of cultured cells with EMCV (Carrasco & Smith, 1976) and with a togavirus (Garry et al., 1979) leads to an increased membrane permeability and to a redistribution of ions in the cellular cytoplasm. In EMCV-infected cells, the synthesis of virus and cellular proteins largely depends on external ionic conditions (Alonso & Carrasco, 1981a). It was of interest to assess the influence of the external ionic conditions on the relative translation of capped or uncapped virus mRNA in cells simultaneously translating both kinds of mRNAs. Fig. 5 shows that in a hypotonic medium these doubly infected cells synthesized mostly the togavirus late proteins, whereas under hypertonic conditions picornavirus-coded proteins were exclusively synthesized. When the synthesis of each kind of virus protein was plotted against the different concentrations of NaCl tested, it was apparent that a hypotonic medium increased the ratio of C/γ and hypertonic media inhibited the synthesis of C, whereas the
Fig. 3. Time course of protein synthesis in EMCV-infected HeLa cells superinfected with SFV at different times. The m.o.i.s. of EMCV and SFV were 5 and 200 respectively. Conditions of infection and protein labelling were as described in Methods. (a) Protein synthesis in HeLa cells infected with each virus alone, estimated at the indicated times after infection (1, 3, 5, 7 and 9 h). (b) Protein synthesis in HeLa cells simultaneously infected with EMCV and SFV. SFV was added at the indicated times after EMCV infection (1, 3, 5 and 7 h); -1 h indicates preinfection with SFV. Protein synthesis was determined at the indicated times after EMCV infection (1, 3, 5, 7 and 9 h).
Fig. 4. Time course of protein synthesis in SFV-infected HeLa cells superinfected with EMCV at different times. The m.o.i.s of SFV and EMCV were 10 and 50 respectively. Conditions of infection and labelling were as described in Methods. (a) Protein synthesis in HeLa cells infected with each virus alone, estimated at the indicated times after infection (1, 3, 5, 7, and 9 h). (b) Protein synthesis in HeLa cells simultaneously infected with SFV and EMCV. EMCV was added at the indicated times after SFV infection (1, 3, 5, and 7 h); -1 h indicates preinfection with EMCV. Protein synthesis was determined at the indicated times after SFV infection (1, 3, 5, 7, and 9 h).
synthesis of the picornavirus proteins was resistant to high salt media. We would like to point out, however, that although the translation of the 26S mRNA was inhibited by hypertonic media, it was more resistant than cellular mRNA translation. In this respect, the SFV 26S mRNA can be considered as being intermediate between the cellular and picornavirus mRNA. These results suggest that in cells doubly infected with SFV and EMCV there are two kinds of virus mRNA present and the relative translation of each kind depends on the external ionic concentration.

It seems unlikely that modification of the ionic conditions exerts an irreversible change in the protein-synthesizing apparatus in a way that could allow preferential translation of the capped or uncapped mRNA. This conclusion is based on the results shown in Fig. 6, which illustrate that after repeated changes from hypotonic to hypertonic medium and vice versa a reversible shut-off of the capped or the uncapped virus mRNA took place.
Fig. 6. Effect of repeated rounds of treatment with hypotonic and hypertonic media on protein synthesis in HeLa cells simultaneously doubly infected with EMCV and SFV. The m.o.i.s of EMCV and SFV were 30 and 5 respectively. The media used were Eagle's medium (control), Eagle's medium with no NaCl (hypotonic medium) and Eagle's medium with a final concentration of 180 mM-NaCl (hypertonic medium). The numbers above each lane indicate the time (h) after infection at which labelling of newly synthesized proteins was carried out.

DISCUSSION

The mechanism by which picornavirus infection leads to an inhibition of host protein synthesis has been studied in several laboratories (Lawrence & Thach, 1974; Carrasco & Smith, 1976; Egberts et al., 1977; Helentjaris & Ehrenfeld, 1978; Jen et al., 1978; Trachsel et al., 1980). However, the mechanism by which picornaviruses shut off cellular protein synthesis is still unclear. We have suggested (Carrasco & Smith, 1976; Carrasco, 1977) that the block of cellular mRNA translation is produced by modification of the ionic concentration in the cellular cytoplasm, as a consequence of the modification of membrane permeability. Evidence from cell-free systems is in agreement with this view. It was found that picornavirus mRNA required a higher concentration of monovalent ions for optimal translation as compared to cellular mRNA translation (Carrasco & Smith, 1976). A similar response is found with those virus mRNAs that are translated in the cell when the shut-off of host protein synthesis has occurred (Carrasco et al., 1979). Several lines of in vivo results
also support this idea. Firstly, it has been found that a hypertonic medium preferentially blocks cellular protein synthesis in a number of cells infected by animal viruses (Saborio et al., 1974; England et al., 1975; Nuss et al., 1975; Cherney & Wilhelm, 1979). On the other hand, transfer of EMCV-infected cells to hypotonic medium reverses the shut-off of host protein synthesis (Alonso & Carrasco, 1981a). Moreover, membrane-active compounds, such as amphotericin B, that disrupt the ionic gradients maintained by the membrane preferentially block cellular protein synthesis in EMCV-infected HeLa cells (Alonso & Carrasco, 1981b). Secondly, a parallel exists between the inhibition of cellular protein synthesis and membrane leakiness to ions in Sindbis virus-infected cells (Garry et al., 1979) and also in cells infected by cardiovirus at low m.o.i. (Carrasco & Smith, 1976). However, when an early shut-off of host translation takes place as a consequence of a high virus input, the ionic conditions in the cytoplasm only change late in infection (Egberts et al., 1977; Nair et al., 1979). Although changes in early permeability to a number of compounds are observed during virus attachment (Fernández-Puentes & Carrasco, 1980; Carrasco, 1981), no modification in the monovalent ion content has been found at that time. We can conclude that in picornavirus-infected cells the ionic changes in the cell take place when the bulk of virus proteins are synthesized.

On the basis that cell-free systems from poliovirus-infected cells translate uncapped mRNAs but not capped mRNAs (Rose et al., 1978; Trachsel et al., 1980), it was suggested that poliovirus infection inactivates a protein factor necessary for the translation of capped mRNAs, but dispensable for the translation of uncapped mRNAs. In agreement with this idea, infection of cells with poliovirus interferes with the translation of herpesvirus or VSV mRNAs (Doyle & Holland, 1972; Saxton & Stevens, 1972; Ehrenfeld & Lund, 1977). However, the infection of monkey cells with poliovirus under conditions that inhibit host protein synthesis has no effect on SV5 protein synthesis (Choppin & Holmes, 1967).

To test whether EMCV infection inactivates a factor necessary for the translation of capped mRNAs, we chose the system of double infection with EMCV and SFV. This system has the advantage that the translation of a single species of uncapped mRNA (the EMCV RNA) can be compared with the translation of a single species of capped mRNA (the subgenomic 26S mRNA from SFV). The results reported in this paper do not support the idea that EMCV infection inactivates an initiation factor activity involved in the translation of capped mRNAs, because both capped and uncapped mRNAs are simultaneously translated in HeLa cells co-infected with EMCV and SFV. These results are in agreement with the recent finding that mengovirus infection allows the translation of VSV mRNAs in L cells (Otto & Lucas-Lenard, 1980).

At present, we are studying the influence of poliovirus infection on the translation of several virus capped mRNAs in mixed infection experiments. Our results indicate that under some conditions HeLa cells can translate both poliovirus RNA and virus capped mRNAs. In particular, the translation of both poliovirus RNA and the SFV 26S mRNA can simultaneously occur in HeLa cells infected with both viruses.

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REFERENCES


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