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Inhibition of Host Translation in Encephalomyocarditis Virus-Infected L Cells: a Novel Mechanism

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Encephalomyocarditis virus induced a rapid shutoff of host translation in mouse L cells shortly after infection and before viral proteins were made in detectable amounts. This kinetic pattern is similar to that seen in poliovirus-infected HeLa cells. However, the mechanisms of host shutoff are different in these two cases, for no reduction in the ability of lysates from encephalomyocarditis virus-infected L cells to translate capped mRNAs was observed. Instead, a change in the subcellular distribution of one or more initiation factors was seen. In particular, cap recognition activity in the high-speed supernatant fraction (S200) prepared from cell lysates increased threefold as a result of virus infection. The significance of this observation in terms of possible shutoff mechanisms is discussed. Inasmuch as the rapid host shutoff is not induced in at least four other cell types by encephalomyocarditis virus infection, it may be concluded that host shutoff mechanisms not only vary within the picornavirus group, but also depend upon the particular cell type employed.

The shutoff of host protein synthesis by picornaviruses has been studied for many years. The mechanism by which this occurs, however, remains only partially understood. One fact that is clear, however, is that not all picornaviruses accomplish host shutoff by the same mechanism. The best illustration of this point is obtained when poliovirus is compared with encephalomyocarditis (EMC) virus and mengovirus. Whereas the former virus inactivates the cellular mechanism for recognizing the 7-methylguanosine cap moiety of host mRNAs (11–13, 29, 33), the latter two viruses do not (1, 9, 15, 19, 32). In addition, the kinetic patterns of total protein synthesis in most types of cells infected with these viruses differ strikingly. For example, in poliovirus-infected HeLa cells, the decline in host translation occurs well before the onset of viral translation, resulting in a bimodal pattern. In contrast, in HeLa cells (15), MOPC ascites (19), Krebs ascites (14), or SC-1 fiboblasts (34; W. E. Walden, T. V. Ramabhadran, and R. E. Thach, unpublished data) infected with EMC virus, the decline in host translation occurs much later, concomitant with the onset of viral translation. This usually results in little or no apparent change in the overall protein synthesis rate until late in the infection cycle.

However, an exception to this general observation has been noted; in EMC virus-infected L cells, the overall kinetic pattern is very similar to that seen in poliovirus-infected HeLa cells (28). Thus, it was of interest to determine whether in this particular case EMC virus infection might also lead to the inactivation of cap recognition factors, in analogy with poliovirus infections. The results of such a study are described in this communication. It was shown that whereas the activity of cap recognition factors is not reduced in EMC virus-infected L cells, their subcellular distribution is altered. The significance of this result and possible mechanisms by which it occurs are discussed.

MATERIALS AND METHODS

Virus infections. The LPA strain of mouse L cells used in these experiments was obtained from Ernest Knight, Jr. (E. I. du Pont de Nemours & Co., Inc.). Suspension cultures of L cells were maintained at a density of 4×10^5 to 1×10^6 cells per ml in Joklik modified essential medium (MEM-J) supplemented with 10% calf serum. The growth and purification of EMC virus have been described previously (14).

The L cells were infected as follows. Actively growing cell cultures were pelleted by centrifugation at $250 \times g$ for 5 min. The harvested medium was saved for use as conditioned medium. The L cells were washed three times with ice-cold MEM-J and were infected in ice-cold MEM-J at a density of 10^7 cells per ml with 20 PFU of EMC virus per cell. Virus attachment proceeded at 4°C for 30 min. At the end of the attachment period the cell suspension was diluted to a density of 2×10^6 cells per ml with a warm mixture of 50% conditioned medium and 50% fresh MEM-J supplemented with 10% calf serum. Zero-hour postinfection refers to the time when the cell suspension was diluted with the warm medium and incubated at 37°C. Mock-infected cells were treated similarly, except that the EMC virus inoculum was omitted.

In vivo radiolabeling of proteins. Cell culture samples were pelleted and washed twice with calcium- and magnesium-free phosphate-buffered saline. Washed cells were suspended to a density of 4×10^6 cells per ml in warm methionine-free MEM-J and labeled with 30 μCi of [^{35}S]methionine per ml (Amersham Corp.; specific activity, 990 Ci/mmol) for 30 min in a 37°C, 5% CO_2 incubator. The label was chased for 10 min in the presence of 10 mM methionine. Labeled cells were lysed by the addition of sodium dodecyl sulfate (SDS) to 1% and 2-mercaptoethanol to 0.1%. The lysates were made 80% in acetone and were stored overnight at −20°C. The precipitates were washed twice with 80% acetone and once with 100% acetone. Dried precipitates were dissolved in SDS sample buffer (50 mM Tris-hydrochloride [pH 6.8], 6 M urea, 2% SDS, 1 mM EDTA, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromphenol blue) and heated for 5 min at 100°C. The amount of label incorporated into proteins was determined by counting 50 μl of this dissolved sample with 200 μl of H_2O in 5 ml of 3a70 scintillator (Research Products International Corp.). The samples were stored at −20°C until analysis by polyacrylamide gel electrophoresis (PAGE).

Preparation of S10 extracts and S200 and P200 fractions. L cell cultures were pelleted and washed twice with ice-cold calcium- and magnesium-free phosphate-buffered saline. Cells were packed by pelleting at 250 × *g* for 5 min and then suspended in twice the packed-cell volume of lysis buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid]–KOH [pH 7.5], 10 mM KCl, 3 mM dithiothreitol [DTT], 1.5 mM $MgCl_2$). The cells were allowed to swell on ice for 10 to 20 min and then were lysed by 30 to 40 strokes in a tight-fitting Dounce homogenizer. The lysates were cleared of debris by two centrifugation steps, the first at 2,500 × *g* for 5 min and the second at 10,000 × *g* for 20 min, both in a Sorvall HB-4 rotor. After the second centrifugation, the fatty layer at the top of the lysate was carefully removed by aspiration. The remaining supernatant (i.e., the S10 extract itself) was pooled, frozen in small portions, and stored at −80°C until used. The S10 extracts varied between 55 and 75 units of absorbance at 260 nm (A_{260}) per ml from preparation to preparation, and the protein concentration, as determined by the assay of Lowry et al. (22), was 0.16 mg per A_{260} unit.

For the preparation of S200 and P200 fractions, S10 extracts were centrifuged at 200,000 × *g* for 1 h in a Spinco 65 rotor. The supernatant (i.e., S200) was carefully removed, divided into portions, and stored at −80°C. The pellet was gently rinsed with HMK buffer (20 mM HEPES–KOH [pH 7.5], 100 mM KCl, 3 mM magnesium acetate, 1.5 mM DTT) and suspended in one-fourth of the original S10 extract volume of HMK buffer. The suspension (i.e., P200) was clarified of debris by centrifugation at 2,500 × *g* for 5 min, divided into portions, and stored at −80°C. On the average, the S200 fraction contained 45% of the protein and A_{260}-absorbing material present in the original S10 extracts.

Preparation of f-[^{35}S]met-tRNA. tRNA from an MOPC 460 solid tumor was prepared as described elsewhere (5). A crude mixture of *Escherichia coli* aminoacyl-tRNA synthetases and methionyl-tRNA transformylase was prepared as previously described (27). The formyl-[^{35}S]methionyl-tRNA (f-[^{35}S]met-tRNA) was prepared according to the procedure of Stanley (31) with the following modifications. The strength of sodium cacodylate solution was increased to 100 mM, folinic acid was used at 1 mM, tRNA from MOPC tumors was charged at a concentration of 1.5 mg/ml, and L-[^{35}S]methionine (Amersham Corp.; specific activity, 990 Ci/mmol) was used at 1 mCi per ml of charging reaction. The activity of the f-[^{35}S]met-tRNA was 3.3×10^5 cpm per μg of tRNA. Thin-layer chromatography analysis showed that greater than 95% of the charged methionine was formylated.

Translations in the S10 extracts and in the S200-P200 reconstituted systems. The S10 translation reaction mixture contained in a 20-μl final volume the following: 0.6 A_{260} units of S10 extract, 3 mM ATP (neutralized with KOH), 0.5 mM GTP, 10 mM creatine phosphate, 300 μg of creatine phosphokinase per ml, 5 μM concentrations of 19 unlabeled amino acids, 2 mM DTT, 0.4 mM spermidine, 90 mM KCl, 4 mM magnesium acetate, and 20 mM HEPES–KOH (pH 7.5). The potassium and magnesium salt concentrations were added in addition to the salts contributed by the S10 extracts, which were estimated to contain approximately 40 mM K^+ ions and 2 mM Mg^{2+} ions. Since the S10 extracts constituted between 0.5 and 0.6 of the volume of the final reaction mixture, the actual potassium and magnesium ion levels of the mixtures were approximately 110 mM and 5 mM, respectively. When [^{35}S]methionine was used, each mixture contained 5 μCi of radiolabel. When f-[^{35}S]met-tRNA was used, each mixture contained 0.45 μCi of radiolabel (3.3×10^5 cpm per μg of tRNA). Globin mRNA was translated at a concentration of 50 μg/ml. Reovirus mRNAs were translated at a concentration of 45 μg/ml. The incubations were for 90 min at 30°C. The reactions were terminated by quenching on ice followed by the addition of 1 volume of pH adjustment buffer (0.1 M Tris-hydrochloride [pH 4.4], 4 mM EDTA, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and 2 volumes of SDS sample buffer. The samples were heated for 5 min at 100°C and stored at −20°C.

The reconstituted translation reaction mixture contained 0.2 A_{260} units of S200 and 0.3 A_{260} units of P200 in 25 μl. The translation conditions were identical to the final conditions used in the S10 translation reaction. Reovirus mRNAs were translated at a concentration of 40 μg/ml.

RNA preparations. Cellular RNA was isolated from S10 extracts by the SDS-phenol-chloroform procedure of Palmiter (25). Globin mRNA, EMC virion RNA, and single-stranded reovirus mRNAs were prepared as described elsewhere (5).

Translation in a wheat germ system. Wheat germ S23 extracts were prepared according to the procedure described by Bruening et al. (6). Endogenous mRNA translation in the S23 extracts was suppressed by pretreatment with micrococcal nuclease (Boehringer-Mannheim Corp.), essentially as described by Pelham and Jackson (26). S23 extract was made 1 mM in $CaCl_2$ and 40 μg/ml in micrococcal nuclease and was incubated for 10 min at 23°C. The digestion was stopped by chilling the mixture on ice and by adding ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid to 3 mM.

Cellular RNAs were translated in the wheat germ S23 system under conditions adapted from those used by Beachy et al. (3). Translation reaction mixtures

contained in 25-μl volumes the following: 5 μl of nuclease-digested S23 extract, 100 mM potassium acetate, 10 mM KCl, 2.5 mM magnesium acetate, 20 mM HEPES–KOH (pH 7.5), 0.5 mM spermidine, 40 μM each of 19 unlabeled amino acids, 80 μM GTP, 80 μM CTP, 1.36 mM ATP, 8.6 mM creatine phosphate, 2 mM DTT, 160 μg of creatine phosphokinase per ml, 1 mM *S*-adenosylhomocysteine, and 5 μCi of [^{35}S]methionine. Cellular RNAs were translated at a concentration of 80 μg/ml. The reactions were carried out at 23°C for 90 min. At the end of the incubation period, labeled proteins were precipitated with 80% acetone and prepared for SDS-PAGE according to procedures described for examination of in vivo labeled proteins.

Translation in a rabbit reticulocyte lysate system. Rabbit reticulocyte lysates were prepared according to described procedures (26). Nuclease pretreatment of the reticulocyte lysates was carried out as described above for the wheat germ S23 extracts, except that 40 μM hemin was included in the digestion reaction. Translation reaction mixtures contained in 25-μl volumes the following: 10 μl of nuclease-treated lysate, 100 mM potassium acetate, 2 mM magnesium acetate, 20 mM HEPES–KOH (pH 7.5), 0.1 mM spermidine, 40 μM each of 19 unlabeled amino acids, 2.5 mM GTP (neutralized with KOH), 15 mM creatine phosphate, 2 mM DTT, 200 μg of creatine phosphokinase per ml, 20 μg of polyinosinate·polycytidylate per ml, and 10 μCi of [^{35}S]methionine. Cellular RNAs were translated at a concentration of 40 μ/ml. The reaction products were processed as described for the wheat germ translations.

Translation in a fractionated murine cell-free system. Cellular RNAs were translated according to a previously described fractionated translation system (5, 8). The ribosomal salt wash (RSW) and pH 5 fractions were pretreated with 40 μg of micrococcal nuclease per ml for 5 min at 23°C according to procedures described above to suppress endogenous translation. RSW was used in the reactions at a concentration of 1 mg/ml. Cellular RNAs were translated at a concentration of 40 μg/ml. The reaction products were prepared for SDS-PAGE analysis as described above.

S200 supplementation of a fractionated cell-free system containing RSW from poliovirus-infected cells. Reaction conditions were identical to those described above, except that the RSW used was from HeLa cells 4 h after poliovirus infection. Because this RSW preparation was devoid of mRNA contamination, treatment with micrococcal nuclease was omitted. The RSW was used in the reactions at a concentration of 0.6 mg/ml. Globin and EMC virus messages were both translated at a concentration of 20 μg/ml. The radiolabel used was f-[^{35}S]met-tRNA at 0.45 μCi per 25-μl reaction mixture. The translation reaction mixtures were supplemented with up to 0.8 mg of S200 proteins per ml. The reaction products were prepared for SDS-PAGE analysis as described above.

SDS-PAGE analysis, fluorography, and densitometry. Processed labeled proteins and translation reaction products were analyzed on slab polyacrylamide gels by using the Laemmli system (17). Depending on the products to be examined, either 7.5 to 15% gradient gels or 15% uniform gels were used.

Two fluorographic systems were used in these studies. Initially, gels were treated for fluorography with EnHance (New England Nuclear Corp.) supplemented with 10% (wt/vol) PPO (2,5-diphenyloxazole), according to the procedures recommended by the manufacturer. During the course of these studies, an alternative fluorographic system was developed for polyacrylamide gels and was used for the remainder of these studies.

We have termed the new fluorographic system APEX, an acronym based on the ingredients used (G. Jen, unpublished data). The essential aspects of the APEX procedures are as follows. Slab gels, 1.4-mm thick, are treated with 10 volumes of 10% acetic acid–30% ethanol in water for 1 h at room temperature with gentle shaking to fix the proteins in the gels, as well as to partially dehydrate the gels. Fixed and dehydrated gels are then infiltrated by gentle shaking with 6 volumes of APEX solution (55% [vol/vol] acetic acid, 0.4% [wt/vol] PPO, 15% [vol/vol] ethanol, and 30% [vol/vol] xylene) for 1 h at room temperature. This is followed by two washes with 20 volumes of water, each for 30 min with gentle shaking. Finally, the treated gels are dried at 80°C under vacuum.

Dried gels were exposed to Kodak X-Omat X-ray film (either XR5 or XRP) after the films were prefogged (18). Film exposures were quantitated by densitometry. Linear dose responses on the X-ray films were obtained for both ^{35}S-labeled and ^{3}H-labeled proteins by using the APEX system.

RESULTS

Protein synthesis in mouse L cells infected with EMC virus began to decline shortly after infection to a minimum level which was generally 30 to 50% of the rate in uninfected cells. This minimum was attained between 2.5 and 3.5 h postinfection (Fig. 1A; 28). Thereafter, the translation rate rose again to a second peak, which was reached by about 5 h postinfection. The majority of the polypeptides made at this time were viral (Fig. 1B). Thus, the initial decline represents a true shutoff of host translation. This pattern is similar to that seen in poliovirus-infected HeLa cells (12, 16), but it differs markedly from that seen in many other EMC virus-infected cells (14, 15, 19, 34).

Analysis of translational activity of host mRNAs isolated from infected cells. A potential cause for the shutoff evident in Fig. 1 is the inactivation or degradation of host mRNA. This possibility was examined by testing the activity of RNAs extracted from infected cells in cell-free translation systems. Although no evidence for message inactivation or degradation has been obtained for several picornavirus infections (10, 16, 29, 32), L cells infected with EMC virus have not been tested. Since recent studies (15) indicate that different picornavirus infections may affect host protein synthesis differently, the results for other systems cannot be assumed to be applicable to the present case.

Total RNA was isolated from EMC virus-infected L cells at various times postinfection,

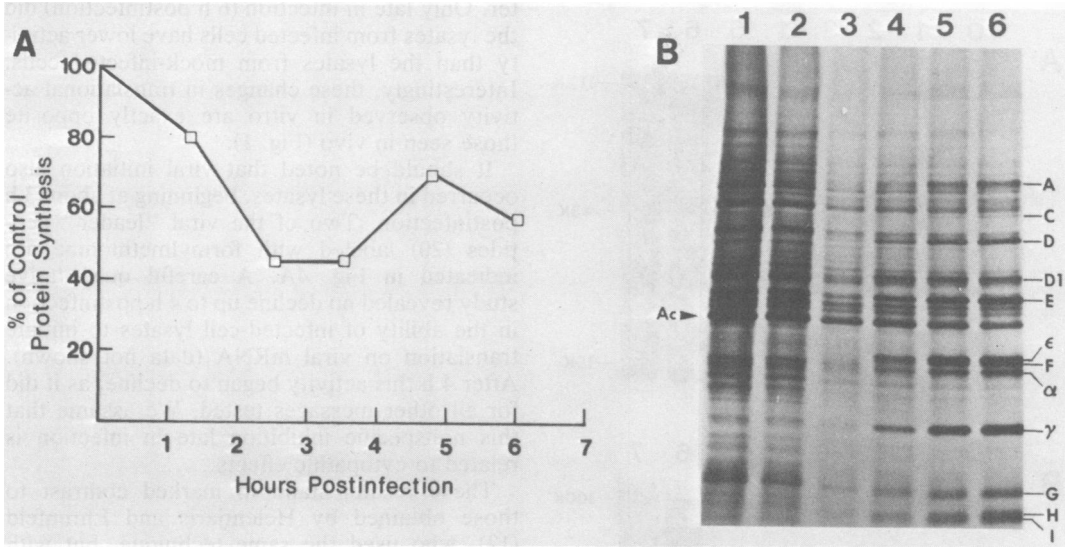

FIG. 1. Protein synthesis in EMC virus-infected L cells. Mock-infected and EMC virus-infected cells were pulse-labeled with [^{35}S]methionine at various times postinfection and then prepared for SDS-PAGE. (Panel A) The rates of protein synthesis were determined as described in the text. These rates were normalized to that of uninfected (control) cells. (Panel B) The labeled proteins were analyzed by SDS-PAGE in a 7.5 to 15% gel and then by fluorography with EnHance. Lane 1, Uninfected cells; lane 2, 1.25-h infected cells; lane 3, 2.5-h infected cells; lane 4, 3.5-h infected cells; lane 5, 4.75-h infected cells; lane 6, 6.0-h infected cells. The positions of actin (Ac) and the various viral proteins are indicated.

and a constant quantity was translated in cell-free systems prepared from wheat germ, rabbit reticulocytes, and Krebs ascites cells (see above). In each case, an amount of RNA from the earliest time point postinfection was chosen so as to be one-half the amount needed to saturate the translation system, and this amount was then used for all subsequent time points. Although the results (Fig. 2) vary somewhat from system to system, reflecting the translational specificities peculiar to each, it is nevertheless clear that there was no substantial loss of mRNA translational activity. The apparent decline in host mRNA activity seen in the rabbit reticulocyte and Krebs ascites systems was probably due to competition by EMC virus RNA which accumulated late in infection. This interpretation is indicated by the lack of any change in the translation of host mRNAs in the wheat germ system (Fig. 2C). These results are in accord with those of others, who have found little, if any, effect of picornavirus infections on host mRNA translatability (10, 16, 19, 32).

Initiation activities of lysates from infected cells. An earlier study showed that host polysomes decreased in size during EMC virus infection of L cells, indicating that initiation of translation is inhibited (28). To determine whether the infection-induced inhibition of initiation could be reproduced in vitro, the translational activities of lysates (S10 extracts) from uninfected and infected cells were examined. In preliminary experiments not shown here, optimal conditions for maximum initiation of translation in the lysates from uninfected cells were determined. These conditions (see above) were used as the standard conditions for assaying the lysates.

When lysates were prepared from EMC virus-infected L cells over the time span indicated (Fig. 3A) and incubated with [^{35}S]methionine under standard translation conditions, the incorporation of radioactivity followed a bimodal pattern nearly identical to the changes seen in the infected cells. However, analysis based on edeine suppression of initiation indicated that the incorporation into endogenous proteins obtained with the [^{35}S]methionine labeling was largely due to completion of preinitiated chains, i.e., "polysome runoff" (data not shown). Therefore, to specifically measure the rate of translational initiation, we employed f-[^{35}S]met-tRNA as the only source of labeled amino acid. Figure 4 shows the results of such an experiment, in which it was clear that the rate of initiation on host mRNAs in vitro was not decreased by viral infection; rather, there was actually an increase in the initiation rate with increasing time postinfection (Fig. 4B). The increase in initiation activity of the lysates peaked around 3.5 h postinfection and reversed thereaf-

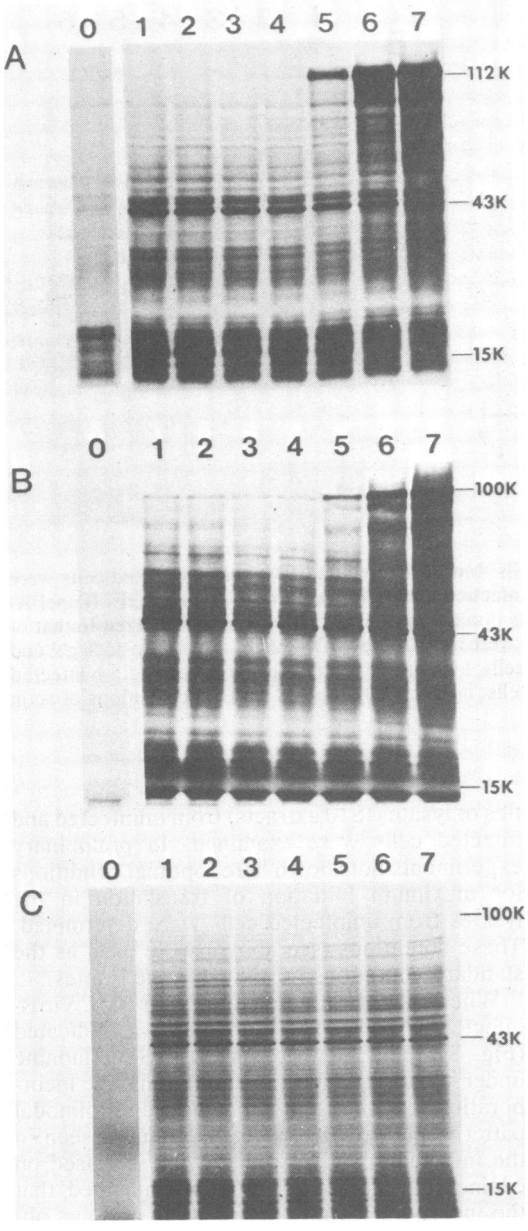

FIG. 2. Translations of RNAs from mock-infected and EMC virus-infected L cells. RNAs were extracted at various times postinfection and were translated in cell-free systems as described in the text. The translation products were analyzed by gel electrophoresis and fluorography with EnHance. (Panel A) Translation products synthesized by the fractionated cell-free system derived from Krebs ascites cells. (Panel B) Translation products synthesized by the rabbit reticulocyte lysate sytem. (Panel C) Translation products synthesized by the wheat germ system in the presence of 1 mM *S*-adenosylhomocysteine. Lane 0, Minus exogenous RNA; lane 1, 2.5-h mock-infected RNA; lane 2, 4.75-h mock-infected RNA; lane 3, 1.25-h infected RNA; lane 4, 2.5-h infected RNA; lane 5, 3.5-h infected RNA; lane 6, 4.75-h infected RNA; lane 7, 6.0-h infected RNA. The positions of various molecular weight markers are shown.

ter. Only late in infection (6 h postinfection) did the lysates from infected cells have lower activity than the lysates from mock-infected cells. Interestingly, these changes in translational activity observed in vitro are exactly opposite those seen in vivo (Fig. 1).

It should be noted that viral initiation also occurred in these lysates, beginning at about 3 h postinfection. Two of the viral "leader" peptides (20) labeled with formylmethionine are indicated in Fig. 4A. A careful quantitative study revealed no decline up to 4 h postinfection in the ability of infected-cell lysates to initiate translation on viral mRNA (data not shown). After 4 h this activity began to decline, as it did for all other messages tested. We assume that this nonspecific inhibition late in infection is related to cytopathic effects.

These results stand in marked contrast to those obtained by Helentjaris and Ehrenfeld (12), who used the same technique, but with lysates prepared from poliovirus-infected HeLa cells. These authors showed that the incorporation of formylmethionine into host polypeptides in vitro diminished rapidly within a few hours after poliovirus infection. Thus it is clear that although the overall in vivo translational kinetic patterns of the two systems are strikingly similar, poliovirus infection involves inactivation of factors required for the translation of host mRNAs, whereas EMC virus infection does not.

Confirmation of this conclusion was obtained when exogenous mRNAs were translated in the lysates prepared from EMC virus-infected L cells. Either rabbit globin mRNA or a mixture of reovirus mRNAs was added to the lysates described above, and the translation products were analyzed and quantitated. Figures 3 and 5 show typical results for globin mRNA, for which the sources of label were [^{35}S]methionine and f-[^{35}S]met-tRNA, respectively. (The paucity of labeled host protein bands in Fig. 5 was due to the fact that the high level of globin mRNA added was more competitive than the endogenous host mRNAs for initiation. This effect is not apparent in Fig. 3 because runoff of preexisting host polysomes is not influenced by competition and because the host proteins are disproportionately labeled with methionine due to their greater length.) From these data it is evident that there was no early decline in the ability of infected-cell lysates to translate exogenous capped mRNA; moreover, as indicated above, there appeared to be a definite increase in initiation activity with increasing time postinfection.

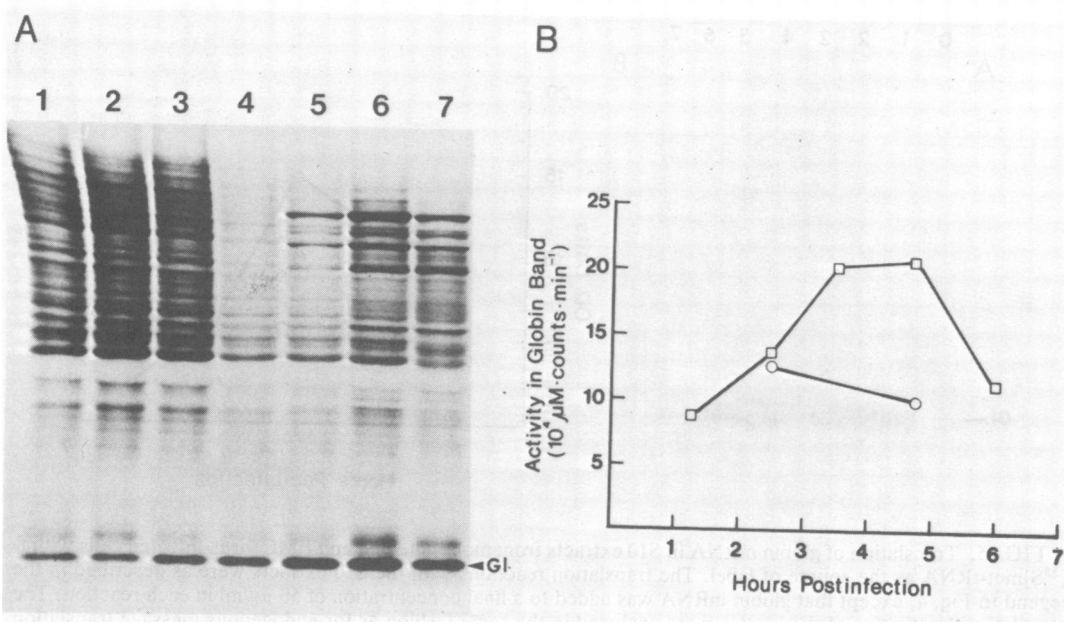

FIG. 3. Translation of endogenous mRNA plus exogenous globin mRNA in infected and control S10 extracts with [^{35}S]methionine as the source of label. The translation reactions, SDS-PAGE, and fluorography with EnHance were carried out as described in the text. (Panel A) Fluorogram of translation products from reactions to which 50 μg of globin mRNA per ml was added. The times after mock or EMC virus infection at which the lysates were prepared are as follows: lane 1, 2.5 h, mock infected; lane 2, 4.75 h, mock infected; lane 3, 1.25 h, in- incorporated into globin (GI) was quantitated by densitometry, corrected for dilution by endogenous methionine and calibrated relative to a standard (to yield micromolar methionine × counts per minute), and plotted in panel B. Symbols: □, S10 extract from infected cells; ○,S10 extract from mock-infected cells.

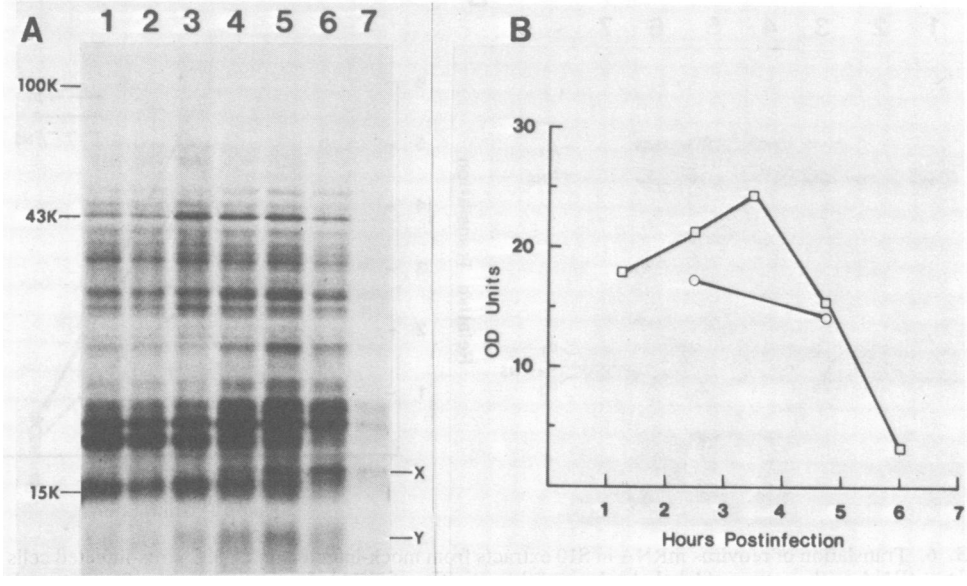

FIG. 4. Translation of endogenous mRNA in infected and control S10 extracts with f-[^{35}S]met-tRNA as the source of label. The translation reactions and the gel analysis of the reaction products were carried out as described in the text. (Panel A) Fluorogram of translation products separated on a 15% gel. The APEX fluorographic system was used in this and all subsequent experiments. The lane designations are as noted in the legend to Fig. 3. The positions of molecular weight markers and of the two EMC virus-specific leader proteins, X and Y (see text), are indicated. (Panel B) Quantitation of lysate activities shown in panel A by densitometry of the fluorogram. Optical densities (OD) for all of the bands in each lane were integrated, and the sum of these is plotted. The symbols are as noted in the legend to Fig 3.

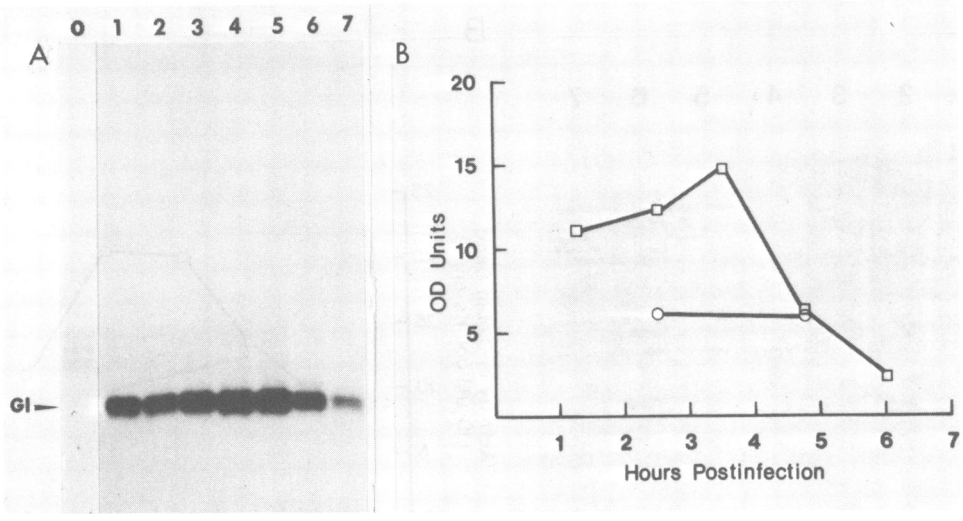

FIG. 5. Translation of globin mRNA in S10 extracts from mock-infected and EMC virus-infected cells with f-[^{35}S]met-tRNA as the source of label. The translation reactions with the S10 extracts were as described in the legend to Fig. 4, except that globin mRNA was added to a final concentration of 50 μg/ml in each reaction. The translational activities of the lysates were analyzed in the same fashion as for endogenous message translation (Fig. 4). (Panel A) Fluorogram of translation products separated on a 15% gel. The lane designations are as noted in the legend to Fig. 3, except that the lane designated 0 is a 2.5-h mock-infected lysate minus globin mRNA. The position of the globin protein (Gl) is noted. (Panel B) Quantitation of lysate activities shown in panel A by densitometry of the globin band. The symbols are as noted in the legend to Fig. 3. OD, Optical density.

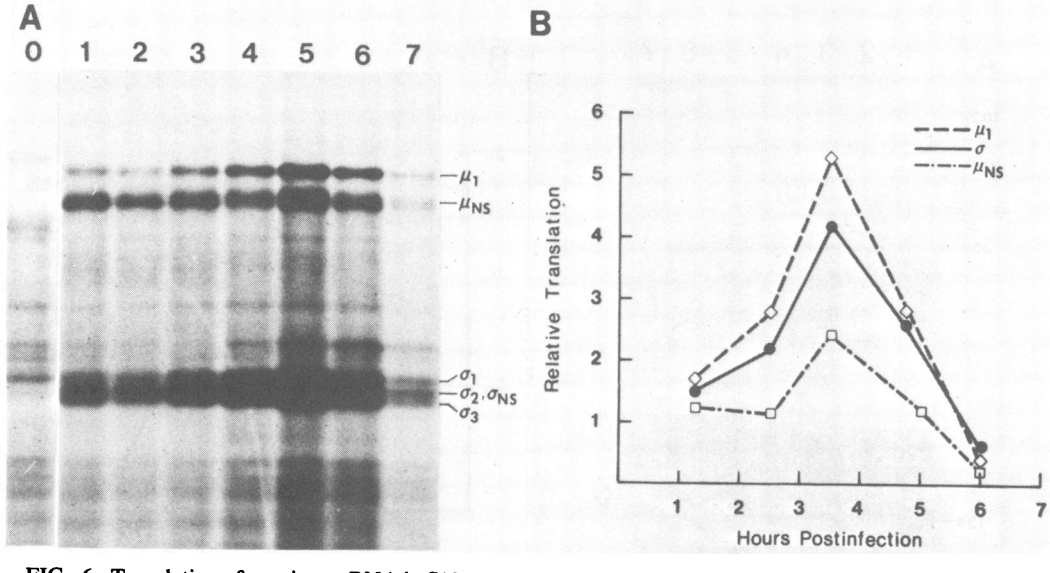

FIG. 6. Translation of reovirus mRNA in S10 extracts from mock-infected and EMC virus-infected cells with f-[^{35}S]met-tRNA as the source of label. Analysis of the activities of lysates for the translation of reovirus mRNAs was identical to that for the translation of globin mRNA (Fig. 5). (Panel A) Fluorogram of translation products separated on a 7.5 to 15% gel. The various reovirus proteins are identified. The lane designations are as noted in the legend to Fig. 5. (Panel B) Activities of infected-cell lysates for the translation of the various reovirus messages. Quantitation of activity was by densitometry of fluorograms. The σ proteins were quantitated as a single group due to incomplete resolution; however, the relative translation of the individual bands was not observed to change. The activities of the infected-cell lysates for synthesis of the reovirus mRNAs are shown in units relative to the average translation seen in the two mock-infected-cell lysates (lanes 1 and 2 of panel A). Symbols: ◇, μ_1 protein; ●, average of four σ proteins; □, μ_{NS} proteins.

Similar results were obtained with reovirus mRNAs (Fig. 6). These data are of particular interest due to the larger increases in translation of M_1 mRNA and the S-class mRNAs as compared with the smaller increase in translation of M_3 mRNA. Inasmuch as saturating, and hence mutually competitive, quantities of reovirus mRNAs were used, these data are consistent with the idea that far more of the message-discriminatory initiation component defined previously (5, 35), becomes available to mRNAs in lysates from EMC virus-infected cells than to those in lysates from control cells. Experiments bearing on this possibility are described below.

Characterization of changes in lysates from infected cells. The results obtained with crude lysates suggested that infection of L cells by EMC virus produces an increased initiation capacity in vitro. To characterize the nature of this infection-induced change, we separated the lysates by centrifugation into ribosomal pellet fractions (P200) and supernatant fractions (S200) and examined their activities in fractionated translation systems. The centrifugation conditions used were such that the P200 contained all molecules and complexes with sedimentation coefficients greater than 20S. This should have included all forms of ribosomes and most of the messenger ribonucleoprotein particles. The S200 should contain only the slower-sedimenting factors.

To identify the fraction responsible for the increased initiation activity, we prepared reconstituted systems by using fractions from mock-infected and infected cells in different combinations and assayed the activities of these systems in translation of reovirus mRNAs. The results of this experiment are shown in Table 1. As expected, the system reconstituted with fractions only from infected cells was more active than that reconstituted with fractions only from mock-infected cells. The systems composed of one mock-infected fraction and one infected fraction had activities intermediate to the two homologous systems. These results indicate that both P200 and S200 from the infected cells possess enhanced activities relative to those from the mock-infected cells.

We next examined the properties of the increased activity present in S200 from infected cells. In particular, we wanted to determine if the increased activity was due to initiation factors, as was suggested by translational studies with reovirus mRNAs. We tested for this possibility by assaying for the presence of the factor which is thought to be inactivated upon poliovirus infection of HeLa cells (29, 33). This factor is a 5 to 7S form of the cap-binding proteins (CBP); it specifically enhances the translation of capped, but not uncapped, mRNAs in control extracts and restores the ability to translate the former in poliovirus-infected extracts (30, 33). Because this restoring activity is specific for capped mRNAs, its presence is easily assayed. To this end, we constructed an in vitro translation system containing RSW from poliovirus-infected HeLa cells (deficient in cap translation activity) plus other components from uninfected mouse cells and f-[^{35}S]met-tRNA as a source of label. The addition of S200 from mock-infected L cells did not noticeably enhance the residual level of globin synthesized in the deficient system (Fig. 7B). Translation of EMC virus RNA was similarly unaffected (Fig. 7B). In contrast, the addition of S200 from EMC virus-infected L cells significantly stimulated, or restored, globin synthesis (Fig. 7C). These same amounts of S200 from infected cells increasingly altered the labeling pattern obtained when EMC virus RNA was used as message, by virtue of their containing EMC viral processing enzyme(s); however, the total number of peptide chains initiated in the system should not be affected by processing, and this was verified by quantitation. Indeed, the constancy of total f-[^{35}S]met-tRNA label in each lane suggests that initiation on EMC virus RNA was not stimulated by S200 from infected cells (Fig. 7E). This suggests that the ability of S200 from infected cells to restore globin synthesis is cap specific and reflects the presence of CBP. As a control, concentrated RSW from uninfected cells was added to the system to demonstrate the fully restored level of globin synthesis (Fig. 7D). As expected, only globin synthesis, and not EMC virus synthesis, was stimulated, confirming the specificity of the assay for capped mRNAs. To quantitate the amount of restoring activity pres-

TABLE 1. Translation of reovirus mRNAs in an S200-P200 reconstituted translation system[a]

Source of components[b]		Activity
S200	P200	
Mock	Mock	42.1
Mock	Infected	65.9
Infected	Mock	62.5
Infected	Infected	88.5

[a] The preparation of S200 and P200 from lysates of 2.5-h mock-infected cells and EMC virus-infected cells and the translation of reovirus mRNAs in the reconstituted translation systems were as described in the text. The radioactive label used was f-[^{35}S]met-tRNA. The labeled polypeptides were analyzed by SDS-PAGE and fluorography. The optical density of the reovirus polypeptides was determined by densitometry. The activity present in each system is given as the sum of optical densities present in μ and σ polypeptides.

[b] Mock, lysate from mock-infected cells; infected, lysate from EMC virus-infected cells.

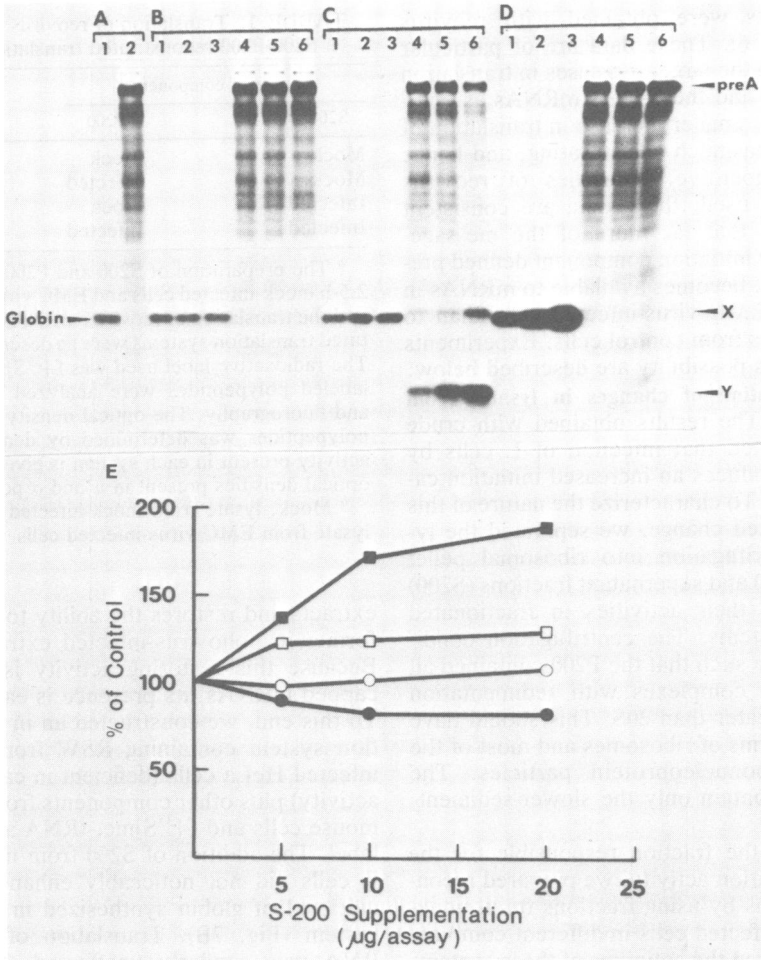

FIG. 7. Cap-specific initiation factor activities of S200 from mock-infected and EMC virus-infected cells. A fractionated cell-free translation system with RSW from 4-h poliovirus-infected HeLa cells and all other biological components (ribosomes, pH 5 fraction, etc.) from mouse ascites tumor cells was prepared as described in the text. The abilities of added S200 or control RSW to stimulate the translation of globin mRNA or EMC virus RNA in this system were examined. Translation products were analyzed by SDS-PAGE on a 15% gel and by fluorography. (Panel A) Translation products of the system without any added RSW or S200. Lane 1, globin mRNA translation; lane 2, EMC virus RNA translation. (Panel B) Effect of supplementation with S200 from 3.5-h mock-infected cells. Lanes 1, 2, and 3, Globin mRNA translation; lanes 4, 5, and 6, EMC virus RNA translation. The amounts of S200 proteins added per assay were 5 μg (lanes 1 and 4), 10 μg (lanes 2 and 5), and 20 μg (lanes 3 and 6). (Panel C) Effect of supplementation with S200 from 3.5-h EMC virus-infected cells. The lane notations are the same as in panel B. (Panel D) Effect of supplementation with RSW from Krebs ascites cells. Lanes 1, 2, and 3, globin mRNA translation; lanes 4, 5, and 6, EMC virus RNA translation. The amounts of Krebs RSW added per assay were 5 μg (lanes 1 and 4), 10 μg (lanes 2 and 5), and 20 μg (lanes 3 and 6). The positions of the two EMC virus-specific leader proteins, X and Y (see text), are indicated. (Panel E) Quantitation of translation seen in panels B and C. The optical densities present in each lane (which were proportional to the number of peptide chains initiated by f-[^{35}S]met-tRNA) were summed and plotted. Symbols: □ and ○, S200 from 3.5-h mock-infected cells; ■ and ●, S200 from 35-h EMC virus-infected cells; □ and ■, translations of globin mRNA; ○ and ●, translations of EMC virus RNA.

ent in S200 from EMC virus-infected L cells, the total radioactivity present in the lanes in Fig. 7B and C was measured and plotted in Fig. 7E. The ordinate of each point is a direct measure of the number of peptide chains initiated in the corresponding lane. The results showed clearly that a cap-specific restoring activity similar to CBP was present in S200 from infected cells, whereas relatively little was present in S200 from control cells. The amounts of this activity represent approximately 50 and 15%, respectively, of the total present in uninfected cells.

DISCUSSION

The results described above establish several interesting points. The first point is that the same virus can affect host translational machinery differently in different cell types. In the case of L cells, EMC virus infection results in a rapid shutoff of host translation which occurs well before the onset of the major viral translation period (Fig. 1). This pattern is quite different from that seen in other cells, such as MOPC ascites (19), Krebs ascites (14), HeLa (15), and SC-1 (34), infected with EMC virus. Whereas mRNA competition can account for the shutoff pattern in these cells (19, 32), it clearly cannot be responsible for the early, rapid decline seen in EMC virus infection of L cells. The fact that different cell types can respond differently to the same virus in terms of translational regulation has been recently noted by other investigators (21, 24). At present all such differences have been described only at the kinetic level. Molecular mechanisms remain to be elucidated. It seems likely that the differences reported merely reflect variation in the timing or order of events in different cell types; thus, a particular mechanism which appears to be absent in a certain cell type may simply occur unusually late, at a time when it cannot readily be detected. Nevertheless, the consequences of such a disturbance in the normal sequence of events may be very significant for virus replication.

The similarity between the translational patterns seen in EMC virus-infected L cells (Fig. 1A) and poliovirus-infected HeLa cells (12, 16) is striking. Thus, it is all the more surprising that different mechanisms appear to be involved in the shutoff of host translation. Whereas inactivation of the cap recognition process is thought to be responsible for shutoff in the latter system (11–13, 29, 33), this is clearly not the case in the former. Indeed, initiation on capped mRNAs is actually increased in lysates from EMC virus-infected L cells relative to lysates from control cells (Fig. 3–6). This observation is in accord with those from a previous study on EMC virus and poliovirus infections of HeLa cells (15). There it was reported that although poliovirus infection resulted in the inactivation of a cap recognition function, EMC virus infection did not.

The mechanism by which EMC virus infection shuts off host translation in L cells is not understood. Several potential mechanisms can be eliminated, however. This study shows that the inactivation of cellular messages, message competition, and the inactivation of a cap recognition function are not responsible for shutoff in this system. One possible mechanism is suggested by the observation that initiation activity is increased in infected cell lysates. The increased activity is associated with both the ribosomal pellet fractions (P200) and supernatant fractions (S200) of the lysate. The increased activity of S200 appears to be due to an increase in the level of a cap-specific component which is barely detectable in S200 from control cells. To reconcile this increased activity with the decrease seen in vivo, we have formulated the following hypothesis. We suggest that EMC virus-induced alterations responsible for reduced initiation on host mRNAs in vivo are reversible in vitro. One mechanism by which this might occur is through EMC virus infection causing an imbalance in small components such as ions, ATP, thiol compounds, etc. Indeed, a 25% decline in the intracellular K^+ concentration does occur concomitant with the shutoff of host translation in this system (28). (Alternatively, or in addition, equilibria in one or more complex reaction systems might be shifted.) These changes or similar alterations or both might disrupt complexes critical for initiation on cellular (but not viral) mRNAs. The disruption of these complexes would result in the breakdown of cellular polysomes and the release of their constituents. However, when lysates are prepared from these infected cells, the conditions are optimized for translation in vitro. This optimization would result in correction of the postulated imbalance in low-molecular-weight components (or reaction equilibria). Under these conditions, the critical complexes could reform in vitro, and the translation of cellular mRNAs would be restored. The increased activities of infected-cell lysates and their derived fractions are explained by this model in that, as a result of the proposed breakdown of host initiation complexes in vivo, these preparations would contain more free translational components than would those from uninfected cells. It should be noted that the postulated imbalance in low-molecular-weight components could also account for the increase in ribosome transit time previously reported in EMC virus-infected L cells (28).

The finding that S200 is enriched for a cap-specific component as a result of infection is consistent with the model presented above. This finding clearly suggests that there is a redistribution of initiation factors, probably including CBP, in infected cells. Alternative explanations, such as activation of cryptic CBP precursors, are difficult to reconcile with the observed shutoff in vivo. The redistribution of a CBP-like component necessarily implies that this is normally associated with faster-sedimenting complexes and is dissociated from these complexes by EMC virus infection. If so, this dissociation may form the basis for the apparent selective inhibition of host translation in vivo, since a

disruption of the component's normal associations could affect the translation of the capped cellular messages, but not that of the uncapped EMC virus message (2).

Carrasco and Smith (7) and Nuss et al. (23) have proposed a model of shutoff in picornavirus infections with features similar to the hypothesis presented above. Specifically, they have suggested that picornavirus infections increase the intracellular concentration of monovalent cations and that the increase selectively inhibits the initiation of translation of cellular mRNAs. However, we have found that inhibition of initiation by hypertonic treatment (35) of L cells significantly reduces the initiation activities of lysates prepared from these cells (G. Jen, unpublished data). Because this effect is opposite the change found in lysates from infected cells, we believe that more than a simple rise in intracellular monovalent cations is involved in EMC virus shutoff of L cell protein synthesis.

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ADDENDUM IN PROOF

It has recently been reported that poliovirus infection of HeLa cells causes the release of a 26,000 M_r form of CBP from a rapidly sedimenting complex (J. Hansen, D. Etchison, J. W. B. Hershey, and E. Ehrenfeld, J. Virol. 42:200–207, 1982). However, this released CBP apparently lacks the ability to stimulate translation in normal or poliovirus-infected lysates. Thus, the relationship of these observations to those reported here is not clear.

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