Translational Elongation Rate Changes in Encephalomyocarditis Virus-Infected and Interferon-Treated Cells

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Infection of mouse L cells with encephalomyocarditis virus results in a rapid inhibition of host protein synthesis before the synthesis of viral proteins. Although no alterations in initiation factor activities have been demonstrated in encephalomyocarditis virus-infected mouse cells, a defect in polypeptide chain elongation has been shown to occur in infected cell extracts. We investigated the significance of this elongation defect in the host shutoff phenomenon *in vivo*. Average polypeptide chain elongation rates were measured at various times after infection. Interferon was used as a reagent to separate temporally the virus-induced alterations. Encephalomyocarditis virus infection of L cells was shown to lead to a progressive reduction in the elongation rate. Whereas interferon pretreatment delayed the decrease in elongation rate in a dose-dependent manner, it failed to alter the kinetics of host shutoff, suggesting that slowing of elongation steps played no significant role in this phenomenon. In addition, interferon pretreatment of either mock-infected or virus-infected cells led to no elongation defect that could be attributed to interferon action.

Infection of mammalian cells by picornaviruses often results in a rapid decrease in the rate of synthesis of cellular proteins (2, 31). In spite of a number of investigations regarding the nature of changes triggered by the infection, the exact mechanism of inhibition of host protein synthesis remains unclear. In the case of poliovirus infection of HeLa cells a loss of initiation factor activity postulated to be specific for capped mRNA's has been demonstrated (19, 36). In contrast, results on encephalomyocarditis (EMC) virus infection of HeLa and other cells have shown that a comparable factor inactivation does not occur (23, 27, 31, 39). Thus, it is clear that the findings from poliovirus-infected cells are not necessarily applicable to other picornavirus infections.

Although the conclusions of these studies are unequivocal, it remained possible that the factor inactivation mechanism employed by poliovirus might be cell specific as well as virus specific, and that in the appropriate host cell EMC virus might effect such an inactivation. Suggestive of this interpretation is the fact that the decline of host protein synthesis in EMC virus-infected HeLa cells occurs several hours later than that induced by poliovirus (23). Comparable kinetics

had previously been observed in EMC virus-infected Krebs and MOPC ascites tumor cells (22, 27), situations in which no factor inactivation had been observed in spite of a careful search. Thus, it remained at least formally possible that cells in which EMC virus induced a rapid, early shutoff of host translation might also be susceptible to factor inactivation by this virus. For this reason we have chosen to investigate such a system.

Infection of mouse L cells or mouse EAT cells with EMC virus or mengovirus leads to a pattern of changes in host protein synthesis rates which closely resemble that seen in the infection of HeLa cells with poliovirus (2, 8, 31): host translation begins a rapid decline shortly after virus infection and is largely suppressed by the time virus translation is maximal. Attempts to demonstrate loss of initiation factor in these cell extracts similar to those caused by poliovirus infection have produced equivocal results (17, 31). However, a significant decrease in the elongation rate of protein synthesis in extracts from such cells has been clearly demonstrated (17). It has been suggested that this phenomenon may play a role in shutting off host translation *in vivo* (17).

To investigate these two issues, the question of cell specificity in host shutoff mechanism, and the question of elongation rate reduction, we

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have chosen to study the kinetics of translation in EMC virus-infected L cells. Results pertaining to the former question will be published elsewhere, where it will be shown that initiation factors are not inactivated. Results pertaining to the role of elongation rate regulation in infected cells are discussed here.

A third issue has been addressed in these studies, which relates to the mechanism by which interferon exerts antiviral specificity. It has been shown previously that antibiotics that inhibit protein synthesis elongation rates, as opposed to initiation rates, specifically inhibit the replication of certain viruses (34, 41). The specificity of these agents can be quite high (34), raising the speculation that interferon might exert at least part of its specificity in a similar manner. To test this hypothesis the effect of interferon pretreatment on translation elongation rate in control and EMC virus-infected cells has been measured.

MATERIALS AND METHODS

Cells and viruses. The LPA strain of mouse L cells kindly provided by Ernest Knight, Jr., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del., was maintained in suspension in Joklik modified minimum essential medium (MEM) supplemented with either 10% bovine calf serum or 7% bovine fetal serum. All experiments were done with monolayers derived from suspension cells which were allowed to attach to plastic substrate and form monolayers for at least 18 h in MEM (Earle) containing either 10% bovine calf serum or 7% fetal bovine serum. Substitution of calf serum for fetal serum did not affect the growth rate of the monolayers and had no effect on viral replication in these cells.

Crude EMC virus stocks were prepared by infecting LPA cell monolayers at 5 PFU/cell with a virus lysate obtained from a low-multiplicity infection of LPA cells. After 30 min of adsorption at room temperature in serum-free MEM, the cells were returned to serum-supplemented MEM and incubated at 37°C. To obtain lysates free of serum components, 3 h after infection the medium was replaced with serum-free MEM. After 24 h the cultures were freeze-thawed twice, and the resulting suspension was centrifuged at $10,000 \times g$ for 5 min. Infecting titers were determined by plaque formation on LPA monolayers as described by Hall and Rueckert (18), and working samples were stored at -70°C.

Interferon. Mouse interferon was induced by infecting primed LPA cells with MM strain of EMC virus (obtained from E. Knight, Jr.) and partially purified to a specific activity of 8×10^6 U/mg through acidification, ammonium sulfate precipitation, and Bio-Gel gel filtration column as described by Knight (24). Working samples in the final column buffer were stored at -70°C after adding 200 µg of bovine serum albumin per ml. Interferon concentrations were assayed by the inhibition of cytopathic effect produced by EMC virus in LPA monolayers as described by Armstrong (1). All concentrations are expressed in

reference units obtained by titrating a National Institutes of Health reference standard in parallel with the samples.

Interferon treatment and infection. LPA cells were resuspended in serum containing MEM at a density of 3×10^5 cells per ml. A 2-ml sample of this suspension was used to seed each 9-cm² well of a Costar 6-well "cluster 6" dish. Cell attachment was complete within 2 h after seeding. After this time medium in the wells was replaced with fresh medium containing desired concentrations of interferon. After overnight incubation (16 to 18 h after interferon additions), cells were washed once with serum-free MEM and infected with 0.3 ml of a suspension of EMC virus diluted in MEM to yield a multiplicity of 10 PFU/cell. After 30 min of adsorption at room temperature, the monolayers were washed twice with MEM and incubated in serum-supplemented MEM at 37°C in a CO₂ incubator.

Determination of protein synthesis rates. At various times after infection, cell monolayers were washed twice with methionine-free MEM, and 0.5 ml of the same medium containing 15 to 30 µCi of [³⁵S]-methionine per ml was added. After 15 min at 37°C, cells were washed twice with complete MEM and lysed immediately in phosphate-buffered saline containing 1% sodium dodecyl sulfate (SDS). Proteins in the lysates were precipitated with 10% trichloroacetic acid, and the resulting precipitates were pelleted, washed twice with acetone, dried, and dissolved in gel sample buffer (20 mM Tris-hydrochloride [pH 6.8], 2% SDS, 20% glycerol). Samples were counted in 3a70 cocktail to determine the incorporated radioactivity. Protein concentrations were determined by using 1:10 or 1:20 dilutions of the dissolved pellet by the method of Lowry et al. (30) with bovine serum albumin standards containing an equivalent amount of SDS. (Interference by this level of SDS was insignificant.) For SDS-polyacrylamide gel analysis, samples were made 5% in β-mercaptoethanol, 0.005% in bromophenol blue, and boiled before loading onto 7.5 to 20% gradient gels.

Determination of elongation rates. Average ribosome transit times (t_{av}) were determined by the method of Fan and Penman (11) incorporating the double-label modification of Palmiter (33) to correct for variations in sample size and recovery. [³H]leucine was used as the constant long-term label, and [³⁵S]-methionine was used for the variable-pulse label. At 1 h after infection, the monolayers were labelled with [³H]leucine (30 µCi/ml) in serum-supplemented MEM containing one-tenth the normal concentration of leucine. After 1.5 to 2 h of incubation at 37°C, the cells were washed twice with serum-supplemented MEM containing one-tenth the normal concentration of methionine (low met medium) and incubated in the same medium until the [³⁵S]methionine pulse.

Incorporation of methionine label was measured at the time chosen for transit time measurement. To facilitate rapid manipulations, the experiment was performed in a 37°C room in an enclosure flushed with 5% CO₂-air mixture. Medium was aspirated, and low met medium containing about 25 µCi of [³⁵S]methionine per ml was added. At various times ranging from 2 to 12 min after label addition, the radioactive medium was replaced with ice-cold hypotonic buffer (10 mM Tris-hydrochloride [pH 7.4], 10 mM KCl, 5 mM

Mg(OAc)₂, 1 mM dithiothreitol) containing 50 μ g of cycloheximide per ml to stop further progress of protein synthesis. After 5 to 10 min on ice, Nonidet P-40 and sodium deoxycholate were added to give 1% final concentration of each. After 10 more min of incubation on ice, KCl and diethylenetriamine were added to 130 mM and 0.1%, respectively. The individual lysates were collected, and wells were washed with the same buffer and pooled. Nuclei were removed by spinning at 2,500 rpm for 5 min. Part of the resulting supernatant was precipitated with 10% trichloroacetic acid, and the acid-precipitable radioactivity was determined. Another sample of the postnuclear supernatant was spun for 3 h at 40,000 rpm in a Beckman type 65 rotor to sediment polysomes, and the acid-precipitable radioactivity in the resulting supernatant was determined. The ³⁵S/³H incorporation ratios obtained were corrected for isotope spillovers by using appropriate single-labeled standards. Transit times were determined as twice the horizontal (time axis) distance between the total and released incorporation ratios (see Fig. 3) as described by Fan and Penman (11).

To obtain the weight-average molecular weight (M_w) of proteins synthesized at the time of transit time determination, identically treated cells (same interferon treatment, infection, etc.) were pulse-labeled during this period in methionine-free MEM containing 15 μ Ci of [³⁵S]methionine per ml for 15 min. Labeled proteins were separated on 7.5 to 20% SDS-polyacrylamide gels. Autoradiograms of the gels were scanned with a Joyce-Loebl densitometer. With labeled EMC virus proteins as markers, the areas under successive 10-kilodalton regions of the scan were determined by cutting and weighing these regions. M_w was determined as $M_w = \sum M_i w_i / \sum w_i$ where w_i is the weight of a segment with midpoint at M_i kilodaltons.

In a short radioactive pulse (without a subsequent cold chase) as described above, a fraction of the incorporated radioactivity remains in nascent chains causing an underestimation of M_w . (A cold chase would obviate this difficulty, but chasing permits the cleavage of high-molecular-weight EMC proteins [4] which in itself skews the protein distribution toward lower molecular weights.) The radioactivity in nascent chains increases as elongation rate decreases. However, even at the lowest elongation rates reported here (see below) it can be shown that the error in M_w does not exceed 25%.

Elongation rates in amino acids per second per ribosome were calculated as $M_w / 110 \times t_{av}$, where 110 is the molecular weight of an average amino acid.

Intracellular potassium levels. Medium was aspirated, and the monolayers were washed for 15 s two times with 0.14 M choline chloride (isotonic) buffered with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid to pH 6.9. Thereafter, the cells were allowed to lyse osmotically at 37°C in 1 ml of flame-photometer lithium standard solution (Instrumentation Laboratory, Inc., Boston, Mass.) containing 15 mM lithium. After 30 min or longer in this lysis solution, cells were freeze-thawed twice and scraped with a rubber policeman. K⁺ ion concentration was determined by using a flame photometer (model 143; Instrumentation Laboratory, Inc.). An aliquot of the same sample was used for the determinations of protein concentrations by the method of Lowry et al. (30).

Polysome profiles. Monolayers grown in 150-cm² T-flasks were rapidly chilled to subzero temperatures in the presence of cycloheximide and lysates were prepared as described elsewhere (W. E. Walden, T. Godefroy-Colburn, and R. E. Thach, submitted for publication). Cell lysates were layered on 15 to 50% (wt/vol) sucrose gradients and spun at 35,000 rpm for 45 min.

RESULTS

Protein synthesis in interferon-treated EMC virus-infected cells. Infection of L cells with EMC virus led to a rapid reduction in the rate of protein synthesis followed by a resumption of protein synthetic activity concomitant with viral replication (Fig. 1). The decline in the incorporation of radioactive methionine was noticeable very early after infection, but the change in rate was maximal at 2 h, reaching the lowest level at around 4 h postinfection (PI). Thereafter, the incorporation rate increased again to a maximum of 5.5 h. Later, a second decrease occurred concomitant with the appearance of drastic cytopathic changes and cell lysis. This pattern is very similar to those reported for the infection of L cells or Ehrlich ascitis tumor cells by mengovirus (8, 31) or of HeLa cells by poliovirus (2, 23). However, it differs from the pattern

FIG. 1. Rates of protein synthesis in interferon-treated, EMC virus-infected L cells. Radioactive methionine incorporated during a 15-min pulse by monolayer cells pretreated for 18 h with the following interferon concentrations: A, none (●), 5 U/ml (Δ), 60 U/ml (■); B, none (●), 600 U/ml (Δ), 4,800 U/ml (■). Monolayers were infected with EMC virus at 0 h. The extrapolation of curves (dotted lines) in A is supported by other experiments carried out for longer periods after infection.

of protein synthesis in EMC virus-infected MOPC or Krebs ascites cells (22, 27).

Also evident in Fig. 1A is the fact that pretreatment of cells with doses of mouse interferon sufficient to reduce virus yields by 50 to 99% did not change the course of the initial decline in protein synthesis. However, the phase of increased viral protein synthesis between 4 and 7 h PI was affected in a dose-dependent manner by interferon. At the very high doses shown in Fig. 1B (600 to 4,800 U/ml), where virus yields were reduced to less than 1% of controls, the initial drop in protein synthesis rate remained unaltered, but no subsequent virus-specific changes were seen. Protein synthesis continued for periods as long as 24 h PI near the lower rate of about 30 to 50% of the control value, which was attained at 4 h PI. Therefore, it is clear that the events that lead to the initial decline of protein synthesis are unaffected by the antiviral activities induced by interferon pretreatment. The difference in the rate of shutoff seen in Fig. 1A and B reflects the variability of the infection process.

Although the fate and the long-term viability of the protected cells are of considerable interest to us, we have not attempted to address this question at this time. However, a recent study using a closely related system has shown that in L cells pretreated with high doses of interferon protein synthesis rate returns to control levels after the rapid drop soon after infection (10). As shown in Fig. 1A and B, we have not observed any increase in the protein synthesis rate as late as 24 h after infection. The reason for this difference is unclear, but the continued presence of interferon in the postinfection medium used in the earlier study (10) may account for the difference.

Figure 2 shows the contributions of host- and virus-directed protein synthesis to total protein synthesis at various times after infection. In Fig. 2a it is shown that intensity of host bands decreased concomitant with the overall decline in translation which occurred between 1 and 4 h PI. The first sign of viral protein synthesis became apparent around 3 h PI with the appearance of viral band A. By 4 h PI all of the major viral bands and cleavage products had appeared, and they continued to increase in intensity concomitant with an overall increase in the rate of protein synthesis. In contrast, in cells pretreated with 60 U of interferon per ml, the EMC virus A band first appeared around 5 h PI, but no other viral band became apparent with increasing time. Host proteins continued to be synthesized, however. In cells treated with 4,800 U of interferon per ml (Fig. 2c), no viral proteins were seen even at 24 h after infection. Thus, interferon

FIG. 2. Host and viral protein synthesis in interferon-treated, EMC virus-infected L cells. Pulse-labeled samples obtained from the experiment shown in Fig. 1 were analyzed on SDS-polyacrylamide gels. Interferon treatment: a, none; b, 60 U/ml; c, 4,800 U/ml. The unmarked lanes on the right and left in each gel are standard EMC virus lysates. Prominent EMC viral proteins are identified on the right. Equal amounts of protein were analyzed in each lane.

treatment delays the appearance of viral proteins and reduces the ultimate rate at which they are synthesized. At sufficiently high doses, it leads to a complete absence of viral protein synthesis. It is also evident from these results that the early shutoff of host protein synthesis is independent of the timing and extent of detectable viral replication.

Polypeptide elongation rates after infection. As noted earlier, a significant deficiency has been reported in the ability of extracts from mengo virus-infected EAT cell extracts to elongate polypeptide chains (17). Before this phenomenon is ascribed a role in the shutoff of host translation, however, it must also be shown to exist *in vivo*. In addition, it is important to determine whether any such rate reduction is truly limiting for protein synthesis. For these reasons we measured the elongation rates *in vivo*. Elongation rates, as measured in amino acids per second, were determined by measuring the average ribosome transit times after infection plus the M_w of proteins synthesized by the cells at the time of transit time measurement (see above). Transit times were determined by the method of Fan and Penman (11) as modified by Palmiter (33), incorporating the use of double label to account for variations in sample sizes and differences in recovery. Values for M_w were estimated from the SDS-polyacrylamide profiles of pulse-labeled proteins. A typical determination of these two parameters is shown in Fig. 3 and 4.

Figure 3 shows the measurement of ribosome transit times in EMC virus-infected cells at 3 h PI and the effect of interferon pretreatment on changes in transit times. We used interferon as an agent to alter the kinetics of viral replication to analyze the correlation between changes in elongation rates and the replication of virus. It

can be seen from Fig. 3a that the ribosome transit time in EMC virus-infected cells was increased in comparison to two types of control cells (other experiments have shown that doses of interferon up to 1,200 U/ml do not lead to a change in the ribosome transit times in uninfected L cells; data not shown). Therefore, the data in Fig. 3 show that EMC virus infection of L cells led to an increase in the average ribosome transit time which was detectable as early as 3 h PI. However, before the significance of this increase can be assessed it must be determined whether changes in the M_w of proteins synthesized in the infected cells have occurred. Since alterations in the spectrum of proteins synthesized are a common feature of all viral infections, it is necessary to account for such changes by converting ribosome transit time data into elongation rate as expressed in amino acids per second. Panels a, b, and c in Fig. 4, respectively, show the pattern of pulse-labeled proteins synthesized at the time when transit times shown in Fig. 3a, b, and c were measured. As described earlier, at 3 h after infection little viral protein synthesis was evident but host protein synthesis was depressed to a significant extent. These changes are reflected in Fig. 4a, where the overall protein pattern is similar to those seen in Fig. 4b and c, except for the 75- to 100-kilodalton region of the gel where EMC bands A, B, C, and D are seen. These viral bands represent less than 10% of the total proteins made at 3 h PI. How-

FIG. 3. Average ribosome transit times in interferon-treated, EMC virus-infected L cells at 3.25 h after infection. Cells prelabeled for 2 h with [^3H]leucine between 1 and 3 h PI were pulsed with [^{35}S]methionine starting at 3.25 h PI (see text). The $^{35}\text{S}/^3\text{H}$ isotope ratio is plotted against time of ^{35}S incorporation. Symbols: total incorporation (●), incorporation into released proteins (▲). Interferon treatment: a, none; b and c, 60 U/ml. Panels: a and b, cells infected with EMC virus; c, mock-infected cells. The horizontal separations between total and released count lines equal one-half the average ribosome transit time.

FIG. 4. Molecular weight distributions of proteins labeled with [^{35}S]methionine at 3.25 h after infection (from experiment shown in Fig. 3) in interferon-treated, EMC virus-infected cells. Interferon levels: a, none; b and c, 60 U/ml. Panels: a and b, EMC virus infected; c, mock infected. Arrows in each panel show the M_w determined by using the molecular weight calibration in panel c. EMC virus protein bands A, B, C, and D are shown in panel a.

ever, the presence of these bands does not significantly alter the M_w values (shown by arrows) in Fig. 4a from those seen in Fig. 4b and c.

The value of M_w of 75 kilodaltons shown in Fig. 4c for interferon-treated, uninfected cells was, within experimental error, close to the values of 80 to 85 kilodaltons measured in cells not treated with interferon. Interferon treatment alone, although known to induce the synthesis of new proteins (15, 25), does not lead to any

significant difference in the spectrum of proteins (as separated by one-dimensional electrophoresis) between interferon-treated and untreated cells. An average value of 80 kilodaltons as the M_w of cellular proteins in our determinations is in agreement with that reported by Fan and Penman (11) for CHO cells, whereas it is higher than the values of about 50 kilodaltons reported by Nielsen and McConkey (32) in HeLa cells. Protein profiles shown in Fig. 4c resemble that published by Fan and Penman to a greater extent than that shown by Nielsen and McConkey. A part of the discrepancy between our data and those of Nielsen and McConkey (32) may arise from real differences in protein patterns between different cell types as well as the inability of these authors to account for contributions from proteins larger than 130 kilodaltons. Higher-molecular-weight proteins affect the M_w to a much greater extent than do those of low molecular weight.

Whereas the spectrum of proteins synthesized after EMC virus infection changes drastically later in infection, the values for M_w are expected to lie close to those in uninfected cells. This result, in spite of the fact that EMC virus codes for a polyprotein larger than 220 kilodaltons, can be attributed to the rapid cleavage of the polyprotein very soon after the ribosomes synthesize a cleavage site (4). Such rapid cleavages occurring soon after ribosomes cross A and F regions of the polyprotein leads to a situation which, for transit time measurements, is kinetically equivalent to the synthesis of the major proteins A, F, C, D, and E by independent initiation events. Based on the model proposed by Butterworth and Rueckert (4), it can be calculated that, in the absence of further slower post-translational processing of these proteins, EMC virus would appear to synthesize a set of methionine-labeled proteins with M_w of 70 kilodaltons (assuming the same mole fraction of methionine residues in each of these proteins). Therefore, the superposition of the EMC virus protein pattern on the host protein with M_w of about 80 kilodaltons is expected to yield a composite M_w between 70 and 80 kilodaltons independent of the extent of viral replication. For reasons described earlier (see above), our measurements in EMC virus-infected cells have yielded values of M_w lower than the expected value by a maximum of about 25%. An error of this magnitude, however, would be insufficient to account for the changes in elongation rates described below.

The changes in elongation rates that ensue upon EMC virus infection are shown in Table 1. A twofold drop in elongation rate was evident by 3 h after infection. This was further decreased by severalfold by 5.5 h PI, when EMC virus-

TABLE 1. *Elongation rates after EMC virus infection*

Treatment	Interferon (U/ml)	Elongation rate at h PI ^a :			Virus yield ^b (% of un- treated)
		0 ^c	3 to 3.5	5.5 to 6	
Mock infection	0	9.4 ± 0.0		9.1 ± 1.6	
	60		11.7 ± 0.4		
	120	8.8			
EMC infection	0		4.8 ± 1.1	1.9	100
	20		8.4 ± 2.5	2.4	50
	60		8.2	3.5 ± 0.9	14
	240			5.2	2

^a Elongation rate (amino acids per second) was calculated as M_w/t_{av} , assuming 110 daltons per amino acid as described in the text. Ranges of observed values are shown whenever more than one determination has been made.

^b Virus yield determined at 12 h after infection.

^c Values in this column were obtained from experiments which involved no infections.

directed translation was at its peak. The elongation rate of 9 or 10 amino acids per s shown in Table 1 for mock-infected cells agrees well with the values reported for reticulocytes (21, 29) and CHO cells (11). Our value of 4 or 5 amino acids per s, which is largely for host proteins in EMC virus-infected cells at 3 h PI, is also in agreement with the elongation rate of 3 or 4 amino acids per s that can be derived from the ribosome transit time (10 min) reported for poliovirus mRNA early in infection (20, 35). This agreement suggests that the virus-induced elongation rate reduction is not selective for host as opposed to virus proteins.

In Table 1, interferon treatment of mock-infected cells is shown not to alter the elongation rates. However, pretreatment of infected cells with interferon delays the virus-induced decrease in elongation rate in a dose-dependent manner. Between 5.5 and 6 h PI, a significant decrease is seen even at doses of interferon sufficient to inhibit viral translation and virus yield by over 95%. This result emphasizes the fact that although the decrease in elongation rate depends on the extent of viral replication, it is not simply a consequence of translation of viral messages alone; ribosomes elongate more slowly on host mRNA's as well. The dependence of elongation rate changes on the extent of viral replication is quite unlike that observed for the rapid reduction in host translation between 2 and 4 h PI (Fig. 1). This leads to the conclusion that the elongation rate changes are not the primary mechanism for the shutoff of host protein synthesis in EMC virus-infected L cells. Rather, the inhibition of host protein synthesis appears to be due to the effects on initiation events as is documented further below.

Polysomes in EMC virus-infected cells. Direct evidence that the shutoff of host translation is due to an initiation block comes from the polysome size determinations. Analysis of

polysome sizes in EMC virus-infected and control cells is shown in Fig. 5. As reported in the mengovirus infection of L cells (31), it is clear that the polysome size in EMC virus-infected L cells decreases with a concomitant increase in the 80S monosome peak. This decrease in polysome size occurs in spite of the two- to threefold decrease in the elongation rates expected by 4.5 h after infection (Table 1). Inasmuch as reduction in the elongation rate would tend to increase polysome size, the data in Table 1 and Fig. 5 indicate that a more substantial reduction in the initiation rates has occurred than can be inferred from the polysome sizes. Thus, it is evident that the virus-induced reduction in elongation rate, substantial though it is, is not the primary mechanism of host translation shutoff. Furthermore, careful studies in our laboratory (G. Jen and R. E. Thach, manuscript in preparation) have failed to detect any decrease in the translatability of host mRNA's isolated from infected cells. Taken together, these two results underscore the importance of initiation rate reduction in the shutoff of L-cell translation by EMC virus.

Intracellular potassium levels after infection. Elongation steps in protein synthesis can be affected by changes in any of the multiple components involved in these steps, ranging from high-molecular-weight components such as ribosomes, tRNA, and elongation factors to low-molecular-weight components such as triphosphates and ions. A number of studies, primarily those of Carrasco and co-workers, have shown that viral infection of mammalian cells can lead to alterations in the intracellular ionic conditions (9, 12, 13). Such ionic changes have been postulated to be the cause of the host translational shutoff, and their effects have been ascribed to an initiation step (6, 26). However, it is also known that K⁺ ions are required for the elongation of protein chains and that elongation steps are more sensitive to changes in K⁺ levels

FIG. 5. Polysome formations in uninfected and EMC virus-infected L cells at 4.5 h after EMC virus infection. A_{260} , absorbancy at 260 nm.

than are the initiation steps (5). Therefore, it was of interest to see whether the decrease in elongation rate we observed was correlated with the loss of K^+ ions from the cells.

Figure 6 shows the changes in intracellular K^+ ion levels after EMC virus infection, as measured by flame photometry. Whereas the K^+ levels in mock-infected cells remained relatively constant, in infected cells they dropped abruptly from control value by about 25% between 2 and 3.5 h PI. This was followed by a further decrease between 6 and 8 h, which coincided with the lysis of cells by the virus. The initial drop that occurred between 2 and 3.5 h PI was not influenced by the pretreatment of cells with interferon, even at a dose sufficient to inhibit virus replication by greater than 99% (600 U/ml). The second phase of decrease of K^+ ions was delayed by interferon in a dose-dependent manner, as would be expected from the ability of interferon to delay the development of cytopathic changes. From these results it is evident that the changes in K^+ levels that occur upon EMC virus infection do not correlate with the decrease in elongation rates described in Table 1. In contrast, an excellent correlation is seen between the 25% change in K^+ ion levels and the early inhibition of host protein synthesis. Both these changes occurred between 2 and 3.5 h PI, and neither was influenced by interferon treatment. However, the change in K^+ ion level is not due to the inhibition of protein or RNA synthesis (or both) that en-

FIG. 6. Changes in intracellular potassium levels in interferon-treated, EMC virus-infected cells. Monolayers pretreated for 18 h with interferon concentrations of 60 U/ml ($\blacktriangle$) or 600 U/ml ($\blacktriangledown$) or not pretreated ($\bullet$) were infected at 0 h. Open circles show mock-infected culture with no interferon pretreatment. A mock-infected culture pretreated with 600 U of interferon per ml yielded results identical to those of the mock-infected culture.

sues upon EMC virus infection, because neither the inhibition of protein synthesis with cycloheximide nor the inhibition of RNA synthesis with actinomycin D caused any changes in the

levels of K^+ ions even after prolonged treatments (data not shown).

DISCUSSION

It has been suggested that changes in translation elongation rates may play important roles in virus-infected or interferon-treated cells (17, 41). For this reason we have measured these rates in mouse L cells infected with EMC virus with or without interferon pretreatment. Interferon has proved to be a valuable reagent for the study of EMC virus replication, inasmuch as it allows various virus-induced changes to be temporally separated. Measurements of host and viral protein synthesis rates in interferon-treated, EMC virus-infected cells show that replication of EMC virus, as judged by the appearance of viral protein bands, is diminished and delayed at lower doses and is abolished at sufficiently high doses of interferon. Similar results have been reported in single cell studies on the ability of interferon-treated, mengovirus-infected L cells to produce infectious virus (14). Also in agreement with previous studies (10, 28), the early inhibition of host protein synthesis that occurs between 0 and 4 h after infection is unaffected by the dose of interferon, indicating that this effect is independent of the development of infection beyond the point at which viral replication becomes affected by interferon. This suggests that the early host shutoff appears to be caused either by virion components or by virus products synthesized early in infection before interferon action is triggered. In cells treated with doses of interferon sufficient to suppress the appearance of any viral proteins, after the initial shutoff host protein synthesis continued unchanged for extended periods at 30 to 50% of the control rate (Fig. 1B). This indicates that the interferon-induced mechanism(s) that abolishes the translation of viral mRNA's does not significantly affect the translation of host messages, in agreement with the recent findings of others (10).

Results summarized in Table 1 show that EMC virus infection of L cells led to a decline in the rate of peptide chain elongation *in vivo*, as has been shown in poliovirus-infected HeLa cells (38). Effects on elongation rate have also been reported in extracts of mengovirus-infected mouse EAT cells (17). Therefore, a decrease in the efficiency of ribosomes in elongation may be a general feature of picornaviral infections. The onset of changes in elongation rate was delayed by interferon in a dose-dependent manner, indicating that products of viral replication may cause this effect.

This result is in striking contrast to the lack

of interferon effects on the early host shutoff, and suggests that the two translational defects may be unrelated. Further support for this view comes from a study of polysome sizes (Fig. 5), which shows that host polysomes became smaller after EMC virus infection. This result, which agrees with much previously published work with other picornaviruses (2, 31), indicates clearly that the primary mechanism of early host shutoff in L cells by EMC virus is at an initiation step. Indeed, initiation inhibition must be more severe than the data in Fig. 5 indicate, since the elongation inhibition would counteract any polysome size decrease caused by initiation inhibition. Although these data appear to suggest that EMC virus-induced early shutoff of host translation in L cells proceeds by a mechanism similar to that produced by other picornaviruses in other cells, this is surprisingly not the case. As will be reported elsewhere, host initiation factors (and more specifically, those that recognize capped mRNA's) are not inactivated by EMC virus infection of L cells.

In a preliminary attempt to determine the cause of the elongation defect resulting from EMC virus infection, we have measured the virus-induced changes in intracellular ionic conditions. It is known that cardiovirus infection of mammalian cells leads to changes in the cell membrane which correlate in time with the levels of low-molecular-weight components within the cells (6, 9, 12, 13). Elongation steps in protein synthesis have been shown to be particularly dependent on the intracellular level of K^+ ions (5). However, our results in EMC virus-infected L cells have failed to show any temporal correlation between the decrease in elongation rate and the decrease in level of intracellular K^+ ions, especially in response to interferon treatment. Moreover, the magnitude of the decrease in K^+ ion levels (20 to 30%) at 3 h after infection (Fig. 6), in light of the data presented by Cahn and Lubin (5), would be insufficient to effect the two- to threefold changes in elongation rates shown in Table 1.

In extracts of mengovirus-infected EAT cells, the decrease in elongation activity has been localized to the pH 5 fraction (17) which comprises high-molecular-weight components such as aminoacyl-tRNA synthetases, elongation factors, and tRNA. Therefore, in the absence of large changes in intracellular ionic conditions, attention must be directed to one or more of these high-molecular-weight components as the agent responsible for the decrease in elongation rate. It has been suggested that the inhibition of host RNA synthesis that follows many picornaviral infections may cause a decrease in the availability of substrates for methyltransferases and,

hence, lead to the hypermethylation of tRNA (40). However, additional investigations would be required to determine whether that is the cause of the elongation defect.

Finally, it should be noted that inactivation of specific tRNA species leading to preferential elongation blocks has been suggested as a component of the antiviral mechanism induced by interferon (7, 16, 34, 37, 41, 42). However, we have been unable to demonstrate any decrease in overall elongation rates which may be ascribed to interferon treatment alone in either mock-infected or EMC virus-infected cells. These results suggest that interferon may not cause a general slowing of elongation, in contrast to the action of antibiotics such as cycloheximide which produce specific antiviral effects in certain cases (34, 41). However, our experiments do not rule out the possible effects of interferon on very early elongation steps, as these would not be detected by the transit time measurements employed here. Inasmuch as the early elongation steps are by far the most important in determining overall translation rates (3), this possibility remains of interest.

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