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Encephalomyocarditis Virus RNA

II. Polyadenylic Acid Requirement for Efficient Translation

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Differentially polyadenylated subpopulations of encephalomyocarditis (EMC) viral RNA were isolated by affinity chromatography on oligodeoxythymidylic acid-cellulose. Translation of these RNA fractions in several in vitro protein-synthesizing systems, isolated from Ehrlich ascites tumor cells, demonstrated that poly(A)$^+$ EMC viral RNA was translated two to three times more efficiently than poly(A)$^-$ EMC viral RNA. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of the polypeptides synthesized by the in vitro system in response to the different RNAs showed no detectable differences in the size or relative amounts of the translational products. mRNA saturation curves indicated that the in vitro systems were stimulated maximally by equivalent amounts of RNA, whether it be poly(A)$^-$ or poly(A)$^+$ EMC viral RNA. Time course experiments showed that the differences in translatability were more pronounced late in the reaction when reinitiation was required, and that by eliminating reinitiation with high salt the apparent effect of poly(A) on translation was diminished. Together, these results suggest that poly(A) may be required for efficient initiation and reinitiation of protein synthesis in the cell-free systems. This interpretation is discussed relative to earlier data.

Many investigators have sought to establish a cytoplasmic function(s) for polyadenylic acid [poly(A)], which is found covalently linked to the 3'-terminus of most RNA molecules capable of being translated by eucaryotic cells (4). Much of this work has been carried out in various eucaryotic cell-free protein-synthesizing systems utilizing de-adenylated mRNA as a probe. These experiments have failed to discern any stringent requirement for poly(A) in the translation of mRNA (2, 15, 27). This may, in part, be due to the inefficiency of the in vitro systems being used or the conditions used for translation. Huez et al. (10) have utilized the *Xenopus* oocyte system, which has been shown to carry out translation of exogenously injected mRNA for long periods of time, to show that poly(A) is important in maintaining the functional stability of globin mRNA. Also, recent careful analysis of ovalbumin message translation has shown that poly(A) facilitates the reutilization of this mRNA in cell-free systems (6). Whether these effects are due to a protection of polyadenylated mRNA from nuclease degradation, as suggested by Hieter et al. (8), or rather a more direct role in protein synthesis is not yet clear.

We have been interested in determining the role(s), if any, of poly(A) in the replication of encephalomyocarditis (EMC) virus. To this end, differentially polyadenylated fractions of EMC viral RNA have been isolated and translated in cell-free extracts from Ehrlich ascites tumor cells. This approach has the advantage of utilizing naturally occurring populations of homogeneous mRNA in which the only apparent difference is the length of the poly(A) moiety and translating these RNAs in a cell-free extract derived from cells that translate this message in vivo. The results reported here indicate that the presence of a poly(A) region stimulates the overall translation of EMC viral RNA two- to threefold. This is in apparent contrast to earlier work on the translation of poliovirus RNA (25) but is explicable due to differences in experimental methods.

MATERIALS AND METHODS

Materials. [8-^{3}H]adenosine (33 Ci/mmol), [5-^{3}H]uridine (28 Ci/mmol), and a ^{14}C-labeled amino acid mixture (algal profile) were obtained from Schwarz/Mann; [^{32}P]orthophosphate and [^{35}S]-methionine (525 Ci/mmol) were from Amersham/Searle; oligodeoxythymidylic acid [oligo(dT)]cellulose, T-3 grade, was from Collaborative Research, Inc.; polyacrylamide gel electrophoresis materials were of electrophoresis purity, purchased from Bio-Rad; Seakem agarose was from Bausch and Lomb; X-ray film and photochemicals were bought from Eastman Kodak.

Virus growth. EMC virus was grown in suspen-

sions of Ehrlich ascites tumor cells and purified as previously described (9). The viral RNA was radioactively labeled and extracted as before (9).

RNA analysis. Oligo(dT)-cellulose chromatography was performed essentially as described by Aviv and Leder (1), with certain modifications (9). Intact 35S EMC viral RNA was isolated by centrifugation through 11.5 ml of 5 to 20% sodium dodecyl sulfate (SDS)-sucrose gradients that were spun at 35,000 rpm for 4.75 h at 24°C in a Beckman SW41 rotor (9).

Gel electrophoresis. (i) Agarose-acrylamide gels (for RNA). Composite gels were run according to the methods of Loening (14). Agarose (0.5%)-acrylamide (1.8%) gels were polymerized for 1 h in acid-washed glass tubes (15 by 0.8 cm). The gels were subjected to electrophoresis at 5.5 mA/gel for 10 min in 0.4 M Tris-hydrochloride, 1 mM EDTA, 0.02 M sodium acetate, 0.1% SDS (pH 5.5). RNA samples to be analyzed were dissolved in 25 μl of 1 mM EDTA, 10 mM Tris-hydrochloride (pH 7.5), 0.2% SDS and then diluted 1:1 with 50% sucrose-0.5% bromophenol blue. Samples were heated at 85°C for 2 min and then applied immediately to the gel. Electrophoresis was at 5.5 mA/gel for 3 to 5 h in running buffer. After electrophoresis, gels were fractionated on a Gilson gel slicer into 2-mm slices. Slices were incubated in 0.5 ml of concentrated ammonia overnight and then counted in 10 ml of Bray solution (5).

(ii) Polyacrylamide gel electrophoresis [for poly(A)]. Polyacrylamide gels were set up and run according to Peacock and Dingman (17). RNA samples were subjected to electrophoresis on 3-mm 10% polyacrylamide slab gels at 200 V at 4°C for 4 h in pH 8.3 buffer. After electrophoresis, the gel was fixed in 1 M acetic acid, stained with methylene blue, and destained with water to locate nonradioactive markers. The gel was then impregnated with PPO (2,5-diphenyloxazole) (3), dried under vacuum, and put on Kodak Royal X-Omat film at −70°C for 2 to 4 weeks. The resulting autoradiogram was traced using the Ortec model 4310 densitometer.

(iii) SDS-polyacrylamide gel electrophoresis (for protein). Ten-microliter samples of cell-free protein synthesis reactions were acetone-precipitated, dried, dissolved in 25 μl of sample buffer, and boiled for 5 min. Samples were then put on a 10%, 1-mm, 0.1% SDS-polyacrylamide slab gel and subjected to electrophoresis at 100 V for 6 h (12). The gel was then stained with Coomassie blue and destained in methanol-acetic acid (5:7.5%, respectively). The gel was then dried under vacuum and put on Kodak Blue Brand film for 1 to 2 weeks.

Cell-free protein synthesis. (i) Fractionated system. An mRNA-dependent in vitro protein synthesizing system, consisting of purified ribosomes and an ammonium sulfate fraction, was prepared from Ehrlich ascites tumor cells as previously described by Sharma et al. (22). Reactions contained, in a volume of 100 μl: 20 mM Tris-hydrochloride (pH 7.5), 1 mM ATP, 0.2 mM GTP, 6 mM creatine phosphate (all adjusted to pH 7.5), 20 μg of creatine kinase, 0.5 μCi of a ^{14}C-labeled amino acid mixture, 0.05 mM each of the six nonradioactive amino acids missing from the ^{14}C-labeled amino acid mixture, 6 mM β-mercaptoethanol, 5 mM $Mg(Ac)_2$, 120 mM KCl, 0.07 optical density (at 260 nm) unit of ammonium sulfate fraction, and 0.33 optical density (at 260 nm) unit of purified ribosomes. Following the reaction, 0.2 ml of 0.1 N KOH was added, and the tubes were incubated at 37°C for 20 min. A 1.0-ml amount of cold 10% trichloroacetic acid was added, and the precipitate was collected on Whatman GF/A glass fiber filters.

(ii) S10-1 system (endogenous protein synthesis reduced by preincubation of the cell-free extract). Ehrlich ascites cells from 8-day tumors were washed with phosphate-buffered saline to remove erythrocytes (20) and suspended to 5% in Fischer medium for leukemic cells of mice (Grand Island Biological Co.), and the suspension was rotated gently at 37°C for 1 h to restore protein synthesis. The ascites cells were then removed by centrifugation and used to prepare an S10 protein-synthesizing system, using the method previously described for L-cells (11). Reactions were carried out in a total volume of 25 μl, which contained: 2.5 μl of S10 extract, 0.125 μCi of the amino acid mixture plus the six nonradioactive amino acids to 0.05 mM, 108 mM KCl, 5 mM $Mg(Ac)_2$, 20 mM HEPES (pH 7.6), 7.2 mM β-mercaptoethanol, 4 μg of creatine phosphokinase, 10 mM ATP, 1 mM GTP, 6 mM CTP, and 14 mM creatine phosphate. Following the reaction, 10-μl samples from each tube were spotted on Whatman 3 MM filter squares and plunged into cold 5% trichloroacetic acid. Filters were heated to 90°C for 15 min and washed twice with cold 5% trichloroacetic acid, twice with cold ethanol, and twice with acetone. Filters were dried and counted by liquid scintillation.

(iii) S10-2 system (endogenous protein synthesis reduced by cell starvation). We have found that by incubating ascites cells in nutrient-free medium we can obtain an active S10 protein-synthesizing system with low endogenous amino acid incorporation without the usual preincubation of the S10 supernatant fraction. Ehrlich ascites cells were washed as above and suspended to 3% in 146 mM NaCl-35 mM Tris-hydrochloride (pH 7.5). The suspension was rotated for 90 min at 37°C, and the cells were collected by centrifugation. An S10 system was prepared as above except that the preincubation of the extract was omitted. Assays were performed as with the S10-1 system.

(iv) S10-3 system (endogenous protein synthesis reduced by preincubation with staphylococcal nuclease). The S10-3 system was prepared using the S10-1 procedure, except that instead of preincubating the S10 extract at 37°C for 45 min, the extract was preincubated briefly with staphylococcal nuclease according to the method of Pelham and Jackson (18). The S10-3 system was assayed as above and showed low background incorporation and good stimulation with EMC viral RNA.

RESULTS

Oligo(dT)-cellulose chromatography. We have previously shown that affinity chromatography of EMC viral RNA on oligo(dT)-cellulose columns separates the RNA into three peak fractions, which differ in both poly(A) content and biological activity (9). These three fractions

were denoted as peak 1 [poly(A)$^-$] RNA, peak 2 [oligo(A)] RNA, and peak 3 [poly(A)$^+$] RNA and were shown to contain RNA molecules with an average of 16, 25, and 74 nucleotides, respectively, in their poly(A) tracts (9). In this communication we will continue to use peaks 1 through 3 as the designation for these differentially polyadenylated subpopulations of EMC RNA.

The EMC viral RNA is not degraded during fractionation on the oligo(dT)-cellulose columns. The RNAs from peaks 1 through 3 migrate as a single band in agarose-acrylamide gels (Fig. 1), thus corroborating the earlier SDS-sucrose analysis, which showed that the RNA from these fractions consisted of undegraded genomic-length EMC RNA (9).

For this study we wanted to determine whether peak 1 EMC RNA contains a uniform population of RNAs with short or missing poly(A) moieties, or whether this RNA consists of poly(A)$^-$ RNA together with a minor contamination of poly(A)$^+$ RNA sufficient to account for the average poly(A) tract size of 16 nucleotides. Accordingly, [^{3}H]adenosine-labeled peak 1 and 3 EMC viral RNAs were treated with a combination of RNases A and T1, and the resistant fraction was isolated and run on 10% polyacrylamide gel electrophoresis. Peak 3 EMC viral RNA poly(A) (Fig. 2B) ran as a relatively homogeneous peak, with a mobility slightly faster than that of 4S RNA. This is similar to the electrophoretic mobility of poliovirus poly(A) regions (24). Virtually no poly(A) sequences were detected in RNase-treated peak 1 EMC viral RNA (Fig. 2A). This eliminates the possibility that peak 1 EMC viral RNA contains a few long poly(A) regions, which account for its residual infectivity, but does not prove that this RNA is truly poly(A)$^-$. One of the steps used to isolate the poly(A) regions is binding to benzoylated cellulose (21), and it is possible that very short oligo(A) tracts from the peak 1 RNA do not bind to benzoylated cellulose and escape detection by this procedure.

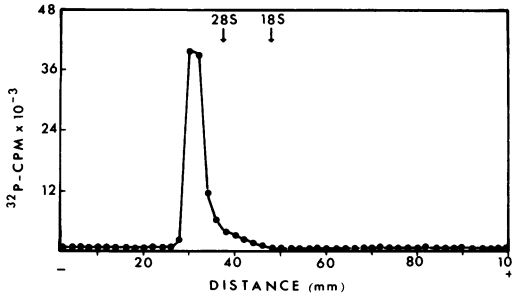

FIG. 1. *Agarose-acrylamide gel electrophoresis of EMC viral RNA. Approximately 100,000 cpm of peak 3 ^{32}P-labeled EMC viral RNA was subjected to electrophoresis through 0.5% agarose-1.8% acrylamide composite gels. Gels were fractionated, and the radioactivity in each slice was determined. The position of the markers was similarly determined on a parallel gel using rRNA from Ehrlich ascites tumor cells. Identical patterns were observed with peak 1 and 2 EMC viral RNA.*

Translation of oligo(dT)-cellulose-fractionated EMC viral RNA. Unlabeled EMC viral RNA was fractionated on oligo(dT)-cellulose as usual. Peaks 1 through 3 were then run on SDS-sucrose gradients, and the RNA in the 35S peak was isolated by ethanol precipitation. The three RNA fractions were assayed by in vitro protein-synthesizing systems prepared from Ehrlich ascites tumor cells using several different methods. These different systems were utilized to investigate the possible effects of changes in preincubation conditions, ions, and relative concentrations of translational components on the translation of the EMC viral RNAs. Reactions were carried out at saturating RNA concentrations: 3.0 μg/assay for the fractionated system; 0.3 μg/assay for each of the S10 systems. Regardless of the method of system preincubation, reaction temperature, ionic conditions (Mg^{2+}-spermidine), or presence of hemin, peak 3 EMC viral RNA always stimulated two- to threefold more radioactive amino acid incorporation than peak 1 EMC viral RNA. Peak 2 EMC viral RNA was usually 90 to

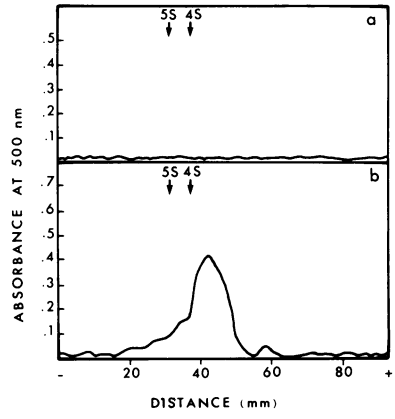

FIG. 2. *Gel electrophoresis of EMC viral RNA poly(A) regions. [^{3}H]adenosine-labeled poly(A) regions were isolated from peak 1 and 3 EMC viral RNA by RNase treatment, followed by chromatography on benzoylated cellulose (21). The poly(A) regions were then subjected to electrophoresis on a 10% polyacrylamide gel along with 4S and 5S RNA markers from Ehrlich ascites tumor cells. Above, the densitometer tracings of the autoradiogram are displayed. (a) Peak 1 EMC viral RNA; (b) peak 3 EMC viral RNA.*

100% as effective as peak 3 EMC viral RNA. The data in Table 1 give the results of several representative experiments. A number of other experiments were performed in which the aforementioned variables were modulated, including translation in the S10-2 and S10-3 systems, and although the overall level of protein synthesis was affected, the relative translatabilities of peak 1, 2, and 3 EMC viral RNA remained constant. This effect has been observed with EMC viral RNAs isolated from five separate virus preparations.

The nature of the polypeptides synthesized by the S10 in vitro system in response to peak 1, 2, and 3 EMC viral RNA was investigated by subjecting the ^{14}C-labeled proteins to electrophoresis on a 10% SDS-polyacrylamide gel. The results of this are shown in Fig. 3. Little, if any, difference in the relative amounts or size of products was detectable. This is in agreement with earlier studies on the translation of deadenylated polioviral RNA (25).

Concentrations of RNA required for optimal translation. EMC viral RNA saturation experiments were carried out to see if the differences in translatabilities could be overcome by simply adding more peak 1 EMC viral RNA to the cell-free system. As can be seen in Fig. 4, this was not the case. Radioactive amino acid incorporation using 2.5 μl of S10 was stimulated maximally by 0.3 μg of EMC viral RNA (12 μg/ml), regardless of whether it was peak 1 or peak 3 EMC viral RNA. Similar experiments were carried out using 5 μl of S10 (or the fractionated system), with identical results, the only difference being in the amount of RNA required to reach saturation (5 μl of S10 = 40 μg/ml; fractionated system = 30 μg/ml).

At this point, it seemed possible that the lowered translatability of peak 1 EMC viral RNA was due to contaminating host cell RNA species, which also sedimented at 35S. To check this, EMC virus was grown in the presence of 5 μg of actinomycin D per ml, which inhibits host cell RNA synthesis, and labeled with [^{3}H]uridine. The RNA was fractionated as usual and then run on SDS-sucrose gradients. Figure 5 shows that the radioactivity and absorbancy at 260 nm profiles of the 35S RNA in peak 1, 2, and 3 EMC viral RNA were coincident. The average specific activities of the peak fractions were virtually identical: peak 1 = 1,140 cpm/μg, peak 2 = 1,170 cpm/μg, and peak 3 = 1,190 cpm/μg. Therefore, it appeared that there was little or no contamination of peak 1 EMC viral RNA by unlabeled host RNA species.

Kinetics of translation. The incorporation of ^{14}C-labeled amino acids into proteins in response to peak 1 and 3 EMC viral RNA was monitored with time (Fig. 6a). Translation of the two RNAs was found to be similar during the first 30 min of incubation; however, after this period the translation of peak 1 RNA slowed considerably, whereas the translation of peak 3 RNA continued at a linear rate for an additional 60 min, resulting in the eventual two- to threefold difference in the extents of translation. Similar kinetic patterns for the translation of poly(A)$^-$ ovalbumin RNA have been obtained by Doel and Carey (6). When translation of the EMC viral RNAs was initi-

TABLE 1. *In vitro translation of oligo(dT)-cellulose-fractionated EMC viral RNA*[a]

In vitro system	Reaction conditions		EMC viral RNA		^{14}C incorporated (cpm)	Stimulation (fold)
	°C	Ions (mM)	Fraction	μg		
Fractionated	37	Mg^{2+}, 5		0	5,100	
		K^+, 120	Peak 1	3	46,800	9.2
			Peak 2	3	128,700	25.4
			Peak 3	3	133,400	26.3
S10-1	30	Mg^{2+}, 2		0	3,800	
		Spd, 0.3	Peak 1	0.3	14,400	3.9
		K^+, 108	Peak 2	0.3	26,500	7.2
			Peak 3	0.3	29,100	7.9
S10-1	30	Mg^{2+}, 5		0	400	
		K^+, 108	Peak 1	0.3	10,700	25.7
			Peak 2	0.3	22,800	54.6
			Peak 3	0.3	23,400	55.9

[a] Oligo(dT)-cellulose-fractionated EMC viral RNAs were further purified by zonal centrifugation and translated in several different in vitro systems prepared from Ehrlich ascites cells. Reactions were allowed to proceed for 120 min, and then ^{14}C-labeled amino acid incorporation was measured as described in the text.

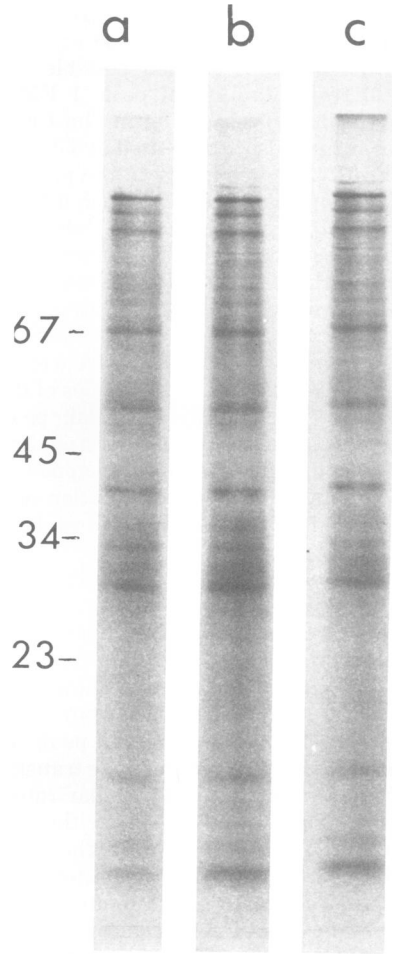

FIG. 3. *SDS-polyacrylamide gel electrophoresis analysis of viral polypeptides synthesized in vitro. Peak 1 (a), peak 2 (b), or peak 3 (c) EMC viral RNA (0.3 μg) was added to a 25-μl protein synthesis assay which contained 2.5 μl of S10-1. After synthesis, approximately 10,000 cpm of each reaction mixture was analyzed by 10% SDS-polyacrylamide gel electrophoresis. Above is displayed the autoradiograph of the polypeptides synthesized in response to the added viral RNA. Numbers refer to the molecular weights of marker proteins (×10⁻³). Note that the largest newly synthesized viral protein corresponds to an approximate molecular weight of 140,000.*

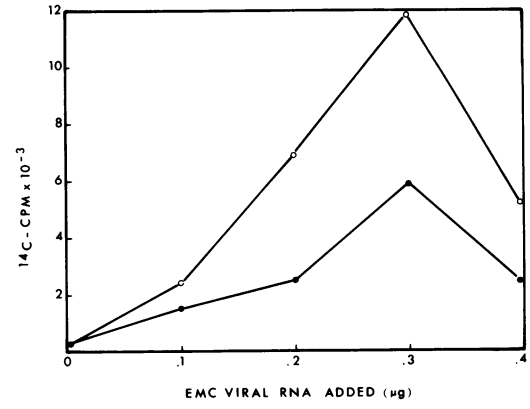

FIG. 4. *RNA saturation curves. Varying amounts of peak 1 or 3 EMC viral RNA were added to 25-μl in vitro protein-synthesizing assays which contained 2.5 μl of S10-2. The reactions were carried out at 5 mM Mg^{2+}-108 mM K^+ and 30°C, for 120 min. Symbols: (○), peak 3 EMC viral RNA; (●), peak 1 EMC viral RNA.*

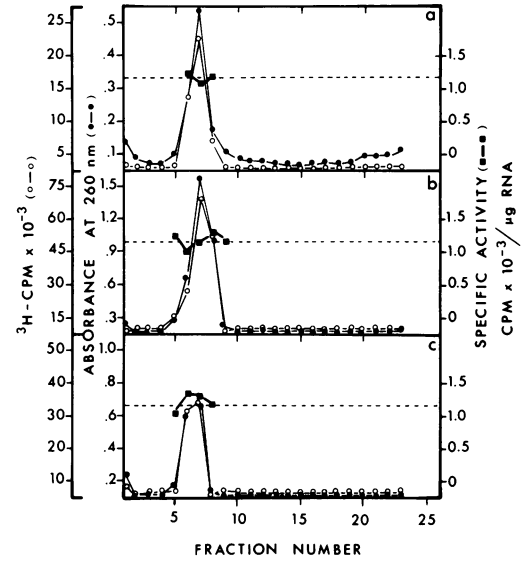

FIG. 5. *Specific activity of [³H]uridine-labeled EMC viral RNA oligo(dT)-cellulose fractions. [³H]uridine-labeled EMC viral RNA was fractionated on oligo(dT)-cellulose. Each of the three fractions was then analyzed on SDS-sucrose gradients. Each gradient was analyzed for absorbance at 260 nm (A_{260}) and trichloroacetic acid-precipitable radioactivity. Specific activities were calculated assuming 1 A_{260} unit equals 40 μg of EMC viral RNA. (a) Peak 1 EMC viral RNA; (b) peak 2 EMC viral RNA; (c) peak 3 EMC viral RNA.*

ated as usual, followed by shifting to high-salt reaction conditions to permit polypeptide elongation but prevent further initiation and reinitiation events (26), much of this difference in the extents of translation disappeared (Fig. 6b).

DISCUSSION

We have never failed to observe a two- to threefold difference in efficiency of translation between poly(A)⁺ and poly(A)⁻ RNAs isolated from five different preparations of EMC virus. This difference was observed under a variety of ionic and temperature conditions used for

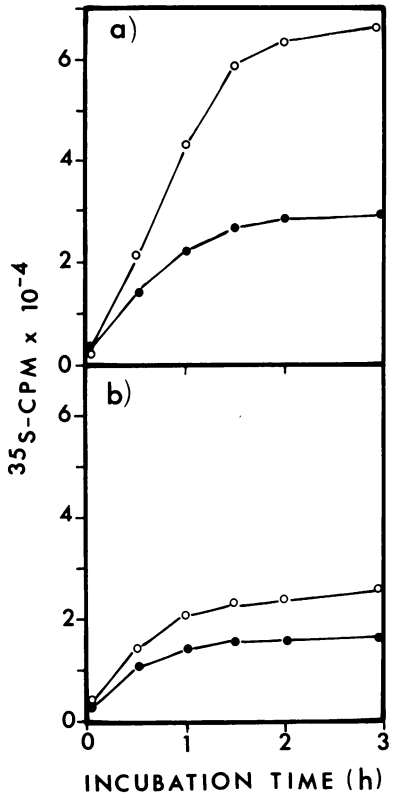

FIG. 6. *Time course of EMC viral RNA translation. A 0.6-μg amount of peak 1 (●) or peak 3 (○) EMC viral RNA was added to a 50-μl S10-1 assay containing 5 μl of S10. At the indicated times, 3-μl samples were removed and processed to determine hot trichloroacetic acid-precipitable radioactivity. (a) Reaction run at 90 mM KCl; (b) reaction started at 90 mM KCl. At 15 min the KCl concentration was shifted to 155 mM.*

translation (Table 1). From the mRNA saturation curves and specific activity analysis it was evident that this difference could not be explained by the presence of contaminating RNA species in the peak 1 EMC viral RNA fraction (Fig. 4 and 5). Also, electrophoresis under denaturing conditions showed the RNAs to be undegraded (Fig. 1).

There are at least two possible explanations for the reduced translatability of poly(A)$^-$ EMC viral RNA. First, it may be that poly(A)-deficient RNA is more susceptible to nuclease degradation (8) and therefore has a lowered probability of remaining intact and interacting productively with the protein synthetic machinery. In preliminary experiments, we have been unable to detect any increased susceptibility of the poly(A)$^-$ RNA to exonucleolytic or endonucleolytic attack during the protein synthesis incubation. However, a more subtle role of the poly(A) moiety, such as affecting the degradation or modification of the 5′ initiation region of the viral RNA, cannot be ruled out. A second possible explanation for the reduced translatability of the poly(A)$^-$ RNA is that poly(A) directly influences one of the protein synthetic reactions required for initiation, elongation, or termination. Since the patterns of polypeptides synthesized in response to peak 1, 2, and 3 EMC viral RNA are identical (Fig. 3), this would indicate that the termination process is not affected. Also, the decreased differential between the translations of poly(A)$^+$ and poly(A)$^-$ RNAs at early times or under high-salt conditions (Fig. 6) suggests that elongation is not involved in the discrimination between the RNAs (13). It seems most likely that the reduced translational capacity of poly(A)$^-$ EMC viral RNA is due to some impairment with the initiation or reinitiation process (6). This impairment could result in an increased functional inactivation of the poly(A)$^-$ RNA whenever it is not engaged with ribosomes in protein synthesis, leading to an accumulating inhibition of protein synthesis, which becomes increasingly apparent late in translation.

There are several possible reasons why we have been able to demonstrate a reduction in the translational capacity of a poly(A)$^-$ message when similar analyses by others (2, 25, 27) have not. One possibility is that the Ehrlich ascites tumor cell-free system carries out protein synthesis for long periods of time in response to EMC viral RNA. Incorporation of radioactive amino acids continues for more than 2 h at 30°C (Fig. 6). This is in contrast to 30 to 60 min of linear incorporation that has been monitored in other studies (2, 15). Huez et al. (10) have been the most successful in establishing a role for poly(A) in the translation of mRNA, and this is probably due to the fact that their studies have been done in the *Xenopus* oocyte system, which carries out many rounds of translation. This is very different from many cell-free systems, such as the wheat germ embryo system, which are very inefficient at reinitiating new rounds of synthesis (19). The Ehrlich ascites system used here continues to reinitiate new rounds of synthesis for more than 90 min (D. E. Hruby, unpublished data), which would facilitate detection of an effect of poly(A) on the accumulating inactivation of mRNA. This is in contrast to the methods of Spector et al. (25), who allowed initiation of poliovirus RNA to proceed for only 15 min before shifting to a higher potassium concentration, which permits elongation but inhibits initiation. Using their procedure, we too can detect little effect of

poly(A) on the translation of viral RNA (Fig. 6b).

In all of the reactions where it was assayed, peak 2 EMC viral RNA was nearly as active as peak 3 EMC viral RNA. This was true even if peak 2 was chromatographed a second time. It may be, as suggested for globin mRNA (16), that there is a critical size for poly(A) function. For globin mRNA this was estimated to be 32 nucleotides. This agrees fairly well with our data, as peak 2 EMC viral RNA has previously been estimated to contain an average of 26 nucleotides in its poly(A) region (9).

Lowered infectivity of poly(A)$^-$ picornaviral RNA molecules has previously been observed by us (9) and others (7, 23). It is not known whether this effect of the poly(A) moiety on infectivity is at the level of penetration, protection against nucleases, translation, or transcription. The lowered efficiency of translation of peak 1 EMC viral RNA may, at least in part, explain its lowered infectivity. Currently, research is in progress to find conditions that potentiate the reduced translational capacity of peak 1 EMC viral RNA in order to better assess the role of poly(A) in translation and infection.

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