Effect of Double-Stranded RNA Associated with Viral Messenger RNA on in Vitro Protein Synthesis†

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ABSTRACT: Vaccinia virus mRNA transcribed in vitro by virions is translated about as efficiently as globin mRNA in the wheat germ cell-free system, but much less efficiently than globin mRNA in a reticulocyte cell free system. In the wheat germ system both mRNAs are optimally translated at the same salt concentration, whereas in the reticulocyte system vaccinia mRNA is optimally translated at higher salt concentrations than globin mRNA. This pattern of translation is due to the presence of an inhibitor in the poly(A)-containing vaccinia mRNA preparations, which is more inhibitory at low salt concentrations than at high salt concentrations. A similar inhibitor is present in RNA transcribed in vitro by reovirus virions. The inhibitor is probably dsRNA since it inhibits protein synthesis in exactly the same manner as synthetic dsRNA. Addition of vaccinia mRNA or synthetic dsRNA to isolated reticulocyte ribosomes causes a loss of Met-tRNA$_f$ binding activity and phosphorylation of two ribosome-associated proteins. The inhibition of protein synthesis in reticulocyte lysates by viral mRNAs can be overcome by the addition of relatively high concentrations of synthetic dsRNA, which are not inhibitory for protein synthesis, or in the case of vaccinia mRNA, by heat-treating the RNA to melt double-stranded regions. The apparent high salt optimum for translation of some viral mRNAs may be explained by the presence of dsRNA in the preparations. As a control we used preparations of encephalomyocarditis virus RNA free of dsRNA and observed the same salt optimum for its translation as for globin mRNA in the reticulocyte cell-free system.

The translation of viral mRNA in cell-free systems has been widely used for investigating the processes involved in viral genes expression and the effect of viral templates on the translation of cellular mRNAs. In such studies, the ionic conditions of the cell-free system are often adjusted to allow optimal translation of viral mRNA, since some viral mRNAs have been found to be optimally translated at higher salt concentrations than cellular mRNAs (Mathews, 1972; Lebleu et al., 1972; Carrasco & Smith, 1976; Weber et al., 1977b).

We show here that the salt optimum for translation of viral mRNAs is influenced by the presence of small amounts of double-stranded RNA (dsRNA)[1] in viral mRNA preparations. The dsRNA acts as a potent inhibitor of protein synthesis (Ehrenfeld & Hunt, 1971; Hunter et al., 1975), but the inhibition decreases with increasing salt concentration. However, the inhibition by the dsRNA associated with viral RNA can be overcome by adding high, noninhibitory concentrations of synthetic dsRNA to the cell-free system or by heat-treating the viral mRNA preparations to melt double-stranded regions.

The inhibition of protein synthesis by dsRNA does not take place in the wheat germ cell-free system (Grill et al., 1976). While the reasons for this lack of effect of dsRNA have not been investigated, it seems likely that the protein kinase activated by dsRNA (Farrell et al., 1977) may be absent in this cell extract. This is particularly convenient in studying the translation of viral mRNAs containing dsRNA. These mRNAs are translated by the wheat germ cell-free system at the same salt optimum as a cellular mRNA, globin mRNA. The optimal translation of these viral mRNAs with high salt concentrations in mammalian cell extracts may therefore be a consequence of the presence of dsRNA.

Materials and Methods

Viral RNAs. Vaccinia virus was prepared from infected HeLa cells as previously described by Joklik (1962). Methylated and unmethylated vaccinia mRNA was synthesized in vitro by detergent-treated virions and purified by phenol extraction as described by Weber et al. (1977b). When indicated, the vaccinia mRNA was further purified by fractionation by oligo(dT)-cellulose chromatography (Pemberton et al., 1975) and gradient centrifugation (Weber et al., 1977b). The poly(A)-containing RNA sedimenting between 7 S and 12 S was used. Reovirus mRNA was a gift of Dr. Aaron J. Shatkin of the Roche Institute of Molecular Biology. This RNA was synthesized by reovirus virions as previously described by Both et al. (1975). EMC virus was grown in ascites cells and virions were prepared as described by Shafritz et al. (1976). The RNA was prepared from virions by phenol extraction and purified by repeated gradient sedimentation (Shafritz et al., 1976).

RNA Translation. The wheat germ cell-free system was prepared according to Roberts & Paterson (1973). The composition of the incubations has been described by Weber et al. (1977b). The reticulocyte cell-free system was prepared as previously reported by Weber et al. (1977a) and made dependent on exogenous mRNA by a short treatment with nuclease according to Pelham & Jackson (1976). The composition of the incubations has been described in detail previously (Weber et al., 1977b). The amounts of mRNA added, the labeled amino acid, and the concentration of added K(OAc) are indicated in the figure legends. Protein synthesis in the reticulocyte lysates translating endogenous mRNA was assayed as described by Weber et al. (1977b). Globin mRNA was prepared from reticulocyte lysates (Hickey et al., 1976) and poly(A)-containing RNA was prepared from HeLa cells (Hickey et al., 1976).

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[1] Abbreviations used: dsRNA, double-stranded RNA; EMC, encephalomyocarditis virus; Hepes, *N*-2-hydroxethylpiperazine-*N'*-2-ethanesulfonic acid.

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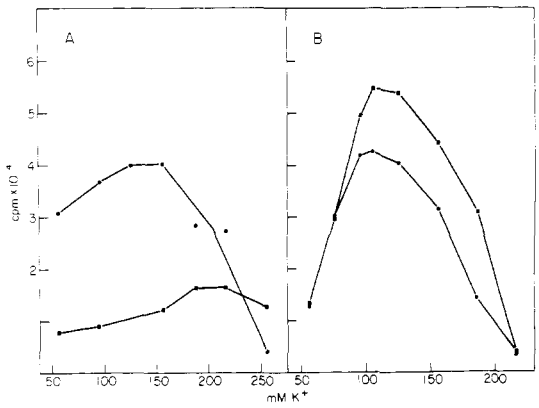

FIGURE 1: Effect of K(OAc) concentration on translation of vaccinia (■) and globin (●) mRNA in the reticulocyte (A) and wheat germ (B) cell-free system. See Materials and Methods for the preparation of the mRNA-dependent reticulocyte lysate, of the wheat germ cell-free system and of the mRNAs. The incubations contained 25 μg/mL of vaccinia mRNA or 12.5 μg/mL of globin mRNA. Control incubations without added mRNA were run at each K^+ concentration. The incorporation obtained in these control incubations was less than 10% of that obtained with added mRNA and was subtracted from the data given. The wheat germ incubations contained 2.5 μCi of [^{3}H]lysine per 50 μL final vol; the incubations with reticulocyte lysate contained 5 μCi or 25 μCi of [^{3}H]lysine for the translation of globin and vaccinia mRNA, respectively. Duplicate 5-μL aliquots were sampled for counting after 60-min incubation as described under Materials and Methods. The concentration of added K(OAc) is indicated for the reticulocyte lysate as mM K^+ in the abscissa. The wheat germ extract contained 36 mM KCl and additional K(OAc) was used to arrive at the final K^+ concentration indicated in the abscissa.

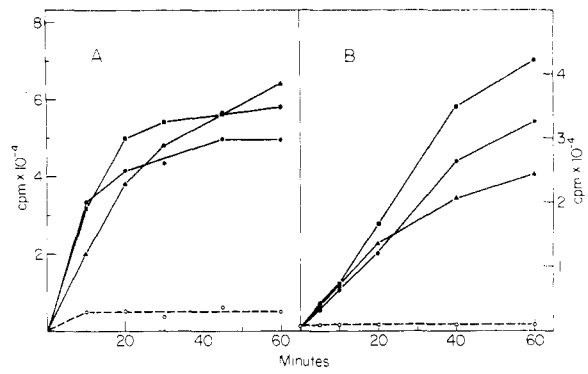

FIGURE 2: Time course of the translation of vaccinia (A) and globin (B) mRNA by reticulocyte lysates with different amounts of added K(OAc). Vaccinia mRNA was added at 25 μg/mL and globin mRNA at 12.5 μg/mL. The incubations contained 7.5 μCi of [^{35}S]methionine (A) or 2.5 μCi of [^{3}H]lysine (B) in 50 μL. Aliquots were sampled for counting at the times indicated as described in Figure 1. Incubations with added 55 mM K(OAc) (●); with added 125 mM K(OAc) (■); and with added 185 mM K(OAc) (▲). Control incubations without added mRNA done at 125 mM K(OAc) (○).

Met-tRNA$_f$ Binding Assay. Ribosomes were obtained from reticulocytes by 3-h centrifugation through 15% sucrose in a solution of 10 mM KCl, 1.5 mM $Mg(OAc)_2$, 20 mM Hepes/KOH, pH 7.4, and 2 mM dithiothreitol as previously described by Lenz & Baglioni (1977). The ribosomes were resuspended in the same solution and stored at −60 °C. The preparation and charging of $tRNA_f^{Met}$ have been previously described by Lenz & Baglioni (1977). The incubations were performed in two stages. First, 40 μg of ribosomes was incubated in 30 μL of 135 mM K(OAc), 2 mM $Mg(OAc)_2$, 20 mM Hepes (pH 7.4), and 1 mM dithiothreitol (buffer A) with 0.4 mM ATP and various RNAs as indicated. Then, after 7 min at 30 °C, an additional 20 μL of buffer A containing 1.2 pmol of [^{35}S]Met-tRNA$_f$ and sufficient GTP with an equimolar concentration of $Mg(OAc)_2$, and unlabeled methionine to give a final concentration of 1 mM, was added and the incubation continued for another 7 min at 30 °C. Reactions were stopped by the addition of 3 mL of ice-cold buffer A, and the Met-tRNA$_f$ bound to ribosomes determined by its retention on nitrocellulose filters as described by Baglioni et al. (1972).

Analysis of Phosphorylated Proteins. Forty micrograms of ribosomes was incubated for 7 min at 30 °C in 30 μL of buffer A with 4 μCi of [γ-^{32}P]ATP, 0.1 mM unlabeled ATP, and various RNAs as indicated by Lenz & Baglioni (1977). Reactions were terminated by the addition of electrophoresis buffer, and fractionated on 12.5% polyacrylamide gels as described by Lenz & Baglioni (1977). Phosphorylated products were determined by autoradiography of the dried gel using a Kodak X-ray intensifying screen and Kodak XR-2 film.

Results

Optimum Ionic Conditions for Translation of Viral and Cellular mRNAs. The viral RNAs used in the experiments reported here were obtained either by in vitro transcription with virions of vaccinia or reovirus, or prepared directly from virions in the case of encephalomyocarditis (EMC) virus. These viral mRNAs have previously been translated in a variety of cell-free protein-synthesizing systems (Beaud et al., 1972; Both et al., 1975; Carrasco & Smith, 1976; Jaureguiberry et al., 1975; Mathews, 1972; McDowell et al., 1972; Nevins & Joklik, 1975; Weber et al., 1977b). We have compared the optimal ionic conditions for translation of mRNA from vaccinia virus with that of a cellular mRNA from reticulocytes, globin mRNA, in both the rabbit reticulocyte cell-free system dependent on exogenous mRNA (Pelham & Jackson, 1976) and in the wheat germ cell-free system (Figure 1). In this and all subsequent experiments, potassium was added as the acetate salt, since Weber et al. (1977a) have observed that chloride salt is inhibitory for protein synthesis at Cl^- concentrations higher than those found in the cell cytoplasm.

Translation of vaccinia and globin mRNA was linearly related to the amount of mRNA added up to concentrations of about 25 μg/mL in both the reticulocyte and wheat germ cell-free system (data not shown). Vaccinia mRNA stimulated [^{3}H]lysine incorporation per μg of input RNA about 70% as efficiently as globin mRNA in the wheat germ cell-free system (Figure 1B). However, in the reticulocyte cell-free system vaccinia mRNA stimulated incorporation less than 10% as well as globin mRNA (Figure 1A). In this cell-free system, optimal translation of vaccinia mRNA was obtained at about 200 mM added K(OAc), whereas optimal translation of globin mRNA was obtained with about 125–150 mM K(OAc) (Figure 1A). However, in the wheat germ cell-free system (Figure 1B), both mRNAs were optimally translated with 105 mM potassium salt.

The following experiments examined the reasons for the different K(OAc) optima for translation of vaccinia and globin mRNA in the reticulocyte cell-free system. We have previously reported that a relatively large number of viral proteins are synthesized during in vitro translation of vaccinia mRNA (Weber et al., 1977b). One possible explanation for the high K(OAc) optimum for translation of this mRNA is that some proteins may be synthesized more efficiently at high salt concentrations. However, electrophoretic analysis followed by autoradiography has revealed that the same proteins are synthesized in approximately the same relative amounts at different potassium salt concentrations (Weber et al., 1977b; Pelham et al., 1978). Moreover, the same proteins are syn-

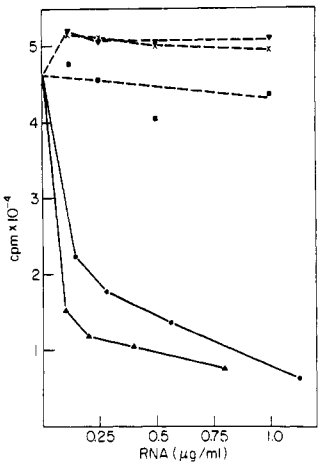

FIGURE 3: Inhibition of protein synthesis by vaccinia mRNA. Methylated (●) or unmethylated (▲) vaccinia mRNA was synthesized as described in Materials and Methods and added at the concentrations indicated on the abscissa to reticulocyte lysate translating endogenous mRNA. Poly(A)-containing RNA from HeLa cells (■), EMC RNA (X), or globin mRNA (▼) were added as controls in parallel incubations. Each 50-μL incubation contained 2.5 μCi of [^{3}H]lysine and 125 mM added K(OAc). Five-microliter aliquots were sampled after 60-min incubation and counted as described in Materials and Methods.

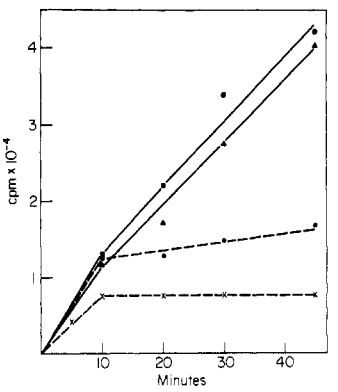

FIGURE 4: Time course of the inhibition of protein synthesis by vaccinia mRNA in the reticulocyte lysate translating endogenous mRNA. Methylated vaccinia mRNA was added at 1 μg/mL to an incubation with added 55 mM K(OAc) (X) or to an incubation with added 155 mM K(OAc) (●). Control incubations were run in parallel with added 55 (▲) or 155 mM K(OAc) (■). The incubations were prepared as described in Figure 3. Aliquots were sampled at the times indicated.

thesized in both the wheat germ and reticulocyte cell-free systems (Weber et al., 1977b).

The time course of translation of vaccinia and globin mRNA was examined at three different K(OAc) concentrations (Figure 2). In order to increase incorporation when translating vaccinia mRNA in the reticulocyte cell-free system, we used a labeled amino acid of higher specific activity, [^{35}S]methionine. In the reticulocyte cell-free system globin mRNA was translated almost linearly for 60 min, at a rate which was dependent on the concentration of added K(OAc) (Figure 2B). Vaccinia mRNA, however, was translated for a much shorter time at low K(OAc) concentrations (Figure 2A). The initial rate of translation of vaccinia mRNA was highest at the K(OAc) concentration optimal for globin mRNA translation. However, the total amount of protein synthesized was greater at higher K(OAc) concentrations, because translation of vaccinia mRNA proceeded for a longer time. This experiment was repeated in the wheat germ cell-free system. Translation of both vaccinia and globin mRNA proceeded linearly for 60 min at four different concentrations of potassium salt between 55 and 125 mM (data not shown). Therefore, the failure to translate vaccinia mRNA longer than 20 to 45 min at low salt concentrations is characteristic only of the reticulocyte cell-free system.

Inhibition of Protein Synthesis by Viral mRNAs. The limited translation of vaccinia mRNA in the reticulocyte cell-free system can either be explained by some property of the mRNA (i.e., a relative instability at low salt concentrations), or by the presence of some inhibitor of protein synthesis in the mRNA preparation. In order to test for the presence of an inhibitor, we added different amounts of poly(A)-containing vaccinia mRNA to a reticulocyte cell-free system translating endogenous mRNA with 125 mM added K(OAc). The addition of even very small amounts of vaccinia mRNA resulted in marked inhibition of protein synthesis (Figure 3). Methylated and unmethylated vaccinia mRNAs were about equally inhibitory. Since unmethylated vaccinia mRNA is translated poorly at K(OAc) concentrations optimal for in vitro protein synthesis (Weber et al, 1977b), the effect of the viral RNA is unlikely to be due to competition with cellular mRNA for translation. To test whether the addition of other cellular or viral RNAs inhibited protein synthesis, we added different amounts of globin mRNA, HeLa cell poly(A)-containing RNA, and of EMC virus RNA to the reticulocyte cell-free system (Figure 3). These RNAs had no effect on protein synthesis.

TABLE I: Inhibition of Protein Synthesis by Reovirus mRNA (Reo-RNA).[a]

RNA added (μg/mL)	counts/min per 5-μL aliquot	% of control
none	27 240	100
Reo-RNA (2.5)	5 280	19
Reo-RNA (1)	5 540	20
Reo-RNA (0.25)	5 280	19
Reo-RNA (0.1)	5 420	20
poly(I)·poly(C) (0.1)	5 380	20
poly(I)·poly(C) (10)	23 180	85
Reo-RNA (2.5) + poly(I)·poly(C) (10)	23 440	86

[a] The incubations were carried out for 60 min as described in Figure 6; 5-μL aliquots were sampled for counting.

The inhibitory activity of poly(A)-containing vaccinia mRNA was determined at two K(OAc) concentrations (Figure 4). The viral RNA inhibited protein synthesis after an initial lag, and the inhibition was more severe at the lower K(OAc) concentration tested. This result suggested that the earlier cessation of translation of vaccinia mRNA in the reticulocyte cell-free system at low K(OAc) concentrations was due to an inhibitor present in the viral RNA preparation, which was more effective at low salt concentrations. Moreover, no such inhibitor was found in cellular RNA or in EMC virus RNA, and the inhibitor was not effective in the wheat germ cell-free system.

A similar inhibitor is present in preparations of RNA synthesized by reovirus virions (Table I). Reovirus mRNA inhibited protein synthesis by more than 80% in the reticulocyte cell-free system at concentrations as low as 0.1 μg/mL. This inhibition of protein synthesis by reovirus mRNA has previously been observed by McDowell et al. (1972) who have suggested that the inhibition might be due to the presence of small amounts of dsRNA. Similarly to vaccinia mRNA, reovirus mRNA did not inhibit protein synthesis in the wheat

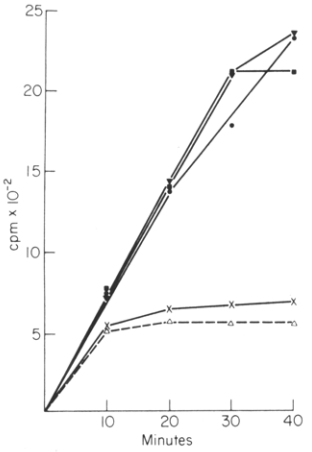

FIGURE 5: The effect of dsRNA on protein synthesis. Reticulocyte lysates were incubated as described in the legend of Figure 3 with 0.1 μg/mL of poly(I)·poly(C) (△), with 2.5 μg/mL of vaccinia mRNA (X), with 10 μg/mL of poly(I)·poly(C) (■), with 2.5 μg/mL of vaccinia mRNA and 10 μg/mL of poly(I)·poly(C) (●), and without any added RNA (▼).

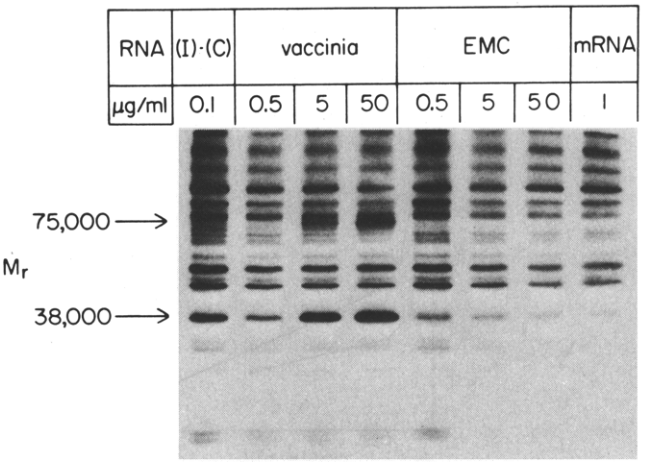

FIGURE 6: Phosphorylation of ribosome-associated proteins induced by various RNAs. Reticulocyte ribosomes were incubated with [γ-^{32}P]ATP and various RNAs at the concentrations indicated. The incubation labeled mRNA contained HeLa poly(A)-rich RNA prepared as previously described (Hickey et al., 1976). Samples were then electrophoresed and autoradiographed as described in Materials and Methods. Arrows denote positions of the two bands whose phosphorylation is induced by dsRNA.

TABLE II: Inhibition of Met-tRNA$_f$ Binding by Vaccinia mRNA.[a]

RNA added (μg/mL)	counts/min	% binding
globin mRNA (1.3)	23 700	100
vaccinia (1.5)	11 600	49
poly(I)·poly(C) (0.1)	10 830	46
none	19 750	83

[a] For each experiment, a preparation of reticulocyte ribosomes was incubated 7 min at 30 °C in the presence of 0.4 mM ATP with the RNA indicated. Met-tRNA$_f$ binding was determined as described under Materials and Methods.

germ cell-free system (result not shown). Interestingly, dsRNA does not appear to inhibit protein synthesis in this cell-free system (Grill et al., 1976).

Identification of the Inhibitory Component in Vaccinia mRNA and Reovirus mRNA as dsRNA. Vaccinia mRNA synthesized in vitro by purified virions or in infected cells has been reported to contain dsRNA (Colby & Duesberg, 1969; Duesberg & Colby, 1969; Colby et al., 1971). Pelham et al. (1978) have also shown that vaccinia cores contain dsRNA which inhibits protein synthesis in reticulocyte lysates. Protein synthesis in reticulocyte lysates is extremely sensitive to low concentrations of dsRNA (Ehrenfeld & Hunt, 1971; Hunter et al., 1975) and the kinetics of inhibition are similar to those we have observed with poly(A)-containing vaccinia mRNA. The inhibition of protein synthesis by dsRNA is characterized by loss of initiation activity and reduced binding of initiator tRNA (Met-tRNA$_f$) to 40S ribosomal subunits. The inhibition by dsRNA occurs only in a defined concentration range and little inhibition is observed at much higher or lower concentrations (Hunter et al., 1975). Moreover, dsRNA added to isolated reticulocyte ribosomes activates a protein kinase which phosphorylates the small subunit of the initiation factor eIF-2 using ATP as substrate (Farrell et al., 1977). If dsRNA in viral mRNA preparations is responsible for the inhibition of protein synthesis, the pattern of inhibition characteristic of dsRNA should be observed with these viral mRNAs. We report here that both poly(A)-containing vaccinia mRNA and reovirus mRNA (which does not contain poly(A)) contain inhibitory amounts of dsRNA. In the case of reovirus, the viral genome is made of dsRNA and fragments of this genomic dsRNA may contaminate mRNA preparations obtained by in vitro transcription. The absence of poly(A) in reovirus mRNA prevents a convenient purification of the mRNA and for this reason most of the subsequent experiments were carried out with poly(A)-containing vaccinia mRNA.

If the inhibition of protein synthesis by viral mRNAs is caused by the presence of dsRNA in the viral RNAs transcribed in vitro, it should be possible to overcome the inhibition by adding large amounts of dsRNA. As previously discussed, high concentrations of dsRNA are not inhibitory for protein synthesis (Hunter et al., 1975). In the presence of 10 μg/mL of the synthetic dsRNA poly(I)·poly(C), the inhibition of protein synthesis by vaccinia and reovirus mRNA was abolished (Figure 5 and Table I). In incubations with only synthetic dsRNA added, poly(I)·poly(C) inhibited protein synthesis at 0.1 μg/mL but not at 10 μg/mL (Figure 5).

Incubation of isolated ribosomes with dsRNA and ATP results in an inhibition of the Met-tRNA$_f$ binding activity of the ribosomes (Farrell et al., 1977). A similar inhibition was observed upon incubation of reticulocyte ribosomes with poly(A)-containing vaccinia RNA transcribed in vitro, whereas globin mRNA used as a control did not cause inhibition of Met-tRNA$_f$ binding (Table II). Poly(I)·poly(C) at 0.1 μg/mL inhibited this reaction to the same extent as vaccinia mRNA.

Further evidence that the inhibitor of protein synthesis in vaccinia mRNA is dsRNA was obtained by studying the phosphorylation of ribosome-associated proteins in incubations with [γ-^{32}P]ATP. In the presence of increasing amounts of poly(A)-containing vaccinia mRNA, two proteins were increasingly phosphorylated (Figure 6). These proteins were also phosphorylated in incubations with poly(I)·poly(C) but not in incubations with either cellular mRNA or with increasing concentrations of EMC virus RNA. They have estimated mol wt's of 38 000 and 75 000, respectively. The first is presumably the small subunit of the initiation factor eIF-2 (Farrell et al., 1977), whereas the identity of other protein has not yet been established. It has, however, been suggested that it may be a kinase activated by dsRNA, which is involved in the phosphorylation of the small subunit of eIF-2 (Farrell et al., 1977). Some phosphorylation of the 38 000 mol wt protein is observed in incubations with contol and EMC RNA. However, this

TABLE III: Effect of Heating on the Inhibition of Protein Synthesis by Vaccinia mRNA Transcribed in Vitro.[a]

RNA added	treatment of the RNA	protein synthesized	% inhibition
none		31 200	
vaccinia	none	10 600	66
vaccinia	20 h at 58 °C, 5 min at 100 °C	24 600	21
vaccinia	5 min at 100 °C, 20 h at 58 °C	10 800	65

[a] The incubations were carried out for 60 min as described in Figure 6. Unmethylated vaccinia RNA was added where indicated at 0.2 μg/mL. The RNA was heat treated at a concentration of 2 μg/mL in 20 mM Hepes/KOH buffer (pH 7.2).

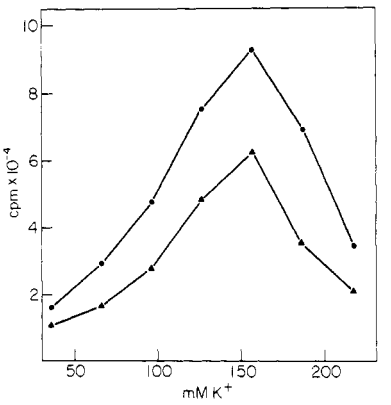

FIGURE 7: Optimum K(OAc) concentration for translation of EMC RNA in reticulocyte lysate. EMC RNA (▲) and globin mRNA (●) were added to a nuclease-treated reticulocyte lysate at 12.5 and 10 μg/mL respectively. Five microliters of [^{35}S]methionine was added to each 20-μL incubation. The concentration of added K(OAc) is indicated in the abscissas as mM K^+.

represents a background level of phosphorylation observed in all samples.

Conclusive evidence that the inhibitor in vaccinia mRNA preparations is dsRNA was obtained by showing that vaccinia mRNA heated 5 min at 100 °C lost its inhibitory activity (Table III). Colby et al. (1971) have previously reported that a small fraction of vaccinia RNA transcribed in vitro is resistant to RNase digestion under conditions in which this enzyme does not hydrolyze dsRNA. Heated RNA, however, is completely digested by RNase. Incubation of this RNA at a temperature which promotes reformation of dsRNA results in the reappearance of the RNase resistant component (Colby et al., 1971). We have digested labeled vaccinia RNA under the same conditions and have confirmed that about 1% is resistant to RNase (data not shown). Reannealing of the melted vaccinia mRNA restores its inhibitory activity (Table III). The vaccinia RNA heated at 100 °C and quickly cooled does not stimulate protein synthesis in reticulocyte lysates (data not shown). The reasons for the failure to translate are not known and are currently being investigated.

The Salt Optimum for Translation of Viral RNA in the Absence of Inhibition by dsRNA. EMC virus RNA does not inhibit protein synthesis when added to reticulocyte lysates at concentrations at which vaccinia or reovirus mRNA are inhibitory (Figure 3 and Table I), and it does not induce the dsRNA-specific phosphorylations (Figure 6). It thus seems unlikely that our preparations of EMC virus RNA contain dsRNA. Therefore, it was of interest to determine the salt optimum for translation of EMC virus RNA in reticulocyte lysates. EMC virus RNA and globin mRNA are both optimally translated at the same salt concentration (Figure 7), and their translation is linearly related to the amount of RNA added up to concentrations of 25 μg/mL (data not shown). The incorporation of [^{35}S]methionine per μg of input of RNA obtained with EMC RNA is approximately 60% of that obtained with globin mRNA (Figure 7).

We have also determined the salt optimum for translation of vaccinia mRNA in the presence of high noninhibitory concentrations of dsRNA. With 10 μg/mL of added poly(I)·poly(C) present in the incubations, vaccinia mRNA is optimally translated with 100 mM added K(OAc) (data not shown).

Discussion

Vaccinia virus and reovirus mRNA prepared by in vitro transcription using viral cores contain an inhibitor of protein synthesis which has been identified as dsRNA. The following lines of evidence support this identification: (1) the inhibitor is active in the reticulocyte cell-free system, which is sensitive to dsRNA, but not in the wheat germ, which is insensitive; (2) in a manner analogous to the effect of authentic dsRNA, the effect of the inhibitor on protein synthesis is not immediate, but takes place after a lag period; (3) like dsRNA, the inhibitor blocks the binding of Met-tRNA$_f$ to ribosomes; (4) viral mRNA preparations containing the inhibitor induce the phosphorylation of the same ribosome-associated proteins as does dsRNA: (5) boiling and quick cooling destroys the inhibitory activity of the viral mRNA preparation, whereas boiling followed by heating at a temperature which favors reannealing of complementary strands restores the inhibitory activity.

The observation that some viral mRNA preparations can contain dsRNA may be an important practical consideration for interpreting in vitro translation data. Furthermore, the observation that high dsRNA concentrations can overcome the inhibitory effect of the dsRNA in viral mRNA preparations may be useful in translating these mRNAs in mammalian cell-free systems.

We have shown that inhibition of protein synthesis by viral and synthetic dsRNA diminishes with increasing salt concentration in the cell-free systems prepared from reticulocytes, ascites cells, L and HeLa cells (unpublished observations). The high salt optimum for translation of viral mRNAs containing dsRNA in these cell-free systems results therefore from the reduced inhibitory activity of dsRNA at high salt concentrations. These results imply that viral mRNAs, which are translated at the same salt optimum as cellular mRNA and are not inhibitory for protein synthesis at low salt concentrations, do not contain significant amounts of dsRNA. Viral mRNAs which have a higher salt optimum for translation than cellular mRNA may contain dsRNA.

EMC virus RNA is translated at a higher salt optimum than cellular mRNA in the ascites cell-free system (Mathews, 1972; Carrasco & Smith, 1976). However, we have found that it is translated at the same salt optimum as globin mRNA in the reticulocyte cell-free system. Therefore, it appears that EMC virus RNA does not contain significant quantities of dsRNA. It is not clear, however, why EMC virus RNA has a higher salt optimum for translation than cellular mRNA in the ascites cell-free system, and why this is not observed in the reticulocyte cell-free system. One possible explanation resides in the differences in the preparation of the cell extracts used for translation experiments. The ascites cell extract is gel-filtered, whereas the reticulocyte lysate is not. Removal of ions and

polyamines by gel filtration may cause a premature termination with ascites extract, similar to that reported for the wheat germ cell-free system by Hunter et al. (1977). An increase of salt concentration would counteract this effect. In this case, a higher salt concentration may strongly favor the translation of large mRNAs, such as those of picornaviruses, while having relatively little effect on the translation of smaller mRNAs.

Vaccinia RNA synthesized in vitro by purified virions or in infected cells has been shown to contain dsRNA (Colby & Duesberg, 1969; Duesberg & Colby, 1969; Colby et al., 1971; Pelham et al., 1978). We have shown here that this dsRNA is associated with poly(A)-containing 7–12S RNA, and that it is active in blocking Met-tRNA$_f$ binding and protein synthesis in vitro. It is unlikely that this dsRNA is an artifact of in vitro transcription since it also accumulates in cells infected with this DNA virus. Colby et al. (1971) have suggested that complementary sequences in viral mRNA might arise by convergent or divergent transcription along opposite DNA strands. These authors find that the amount of dsRNA can be increased by incubating vaccinia mRNA under conditions which favor annealing.

The functional significance of the dsRNA associated with vaccinia mRNA is not clear. In cells infected by vaccinia virus, the synthesis of host proteins is rapidly shut off despite the persistence of cellular mRNA (Rosemond-Hombeak & Moss, 1975). Perhaps the viral dsRNA may limit the rate of initiation of protein synthesis during infection thereby favoring the translation of more efficient viral mRNAs. The viral dsRNA is also involved in the induction of interferon (Colby & Morgan, 1971).

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