mRNA Methylation and Protein Synthesis in Extracts from Embryos of Brine Shrimp, *Artemia salina*

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S. Muthukrishnan, Witold Filipowicz,* José M. Sierra,‡ Gerald W. Both, Aaron J. Shatkin, and Severo Ochoa

From the Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Cell-free protein-synthesizing extracts prepared from the brine shrimp, *Artemia salina*, translate methylated mRNAs. Reovirus unmethylated mRNA is inactive as a template when methylation is prevented by the inhibitor, S-adenosylhomocysteine.

*A. salina* mRNAs from both undeveloped and developed embryos contain 5'-terminal 7-methylguanosine in an inverted 5'-5' linkage through three phosphate groups to the rest of the polynucleotide chain. Removal of the 7-methylguanosine by β elimination converts the mRNA from an active form to one inactive in protein synthesis in extracts of *A. salina* or wheat germ.

Extracts of undeveloped and developed embryos methylate reovirus unmethylated mRNA at the 5' ends to form 5'-terminal structures of the type, m'G(5')ppp(5')G and m'G(5')ppp(5')Gm.

Unfertilized eggs of sea urchin (1-3) and *Xenopus laevis* (4, 5) contain latent "maternal" mRNA that is synthesized during oogenesis. Despite the presence of mRNA, there is little protein synthesis in mature unfertilized eggs or in egg homogenates (1). Upon fertilization and during the early stages of development, the stored mRNA becomes an active template for protein synthesis. A similar activation of protein synthesis has been observed in dry wheat embryos (6, 7). Ungerminated soybean seeds (8) and encysted embryos of the brine shrimp, *Artemia salina* (9-11), were also found to contain stored mRNA.

Several mechanisms for regulating maternal mRNA expression during development have been proposed. For example, it has been suggested that latent mRNA may be complexed with proteins that can be removed after fertilization (1, 12). In sea urchin eggs, poly(A) synthesis increases immediately after fertilization, and it was proposed that the length of the poly(A) in maternal mRNA may be related to its ability to function in protein synthesis (13-15).

Recently, the mRNAs of a variety of different eukaryotic cells and viruses were found to be methylated and to contain blocked 5'-terminal structures of the type, m'G(5')ppp(5')N' (16-24). These methylated sequences may play a role in mRNA biogenesis (24). In addition, 5'-terminal 7-methylguanosine in vesicular stomatitis virus (VSV), reovirus, and globin mRNAs is required for translation in wheat germ cell-free extracts (25, 26). Chemical removal of 7-methylguanosine from the 5' ends of reovirus and globin mRNAs abolished their ability to stimulate polypeptide synthesis in vitro (26).

By analogy, it seemed possible that methylation of mRNA in dormant cells could serve as a simple mechanism for controlling protein synthesis, i.e. induction of a 5'-terminal methylase activity could be responsible for converting inactive, unmethylated mRNA to an active, methylated form in developing cells. To test this hypothesis, we have investigated whether the presence of 5'-terminal 7-methylguanosine in mRNA is important for protein synthesis in *A. salina*. We studied the ability of cell-free extracts to translate methylated and unmethylated reovirus mRNA. In addition, the mRNA 5'-terminal structures and the mRNA-methylating activities of dry cryptobiotic cysts and partially developed embryos of *A. salina* were compared.

MATERIALS AND METHODS

Preparation of Cell Fractions and mRNAs from *Artemia salina*—High speed supernatant fraction (S105) and ribosomes or polysomes washed with 0.5 M KCl were prepared from undeveloped and partially developed *A. salina* embryos as described previously (27). Dry cysts of *A. salina* were allowed to develop partially by incubation in 4% NaCl at 28°C for 16 hours. Details of this procedure have been described (27). "Developed mRNA" was prepared from salt-washed polysomes that were isolated from partially developed *A. salina* embryos. RNA was extracted from polysomes by a modification of the method of Lee et al. (28) as follows. Polysomes were suspended in 0.1 M Tris·HCl buffer (pH 9.0) containing 5 mM EDTA and 0.05% Na deoxycholate at a concentration of 100 A260 units/ml and shaken for 10 min at 4°C with an equal volume of redistilled 80% aqueous phenol. The phases were separated by centrifugation, and the phenol phase was re-extracted with 1 volume of 0.1 M Tris·HCl buffer (pH 9.0)/5 mM EDTA. The combined water phases were extracted three additional times with 80% phenol, the final H2O phase was made 0.2 M
with respect to potassium acetate (pH 5.5), and the RNA was precipitated by addition of 2 volumes of ethanol. The poly(A)-containing mRNA fraction was selected by oligo(dT)-cellulose chromatography (29).

Because dry cysts of undeveloped A. salina contain very few, if any, polysomes (30-32), the postribosomal supernatant fraction (S105) was assayed for extracts of undeveloped embryos was used as a source of "undeveloped mRNA" (11). S105 was prepared according to Sierra et al. (27) with the exception that cysts were disintegrated in a French press according to Zasloff and Ochoa (33) instead of grinding them in a mortar. Following ultracentrifugation, the supernatant fraction was applied to a DEAE-cellulose (DE52, Whatman) column equilibrated with Buffer A containing 1 M KCl and precipitated with 2 volumes of ethanol. The polv(A)-containing mRNA fraction was selected by oligo(dT)-cellulose chromatography.

**Translation in A. salina Extracts**—Standard incubation mixtures contained the following components in a volume of 0.1 ml: 20 mM N 2 hydroxyethylpiperazine-N 2-ethanesulfonic acid (Hepes) buffer adjusted to pH 7.6 with KOH, 80 mM KCl, 3 mM magnesium acetate, 1 mM dithiothreitol, 1.3 mM ATP, 0.25 mM GTP, 7.7 mM creatine phosphate, 0.6 µg (0.9 unit) of creatine phosphokinase (EC 2.7.3.2), 28 µg of A. salina tRNA, 0.4 µCi of L-[3H]leucine (specific activity = 286 Ci/mmol), 0.04 µM each of the remaining 19 l-amino-acids, 2.0 A 160 units of 0.5 M KCl-washed ribosomes from undeveloped A. salina, S105 supernatant fraction from developed A. salina (1.40 mg of protein), and, when indicated, 4 µg of mRNA from developed A. salina or 8 µg of reovirus mRNA synthesized in vitro (34), 160 µM S-adenosylhomocysteine, or 4 µM S-adenosylmethionine. Assay mixtures were incubated for 45 min at 30°C. For determination of the incorporation of radioactivity into hot acid-insoluble material, samples were applied to Whatman No. 3MM filter squares which were processed according to the method of Mans and Novelli (35).

When translation by extracts of undeveloped A. salina or 8 µg of reovirus mRNA synthesized in vitro (34), 160 µM S-adenosylhomocysteine, or 4 µM S-adenosylmethionine. Assay mixtures were incubated for 45 min at 30°C. For determination of the incorporation of radioactivity into hot acid-insoluble material, samples were applied to Whatman No. 3MM filter squares which were processed according to the method of Mans and Novelli (35).

**RESULTS**

**Preferential Translation of Methylated mRNA by Cell Free Extracts of Artemia salina**—Protein-synthesizing extracts prepared from wheat germ embryos and mouse L-cells translate the methylated mRNAs of reovirus but not the corresponding unmethylated mRNAs under conditions where methylation is blocked (25, 26). Translation of VSV mRNAs in wheat germ extracts is also methylation-dependent (25). The methylated residue in mRNA essential for translation is the 7-methylguanosine in the 5'-terminal structure, m[G(5')ppp(5')N] (26). In wheat germ, this requirement is manifested at an early step in the initiation process of protein synthesis, at or prior to the level of binding of the 40 S ribosomal subunit to mRNA (39). To test whether there is a similar requirement for mRNA methylation in protein synthesis of lower eukaryotes, cell-free extracts containing 80 S ribosomes from undeveloped embryos and S105 supernatant from developing embryos of the crustacean, Artemia salina (27), were programmed with methylated and unmethylated reovirus mRNA and with the homologous mRNA. Methylated reovirus mRNA stimulated polypeptide synthesis in these extracts, and the stimulation was unaffected by the addition of the methyl donor, S-adenosylmethionine, or its analog, S-adenosylhomocysteine, an inhibitor of methylation (Table I). Similar results were obtained with A. salina poly(A)-containing mRNA isolated from undeveloped embryos of A. salina.

**Table I**

| Additions | A. salina mRNA* | Methylated reovirus mRNA | Unmethylated reovirus mRNA |
|-----------|-----------------|--------------------------|---------------------------|
| S-adenosylhomocysteine | 8.0 | 17.1 | 12.5 |
| (180 µM) | 9.6 | 16.5 | 2.6 |
| S-adenosylmethionine | 10.2 | 17.8 | 13.8 |
| (4 µM) | | | |
*Prepared from developing embryos of A. salina.
polysomes of developing embryos. Unmethylated reovirus mRNA was also translated in Artemia extracts, but to a lesser extent than methylated mRNA; again, addition of S-adenosylmethylthionine had little, if any, effect. However, in the presence of S-adenosylhomocysteine, polypeptide synthesis directed by unmethylated reovirus mRNA was inhibited by 90%. The results indicate that A. salina extracts can translate unmethylated viral mRNAs only after they are methylated, and suggest, as demonstrated below, that they contain an mRNA-methylating activity that converts unmethylated reovirus mRNA to the methylated form which is active in protein synthesis. Presumably, A. salina extracts also contain a level of methyl donor(s) sufficient to methylate the exogenous unmethylated viral mRNA because addition of S-adenosylmethylthionine to cell extracts had little effect on protein synthesis.

5’-Terminal 7-Methylguanosine in mRNA from Developed A. salina—As observed for methylated reovirus mRNA, the addition of S-adenosylmethylthionine or S-adenosylhomocysteine to A. salina extracts did not alter their ability to translate homologous mRNAs (Table I). These results suggested that mRNAs from developing embryos may already be methylated and contain 5’-terminal 7-methylguanosine in a structure similar to that present in methylated reovirus mRNA, i.e. m’G(5’)ppp(5’)G’. This possibility was tested as follows. Poly(A)-containing mRNA from developing embryos was treated with periodate to oxidize free 2’,3’-hydroxyl groups contained in 3’-terminal nucleotides and in presumptive 5’-terminal structures (17). The RNA was reduced with [3H]-borohydride, digested with Penicillium nuclease (P,) and bacterial alkaline phosphatase, and analyzed by high voltage paper electrophoresis at pH 3.5 (26). Methylated reovirus and globin mRNAs treated under the same conditions yielded the 5’-terminal trialcohol derivative of m’G(5’)ppp(5’)N as the only 3H-labeled digestion product migrating in the direction of the anode (26). Similarly, only one 3H-labeled component with a net negative charge was obtained from the digested A. salina mRNA. It migrated in the position of PA and comprised a small fraction (1%) of the total radioactivity. Most of the radioactivity (95%) stayed at the origin and was not a nucleotide derivative. It may correspond to a derivatized carbohydrate contaminant since high concentrations of trehalose and glycogen have been found in A. salina embryos (40). The remaining 3H-labeled components containing 3% and 1% of the total 3H radioactivity corresponded in mobility to adenosine and cytidine, respectively; presumably they were derived from the 3’ ends of the mRNAs. The 3H-labeled, P,, and phosphatase-resistant anionic component was eluted and reanalyzed by descending paper chromatography (Fig. 1A). More than 90% of the radioactivity chromatographed in one peak. This 3H-labeled material was eluted and an aliquot digested with nucleotide pyrophosphatase followed by alkaline phosphatase was reanalyzed by chromatography. The radioactivity was quantitatively recovered as the 3H-labeled trialcohol derivative of 7-methylguanosine (Fig. 1B), which was identified further by paper chromatography. The results indicate that the 5’ termini of mRNA from developing embryos of A. salina contain “capped” methylated structures similar to those in other viral and cellular mRNAs, i.e. 7-methylguanosine linked by an inverted 5’-5’ pyrophosphate bridge to the rest of the RNA molecule.

In order to determine the number of phosphate groups in the cap structures, an aliquot of the [3H]borohydride-labeled RNA was digested with Penicillium nuclease and alkaline phosphatase and analyzed by paper chromatography. The cap material was recovered together with 3H-labeled nucleosides derived from the 3’-termini and analyzed by DEAE-cellulose column chromatography (17). As shown in Fig. 2, the nucleosides elute in the position of zero charge. The cap structures clute between the mononucleotide and dinucleotide optical density markers with a net charge of approximately -2.5. This value is consistent with the presence in the 5’ termini of three phosphates, one partially neutralized by the positively charged 7-methylguanosine (17, 26).

Effect of β Elimination on Translation of A. salina mRNA—Previously, it was found that removal of the 5’-terminal 7-methylguanosine from reovirus and globin mRNAs resulted...
S-adenosylhomocysteine extracts, respectively) were subtracted.
['Clleucine incorporated. Blank values without mRNA added (2.7 to 3.0 pmol/assay and 3.3 to 3.5 pmol/assay in wheat germ and Artemia translation, poly(A)-containing mRNA from polysomes of developing embryos was subjected to β elimination (37, 38). After treatment, its template activity was compared with control mRNA in cell free protein synthesizing extracts from A. salina and wheat germ (Table II). Control mRNA, treated under the same conditions but without addition of periodate and aniline, was translated in both systems, although net synthesis of polypeptides was 3-fold greater in wheat germ extracts. Addition of S-adenosylmethionine or S-adenosylhomocysteine to either extract had little or no effect on translation of control mRNA, as observed for reovirus mRNAs (Table I) and methylated VSV and globin mRNAs in wheat germ extracts (25, 26). After β elimination under conditions that removed 80 to 85% of the 5'-terminal 7-methylguanosine from reovirus mRNA without chemical degradation (26), the translational capacity of A. salina mRNA was reduced by 67% and 72% in the heterologous and homologous systems, respectively (Table II). The loss of activity apparently is not due to internal cleavage of the mRNA because sedimentation profiles under denaturing conditions of reovirus and silk fibroin mRNAs were unchanged by β elimination.14 Furthermore, methylated reovirus mRNA which is inactivated by β elimination can, in the presence of S-adenosylmethionine, stimulate protein synthesis in wheat germ extracts (26). This restoration of activity was shown to be due to the presence of some mRNA molecules with 5'-terminal pppG which were converted to 5'-m'GpppG. Those molecules with 5'-terminal pppG were not converted to active mRNA (26). A strict requirement for 5'-terminal diphosphates by the vaccinia-derived guanylyltransferase has also been described recently (41). The results suggest that β elimination per se does not nonspecifically inactivate mRNA, and that 5'-terminal 7-methylguanosine in A. salina mRNA is required for translation in vitro.

5'-Terminal 7-Methylguanosine in mRNA from Undeveloped Embryos—The results obtained with mRNA from developing embryos demonstrate that 5'-terminal 7-methylguanosine in mRNA is important for protein synthesis in brine shrimp. To determine whether the low level of protein synthesis in dormant cysts is due to an absence of 5'-terminal 7-methylguanosine in mRNA, the in vitro template activities of mRNA derived from undeveloped and developed embryos were compared. As previously found for reovirus unmethylated mRNA in wheat germ extracts (25), the translation of mRNA isolated from undeveloped embryos should be low in the presence of S-adenosylhomocysteine if 5'-terminal 7-methylguanosine is absent from these mRNAs. However, as shown in Fig. 3, the kinetics of incorporation of [35S]methionine into acid-precipitable material directed by mRNA preparations from both developed and undeveloped embryos was unaffected by S-adenosylhomocysteine, although the activity of the former mRNA was 2- to 3-fold greater.

The findings in Fig. 3 suggest that mRNA isolated from undeveloped embryos also contains 5'-terminal 7-methylguanosine. Poly(A)-containing mRNA prepared from dry cysts was oxidized with periodate, reduced with [3H]borohydride, digested with *Penicillium* nuclease and alkaline phosphatase, and analyzed by paper electrophoresis at pH 3.5 as in Fig. 1. A single peak of [3H]-labeled anionic material was obtained, and it was further purified by paper chromatography (Fig. 4A). The predominat [3H]-labeled component (Fractions 22 and 23) was eluted, an aliquot digested with nucleotide pyrophosphatase and alkaline phosphatase, and reanalyzed by electrophoresis. More than 85% of the [3H] was obtained as the triethylOrthovaterivative of 7-methylguanosine (Fig. 4B). Similarly, the minor [3H]-labeled component (Fractions 13 and 14) yielded 7-methylguanosine trimethylammonium as the only radioactive material. The results indicate that mRNA of undeveloped embryos also

**Table II**

| Additions | Wheat germ extract | Artemia salina extract |
|-----------|-------------------|-----------------------|
|           | mRNA              | Inhibition%            | mRNA              | Inhibition% |
|           | Control            | β eliminated          | Control            | β eliminated |
| S-adenosylhomocysteine | 17.0 | 5.7 | 67 | 5.0 | 1.4 | 72 |
| (160 µM) | 14.4 | 5.8 | 60 | 4.5 | 0.8 | 83 |
| S-adenosylmethionine | 17.0 | 5.7 | 67 | 4.3 | 0.9 | 78 |

*Unpublished results.

**P. Gage, personal communication.**
mRNA methylase activities that specifically methylate the 5' termini of reovirus mRNA to form structures of the type m,GpppG... However, under the in vitro conditions developing Artemia embryos, like mouse L-cells (26), contain phosphatase treatment (Fig. 5A). The results indicate that the major SH-labeled component of lower mobility yielded only 7-methylguanosine after nucleotide pyrophosphatase and alkaline phosphatase and analyzed by paper electrophoresis at pH 3.5. As shown in Fig. 5A, a predominant [3H]methyl-labeled component migrating in the position of pG was obtained only from the digest of RNA isolated from the reaction mixture to which exogenous unmethylated reovirus mRNA was added. The [3H]labeled material in this peak was eluted and analyzed by paper chromatography. Two components were resolved, a minor one which migrated in the position of marker m'GpppG obtained from the 5'termini of the viral mRNA. As a direct test of this possibility, unmethylated reovirus mRNA was incubated for 10 min under conditions of protein synthesis in an A. salina cell-free system containing developed S105 supernatant and [3H]-methyl-S-adenosylmethionine. Reaction mixtures containing no exogenous viral mRNA were treated identically. The incubation mixtures were extracted with phenol, and the RNA was recovered by alcohol precipitation, digested with P1 nuclease followed by alkaline phosphatase and analyzed by paper electrophoresis at pH 3.5. As shown in Fig. 5, a predominant [3H]methyl-labeled component migrating in the position of pG was obtained only from the digest of RNA isolated from the reaction mixture to which exogenous unmethylated reovirus mRNA was added (Fig. 5A). The [3H]labeled material in this peak was eluted and analyzed by paper chromatography. Two components were resolved, a minor one which migrated in the position of marker m'GpppG obtained from the 5'termini of reovirus mRNA, and a major one which migrated more slowly in the position of m'GpppG (Fig. 5B). The latter marker compound was prepared from reovirus mRNA methylated in wheat germ extract (26). Each component was eluted, treated with nucleotide pyrophosphatase and alkaline phosphatase, and reanalyzed by paper electrophoresis at pH 3.5. The [3H]labeled component that migrated with the authentic m'GpppGm yielded two equal peaks of radioactivity, one which migrated with 7-methylguanosine, and another which was close to the origin, the position of 2'-O-methylguanosine (Fig. 5D). The latter was further identified as 2'-O-methylguanosine on the basis of its migration during chromatographic analysis in isobutyric acid/NH4OH of the material eluted from the region indicated by the bracket in A. C, the material in the region of the marker m'GpppG in B was eluted, digested with nucleotide pyrophosphatase and alkaline phosphatase, and analyzed by paper electrophoresis at pH 3.5. D, material in the region of the marker m'GpppGm in B was analyzed as described for Part C. G = guanosine; U = uridine; pC, pG, pU = 5'-monophosphates of cytidine, adenosine, guanosine, and uridine, respectively.

The same procedures were used to determine whether mRNA-methylating activities are also present in undeveloped A. salina. The results were very similar to those obtained with S105 supernatant fraction from developed A. salina and ribosomes from undeveloped embryos in the presence of [3H]methyl-S-adenosylmethionine as described under "Materials and Methods." An identical control incubation mixture contained no added viral mRNA. RNA was recovered from the incubation mixtures by phenol extraction and alcohol precipitation and digested with Penicillium nuclease, followed by alkaline phosphatase. A, paper electrophoresis of the enzyme digests with (----) and without (---) reovirus mRNA. The profiles from separate analyses are superimposed. B, paper chromatography in isobutyric acid/NH4OH of the material eluted from the region indicated by the bracket in A. C, the material in the region of the marker m'GpppG in B was eluted, digested with nucleotide pyrophosphatase and alkaline phosphatase, and analyzed by paper electrophoresis at pH 3.5. D, material in the region of the marker m'GpppGm in B was analyzed as described for Part C. G = guanosine; U = uridine; pC, pG, pU = 5'-monophosphates of cytidine, adenosine, guanosine, and uridine, respectively.

Fig. 5. Methylation of reovirus mRNA by cell-free extracts containing 80 pg of reovirus mRNA (40 pg) synthesized in vitro as described previously (34) was incubated for 10 min in an extract containing S105 supernatant fraction from developed A. salina and ribosomes from undeveloped embryos in the presence of [3H]methyl-S-adenosylmethionine as described under "Materials and Methods." An identical control incubation mixture contained no added viral mRNA. RNA was recovered from the incubation mixtures by phenol extraction and alcohol precipitation and digested with Penicillium nuclease, followed by alkaline phosphatase. A, paper electrophoresis of the enzyme digests with (----) and without (---) reovirus mRNA. The profiles from separate analyses are superimposed. B, paper chromatography in isobutyric acid/NH4OH of the material eluted from the region indicated by the bracket in A. C, the material in the region of the marker m'GpppG in B was eluted, digested with nucleotide pyrophosphatase and alkaline phosphatase, and analyzed by paper electrophoresis at pH 3.5. D, material in the region of the marker m'GpppGm in B was analyzed as described for Part C. G = guanosine; U = uridine; pC, pG, pU = 5'-monophosphates of cytidine, adenosine, guanosine, and uridine, respectively.

The same procedures were used to determine whether mRNA-methylating activities are also present in undeveloped A. salina. The results were very similar to those obtained with S105 supernatant fraction from developed embryos, with the exception that lesser amounts of 2'-O-methylated 5'-terminal structures were observed in the mRNA methylated by S105 from undeveloped embryos (data not shown). The findings indicate that similar, possibly identical, mRNA-methylating activities are present in developed and undeveloped A. salina embryos, and that both convert reovirus unmethylated mRNA to molecules containing 5'-terminal m'GpppGm or m'GpppGm.
cell-free extracts prepared from hydrated developing embryos have a high level of endogenous protein synthesis activity, indicating that they contain mRNA and all of the components necessary for translation (27, 30–32). The low number of polysomes observed in undeveloped embryos is apparently not due to a lack of mRNA or some other factor(s) necessary for elongation of polypeptide chains. Thus, undeveloped embryos contain preformed mRNA not associated with ribosomes (9–11), and within minutes after hydration and incubation of dormant cysts at 30°C, the amount of polysomes increases dramatically (27, 30, 31). In addition, extracts from undeveloped embryos translate poly(U) efficiently (32).

Because 5’-terminal 7-methylguanosine in VSV and reovirus mRNAs (and possibly globin mRNA) is important for translation (25, 26), we have investigated the possibility that the failure of A. salina mRNA to associate with ribosomes in undeveloped embryos may be due to the absence of blocked, methylated 5’ termini. The results indicate that A. salina protein-synthesizing extracts discriminate between methylated and unmethylated mRNAs, and that mRNAs from both undeveloped and developed embryos contain 5’-terminal 7-methylguanosine. Furthermore, mRNA methylase activities are present in extracts of both. Thus, the absence of protein synthesis in dormant embryos cannot be simply due to its regulation at the level of mRNA methylation.

From our results, the dependence on 5’-terminal 7-methylguanosine in mRNA for translation can be extended to the brine shrimp, A. salina, a lower eukaryote. Evidence is accumulating that this may be a requirement common to the translation of most eukaryotic mRNAs. For example, the mRNA of the slime mold, Dictyostelium discoideum,4 and the plant virus, Brome mosaic virus (42), have recently been found to have 5’-terminal 7-methylguanosine in addition to numerous examples of other viral and cellular mRNAs containing blocked, methylated 5’ ends (16–24). The requirement for 7-methylguanosine appears to be at the level of binding of mRNA to 40 S ribosomal subunits during initiation of protein synthesis (39). In addition, it is of interest that a 32–40 nucleotide, 7-methylguanosine-containing fragment from the 5’ termini of reovirus mRNAs, is partially protected against digestion by RNase when methylated reovirus mRNA was bound in 80 S ribosome complexes (39). It was suggested from these results that the 7-methylguanosine and the ribosome-binding site in these viral mRNAs may be required together for the correct initiation of protein synthesis. It is possible that some ribosomal protein(s) and/or initiation factor(s) can specifically recognize the 7-methylguanosine at the 5’ end of mRNA. A deficiency of these proteins might result in a lower efficiency of translation of mRNA. Recent results indicate that the activity of several protein factors necessary for initiation of natural mRNA translation is very low in undeveloped A. salina embryos* (27). The activity of these factors shows a pronounced increase with development; they were found to correspond to Factors IF-MP, IF-M2A, IF-M2B, and IF-M3 of reticulocytes (43). It will be of interest to determine whether they interact with the 5’-terminal 7-methylguanosine in mRNA.

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