Translational Control by Messenger RNA Competition for Eukaryotic Initiation Factor 2*

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Translation of globin mRNA in a micrococal nuclelease-treated reticulocyte lysate was studied in the presence of increasing amounts of Mengovirus RNA, under conditions in which the number of translation initiation events remains constant as judged by the transfer of label from N-formyl[35S]methionyl-tRNA, into protein. The translation of globin mRNA is progressively inhibited by low concentrations of Mengovirus RNA, free of detectable traces of double-stranded RNA, concomitant with the increasing synthesis of Mengovirus RNA-directed products. On a molar basis, Mengovirus RNA apparently competes about 35 times more effectively than globin mRNA for a critical component in translation. The competition is relieved by the addition of highly purified eukaryotic initiation factor 2 (eIF-2). Addition of eIF-2 does not stimulate overall protein synthesis, but shifts it in favor of globin synthesis. No stimulation of globin mRNA translation by eIF-2 is seen when Mengovirus RNA is absent. These experiments show that Mengovirus RNA competes, directly or indirectly, with globin mRNA for eIF-2.

In direct binding experiments using isolated mRNA and eIF-2, Mengovirus RNA is shown to compete with globin mRNA for eIF-2 and to exhibit a 30-fold higher affinity for this factor. The binding of Mengovirus RNA to eIF-2 is much more resistant to increasing salt concentrations than is the binding of globin mRNA, again reflecting its high affinity.

These results reveal a direct correlation between the ability of these mRNA species to compete in translation and their ability to bind to initiation factor eIF-2. They suggest that the affinity of a given mRNA species for eIF-2 is essential in determining its translation, relative to that of other mRNA species. Messenger RNA competition for eIF-2 may contribute significantly to the selective translation of viral RNA in infected cells.

Gene expression in eukaryotic organisms often involves the selective translation of certain mRNA templates over other ones. This type of regulation is used frequently in virus infection and cellular differentiation. Messenger RNA discrimination is thought to occur mainly at the initiation step, which involves the recognition of mRNA and its binding of ribosomes. Thus, translational competition between host mRNA and picornavirus RNA in a cell-free system from mouse ascites cells occurs at the level of initiation (Lawrence and Thach, 1974; Golini et al., 1976). During erythroid development, protein synthesis on β-globin mRNA is initiated more frequently than on α-globin mRNA, because β-globin mRNA competes more effectively for a critical component in translation (Lodish, 1971, 1974; Lodish and Jacobson, 1972). The translational competition between the α- and β-globin mRNA species occurring in a micrococal nuclelease-treated reticulocyte lysate is relieved by the addition of eukaryotic initiation factor 2 (Di Segni et al., 1979). In addition to its indispensable role in the binding of Met-tRNA, to 40 S ribosomal subunits during initiation (Trachsel et al., 1977), this initiation factor possesses mRNA-binding properties (Kaempfer, 1974; Barrieux and Rosenfeld, 1977, 1978; Kaempfer et al., 1976, 1979; Rosen and Kaempfer, 1979). Indeed, β-globin mRNA binds to eIF-2 with higher affinity than does α-globin mRNA (Di Segni et al., 1979); the results of that study suggest strongly that mRNA interacts directly with eIF-2 during translation.

Here we show that a molecule of Mengovirus RNA apparently competes 35 times more effectively than a molecule of globin mRNA for a component at the initiation step of translation. The competition is relieved by eIF-2. We demonstrate, in binding experiments, that Mengovirus RNA and globin mRNA compete directly for eIF-2, and that Mengovirus RNA binds directly to this protein with a 30-fold higher affinity. These experiments reveal a quantitative correlation between the ability of a given mRNA species to compete in translation, and its affinity for initiation factor eIF-2.

Direct evidence for a specific interaction between eIF-2 and mRNA comes from studies with STNV RNA, as well as Mengovirus RNA. In the 1,239-nucleotide-long STNV RNA, eIF-2 recognizes specifically the 5'-terminal 44-nucleotide sequence that contains the ribosome binding site (Kaempfer et al., 1981). In Mengovirus RNA, eIF-2 by itself protects specific sequences against nuclease attack that are identical with those protected in 40 S and 80 S initiation complexes. These sequence studies point to an essential role for eIF-2 in directing the binding of ribosomes to mRNA during initiation of translation. The present results support the concept that this direct and specific interaction between eIF-2 and mRNA occurs during protein synthesis and reveal its importance for translational control.

**EXPERIMENTAL PROCEDURES**

**Reticulocyte Lysate—**Preparation of micrococal nuclelease-treated rabbit reticulocyte lysate was as described by Di Segni et al. (1979).

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1 The abbreviations used are: eIF, eukaryotic initiation factor; Met-tRNA, methionyl-tRNA; STNV, satellite tobacco necrosis virus; dsRNA, double-stranded RNA; Tris, tris(hydroxymethyl)aminomethane; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

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mRNA—Globin mRNA was prepared as described (Kaempfer, 1979). Mengovirus RNA was a gift of Dr. F. Brown, The Animal Virus Research Institute, Pirbright, England.

Cell-free Protein Synthesis—Cell-free translation mixtures of 25 μl contained, besides mRNA, 15 μl of lysate, 3 μg of mouse liver tRNA (Kaempfer, 1979), 90 mM of added KCl, 4 mM creatine phosphate, 20 mM Hepes buffer, pH 7.5, and [35S]methionine (700 Ci/mmol; Amer-sham). Mixtures were incubated for 45 min at 30°C and frozen until analysis of the products. Maximal translation of globin mRNA was seen at about 90 mM of added KCl. The salt concentration contributed by the lysate is about 55 mM, as estimated from translation requirement of a gel-filtered lysate (not shown). Maximal translation of Mengovirus RNA was obtained at 125 mM of added KCl, but the optimum is broad and translation at 90 mM of added KCl is nearly as extensive. Hot CCl4COOH-precipitable radioactivity was determined in aliquots spotted onto 1-inch square filters (Whatman No. 1).

In the conditions used (Kaempfer, 1979), the addition of ATP or GTP to the reaction mixtures did not enhance translation, as reported previously (Pelham and Jackson, 1976), nor did the addition of mixed amino acids.

Cellulose Acetate Electrophoresis—Products of cell-free translation were analyzed by electrophoresis on cellulose acetate in buffer containing urea and 2-mercaptoethanol, as described by Di Segni et al. (1979).

Preparation of [35S]Met-tRNA, and Assay of Ternary Complex Formation—[35S]Met-tRNA, was prepared and ternary complex formation with eIF-2 and GTP was assayed as described (Kaempfer et al., 1978a). N-Formyl-[35S]Met-tRNA was prepared as described by Rosen et al. (1978).

Preparation of eIF-2—The procedure is described by Kaempfer (1979), Di Segni et al. (1979), and Rosen and Kaempfer (1979). eIF-2 purified by this procedure is at least 98% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and is free of detectable protein at 80,000, the molecular weight assigned to factor eIF-2B; its mRNA-binding activity is completely sensitive to competitive inhibition by Met-tRNA, and GTP but not by uncharged tRNA, showing that the only mRNA-binding component in the preparation is eIF-2 itself (Rosen and Kaempfer, 1979). The purified eIF-2 bound 0.3 to 1.0 pmol of Met-tRNA/μg of protein.

Radioiodination of mRNA—The method of Commerford (1971) was followed, with the modifications described by Di Segni et al. (1979). The 125I-labeled mRNA obtained in this procedure is labeled to a relatively low specific activity, up to 2 x 106 cpm/μg. This low specific activity is important in determining the properties of the labeled RNA. The RNA is fully intact as judged, for globin mRNA, by polyacrylamide gel electrophoresis and autoradiography. Both globin mRNA and Mengovirus RNA labeled in our procedure bind to eIF-2 with an affinity equal to that of the unlabeled, native RNA species, as determined by self-competition (Fig. 9; Kaempfer, 1979; Kaempfer et al., 1979).

Binding of mRNA to eIF-2—Reaction mixtures of 50 μl containing 150 mM KCl, 20 mM Tris-HCl, pH 7.8, 2 mM Mg-acceptase, 6 mM 2-mercaptoethanol, eIF-2, and RNA, were incubated for 10 min at 25°C and then cooled for 10 min at 0°C before the addition of 1 ml of ice-cold buffer A (20 mM Tris-HCl, pH 7.8, 6 mM 2-mercaptoethanol, 50 mM KCl, 2 mM Mg-acceptase). The samples were passed through 25-mm nitrocellulose filters (0.45 μm pore diameter) at a flow rate of 1 ml/min, and washed three times with 1 ml of buffer A. Dried filters were counted by γ radiation spectrometry. For a more detailed description, see Kaempfer (1979).

Phosphorylation of eIF-2 and M. = 67,000 Polypeptide—Double-stranded RNA-dependent phosphorylation was studied in the ribosomal system, as described by Rosen et al. (1981).

RESULTS

Translational Competition between Globin mRNA and Mengovirus mRNA—The micrococcal nuclease-treated rabbit reticulocyte lysate (Pelham and Jackson, 1976) was used to study translational competition between globin mRNA and Mengovirus RNA. The products of translation were analyzed by cellulose acetate electrophoresis in the presence of urea and 2-mercaptoethanol. Fig. 1 illustrates the autoradiogram of the products synthesized in a series of lysates containing globin mRNA and increasing amounts of Mengovirus RNA. It is seen that α- and β-globin are well resolved from each other and from the Mengovirus RNA-directed products of translation which move to the opposite electrode. The protein products synthesized under the direction of Mengovirus RNA move as a single, broad band. By varying the conditions of electrophoresis, it can be shown that this broad band includes all the products of Mengovirus RNA translation. Mengovirus RNA, when translated alone, directed the synthesis of products that all moved with the latter band; these products are M, = 60,000 to 100,000, as judged by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (not shown).

Fig. 2 depicts densitometer scans of the autoradiogram shown in Fig. 1. It is seen that increasing amounts of Mengovirus RNA progressively inhibit the synthesis of α- and, to a smaller extent, β-globin, concomitant with the synthesis of Mengovirus protein in progressively greater amounts. These results are quantitated in Fig. 3. This figure also shows that the addition of Mengovirus RNA does not significantly affect the total incorporation of [35S]methionine into protein, although some inhibition is observed at high levels of Mengovirus RNA. It has been observed previously that high concentrations of mRNA can be inhibitory in this system (Pelham and Jackson, 1976; Di Segni et al., 1979).

The addition of increasing amounts of either Mengovirus RNA or globin mRNA to a lysate programmed with globin mRNA does not lead to a significant change in the amount of label transferred from N-formyl[35S]methionyl-tRNA into protein (Fig. 4). This result shows that the number of translation initiation events remains essentially constant in the presence of increasing amounts of either mRNA species. Hence, the decrease in globin mRNA translation that is observed in Figs. 1 to 3 cannot be explained by a general inhibitory effect of Mengovirus RNA on translation. Since the number of initiation events remains essentially constant in conditions where Mengovirus RNA-directed translation increases at the expense of globin synthesis, these results...
alone directed the synthesis of 5,500 cpm. The total amount of gmvirus RNA are from the same experiment; this amount of RNA the globin chains (not shown). Values obtained with 1.5 pg of Mengovirus RNA only gave no discernible background in the positions of the globin chains (not shown). Values obtained with 1.5 pg of Mengovirus RNA are from the same experiment; this amount of RNA alone directed the synthesis of 5,500 cpm. The total amount of [35S]methionine incorporated into protein is presented as hot CCl4COOH-precipitable material per 5 µl of reaction mixture.

Relief of Translational Competition by eIF-2.—The effect of addition of highly purified initiation factor eIF-2, free of detectable traces of other initiation factors (Rosen and Kaempfer, 1979), to a reaction mixture for translation containing both Mengovirus RNA and globin mRNA is illustrated in Fig. 5. The addition of eIF-2 does not lead to any increase in total protein synthesis, but does lead to an increase in the amount of globin synthesis, concomitant with a decrease in the synthesis of Mengovirus protein. The total globin/Mengovirus protein synthetic ratio increases from 0.74 to 1.55. Comparison of Fig. 5 with Fig. 2 shows that the addition of eIF-2 to a lysate containing 1 µg of Mengovirus RNA caused a shift in the pattern of proteins synthesized to one resembling that seen in the presence of 0.5 µg of Mengovirus RNA. Therefore, the amount of eIF-2 added in the experiment of Fig. 5 reduced the inhibitory effect of 1 µg of Mengovirus RNA to that caused by about one-half this amount. The finding that the eIF-2-dependent increase in globin synthesis is coupled with a concomitant decrease in Mengovirus RNA translation, while overall translation remains constant, supports the explanation that eIF-2 acts to relieve mRNA competition.

Low concentrations of dsRNA lead to the inactivation of eIF-2 and the resulting inhibition of translation can be relieved by the addition of eIF-2 (Kaempfer, 1974; Clemens et al., 1975). Since translation of Mengovirus RNA, in contrast to translation of globin mRNA, is resistant to the inhibitory effect of dsRNA (Rosen et al., 1981), and since RNA from vaccinia, reo- or vesicular stomatitis virus was shown to contain contaminating traces of dsRNA (Lenz and Baglioni, 1978), it was necessary to eliminate the possibility that both the inhibitory effect of Mengovirus RNA on translation of globin mRNA, and its relief by eIF-2 are accounted for by the presence of contaminating traces of dsRNA in the Mengovirus RNA preparation.

To examine this point, the dsRNA-dependent phosphorylation of the Mr = 38,000 subunit of eIF-2 and of a Mr = 67,000 polypeptide was studied by incubating ribosomes isolated from a micrococcal nuclease-treated reticulocyte lysate with [γ-32P]ATP (Rosen et al., 1981). The reaction mixtures were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As seen in the autoradiogram of Fig. 6, extensive phosphorylation of the Mr = 38,000 and 67,000 bands does occur in the presence of dsRNA (lane b), but it is not supported by increasing amounts of either globin mRNA (lanes c, d) or Mengovirus RNA, even in amounts well in excess of those needed to inhibit globin mRNA translation (lanes e, f, g).

Fig. 7 shows that the activity that relieves translational
The autoradiogram was incubated without RNA and with sogenum in dsRNA-dependent phosphorylation polypeptides are indicated. ATP and crude initiation factors (see "Experimental Procedures"), 67,000 reaction mixtures is shown. The positions of globin mRNA is indicated. Aliquots of eIF-2, see "Experimental Procedures." The gradient portion of the phosphocellulose column is shown. Aliquots of 3 μl of reaction mixture containing 1 μg of globin mRNA and 0.5 μg of Mengovirus RNA, the KC1 concentration was adjusted to 95 mM in each sample. Incorporation of [35S]methionine into total protein was determined in 3 μl aliquots. The products were analyzed as in Figs. 1 and 2.

Fig. 6. Failure of Mengovirus RNA to substitute for dsRNA in dsRNA-dependent phosphorylation of eIF-2 and a M₄, = 67,000 polypeptide. The ribosomal system (25 μl), containing [γ-³²P]ATP and crude initiation factors was incubated without RNA (a), with 20 ng/ml of Penicillium chrysospernum dsRNA (b), with 0.1 μg (e) and 0.25 μg (d) of globin mRNA, and with 0.1 μg (e), 0.25 μg (f), and 0.5 μg (g) of Mengovirus RNA. The autoradiogram of the NaDodSO₄-polyacrylamide gel of these reaction mixtures is shown. The positions of M₄, = 38,000 and 67,000 polypeptides are indicated.

Fig. 7. Co-purification of the activity that relieves translational competition with eIF-2. For purification of eIF-2, see "Experimental Procedures." The gradient portion of the phosphocellulose column is shown. Aliquots of 5 μl were used to assay GTP-dependent binding of 35S-labeled Mengovirus RNA (8 × 10⁷ cpm/μg; input, 7,100 cpm). No background was subtracted in either assay. Aliquots of 3 μl were added to reaction mixtures for protein synthesis containing 1 μg of globin mRNA and 0.5 μg of Mengovirus RNA; the KC1 concentration was adjusted to 95 mM in each sample. Incorporation of [35S]methionine into total protein was determined in 2 μl of reaction mixture. The products of translation were analyzed in Figs. 1 and 2. The total amount of globin formed is depicted. Triangles on right indicate globin synthesis in reaction. Arrows indicate amount of globin synthesized in reaction mixtures lacking Mengovirus RNA, incubated with (•) and without (○) 3 μl of material from tube 11; total [³⁵S] incorporated into protein in these controls was 3,900 cpm and 3,700 cpm, respectively.

Fig. 8. Effect of increasing amounts of eIF-2 on translation of globin mRNA and Mengovirus RNA. Reaction mixtures were incubated at 37°C for 949 messenger RNA competition for eukaryotic initiation factor 2

Incorporation of [35S]methionine into total protein was determined in 3 μl aliquots. The products were analyzed as in Figs. 1 and 2.

competition between Mengovirus RNA and globin mRNA co-purifies with eIF-2. The experiment illustrates the phosphocellulose chromatography step in the purification, which involves elution of eIF-2 with a linear gradient from 0.4 to 0.8 M KC1. Two characteristic activities of eIF-2, its ability to form a ternary complex with [³⁵S]Met-tRNA and GTP, and its ability to bind mRNA, assayed by the retention of [³²P]labeled Mengovirus RNA on nitrocellulose filters, are seen to co-elute in a single peak near 0.6 M KC1. The fractions of this gradient were tested for their ability to stimulate globin synthesis in reaction mixtures containing globin mRNA and 0.5 μg of Mengovirus RNA. As seen in Fig. 7, this amount of Mengovirus RNA caused a 2- to 2.5-fold reduction in globin synthesis when compared to a control sample containing only globin mRNA. Although there is no stimulation of total protein synthesis by any fraction across the gradient, the synthesis of globin is stimulated to the level seen in the control lacking Mengovirus RNA by an activity that elutes precisely in the position of eIF-2. It should be noted that other basic proteins do elute ahead of eIF-2 or behind it, yet these fail to stimulate globin synthesis in conditions of competition. The stimulation of globin mRNA translation by eIF-2 is seen only in the presence of competing amounts of Mengovirus RNA, but not in the control containing only globin mRNA. Thus, the effect of eIF-2 is to relieve competition, and not to stimulate globin mRNA translation specifically.
tion of the amount of eIF-2 added. This increase is more pronounced, the lower the concentration of Mengovirus RNA, a finding also evident from the results of Figs. 5 and 7. Indeed, the slopes of the eIF-2 dose-response curves shown in Fig. 8 are approximately inversely proportional to the amount of Mengovirus RNA present. This is the result expected if Mengovirus RNA and globin mRNA compete for eIF-2 in translation.

**Preferential Binding of Mengovirus RNA to eIF-2**—If the translational competition observed between Mengovirus RNA and globin mRNA involves direct competition for initiation factor eIF-2, then one would predict Mengovirus RNA to possess a greater affinity than globin mRNA for eIF-2. To examine this point, we have labeled Mengovirus RNA and globin mRNA with \(^{125}\)I, and have studied the binding of these labeled RNA species to eIF-2 in the presence of competing amounts of the unlabeled ones. In the experiment of Fig. 9A, \(^{125}\)I-labeled Mengovirus RNA was incubated with a limiting amount of eIF-2 and increasing amounts of unlabeled Mengovirus RNA or globin mRNA. It is seen that, on a molar basis, Mengovirus RNA competes 30 times more effectively than globin mRNA. Unlabeled Mengovirus RNA competes with labeled Mengovirus RNA on an equimolar basis, showing that radioiodination did not affect the affinity of this RNA for eIF-2 (Kaeppfer, 1979). The reciprocal experiment, using \(^{125}\)I-labeled globin mRNA, is presented in Fig. 9B. Again, Mengovirus RNA is seen to compete 30 times more effectively than globin mRNA for eIF-2.

In Fig. 10, the extent of complex formation between eIF-2 and labeled globin mRNA or Mengovirus RNA is analyzed as a function of increasing KCl concentration. Binding of globin mRNA to eIF-2 decreases sharply above 100 mM KCl, and is inhibited 50% at 160 mM KCl. By contrast, binding of Mengovirus RNA to eIF-2 is much more resistant to increasing salt concentrations. This result provides independent evidence that Mengovirus RNA binds more strongly to eIF-2.

**DISCUSSION**

The major result of these experiments is that Mengovirus RNA and globin mRNA compete during translation in a manner that can be relieved by eIF-2, and that each of these mRNA species binds to this initiation factor with an affinity that closely reflects its ability to compete in translation. Direct binding experiments show that Mengovirus RNA possesses a 30-fold greater affinity for eIF-2 than does globin mRNA. This affinity ratio is in striking agreement with translation competition experiments that reveal about a 35-fold greater apparent affinity of Mengovirus RNA for a critical component in protein synthesis. Together with our finding that eIF-2 relieves the translational competition between Mengovirus RNA and globin mRNA, these experiments strongly support the concept that Mengovirus RNA and globin mRNA compete directly for eIF-2 in protein synthesis. More generally, these results reveal a direct correlation between the ability of a given mRNA molecule to compete in translation and its ability to bind to initiation factor eIF-2. Such messenger RNA competition for eIF-2 may contribute significantly to the selective translation of viral RNA in infected cells.

**Mengovirus RNA Competition for eIF-2**—The concept that mRNA species differ in their efficiency of translation, apparently because of a different affinity for one or more critical components in the initiation step, was first suggested by Lodish (1974, 1976) who showed that initiation of protein synthesis on a molecule of \(\alpha\)-globin mRNA occurs with lower frequency than that on a molecule of \(\beta\)-globin mRNA (Lodish, 1971). Indeed, the addition of eIF-2 leads to relief of the
translational competition between α- and β-globin mRNA occurring in a micrococcal nuclease-treated lysate identical with that used in these experiments (Di Segni et al., 1979). Such relief is observed both when competition is caused by a high concentration of globin mRNA and when it is sharpened by a high concentration of KCl. Results of that study suggest strongly that mRNA interacts directly with eIF-2 during protein synthesis, and that α-globin mRNA possesses a lower affinity for eIF-2 than does β-globin mRNA.

In the present experiments, the result of translational competition analysis between globin mRNA and Mengovirus RNA are shown to correlate quantitatively with the results of direct binding competition analysis between these mRNA species and isolated eIF-2. Mengovirus RNA competes directly with globin mRNA for this factor, and binds it with a 30-fold higher affinity (Fig. 9). Independent verification that eIF-2 itself binds 30-fold more tightly to Mengovirus RNA than to globin mRNA is provided by the finding that Mengovirus RNA is 30 to 40 times more effective than globin mRNA as a competitive inhibitor of ternary complex formation between Met-tRNAf, GTP, and eIF-2 (Rosen et al., 1981). Mengovirus RNA acts as a powerful inhibitor of globin mRNA translation (Figs. 1 to 3), whereas the overall number of initiation events remains constant (Fig. 4). This inhibition of globin mRNA translation is relieved by the addition of highly purified eIF-2 (Figs. 6 to 8). Yet, addition of this initiation factor does not stimulate total translation, and it stimulates globin mRNA translation only when Mengovirus RNA is present, but not in its absence (Fig. 7). This explains why, in the presence of competing amounts of Mengovirus RNA, the increase in globin mRNA translation caused by addition of eIF-2 is matched by a decrease in Mengovirus RNA translation (Fig. 5). This observation suggests strongly that the addition of eIF-2 does not lead to an increase in the total number of initiation events, a point shown already for the case of α- and β-globin mRNA competition studied in the same circumstances (Di Segni et al., 1979). The fact that eIF-2 acts to shift translation in favor of globin synthesis shows clearly that globin mRNA and Mengovirus RNA compete for eIF-2. The addition of eIF-2 leads to relief of competition because it increases the number of eIF-2 molecules available, allowing thereby a relatively greater number of the more weakly binding, but more numerous, globin mRNA molecules to interact with eIF-2. Thus, although the total number of initiation events does not increase, the proportion of globin mRNA molecules entering initiation complexes will increase, up to the point where binding of globin mRNA and Mengovirus RNA is exactly according to their molar ratio, and there no longer is competition.

The Interaction between mRNA and eIF-2—The present results support the concept that a direct and specific interaction between eIF-2 and mRNA occurs during protein synthesis and contributes significantly to translational control. A critical role for the affinity of mRNA for eIF-2 in protein synthesis is also suggested by the observation that dsRNA, an agent known to inactivate eIF-2 (Kaempfer and Kaufman, 1973; Kaempfer, 1974; Clemens et al., 1975), fails to inhibit the translation of Mengovirus RNA in conditions where translation of globin or mouse ascites cell mRNA is blocked completely; direct RNA-binding studies reveal that dsRNA competes with mRNA for eIF-2, binding this factor more strongly than globin mRNA, but more weakly than Mengovirus RNA (Rosen et al., 1981).

Evidence for a specific interaction between eIF-2 and mRNA is furnished by a number of observations. Binding of all mRNA species examined, including species lacking the 5'-terminal cap or 3'-terminal poly(A) moieties, is first order in eIF-2, pointing to the existence of a high affinity binding site in mRNA (Kaempfer et al., 1978a, 1981). By contrast, mRNA species not serving as mRNA, such as negative strand RNA (Kaempfer et al., 1978a), RNA (Barrieux and Rosenfeld, 1977; Rosen and Kaempfer, 1979) and rRNA (Barrieux and Rosenfeld, 1977) bind much more weakly. eIF-2 forms an equimolar complex with globin mRNA, possessing an apparent $K_d$ of less than $10^{-9}$ M at physiological salt concentrations, at a site that involves neither the poly(A) tail nor the 3'-untranslated sequence of this mRNA (Kaempfer et al., 1979). Analogs of the cap that inhibit translation also inhibit the binding of mRNA to eIF-2, as well as ternary complex formation between eIF-2, Met-tRNAf, and GTP, suggesting an interaction of eIF-2 with the modified 5'-end of mRNA (Kaempfer et al., 1978b). However, several types of results indicate that binding of eIF-2 to mRNA is primarily at an internal sequence and secondarily through the cap (Kaempfer et al., 1978a, 1981). Direct evidence for the existence of specific binding sites for eIF-2 in mRNA comes from nucleotide sequence analysis. eIF-2 binds specifically to the 5'-end of STNV RNA, a 1,239-nucleotide-long, noncapped messenger, but not to the 5'-ends of fragments of internal origin (Kaempfer et al., 1981). The factor does not bind detectably to the 32-nucleotide 5'-terminal fragment ending in the AUG initiation codon, or shorter ones, but does bind specifically to the 44-nucleotide 5'-terminal fragment that contains the ribosome binding site. These experiments reveal not only that eIF-2 binds to a specific site in STNV RNA, but also that this site overlaps closely with the 40 S ribosome binding site (Kaempfer et al., 1981).

Studies of the interaction between Mengovirus RNA and eIF-2 lead to a similar conclusion. Upon digestion with ribonuclease T1, the RNA protected in either 40 S or 80 S initiation complexes yields four unique, large oligonucleotides, 17 to 28 bases in length, all located between the poly(C) tract and the 3'-end. Fingerprint analysis of the Mengovirus RNA sequences protected by eIF-2 against ribonuclease T1 digestion yields oligonucleotides that are identical with those protected in either 40 S or 80 S initiation complexes. Since Mengovirus RNA is approximately 7,500 nucleotides long, these results demonstrate high specificity in the recognition of this RNA by eIF-2.

Considering that in the studies with STNV and Mengovirus RNA the interaction between eIF-2 and mRNA was examined in the absence of Met-tRNAf, ribosomes or other components of the protein-synthesizing machinery, the striking resemblance between the binding site for isolated eIF-2 on the one hand, and for the 40 S ribosomal subunit in the presence of all initiation factors on the other, supports the concept that during initiation of translation, binding of a ribosome to mRNA is guided to a significant extent by eIF-2. The findings reported here, showing that Mengovirus and globin mRNA compete in initiation of translation as they do in direct binding to eIF-2 are in harmony with this concept. Competition between mRNA species most likely is for eIF-2 molecules located in 40 S/Met-tRNAf/eIF-2 complexes (see Di Segni et al., 1979).

The implication of the present findings is that the affinity of a given mRNA species for eIF-2 directly influences its translation, relative to that of other mRNA species.

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REFERENCES
Barrieux, A. & Rosenfeld, M. G. (1977) J. Biol. Chem. 252, 3843-3847
Barrieux, A. & Rosenfeld, M. G. (1978) J. Biol. Chem. 253, 6311-6314
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Clemens, M. J., Safer, B., Merrick, W. C., Anderson, W. F. & London, I. M. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1286-1290
Commerford, S. L. (1971) Biochemistry 10, 1993-2000
Di Segni, G., Rosen, H. & Kaempfer, R. (1979) Biochemistry 18, 2847-2854
Golini, F., Thach, S., Birge, C., Safer, B., Merrick, W. & Thach, R. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3040-3044
Kaempfer, R. (1974) Biochem. Biophys. Res. Commun. 61, 591-597
Kaempfer, R. (1979) Methods Enzymol. 60, 380-392
Kaempfer, R. & Kaufman, J. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1222-1226
Kaempfer, R., Hollender, R., Abrams, W. R. & Israeli, R. (1978a) Proc. Natl. Acad. Sci. U. S. A. 75, 208-213
Kaempfer, R., Rosen, H. & Israeli, R. (1978b) Proc. Natl. Acad. Sci. U. S. A. 75, 650-654
Kaempfer, R., Hollender, H., Soreq, H. & Nudel, U. (1979) Eur. J. Biochem. 94, 581-600

Kaempfer, R., van Emmelo, J. & Fiers, W. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 1543-1546
Lawrence, C. & Thach, R. (1974) J. Virol. 14, 598-610
Lenz, J. R. & Baglioni, C. (1978) J. Biol. Chem. 253, 4219-4223
Lodish, H. F. (1971) J. Biol. Chem. 246, 7131-7138
Lodish, H. F. (1974) Nature (Lond.) 251, 385-388
Lodish, H. F. (1976) Annu. Rev. Biochem. 45, 29-72
Lodish, H. F. & Jacobsen, M. (1972) J. Biol. Chem. 247, 3622-3629
Pelham, H. R. B. & Jackson, R. J. (1976) Eur. J. Biochem. 67, 247-256
Rosen, H. & Kaempfer, R. (1979) Biochem. Biophys. Res. Commun. 91, 449-455
Rosen, H., Knoiler, S. & Kaempfer, R. (1981) Biochemistry 20, 3011-3020
Trachsel, H., Erni, B., Schreier, M. H. & Staehelin, T. (1977) J. Mol. Biol. 116, 755-767
Translational control by messenger RNA competition for eukaryotic initiation factor 2.
H Rosen, G Di Segni and R Kaempfer

J. Biol. Chem. 1982, 257:946-952.

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