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Specific binding of messenger RNA and methionyl-tRNA$_f^{Met}$ by the same initiation factor for eukaryotic protein synthesis

(eukaryotic initiation factor 2/RNA affinity chromatography/mRNA-cellulose/double-stranded RNA/negative-strand RNA)

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ABSTRACT **Affinity chromatography on columns containing globin mRNA, R17 phage mRNA, or double-stranded RNA linked to cellulose is used to demonstrate unequivocally that the eukaryotic initiation factor (eIF-2) that forms a ternary complex with Met-tRNA$_f$ and GTP also binds tightly to these RNA species. Affinity chromatography of reticulocyte ribosomal wash yields over 100-fold purification of Met-tRNA$_f$-binding factor. This factor is eluted as one of the most tightly bound proteins, and is active in protein synthesis even after passage over a column of double-stranded RNA-cellulose. eIF-2 binds mRNA and double-stranded RNA in distinctly different modes, protecting essentially all sequences in double-stranded RNA, but very few in mRNA, against digestion with ribonuclease. Apparently, eIF-2 recognizes the A conformation of double-stranded RNA, but not its sequence. By contrast, globin, Mengo virus, R17 and vesicular stomatitis virus mRNA are shown to possess a high-affinity binding site for eIF-2 that is absent in negative-strand RNA of vesicular stomatitis virus, an RNA that cannot serve as messenger. The results support the concept that eIF-2, the initiation factor that binds Met-tRNA$_f$, recognizes an internal sequence in mRNA essential for protein synthesis.**

The recognition of mRNA and its binding to ribosomes is an essential point of regulation in eukaryotic gene expression. Two of the clearest instances of translational control that can be studied *in vitro* are the block in initiation of protein synthesis occurring in rabbit reticulocyte lysates in the absence of added hemin (1) or in the presence of double-stranded RNA (2). In both cases, the inhibition can be relieved completely by addition of an initiation factor for protein synthesis (3–5) that has been identified (6, 7) with eukaryotic initiation factor 2 (eIF-2), the protein that forms a ternary complex with Met-tRNA$_f$ and GTP (8–11). In addition to having these properties, preparations of this factor are able to retain globin mRNA (3, 12), R17 phage RNA (3, 6), and double-stranded RNA (dsRNA) (4, 6) on membrane filters. These RNA-binding properties suggest that besides binding Met-tRNA$_f$, eIF-2 may be involved directly in the binding and recognition of mRNA. Such a dual function for a single initiation factor would be unique, and would have important implications for understanding translational control. However, the early demonstrations of mRNA-binding properties in eIF-2 preparations were based on membrane-filtration studies using labeled RNA, leaving open the possibility that this binding was due to contaminating protein. Hence, we have sought more direct evidence for this dual function.

Here, we report that the Met-tRNA$_f$-binding factor can be purified by affinity chromatography on columns containing globin mRNA, R17 RNA, and dsRNA linked to cellulose. This provides unequivocal evidence that the same initiation factor binds to Met-tRNA$_f$, mRNA, and dsRNA. Binding to dsRNA is not restricted to specific sequences. By contrast, we show that binding to mRNA is at a high-affinity site present in all mRNA species examined, but absent in negative-strand RNA. The results support the concept that eIF-2 recognizes an internal sequence in mRNA that is essential for protein synthesis.

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MATERIALS AND METHODS

RNA. Poly(A)-containing rabbit reticulocyte RNA (hereafter called globin mRNA) was purified according to Krystosek *et al.* (13) with these modifications: Polysomes were first washed with 0.4 M KCl (3), and no carrier RNA was added. Phage R17 RNA (14) and dsRNA from *Penicillium chrysogenum* (4) and phage ϕ6 (15) were prepared as described. Vesicular stomatitis virus (VSV) negative-strand RNA was extracted from purified virions (a gift of A. S. Huang) with phenol/cresol. VSV mRNA was prepared (16) from baby hamster kidney (BHK) cells at 4.5 hr after infection. Translation studies have shown that such mRNA is essentially free of host mRNA (17). Iodination (18) of globin mRNA and Mengo virus RNA (a gift of F. Brown) was to low specific activity (about 5×10^5 cpm/μg); native and iodinated mRNA bind with equal affinity to eIF-2 (not shown).

RNA-Cellulose Chromatography. RNA-cellulose is prepared (19) by irradiating mixtures containing 0.3 mg of RNA and 0.3 g of Whatman CF-11 cellulose with a UV dose of 4×10^5 ergs/mm^2 (20). Before use, RNA-cellulose columns (0.5 × 6 cm) are washed extensively with buffer A [20 mM Tris·HCl (pH 7.8)/6 mM 2-mercaptoethanol] containing 0.8 M KCl, and equilibrated with 0.08 M or 0.02 M KCl in Buffer A. The column is washed with loading buffer and developed with a 0.08–0.6 M or 0.02–0.6 M KCl gradient (12–15 ml) in Buffer A, at a flow rate of 6 ml/hr.

Met-tRNA$_f$-Binding Assay. Assay mixtures (50 μl) containing 150 mM KCl, 20 mM Tris·HCl (pH 7.4), 0.1 mM GTP, 0.1 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 0.02–0.1 pmol of [^{35}S]Met-tRNA$_f$ (21) are incubated for 10 min at 25°. Samples are diluted with cold Buffer B [20 mM Tris·HCl (pH 7.4)/150 mM KCl] and passed through Millipore HA 0.45-μm pore diameter filters, washing three times with 1 ml of Buffer B. Filters are dried and assayed for radioactivity.

mRNA-Binding Assay. Retention of labeled mRNA on nitrocellulose filters is assayed (6) in reaction mixures (100 μl) containing 50 mM KCl, 20 mM Tris·HCl (pH 7.8), 6 mM 2-mercaptoethanol (Buffer C). Samples are incubated for 10 min at 30°, for 10 min at 0°, diluted with 1 ml of cold Buffer C, and passed through Millipore HA 0.45-μm filters, washing thrice with 1 ml of Buffer C.

RESULTS

Affinity Chromatography of eIF-2 on Globin mRNA-Cellulose. When the 0–50% saturated ammonium sulfate

Abbreviations: eIF-2, eukaryotic initiation factor 2; tRNA$_f$, tRNA$_f^{Met}$; dsRNA, double-stranded RNA; VSV, vesicular stomatitis virus.

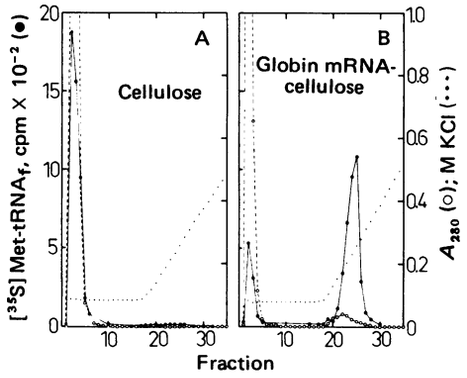

FIG. 1. Chromatography of Met-tRNA$_f$-binding factor on globin mRNA-cellulose. Reticulocyte ribosomal salt wash protein (3, 5) [0–50% saturated $(NH_4)_2SO_4$ fraction, 3.1 A_{280} units] was applied to a column of pure cellulose (*A*) or globin mRNA-cellulose (*B*). The columns were washed with 0.08 M KCl buffer and developed with a 0.08–0.6 M KCl gradient (12 ml). Aliquots (35 μl/0.5-ml fraction) were assayed for binding of [^{35}S]Met-tRNA$_f$ (input, 3800 cpm).

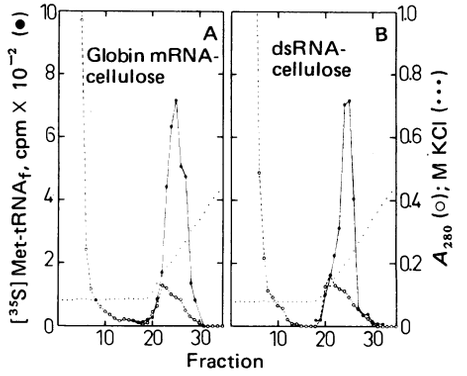

FIG. 3. RNA-affinity chromatography of Met-tRNA$_f$-binding factor from reticulocyte supernatant. S-100 protein [0–50% saturated $(NH_4)_2SO_4$ fraction; 149 A_{280} units] was applied to globin mRNA-cellulose (*A*) or ϕ6 dsRNA-cellulose (*B*) columns that were developed and assayed as in Fig. 1.

fraction of proteins washed off rabbit reticulocyte ribosomes by 0.4 M KCl is passed over a column consisting of pure cellulose, neither protein nor material able to form ternary complexes with ^{35}S-labeled Met-tRNA$_f$ and GTP is retained (Fig. 1*A*). By contrast, a column of globin mRNA linked to cellulose retains most of the Met-tRNA$_f$-binding activity, but little protein (Fig. 1*B*). When a gradient of increasing salt concentration is applied, most of the 280-nm-absorbing material that was bound to the column is eluted ahead of the peak of Met-tRNA$_f$-binding activity. Among the proteins present in the ribosomal salt wash fraction, therefore, initiation factor eIF-2 is one with the highest affinity for globin mRNA.

Affinity Chromatography on R17 and dsRNA-Cellulose. The selective retention of the Met-tRNA$_f$-binding factor on columns containing R17 RNA or dsRNA is demonstrated in Fig. 2. R17 RNA is translated faithfully, although less efficiently than globin mRNA, in a mammalian cell-free system (22). In the stepwise elution profile of Fig. 2*A*, most of the 280-nm-absorbing material retained by the column is eluted at 0.2 M KCl, while Met-tRNA$_f$-binding activity is eluted predominantly at 0.3 M KCl. Material with the ability to retain ^{32}P-labeled R17 RNA on nitrocellulose filters is eluted at both steps, but in the peak eluted at 0.3 M KCl the ratio of Met-tRNA$_f$-binding activity to R17 RNA-binding activity is significantly greater.

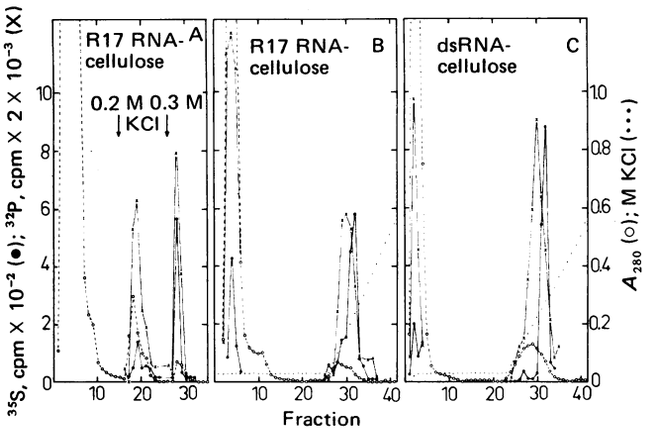

FIG. 2. Chromatography of Met-tRNA$_f$-binding factor on R17 RNA-cellulose and dsRNA-cellulose. (*A*) Reticulocyte S-100 protein [0–50% saturated $(NH_4)_2SO_4$ fraction; 145 A_{280} units] was applied to an R17 RNA-cellulose column that was washed with 0.08 M KCl buffer and stepwise eluted. Ribosomal salt wash protein [0–50% saturated $(NH_4)_2SO_4$ fraction; 7 A_{280} units] was applied to R17 RNA-cellulose (*B*) and ϕ6 dsRNA-cellulose (*C*) columns that were washed with 0.02 M KCl buffer and eluted with a 0.02–0.6 M KCl gradient. Aliquots were assayed for binding of [^{35}S]Met-tRNA$_f$ (input, 2300 cpm) and R17 [^{32}P]RNA (input, 9000 cpm).

Gradient elution profiles of ribosomal salt wash proteins applied to R17 RNA- and dsRNA-cellulose columns are presented in Fig. 2 *B* and *C*. In each case, most of the 280-nm-absorbing material and ^{32}P-labeled R17 RNA-binding activity retained on the column are eluted ahead of the peak of Met-tRNA$_f$-binding activity. Note that the flow-through fraction also contains a considerable amount of protein able to retain R17 RNA on filters, but relatively little Met-tRNA$_f$-binding activity. Clearly, eIF-2 is retained preferentially on these columns.

Affinity Chromatography of eIF-2 from Postribosomal Supernatant. When the 0–50% saturated ammonium sulfate fraction of rabbit reticulocyte S-100 (100,000 × *g* supernatant) is subjected to chromatography on globin mRNA-cellulose or dsRNA-cellulose columns (Fig. 3), Met-tRNA$_f$-binding activity is retained in each case, and elutes trailing a peak of absorbance that represents approximately 0.5% of the material applied to the column. Met-tRNA-hydrolase activity in the flow-through fraction of supernatant proteins prevents measurement of unadsorbed Met-tRNA$_f$-binding activity. Essentially the same result is obtained with R17 RNA-cellulose, as shown in Fig. 2*A*, where supernatant fraction was used. These RNA-affinity columns, therefore, are able to select eIF-2 from a mixture of ribosomal wash or supernatant proteins.

Recovery of Protein-Synthetic Activity from dsRNA-Cellulose. Initiation of protein synthesis in rabbit reticulocyte lysates is inhibited during heme deprivation (1) or upon the addition of dsRNA (2). This block in initiation can be relieved specifically by the addition of eIF-2 (3, 4, 6). The function of this factor in protein synthesis is inhibited by dsRNA, but the mechanism of inhibition is not yet understood. Met-tRNA$_f$-binding activity, we have seen, is recovered intact from dsRNA-cellulose columns. It is of interest to know if the protein-synthetic activity of eIF-2 likewise can be recovered.

Ribosomal wash fraction was subjected to chromatography on dsRNA-cellulose and each fraction was assayed for its ability to support protein synthesis in a reticulocyte lysate lacking added hemin. As seen in Fig. 4, fractions preceding the salt gradient support ^{14}C-labeled amino acid incorporation to the level obtained in a control lysate, but a peak of stimulatory activity is eluted by the gradient, behind most of the absorbance. To show that this stimulation of protein synthesis is due to protein and not to KCl in the gradient, we have taken advantage of the observation that the protein-synthetic activity

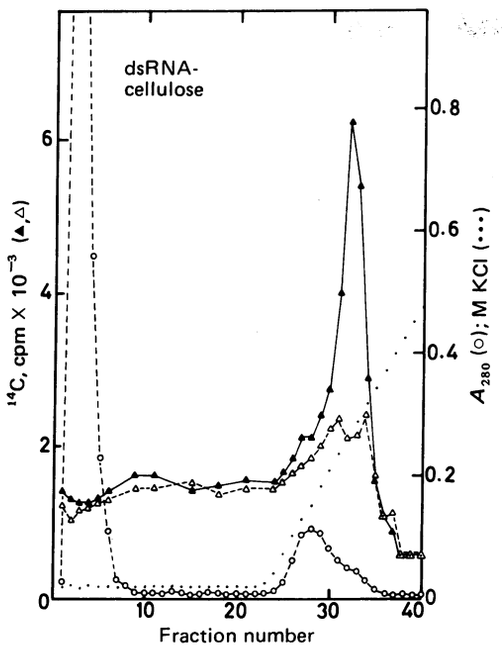

FIG. 4. Recovery of factor active in protein synthesis from dsRNA-cellulose. Ribosomal salt wash proteins [0–50% saturated $(NH_4)_2SO_4$ fraction; 7.7 A_{280} units] were applied to a *P. chrysogenum* dsRNA-cellulose column that was developed as in Fig. 2*C*. Aliquots of 30 μl were incubated at 30° in a 50-μl reaction mixture for protein synthesis (3) containing 10 μl of reticulocyte lysate and ^{14}C-labeled amino acids, but no added hemin. CCl_3COOH-insoluble radioactivity was determined after 60 min. The control that received no column sample gave 1420 cpm. △, Aliquots were incubated in 20 mM *N*-ethylmaleimide for 30 min at 37° and received 22 mM 2-mercaptoethanol before assay as above.

of the Met-tRNA$_f$-binding factor is abolished by treatment with the SH-reagent, *N*-ethylmaleimide (6). As shown in Fig. 4, such treatment severely reduces the activity of fractions that stimulate protein synthesis but has no effect on other fractions.

Purification of eIF-2 by Affinity Chromatography. We calculate that a single passage of the 0–50% saturated ammonium sulfate fraction of ribosomal wash protein over globin mRNA-cellulose results in at least 100-fold purification of eIF-2; passage over a dsRNA-cellulose column yields at least 125-fold purification. These properties are advantageous because eIF-2 tends to be unstable during purification, being very sensitive to oxidation. A rapid and convenient purification procedure was developed by combining conventional methods with affinity chromatography.

Ribosomal wash proteins were subjected to ammonium sulfate fractionation and DEAE-cellulose chromatography and then eluted stepwise from phosphocellulose (Fig. 5*A*). Material from the peak of Met-tRNA$_f$-binding activity was applied to a column of dsRNA-cellulose (Fig. 5*B*). Met-tRNA$_f$-binding activity is recovered in a peak coincident with the absorbance and the protein-synthetic activity, assayed in a lysate lacking added hemin. Protein-synthetic activity again is nearly abolished by treatment with *N*-ethylmaleimide. Analysis of columns run without a sample shows that the slight residual stimulation of protein synthesis seen in Figs. 4 and 5*B* is due to salt in the gradient, as is the inhibition observed in later fractions.

Specificity of Interaction between Met-tRNA$_f$-Binding Factor and mRNA. How specific is the interaction of eIF-2 with mRNA? Fig. 6 shows the extent of complex formation between purified eIF-2 and various labeled mRNA species, plotted as a function of the ratio of protein to mRNA molecules. The saturation binding curves of globin mRNA, VSV mRNA,

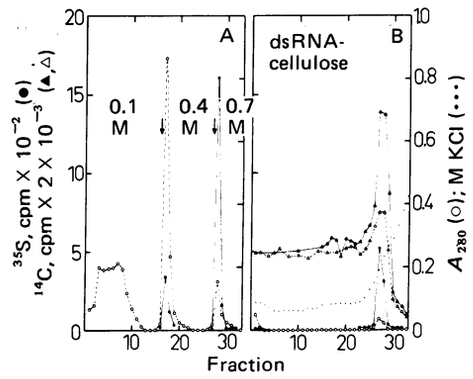

FIG. 5. Purification of eIF-2. Ribosomal salt wash protein (see Fig. 1) was applied to a DEAE-cellulose column equilibrated with 0.01 M Tris·HCl (pH 7.4)/0.1 mM EDTA/0.05 M KCl/10 mM 2-mercaptoethanol/10% (vol/vol) glycerol, and eluted stepwise with buffer containing 0.05 M, 0.1 M, and 0.22 M KCl. Material eluted at 0.22 M KCl was diluted and applied to a phosphocellulose column equilibrated with 0.05 M Tris·HCl (pH 7.8)/0.1 mM EDTA/0.1 M KCl/1 mM dithiothreitol/10% glycerol, and eluted stepwise as shown (*A*). An aliquot of 300 μl of fraction 28 was diluted and applied to a φ6 dsRNA-cellulose column (*B*) that was developed as in Fig. 1. Aliquots of 20 μl were assayed for binding of [^{35}S]Met-tRNA$_f$ (input, 5400 cpm). Incorporation of ^{14}C-labeled amino acids before (▲) or after (△) treatment with *N*-ethylmaleimide was as in Fig. 4. Control that received no column sample gave 2480 cpm.

Mengo virus RNA, and R17 RNA are all typical of a first-order reaction. As in the case of the *lac* repressor and its operator DNA (23), this property indicates that binding of one initiation factor molecule to a molecule of mRNA is sufficient to cause retention of this RNA molecule on the filter. These mRNA species, therefore, all possess a high-affinity binding site for eIF-2. Neither the 7-methylguanosine 5′-phosphate moiety (cap) at the 5′ end of mRNA, nor the poly(A) sequence at the 3′ end seems to be required for binding, because Mengo virus RNA (which lacks the cap) and R17 RNA [which lacks both cap and poly(A)] are bound efficiently.

If the interaction between eIF-2 and mRNA involves recognition of a site essential for protein synthesis, then RNA species lacking such a site should exhibit different binding properties. To test this point, we have examined the interaction between eIF-2 and negative-strand RNA extracted from VSV virions. This RNA cannot serve as messenger but it is transcribed into complementary mRNA sequences during infection (24). As seen in Fig. 6, VSV negative-strand RNA indeed is different: the saturation binding curve has an extremely pronounced sigmoid shape, typical of a multi-order interaction. At saturation, however, nearly all these RNA molecules are able to form complexes. It is unlikely that the higher molecular weight of VSV negative-strand RNA (molecular weight 3.5×10^6) (16) is responsible for this sigmoid shape, because R17 RNA (molecular weight 1.1×10^6) and Mengo virus RNA (molecular weight 2.6×10^6) bind just as well as the much smaller globin or VSV mRNA species, and binding of Mengo virus RNA is particularly efficient (Fig. 6). Instead, the sigmoid shape indicates that VSV negative-strand RNA possesses multiple, weak binding sites for eIF-2, but lacks the high-affinity binding site present in VSV mRNA and other mRNA species.

Distinct Binding Modes for dsRNA and mRNA. In the experiment of Fig. 7, ^{32}P-labeled R17 RNA and φ6 viral dsRNA were incubated with increasing amounts of purified eIF-2, and the extent of protection of RNA sequences then was measured by ribonuclease digestion in 0.01 M KCl. At this salt concentration, the RNA·eIF-2 complexes have a half-life of at least 1 hr in the absence of ribonuclease (W. R. Abrams and R.

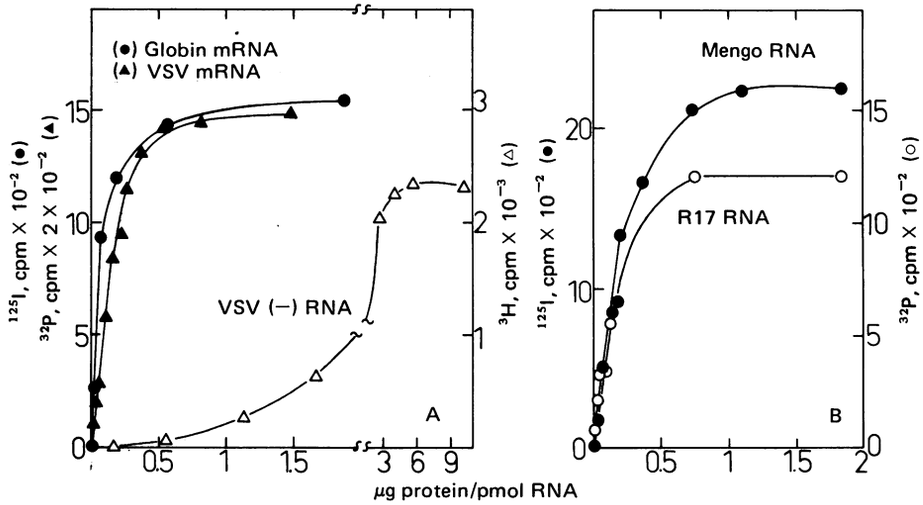

FIG. 6. Relative affinities of mRNA and VSV negative-strand RNA for eIF-2. ^{125}I-Labeled purified globin mRNA (0.031 pmol; 2300 cpm), ^{32}P-labeled VSV mRNA [3.6 pmol, assuming average molecular weight 0.55×10^6 (see ref. 16); 800 cpm], ^{3}H-labeled RNA extracted from VSV virions (0.21 pmol; 2800 cpm), ^{125}I-labeled Mengo virus RNA (0.0023 pmol; 2630 cpm), and ^{32}P-labeled R17 RNA (2.25 pmol; 1660 cpm) were incubated with increasing amounts of eIF-2 purified as in Fig. 5, and RNA binding was assayed. Background without protein was subtracted; for all RNA species, this background was less than 3%. Note scale change in curve of VSV negative-strand RNA.

Kaempfer, unpublished data). Fig. 7 shows that in the absence of eIF-2 most dsRNA sequences are digested, while in the presence of increasing amounts of this factor significant protection is observed. Indeed, virtually all the sequences in dsRNA can be protected by eIF-2 against digestion. By contrast, no such protection is evident in the case of R17 RNA, even at relatively high levels of eIF-2. In each curve, arrows indicate the amount of factor sufficient to give plateau binding when complex formation is assayed as in Fig. 6. In view of the stability of the complexes, a reasonable interpretation of these results is that binding of eIF-2 to dsRNA is not specific with respect to sequence and that there are many factor-binding sites on dsRNA, while binding to R17 RNA is at one, or perhaps several sequences, apparently comprising less than 1–2% of this RNA.

Consistent with this interpretation is our finding that the capacity of R17 or globin mRNA columns to retain Met-tRNA$_f$-binding activity is distinctly lower than that of columns containing an equal weight of dsRNA. For preparative purposes, therefore, affinity chromatography on dsRNA-cellulose is the method of choice.

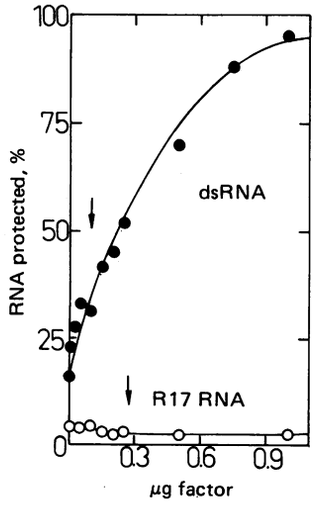

FIG. 7. Ribonuclease sensitivity of complexes between eIF-2 and mRNA or dsRNA. ^{32}P-Labeled ϕ6 dsRNA [0.63 pmol (15); 2730 cpm] and ^{32}P-labeled R17 RNA (1.7 pmol; 9600 cpm) were incubated for 10 min at 37° in the RNA-binding assay, with increasing amounts of purified eIF-2 (see Fig. 5). Pancreatic ribonuclease was then added to a concentration of 5.4 μg/ml, and incubation at 37° was continued for another 30 min. The KCl concentration during binding and digestion was 0.01 M. After cooling, cold CCl_3COOH-precipitable radioactivity was determined. No background was subtracted.

DISCUSSION

These experiments provide unequivocal evidence that the same initiation factor for eukaryotic protein synthesis that recognizes and binds to initiator Met-tRNA$_f$ also binds with high affinity to mRNA and dsRNA. Binding to mRNA molecules is at a high-affinity site present in all mRNA species tested, including VSV mRNA, but absent in negative-strand VSV RNA. That binding is at a specific site in mRNA is further supported by the finding that saturating amounts of eIF-2 do not protect the sequences in mRNA to an appreciable extent against ribonuclease digestion. By contrast, binding to dsRNA can occur at many sites, because eIF-2 is able to protect essentially all sequences against digestion. Binding of eIF-2 to mRNA does not require a 7-methylguanosine phosphate at the 5′ end of mRNA, or poly(A) at the 3′ end. These results provide evidence for the concept that eIF-2 recognizes an internal sequence in mRNA that is essential for protein synthesis.

Chromatography on columns of mRNA or dsRNA linked to cellulose shows that eIF-2 is eluted as one of the most tightly bound proteins among those in the ribosomal wash and post-ribosomal supernatant fractions of rabbit reticulocytes. The good correlation between the high capacity of dsRNA columns in retaining eIF-2, as opposed to the much lower capacity of mRNA columns, with the results of the ribonuclease protection experiment of Fig. 7 supports the interpretation that fractionation on these columns is based on affinity for RNA, rather than on ion exchange. This is further supported by equilibrium binding studies showing that eIF-2 binds R17 or globin mRNA at 0.15 M KCl with an apparent dissociation constant of 5 nM or less (R. Kaempfer, R. Hollender, H. Soreq, and U. Nudel, unpublished data).

These properties of eIF-2 allow its purification by affinity chromatography. The eluted factor not only binds Met-tRNA$_f$, but also is active in protein synthesis (Figs. 4 and 5). A rapid and convenient purification procedure for eIF-2 is obtained by

combining conventional methods with affinity chromatography (Fig. 5).

The present results provide cogency to the earlier finding that the protein-synthetic activity of eIF-2 copurifies with the ability to retain labeled globin mRNA (3), R17 RNA (3, 6), or dsRNA (4, 6) on filters. Hellerman and Shafritz (12) found Met-tRNA$_f$-binding activity in association with globin messenger ribonucleoprotein particles and also suggested a relationship between binding of Met-tRNA$_f$ and mRNA. However, because they showed that preparations of this factor not only bind readily to globin mRNA, but also to free poly(A), it was possible that binding to globin mRNA was in fact to poly(A). This possibility is made unlikely by the affinity chromatography data with R17 RNA-cellulose, and by the results of Fig. 6 showing that R17 RNA is bound in a first-order reaction closely resembling that for globin, Mengo virus, and VSV mRNA, species that do contain poly(A). Indeed, globin mRNA preparations lacking poly(A) bind the factor with an affinity equal to that of native globin mRNA (R. Kaempfer, R. Hollender, H. Soreq, and U. Nudel, unpublished data).

Our finding that VSV negative-strand RNA, in contrast to mRNA, does not possess a high-affinity binding site for eIF-2 strongly suggests that eIF-2 recognizes a specific site in mRNA that is essential for protein synthesis. The 7-methylguanosine phosphate moiety at the 5′ end of mRNA clearly is not absolutely required for binding of eIF-2, because R17 and Mengo virus RNA, which lack that end group, are bound efficiently (Fig. 6). It follows, therefore, that eIF-2 binds at an internal site in mRNA. We shall show elsewhere, however, that in addition to binding to an internal sequence, eIF-2 recognizes the methylated 5′ end of eukaryotic mRNA with high specificity (25).

The concept that in eukaryotic protein synthesis the same initiation factor that binds Met-tRNA$_f$ also recognizes and binds mRNA, is novel and stands in contrast to the situation in prokaryotes, where two separate proteins, IF-2 and IF-3, direct the successive binding of fMet-tRNA$_f$ and mRNA, respectively, to the small ribosomal subunit (26, 27). However, this concept fits well with the fact that in both prokaryotic (26, 27) and eukaryotic (9, 10, 28) initiation, binding of Met-tRNA$_f$ to the small ribosomal subunit not only precedes, but is necessary for the binding of mRNA. In eukaryotes, accordingly, binding of mRNA may be directed both by initiation factor eIF-2 and by the anticodon of the Met-tRNA$_f$ molecule bound to it. Additional proteins are necessary to stabilize mRNA on the 40S ribosomal subunit, but these cannot act in the absence of the Met-tRNA$_f$-binding factor (29). Hence, the requirement for additional proteins is not in conflict with the concept, supported by our results, that the initiation factor that binds Met-tRNA$_f$ has a direct function in the recognition and binding of mRNA.

The finding that dsRNA molecules possess many binding sites for eIF-2 (Fig. 7) suggests that this factor recognizes the A conformation of dsRNA, and not its base sequence, and hence can bind anywhere along the dsRNA molecule. This could explain the effective inhibition of initiation of protein synthesis by low doses of dsRNA (2). We have shown previously that as more dsRNA is added, increasing doses of eIF-2 are needed to overcome translational inhibition (4, 6). This suggested that dsRNA might inhibit the function of eIF-2 by a direct interaction (4). The data of Figs. 2–5 show that both the protein-synthetic and Met-tRNA$_f$-binding activities of eIF-2 can be recovered intact from complexes with dsRNA. Thus, eIF-2 can bind reversibly to dsRNA without losing its activity. Inhibition of protein synthesis may yet occur because dsRNA, by binding to eIF-2, interferes directly with the functions of this factor. Alternatively, or in addition to this direct effect, binding of dsRNA to eIF-2 may induce a conformational change in the factor molecule that renders it susceptible to irreversible inactivation, perhaps by a protein kinase (30). More work is needed to clarify this point, but we favor the interpretation that binding of eIF-2 to dsRNA is essential for inhibition of translation. Indeed, the present results, which demonstrate that eIF-2 is one of the proteins with the highest affinity for dsRNA, strengthen the possibility that the sequence in mRNA recognized by eIF-2 has double-stranded properties (4).

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