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Interaction of Poly(A) and mRNA with Eukaryotic Initiator Met-tRNA$_f$ Binding Factor: Identification of This Activity on Reticulocyte Ribonucleic Acid Protein Particles

[poly(A)-protein complexes/eukaryotic initiation factors/binding/nitrocellulose filter assay]

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ABSTRACT **Messenger ribonucleoprotein particles (mRNPs) have been isolated from rabbit reticulocyte polysomes. One of the proteins of the mRNP complex has many properties of a specific eukaryotic initiation factor, the soluble Met-tRNA$_f$ binding protein. A purified preparation of this factor, in addition to binding Met-tRNA$_f$, binds poly(A) and globin mRNA. The binding of these substrates is greater than that obtained with other natural or artificial polyribonucleotides. The mRNP fraction also binds poly(A) and Met-tRNA$_f$. Since the factor which binds initiator tRNA also binds poly(A) and mRNA, and this activity can be found on mRNPs, there may be a relationship between initiator tRNA binding and messenger binding in early events of eukaryotic initiation.**

Various laboratories have reported the isolation and characterization of a protein factor from eukaryotic cells which forms a ternary complex with Met-tRNA$_f$ (initiator tRNA) and GTP (1–5). Formation of this complex is followed by joining of Met-tRNA$_f$ to the 40S ribosomal subunit. This latter reaction requires additional initiation factors but does not require added mRNA (1, 4). Other studies have reported an association of mRNA with proteins in mammalian cells. Such complexes, termed messenger-ribonucleoprotein particles (mRNPs), have been isolated from nucleoplasmic, cytoplasmic, and polysomal fractions (6–21). Depending on tissue source and preparative methods, polypeptides of varying molecular weights have been described in these particles (6–21). Although a number of functions for these proteins in messenger storage, transport, protection, or initiation have been suggested, their precise role in eukaryotic regulation has remained unclear (for general reviews, see refs. 22–24).

In the present study, we have isolated the Met-tRNA$_f$ binding factor and reticulocyte mRNPs and have studied their interaction with various polyribonucleotides. Our results indicate that reticulocyte mRNPs bind Met-tRNA$_f$ in a reaction similar to that obtained with purified Met-tRNA$_f$ binding factor. In addition, both the purified factor and the mRNP are capable of interacting with poly(A), as demonstrated by retention of the poly(A)-protein complex on a nitrocellulose filter.

METHODS

Reticulocyte lysate, polysomes, 105,000 × *g* supernatant protein, and a 0.5 M KCl ribosomal wash fraction were prepared as previously reported (25). A purified preparation of the Met-tRNA$_f$ binding factor was obtained from the ribosomal wash fraction by ammonium sulfate precipitation, ion exchange chromatography on DEAE cellulose and phosphocellulose, and gel filtration through Sephadex G-200 (to be reported separately). This material was analogous in biological activity to the Met-tRNA$_f$ binding protein referred to by Levin *et al.* as *IF-L3* (1), by Dettman and Stanley as *IF-1* (2), by Gupta and coworkers as *IF-1* (3), by Schreier and Staehelin as *IF-E2* (4), and by Anderson and coworkers as *IF-MP* (5). A preparation of homogeneous IF-MP was kindly supplied by Safer, Merrick, and Anderson, Molec. Hemat. Br., NHLI, NIH, Bethesda, Md. Rabbit reticulocyte [^{3}H]Met-tRNA$_f$ and [^{3}H]Met-tRNA^{Met} (s.a. 1600 cpm/pmol), rabbit liver [^{14}C]Phe-tRNA (s.a. 800 cpm/pmol) and rabbit reticulocyte 4S-tRNA, 9S-mRNA, 18S-rRNA, and 28S-rRNA all *in vivo* labeled with ^{3}H, were prepared as previously reported (25–27). [^{3}H]Poly(A) (s.a. 18.7 mCi/mmol), [^{3}H]poly(U) (s.a. 21 mCi/mmol) and [^{3}H]poly(C) (s.a. 8.2 mCi/mmol) were obtained from Schwarz/Mann, Orangeburg, N.Y.

Preparation of Messenger-Ribonucleoprotein Particles (mRNPs). Messenger RNPs were isolated from rabbit reticulocyte lysate essentially according to the method of Lebleu *et al.* (19). In this procedure polysomes are dissociated by addition of 33 mM EDTA, and a fraction containing mRNA in association with protein is isolated by sucrose gradient centrifugation. See legend of Fig. 1 for experimental details. Messenger RNPs were also prepared by a modification of procedure of Blobel (20). In this method polyribosomes are dissociated by incubation with puromycin and 0.5 M KCl and a fraction containing mRNA in association with protein is isolated by sucrose gradient centrifugation. See legend of Fig. 2 for experimental details.

Binding of [^{3}H]Met-tRNA to Soluble Protein. The binding of [^{3}H]Met-tRNA to soluble protein was determined by collection of the Met-tRNA–GTP–protein complex on a nitrocellulose filter (1–5). Incubations in a final volume of 100 μl were performed at 23° for 5 min and contained 20 mM Tris·HCl at pH 7.4, 100 mM KCl, 1 mM GTP, 1 mM dithiothreitol, 10 pmol of [^{3}H]Met-tRNA or 8 pmol of [^{3}H]Met-tRNA$_f$ (s.a. 1600 cpm/mol) and protein as noted in the appropriate figure legends. Reactions were stopped by the addition of 3 ml of ice-cold buffer solution (20 mM Tris·HCl at pH 7.4–100 mM KCl). Reaction mixtures were filtered onto HAWP 0.45 μm pore size, 25 mm diameter, nitrocellulose filters (Millipore Corp., Bedford, Mass.) and rinsed three times with 3 ml of the same buffer. The filters were dissolved in 10 ml Bray's Solution (28) and counted by liquid scintillation spectroscopy at 23% efficiency for ^{3}H.

Abbreviation: mRNPs, messenger ribonucleic acid protein particles.

TABLE 1. *Requirements for complex formation between Met-tRNA$_f$ and eukaryotic initiator tRNA binding factor*

Additions	[^{3}H]Met-tRNA	[^{3}H]Met-tRNA$_f$	[^{3}H]Met-tRNA^{Met}
	(pmol substrate bound)		
Complete system	1.61	4.36	0.02
− Met-tRNA$_f$ binding factor	0.04	0.01	0.01
− GTP	0.18	0.68	—
+ aurintricarboxylic acid, 5×10^{-5} M	0.01	0.01	—
+ Heated Met-tRNA$_f$ binding factor	0.09	0.19	—

Incubations in a total volume of 100 μl were performed at 23° for 5 min and for the complete system contained 20 mM Tris·HCl at pH 7.4, 100 mM KCl, 1 mM GTP, 1 mM dithiothreitol, 10 pmol of [^{3}H]Met-tRNA, 8 pmol of [^{3}H]Met-tRNA$_f$ or 8 pmol of [^{3}H]Met-tRNA^{Met} (1600 cpm/pmol) and 6.2 μg of protein of the Met-tRNA$_f$ binding factor. Where indicated, Met-tRNA$_f$ binding factor or GTP was deleted, 5×10^{-5} M aurintricarboxylic acid was added, or Met-tRNA$_f$ binding factor was heated to 60° for 5 min before incubation. Reactions were stopped by the addition of 3 ml of ice-cold buffer solution (20 mM Tris·HCl at pH 7.4–100 mM KCl), the tRNA–protein complex was collected on a nitrocellulose filter, the filter was washed three times with cold buffer solution and counted by liquid scintillation spectrophotometry. See *Methods* for further experimental details.

Polyribonucleotide Binding Assay. The binding of various ^{3}H-labeled polyribonucleotides to soluble protein was performed by the nitrocellulose filter technique. Incubations in a total volume of 100 μl were performed at 23° for 5 min and contained 20 mM Tris·HCl at pH 7.4, 50 mM KCl, 1 mM dithiothreitol, 0.2 A_{260} units of [^{3}H]poly(A) (12,000 cpm) or other ^{3}H-labeled polyribonucleotide, and soluble protein as indicated in the appropriate tables and legends. For ^{3}H-labeled reticulocyte 4S, 9S, 18S, and 28S RNAs, 0.2 A_{260} units or approximately 2000–2500 cpm were used as substrate. For the binding of [^{3}H]poly(U), reaction mixtures were filtered at 23° to reduce background. Reactions were stopped by addition of 3 ml ice-cold buffer solution (20 mM Tris·HCl at pH 7.4–50 mM KCl); the reaction mixtures were filtered onto nitrocellulose filters, washed three times with buffer, and counted by liquid scintillation spectroscopy.

Identification of the Reticulocyte mRNP by Hybridization with Poly(U). The position of the globin mRNP in sucrose gradient fractions was determined by hybridization with high specific activity [^{3}H]poly(U), according to the method of Jeffery and Brawerman (29). This assay is based on the ability of [^{3}H]poly(U) to form stable hybrids with poly(A) segments found at the 3′ end of most eukaryotic mRNAs and can be used effectively with globin mRNPs as well as globin mRNA. Incubations in a total volume of 500 μl were performed at 30° for 15 min and contained 10 mM Tris·HCl at pH 7.4, 200 mM NaCl, 5 mM $MgCl_2$, 2500–3000 cpm of [^{3}H]poly(U) (s.a. 7.76 mCi/mmol of phosphorous) and appropriate gradient fractions or control reticulocyte mRNA. Pancreatic ribonuclease A (Worthington Biochemicals, Inc.), 0.25 μg of protein, was then added and incubation continued for an additional 30 min. Carrier rabbit liver tRNA, 1.0 A_{260} unit, was added and this was followed immediately by the addition of 2 ml cold 10% trichloroacetic acid. The samples were placed in ice for 10 min and were filtered onto nitrocellulose filters. The filters were washed four times with 4 ml of ice cold 0.1% trichloroacetic acid and counted by liquid scintillation spectroscopy.

TABLE 2. *Binding of poly(A), reticulocyte mRNA, and other polyribonucleotides to eukaryotic initiator tRNA binding factor*

Substrate	cpm bound	pmol bound*
[^{3}H]Met-tRNA$_f$	8074	5.05
[^{14}C]Phe-tRNA	12	0.01
[^{3}H]Poly(A)	2499	—
[^{3}H]Poly(U)	544	—
[^{3}H]Poly(C)	566	—
[^{3}H]ATP	105	0.10
[^{3}H]RNA, 4S	63	—
[^{3}H]mRNA, 9S	393	10.6
[^{3}H]RNA, 18S	212	1.6
[^{3}H]RNA, 28S	271	0.86

Incubations in a total volume of 100 μl were performed at 23° for 5 min and contained 20 mM·Tris HCl at pH 7.4, 50 mM KCl, 1 mM dithiothreitol, and 6.2 μg of protein of Met-tRNA$_f$ binding factor. The various substrates were used in the following amounts: 8 pmol of [^{3}H]Met-tRNA$_f$ (1600 cpm/pmol) or 10 pmol of [^{14}C]Phe-tRNA (800 cpm/pmol); 0.2 A_{260} units (approximately 10,000 cpm) of [^{3}H]poly(A), [^{3}H]poly(U), or [^{3}H]poly(C) (molecular weights > 100,000); 50 μM [^{3}H]ATP (1050 cpm/pmol); 0.2 A_{260} units (2000–2500 cpm) [^{3}H]RNA, 4S; [^{3}H]mRNA, 9S; [^{3}H]RNA, 18S, or [^{3}H]RNA, 28S (each labeled to approximately the same specific activity.) Reactions were stopped by the addition of 3 ml of ice-cold buffer solution (20 mM Tris·HCl at pH 7.4–50 mM KCl). RNA–protein complexes were collected on nitrocellulose filters and were processed as noted in *Methods* and Table 1.

* Since binding of reticulocyte [^{3}H]RNA, 4S was rather low and this material was heterogeneous, consisting of a large number of tRNA components, 5S RNA and possibly degradation fragments of higher molecular weight RNAs, a calculation of pmol bound was not performed. In view of limited information regarding the average molecular weights of [^{3}H]poly(A), [^{3}H]-poly(U), and [^{3}H]poly(C), pmol bound was not computed.

RESULTS

The eukaryotic initiator Met tRNA$_f$ binding factor, prepared from reticulocyte 0.5 M KCl ribosomal wash protein (see *Methods*), had an apparent molecular weight greater than aldolase (160,000) by gel filtration thru Sephadex G-200. Table 1 gives the requirements for formation of a stable complex, collected on a nitrocellulose filter, between this factor and Met-tRNA$_f$. As reported by other investigators (1–5), this reaction requires GTP*, occurs specifically with Met-tRNA$_f$ as opposed to Met-tRNA^{Met}, and is inhibited by low concentrations of aurintricarboxylic acid, a potent inhibitor of initiation in both bacterial and mammalian systems (30). Binding activity is also abolished by heating the protein fraction to 60° for 5 min.

To investigate further the specificity of interaction of this protein with nucleic acids, we tested a series of natural and artificial polyribonucleotides for binding activity. As shown in Table 2, [^{14}C]Phe-tRNA was not bound by the factor. There was considerable binding of [^{3}H]poly(A), i.e., ap-

* Similar binding activity can be obtained with GDPCP (1, 2), a methylene analogue of GTP in which the terminal phosphate group is resistant to hydrolysis.

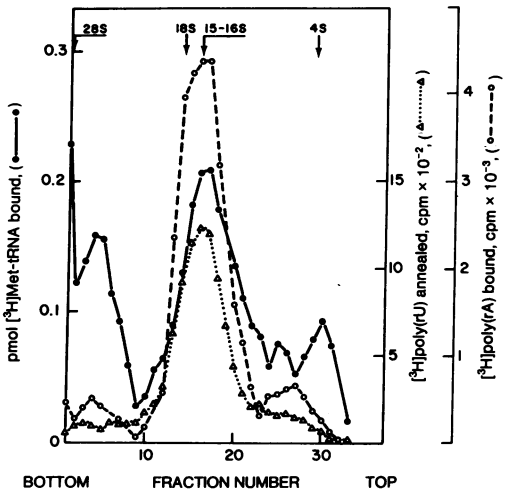

FIG. 1. Sucrose gradient centrifugation of reticulocyte mRNPs isolated by the EDTA method. Reticulocyte mRNPs were isolated from polysomes by the EDTA method of Lebleu *et al.* (19). After initial treatment of polysomes with 33 mM EDTA, a crude mRNP fraction was isolated by zone sedimentation in a 36 ml, 15–30% (w/v) linear sucrose gradient containing 20 mM Tris·HCl (pH 7.4)–1 mM dithiothreitol. Centrifugation in a Beckman SW27 rotor was at 24,000 rpm for 40 hr at 2°. One milliliter fractions were collected from each gradient and absorbance at 260 nm determined on aliquots of each fraction using a Zeiss PQ 11 spectrophotometer. The mRNP fractions were pooled (a total of 6 A_{260} units), concentrated by vacuum dialysis to 2 ml, dialyzed for 6 hr against 10 mM Tris·HCl (pH 7.4)–100 mM KCl–1 mM dithiothreitol—0.1 mM EDTA and recentrifuged at 24,000 rpm for 38 hr at 2° in a second 36 ml, 15–30% (w/v) linear sucrose gradient containing 10 mM Tris·HCl (pH 7.4)–10 mM KCl–1 mM dithiothreitol–0.1 mM EDTA. One milliliter fractions were collected. [^{3}H]Met-tRNA binding and [^{3}H]poly(A)-binding was determined on 150 μl aliquots of each fraction as noted in *Methods*, except that 500 μl reaction volumes were used. Blanks of 0.05 pmol for [^{3}H]Met-tRNA binding and 311 cpm for [^{3}H]poly(A)-binding were subtracted from each value. [^{3}H]Poly(U) annealing was performed with 100 μl aliquots of each fraction as described in *Methods*. A blank of 105 cpm, representing [^{3}H]poly(U), incompletely degraded by pancreatic RNAse in the absence of added mRNP or mRNA, was subtracted from each value. The positions of marker 4S-RNA, 18S-RNA, and 28S-RNA were determined in a duplicate gradient.

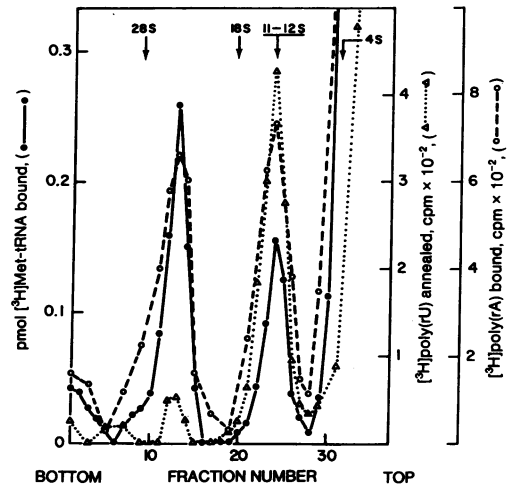

FIG. 2. Sucrose gradient centrifugation of reticulocyte mRNPs prepared by the puromycin method. Reticulocyte mRNPs were isolated from 0.5 M KCl washed ribosomes by the puromycin–0.5 M KCl method of Blobel (20). After initial treatment of polysomes with 0.5 M KCl and centrifugation through a cushion of 30% sucrose–50 mM Tris·HCl (pH 7.4)–0.5 M KCl–5 mM $MgCl_2$–1 mM dithiothreitol, the ribosomes were suspended in 50 mM Tris·HCl (pH 7.4)–0.5 M KCl–2 mM $MgCl_2$–1 mM dithiothreitol and adjusted to a final concentration of 1 mM puromycin dihydrochloride (adjusted to pH 7.0 with KOH). The ribosomal suspension was incubated at 37° for 10 min and the resulting mRNP fraction isolated by zone sedimentation in duplicate 37 ml, 15–30% (w/v) linear sucrose gradients containing 50 mM Tris·HCl (pH 7.4)–0.5 M KCl–3 mM $MgCl_2$–1 mM dithiothreitol. Centrifugation in a Beckman SW27 rotor was at 24,000 rpm for 40 hr at 2°, and 1 ml fractions were collected from each gradient. The mRNP zone from six gradients was pooled (a total of 4 A_{260} units), concentrated by vacuum dialysis to 2 ml, dialyzed for 6 hr against 50 mM Tris·HCl (pH 7.4)–0.5 M KCl–1 mM dithiothreitol, centrifuged at 10,000 × *g* for 10 min to remove aggregated material and recentrifuged in a second 36 ml, 15–30% (w/v) linear sucrose gradient containing 10 mM Tris·HCl (pH 7.4)–10 mM KCl–1 mM dithiothreitol. Centrifugation was at 23,000 rpm for 26 hr at 2° and 1 ml fractions were collected. [^{3}H]Met-tRNA binding, [^{3}H]poly(A)-binding and [^{3}H]poly(U)-annealing were performed as noted in Fig. 1. The positions of marker 4S-RNA, 18S-RNA, and 28S-RNA were determined in a duplicate gradient.

proximately four to five times greater than activity obtained with [^{3}H]poly(U) or [^{3}H]poly(C). [^{3}H]ATP was virtually inactive. Binding of [^{3}H]poly(A) was not dependent on GTP, was inhibited completely by 5×10^{-5} M aurintricarboxylic acid and was inactivated 72% by heating to 60° for 5 min (data not shown). Essentially, the same results were obtained with homogeneous Met-tRNA$_f$ binding factor (IF-MP) supplied by Safer, Merrick, and Anderson. With ^{3}H-labeled reticulocyte RNAs, there was considerable binding of "9S" mRNA, lesser binding of 18S RNA and 28S RNA, and little binding of 4S RNA. Assuming molecular weights of 2×10^5 for 9S globin mRNA, 7×10^5 for 18S RNA, and 1.7×10^6 for 28S RNA, the number of moles of RNA bound to a given quantity of protein factor was considerably higher for 9S mRNA as compared to ribosomal RNA.

To examine further the possible relationship between Met-tRNA binding and poly(A) or mRNA binding, we prepared messenger ribonucleoprotein particles (mRNPs) from reticulocyte polysomes. Messenger RNPs, released from polysomes either by EDTA or puromycin–0.5 M KCl, were isolated and characterized by zone sedimentation in sucrose gradients. For further purification, the mRNP fractions were recentrifuged through a second sucrose gradient. Gradient fractions were tested for both [^{3}H]poly(A) and [^{3}H]Met-tRNA binding. The position of mRNA in the gradients was determined by annealing high specific activity [^{3}H]poly(U) to the mRNP. As shown in Fig. 1, mRNPs obtained by EDTA treatment sedimented at 15–16 S, as reported previously by Lebleu *et al.* (19). Peaks of binding activity for both [^{3}H]Met-tRNA and [^{3}H]poly(A) corresponded to the position of mRNA ([^{3}H]poly(U) annealing). Similar results were obtained with mRNPs obtained by the puromycin–0.5 M KCl method (Fig. 2). In this case, the mRNP sedimented at 11–12 S, as previously reported by Blobel (20). There was an additional peak of [^{3}H]Met-tRNA and [^{3}H]poly(A) binding, but little detectable messenger RNA, in the 25S region of the gradient (Fig. 2). This probably represents residual activity on the small ribosomal subunit. Additional activity at the top of the gradient is thought to represent dissociated and/or

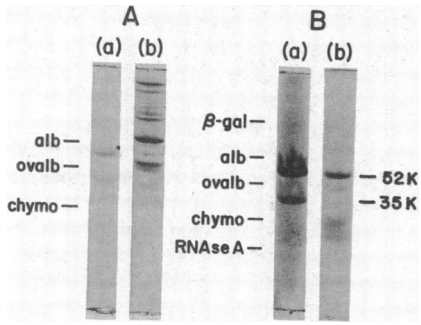

FIG. 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of reticulocyte mRNPs prepared by the EDTA and the puromycin method. Acrylamide–methylenebisacrylamide gels (6.5%) were cast in 5 × 75 mm cylindrical glass tubes in a buffer solution containing 40 mM Tris·HAc (pH 7.4), 2 mM EDTA–0.5% sodium dodecyl sulfate as previously reported (47). Samples of 50–75 μl, containing 40 mM Tris·HAc (pH 7.4), 2 mM EDTA, 1% sodium dodecyl sulfate, 5% sucrose, 2% β-mercaptoethanol, pyronin Y (tracking dye), and the various protein or mRNP fractions, were heated to 95° for 1 min and layered over parallel gels. Electrophoresis was performed in 40 mM Tris·HAc (pH 7.4)–2 mM EDTA–1% sodium dodecyl sulfate at room temperature for 1.5–2 hr in an apparatus from Buchler Inst., using a current of 5 mA per gel. Gels were extruded, fixed, and stained with 25% isopropanol–10% acetic acid–0.2% Coomassie Brilliant Blue and destained with 10% acetic acid. A. (a) Purified Met-tRNA$_f$ binding factor, (b) mRNP fraction obtained by EDTA method. B. (a) Homogeneous Met-tRNA$_f$ binding factor supplied by Safer, Merrick, and Anderson, (b) mRNP fraction obtained by the puromycin–0.5 M KCl method. The positions of protein standards, β-galactosidase (130,000 daltons), bovine serum albumin (68,000 daltons), ovalbumin (45,000 daltons), chymotrypsinogen A (25,000 daltons), and pancreatic ribonuclease A (13,700 daltons), were determined in parallel gels.

degraded mRNP components. Under these conditions, deproteinized globin mRNA sediments at 9 S.

The mRNP fraction obtained by puromycin treatment was isolated and tested for its specificity in Met-tRNA binding (Table 3). As with the purified binding factor, the reaction was specific for Met-tRNA$_f$, but dependence on GTP was only partial. The explanation for this reduced GTP requirement is not apparent.

Banding patterns of the mRNP proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis are shown in Fig. 3. Messenger RNPs, prepared by both the EDTA method (Fig. 3A) and the puromycin–0.5 M KCl method (Fig. 3B), were subjected to electrophoresis in parallel gels with purified Met-tRNA$_f$ binding factor and molecular weight standards. Under the conditions used, the purified factor contained 2 nonidentical subunits with molecular weights of approximately 35,000 and 52,000, respectively. The 52,000 subunit was present in both preparations of mRNPs. This band may represent the 52,000 polypeptide reported by Blobel (20). Identification of the 35,000 subunit of Met-tRNA$_f$ binding protein was less certain, although a diffuse band in this region was present on EDTA-released mRNPs (Fig. 3A). With mRNPs prepared by the puromycin method (Fig. 3B), this band was markedly reduced in intensity compared to the 52,000 polypeptide.

TABLE 3. *Binding of [^{3}H]Met-tRNA$_f$ to reticulocyte messenger-ribonucleoprotein particle (mRNP)*

Additions	[^{3}H]Met-tRNA$_f$ (pmol substrate bound)	[^{3}H]Met-tRNA^{Met} (pmol substrate bound)
Complete system	0.51	0.06
− mRNP	0.02	0.01
− GTP	0.34	—

Incubation conditions and assay procedures were as noted in Table 1, except that 0.033 A_{260} units of reticulocyte mRNPs, isolated from polysomes by the puromycin method, were substituted for Met-tRNA$_f$ binding factor. See *Methods* for further experimental details.

DISCUSSION

Reticulocyte mRNPs have been reported by various investigators to contain specific proteins, the functions of which are largely undetermined. Since nonspecific complexes between RNA and basic proteins can be formed, especially at low ionic strength (32, 33), the significance of the mRNP has remained unclear. Recently, Kwan and Brawerman (34) reported a complex between protein and the 3′-poly(A) segment of mouse sarcoma 180 mRNA. This complex was identified by retention of labeled RNP on a nitrocellulose filter under conditions in which deproteinized or free polyribonucleotide passed through the filter (34). The same RNP could be identified by sedimentation in a sucrose gradient, and a similar complex of lower sedimentation value was obtained by incubating synthetic [^{3}H]poly(A) with crude cytoplasmic protein. These results suggested a role for the 3′-poly(A) segment of mRNA as a specific protein binding site (34), and other investigators have reported poly(A)–protein complexes with various partially purified cell fractions (35, 36).

Lebleu *et al.* (19) have characterized proteins of reticulocyte mRNPs released from polysomes by 33 mM EDTA. Under these conditions, the major components have apparent molecular weights of 45,000, 68,000, and 130,000, respectively. A number of other minor bands are also present, but these have not been further characterized. Blobel (20) has obtained a preparation of reticulocyte mRNPs containing only two polypeptide components of molecular weights 52,000 and 78,000. This has been accomplished by treating polysomes with puromycin and 0.5 M KCl and collecting the mRNP zone from a sucrose gradient containing 0.5 M KCl. In later studies, the same author found similar polypeptides in mRNPs from liver and L-cells (31) and observed that the larger of these polypeptides is associated with the 3′-poly(A) region of messenger RNA.

In the present study, poly(A) binding activity has been identified in one of the known reticulocyte initiation factors, the Met-tRNA$_f$ binding protein. This factor also preferentially binds mRNA compared to other reticulocyte RNAs. In other studies, we have separated and partially characterized a series of proteins which bind to poly(A) but are distinct from poly(U) and poly(C) binding proteins (Hellerman and Shafritz, unpublished). None of these other poly(A) binding proteins, however, binds Met-tRNA$_f$. Reticulocyte mRNPs also bind Met-tRNA$_f$ and poly(A) and contain polypeptide components of the purified Met-tRNA$_f$ binding factor. The 52,000 dalton mRNP polypeptide reported previously by Blobel (20) may correspond to the larger subunit of this factor. Conditions employed in the present study for isolation of globin mRNPs have included varied techniques for polysomal disaggregation and repeated sucrose gradient centrifugation under conditions

of both high and low ionic strength. Under these circumstances, variations may occur in the amount of specific proteins remaining attached to mRNA. However, the likelihood of nonspecific RNA–protein interaction accounting for the binding activities observed with these particles has been reduced considerably. In preliminary studies, we have also found Met-tRNA$_f$ binding activity with mRNPs isolated from the reticulocyte supernatant fraction (data not shown).

According to current views on the mechanism for initiation of eukaryotic protein synthesis (1–4), the first step is formation of a ternary complex between Met-tRNA$_f$, the Met-tRNA$_f$ binding factor, and GTP. This is followed by joining of the ternary complex to the 40S subunit, without a requirement for added mRNA. Thus, a role for mRNA in early initiation events has not been established. Since we have been able to show an interaction of mRNA with the Met-tRNA$_f$ binding factor, it is tempting to speculate an association between these events in the initiation process. A relationship between initiator tRNA and messenger RNA binding has been reported previously in bacterial systems (37–39). *Escherichia coli* IF-2, the prokaryotic initiation factor which forms a ternary complex with Met-tRNA$_f$ (40–42), also participates with IF-3 in binding mRNA to the 30S ribosomal subunit (37–42). The bacterial system does not require poly(A). Since eukaryotic mRNAs that do not contain poly(A) may also bind to Met-tRNA$_f$ binding factor (this possibility has not yet been tested), the present results do not necessarily imply a function for poly(A) in eukaryotic initiation. This is particularly important in view of the absence of poly(A) in histone mRNAs (43), and recent studies reporting translation of eukaryotic mRNAs from which the 3′-poly(A) segment has been removed (44, 45) or blocked by poly(U) (46). Therefore, additional studies will be needed to establish a direct link between binding of mRNA to the Met-tRNA$_f$ binding factor and a specific function for the mRNP in eukaryotic translation.

Note Added in Proof. A similar complex between IF-MP and R17-RNA has recently been reported by Kaempfer, R. (1974) *Biochem. Biophys. Res. Commun.* **61**, 591–597.

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