Characterization of the Association of Specific Proteins with Messenger Ribonucleic Acid†

Alice Barrieux,* Holly A. Ingraham, Steven Nystul, and Michael G. Rosenfeld‡

ABSTRACT: Isolation of messenger ribonucleic acid (mRNA)-protein particles from cytosol or dissociated polyribosomes yielded complexes in which several proteins were consistently associated with mRNA. Some of the mRNA-associated proteins appeared to have a high affinity for mRNA since they remained complexed to mRNA during centrifugation in CsCl gradients. Quantitation of RNA and protein in polyribosomal mRNPs suggested that each molecule of mRNA bound a molecule of each of the two major proteins of 78 000 and 52 000 apparent molecular weights and/or one or more of several minor proteins found in mRNPs. Of the several mRNP proteins, only the protein of 78 000 apparent molecular weight appeared to form a stable complex with the polyriboadenylic acid [poly(A)]-tract of mRNA, suggesting that the remaining mRNA-associated proteins bind to other regions which may be common to many or all mRNAs. Binding of [^{3}H]poly(A)-rich RNA to mRNP proteins was effectively inhibited by unlabeled poly(A)-rich RNA or the homopolymer polyriboguanylic acid [poly(G)], but not by poly(A) or other natural or synthetic mRNAs. The properties of non-poly(A)-dependent binding of mRNA by mRNP protein were similar to those of mRNA binding by the guanosine triphosphate dependent Met-tRNA^{fMet}-binding protein.

Although mRNA[1] may interact nonspecifically with cytoplasmic proteins (Baltimore and Huang, 1970), many studies have suggested that specific mRNA-protein complexes (mRNPs) occur in both the nucleus and cytoplasm (Perry and Kelley, 1968; Henshaw, 1968; Cartouzou et al., 1969; Olsnes, 1970; Spohr et al., 1970; Lebleu et al., 1971; Morel et al., 1973) and may be of functional significance in mRNA transport or translation (Kohler and Arends, 1968; Mantieva et al., 1969; Ilan and Ilan, 1973; Cashion and Stanley, 1974). While there has been variation in the number and molecular weights of the proteins observed, most reports have indicated that there are at least two major proteins of apparent molecular weights 49 000 to 52 000 and 68 000 to 78 000 (Lebleu et al., 1971; Blobel, 1972, 1973; Bryan and Hayashi, 1973; Scherrer et al., 1966). Evidence indicating that the larger protein is associated with the poly(A) region in polyribosomal mRNPs (Blobel, 1973) and nuclear RNPs (Kish and Pederson, 1975) has been presented. Irrespective of the method of preparation used, it is uncertain whether proteins present in mRNPs represent unspecific proteins bound during isolation, specific proteins associated with mRNA in the nucleus or cytosol to form particles analogous to ribosomal subunits, or factors involved in the process of mRNA translation and exhibiting specific and high affinity for mRNA (Hellerman and Shafritz, 1975; Rosenfeld and Barrieux, submitted for publication). The failure of high ionic strength to dissociate such proteins from mRNA has suggested that they represent tightly bound proteins common to eukaryotic mRNAs.

The studies reported here provide further evidence that (i) the several proteins present in mRNP complexes exhibited a high affinity for mRNA, but only the 78 000 molecular weight protein appeared to be associated with poly(A); each molecule of mRNA could associate with one molecule of each of the two major proteins and/or several minor proteins, and (ii) a non-poly(A)-dependent mRNA binding to mRNP particles exhibited properties similar to those observed for mRNA binding to a specific initiation factor, suggesting that some of the proteins present in mRNPs complexes may be translation factors.

Experimental Procedures

Materials

[^{3}H]Uridine (55 Ci/mmol) and [^{3}H]adenosine (55 Ci/mmol) were obtained from Schwarz/Mann; carrier-free ^{125}I and [^{3}H]leucine (60 Ci/mmol) were purchased from New England Nuclear. Synthetic ribohomopolymers, [^{3}H]polyriboadenylic acid (10 mCi/mmol P) and [^{3}H]polyriboguanylic acid (38 mCi/mmol P) were purchased from Miles Laboratories, Inc. Prior to use the homopolymers were applied to preformed cesium chloride gradients and fractions of buoyant density >1.8 g cm^{-3} were precipitated with three volumes of ethanol and redissolved in sterile water. Oligo(dT)-cellulose (T_3) was purchased from Collaborative Research and electrophoretically purified pancreatic ribonuclease from Worthington Biochemical Corporation.

Methods

Preparation of Ribonucleoprotein Particles. Subribosomal- and polyribosome-associated mRNPs were obtained from Ehrlich ascites tumor cells, using procedures previously described (Barrieux et al., 1975). Briefly, mRNPs separated from ribosomal subunits by sucrose gradients were collected by

† From the Division of Endocrinology, Department of Medicine, University of California, San Diego, School of Medicine, La Jolla, California 92092. *Received September 22, 1975.* This investigation was supported by National Institutes of Health Research Grant No. AM18477 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and American Cancer Society Grant No. BC-169.

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[1] Abbreviations used are: MEM, Minimal Essential Medium; oligo(dT), oligodeoxyribothymidylic acid; poly(G), polyriboguanylic acid; poly(A), polyriboadenylic acid; poly(C), polyribocytidylic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; GTP, guanosine triphosphate; Met-tRNA^{fMet}, formylmethionine-specific Met-tRNA; RNP, ribonucleoprotein; DEAE, diethylaminoethyl.

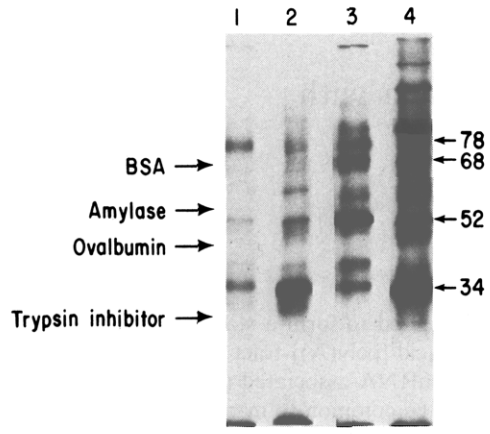

FIGURE 1: Gel electrophoresis of iodinated mRNP proteins. mRNPs were prepared, iodinated, reduced in sodium dodecyl sulfate, and subjected to slab gel electrophoresis as described under Methods. The migrations of marker proteins are indicated: bovine serum albumin (BSA), 68 500; α-amylase, 56 000; ovalbumin, 43 000; and soybean trypsin inhibitor, 21 500. Two different polyribosomal mRNP preparations are shown in samples 1 and 2; different preparations of subribosomal mRNPs are shown in samples 3 and 4.

filtration through glass filters and repeatedly washed with 0.5 M KCl buffer before elution from the filters. Alternatively, they were collected by centrifugation at 4 °C for 12 h at 15 000g in 20% sucrose containing 0.5 M KCl. Radiolabeled RNPs were obtained from Ehrlich ascites tumor cells incubated for 14 h with [^{3}H]uridine as previously described (Barrieux et al., 1975).

RNase Treatment of mRNPs. Immobilized RNase was prepared by incubating RNase (50 mg/ml) with 0.1 g of Affigel beads (Bio-Rad Laboratories) in 0.1 M potassium phosphate (pH 7.0) for 120 min at 4 °C. Ethanolamine was added to a concentration of 0.1 M and the incubation was continued for 4 h. Then the beads were washed successively with 0.1 M sodium acetate (pH 4.0), 0.1 M Tris-HCl (pH 9.0), and distilled water. Four hundred microliters of beads thus prepared rendered at least 93% of radiolabeled ribosomal RNA (100 μg/ml) Cl_3CCOOH soluble when incubated for 20 min at 37 °C in buffer containing 150 mM KCl.

Iodination of Proteins and Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. All samples were adjusted to 0.1% sodium dodecyl sulfate. Iodination was accomplished using the solid-state lactoperoxidase method of David and Reisfeld (1974) using 250 μCi carrier-free ^{125}I (New England Nuclear). The iodinated samples were dialyzed extensively before analysis by slab polyacrylamide gel electrophoresis as previously described (Barrieux et al., 1975).

Sedimentation of RNPs on Preformed Cesium Chloride Gradients. Samples were applied to preformed CsCl gradients in 10 mM triethanolamine and 0.5% Brij 58 and centrifuged at 22 °C for 45 h at 45 000 rpm using a SW 56 rotor. The gradients were collected from the top by pumping saturated CsCl from the bottom and the density across the gradient was determined by weighing an aliquot of every fifth fraction. Trichloroacetic acid precipitable counts were determined by adding 10 μg of albumin before the addition of 1 volume of 20% Cl_3CCOOH. After cooling at 5 °C for 30 min, samples were applied to nitrocellulose filters (Millipore, 0.45 nm) extensively washed with ice-cold 5% Cl_3CCOOH and 95% ethanol and counted in Bray's solution (Bray, 1960).

Quantitation of RNA and Protein. Protein was quantitated by the fluorescamine method of Bohlen et al. (1973) using bovine serum albumin as a standard. RNA or nucleotides added to the standard solutions in concentrations comparable to those present in mRNPs did not interfere with protein determination. RNA was assayed by the orcinol procedure (Schneider, 1957; Zamenhof, 1957) using rabbit liver tRNA as standard. Prior to the orcinol determination, samples were adjusted to 10% Cl_3CCOOH, and the precipitated RNA was collected by centrifugation at 20 000g.

TABLE I: Analysis of RNA and Protein Content of mRNPs.[a]

Source of RNP	RNA (μg/ml)	Protein (μg/ml)	Protein/RNA
Polyribosomal	152	67	0.44
Polyribosomal	108	51	0.47
Polyribosomal	150	84	0.56
Polyribosomal	69	36	0.52
Polyribosomal	32	13	0.41
Subribosomal	45	191	4.2
Subribosomal	44	178	4.0

[a] Different preparations of subribosomal and polyribosomal mRNPs were prepared, pelleted through buffer containing 0.5 M KCl, and analyzed for RNA and protein content as described under Methods; the ratio of RNA to protein (μg:μg) is shown for each group.

Binding of [^{3}H]mRNA by mRNA-Associated Proteins. [^{3}H]Poly(A)-rich mRNA (20 000–25 000 cpm/μg RNA) was prepared as previously described (Barrieux et al., 1975) from cultured Ehrlich ascites tumor cells using oligo(dT)-cellulose chromatography (Aviv and Leder, 1972). mRNPs were dissolved in water and added to 50 μl of buffer containing 20 mM Tris-HCl (pH 7.6), 30 mM KCl, 1 mM $MgCl_2$, and 0.4 μg of [^{3}H]poly(A)-rich mRNA. Following incubation for 20 min at 30 °C, 2 ml of mRNA-free incubation buffer was added, and the solution was filtered slowly through a nitrocellulose filter (Millipore, 0.45 nm). The filter was dried and counted in Liquifluor–toluene.

Results

Stability of the Protein:RNA Interaction. Analysis of RNPs by electrophoresis and protein staining consistently revealed two major protein bands of 78 000 and 52 000 apparent molecular weight, and two less prominent proteins of 34 000 and 130 000 apparent molecular weights. When proteins were iodinated prior to electrophoresis, and the film was exposed beyond the period of linear radioautography for the more prominent bands, minor protein bands of 130 000, 68 000, and 56 000 apparent molecular weights were consistently visualized (Figure 1). Tyrosine available for iodination was apparently limited in the 78 000 molecular weight protein, compared with other proteins, since it was relatively more prominent by protein staining than iodination and radioautography. In contrast, the 34 000 molecular weight protein, which was barely detectable by staining, became one of the prominent proteins after iodination. Although the relative intensity of the minor bands varied from preparation to preparation, the identity of these bands was constant in all mRNPs preparations (Barrieux et al., 1975). Quantitation of RNA and protein content in polyribosomal mRNPs revealed a 2:1 RNA to protein ratio (μg:μg) (Table I). Subribosomal mRNPs which have been suggested to contain at least two additional proteins (Barrieux et al., 1975), or different proteins (Gander et al., 1973) exhibited a considerably higher protein to RNA ratio (Table I).

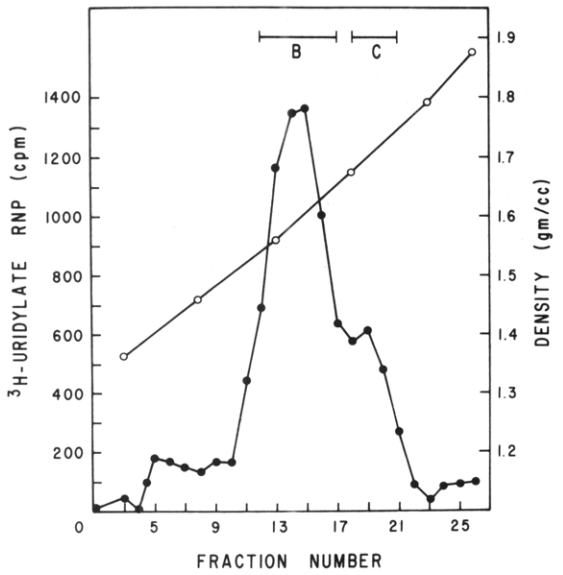

FIGURE 2: Sedimentation of RNPs on cesium chloride gradients. [^{3}H]-Uridylate-labeled subribosomal mRNPs were prepared as described under Methods from Ehrlich ascites tumor cells. The RNPs were applied in 0.2 ml of water to a preformed cesium chloride gradient (see Methods) and sedimented for 36 h at 45 000 rpm using an SW 56 rotor. The gradient was collected in 0.1-ml aliquots from the top, and the trichloroacetic acid precipitable radioactivity was determined for every supply and buoyant density for every fifth sample. The regions indicated by bars were pooled and dialyzed against 7 l. of distilled water with multiple changes for 48 h. The samples were lyophilized to a volume of 0.1–0.2 ml, adjusted to 0.1% sodium dodecyl sulfate, iodinated, and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described under Methods.

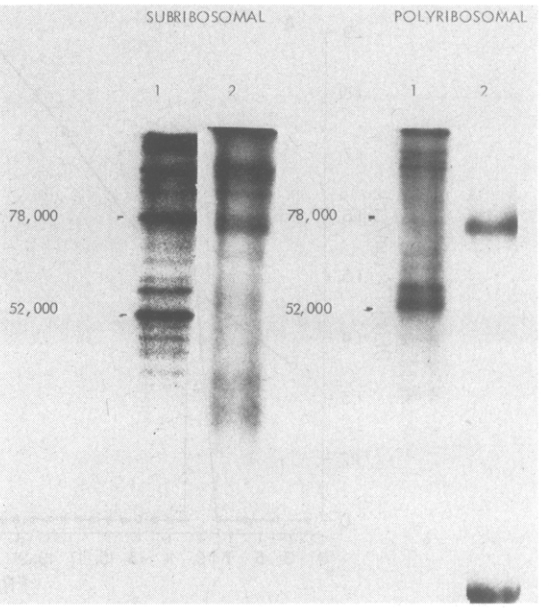

FIGURE 3: Polyacrylamide gel electrophoresis of mRNP proteins. Polyribosomal and subribosomal RNPs, sedimented unfixed through cesium chloride gradients and collected as described in the legend to Figure 2, were analyzed by polyacrylamide gel electrophoresis following iodination in sodium dodecyl sulfate and reduction. Subribosomal RNPs: (1) fraction B, Figure 2; (2) fraction C, Figure 2. Polyribosomal mRNPs: (1) material sedimenting at 1.55–1.65 g cm^{-3}; (2) material sedimenting at 1.65–1.75 g cm^{-3}.

In order to determine the affinity of these proteins for mRNA, the behavior of unfixed mRNPs was analyzed in CsCl gradients. As seen in Figure 2, the sedimentation of unfixed [^{3}H]uridylate-labeled subribosomal RNPs resulted in a broad distribution of the radiolabeled material. Proteins associated with RNPs were iodinated and radioautography was allowed to proceed for an extended period of time in order to detect the presence of minor components. With the exception of the 34 000 molecular weight protein which was no longer associated with mRNA after sedimentation through CsCl (Figure 2), most proteins present in subribosomal mRNPs before sedimentation were identified in the material banding at a buoyant density ranging from 1.6 to 1.78 g cm^{-3} (Figure 3). The denser material was greatly enriched in the 78 000 molecular weight protein which, in the presence of lesser amounts of other proteins, was relatively more prominent following iodination (Figure 3). Similar broad distribution of polyribosomal mRNPs was observed on sedimentation through CsCl, most of the major proteins remained associated with mRNA (Figure 3).

Binding of an mRNA-Associated Protein to Synthetic Poly(A). When exposed to acidic pH, mRNPs rapidly dissociate into RNA and proteins as demonstrated by their behavior in CsCl gradients (data not shown). In order to determine whether one or several of the mRNA-associated proteins can bind to poly(A), RNPs were first exhaustively digested with immobilized pancreatic ribonuclease in water so that the poly(A) tract was also degraded. Following removal of the immobilized ribonuclease, the pH was adjusted to 2.6 and synthetic [^{3}H]polyriboadenylic acid was added. The pH of the solution was then readjusted to 7.6, and the solution was incubated for 20 min at 37 °C and 4 h at 4 °C. As seen in Figure 4, a portion of the [^{3}H]poly(A) now sedimented at a buoyant density characteristic of an RNA–protein complex. Addition of a 100-fold excess of unlabeled synthetic poly(A) at the time of addition of [^{3}H]poly(A) virtually abolished the observed shift in the buoyant density of radiolabeled ribohomopolymer. A single protein of 78 000 apparent molecular weight was associated with the poly(A) sedimenting at a buoyant density characteristic of an RNA–protein complex (Figure 5). Analysis of samples of [^{3}H]poly(A) subjected to the same procedure failed to reveal any protein in the fractions of buoyant density analogous to those at which the poly(A)–protein complex banded.

Poly(A)-Independent Binding of mRNA-Associated Proteins to [^{3}H]mRNA. The observation that only one mRNA-associated protein bound to poly(A) suggested that other regions of mRNA were involved in binding of the other mRNA-associated proteins. Incubation of mRNPs with [^{3}H]poly(A)-rich mRNA resulted in the formation of a putative [^{3}H]mRNA–protein complex which was retained on a nitrocellulose filter under conditions when <0.5% of [^{3}H]-mRNA was retained in the absence of RNPs. The ability of various natural and synthetic RNAs to compete with [^{3}H]-mRNA for binding to mRNPs was compared. As shown in Figure 6, addition of unlabeled mRNA effectively competed for binding of radiolabeled mRNA; in contrast, poly(A) was an ineffective competitor, suggesting that protein(s) other than the 78 000 molecular weight protein were responsible for binding of the mRNA. Poly(A)-rich mRNA from rabbit reticulocytes or Ehrlich ascites tumor cells was equally effective in competing for binding. Poly(C), the hybrid poly(U)·poly(A), tRNA, or 18S RNA was 100- to 150-fold less effective than mRNA in competing for binding of [^{3}H]mRNA. The RNA–RNA hybrid poly(C)·poly(G) was an effective competitor (Figure 6), while synthetic polyriboguanylic acid was tenfold more effective than poly(A)-rich mRNA in competing for binding. This appeared to be due to preferential binding of

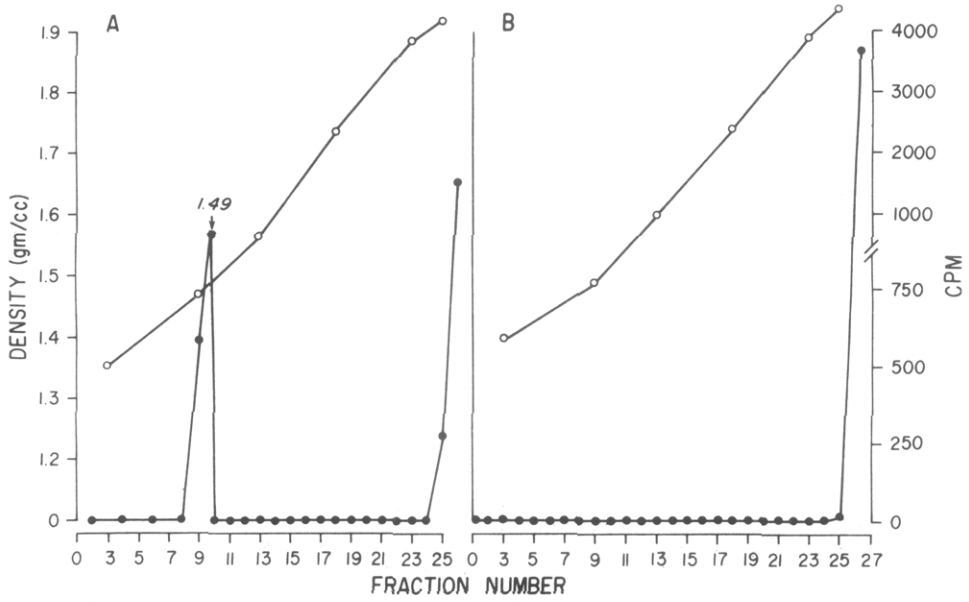

FIGURE 4: Binding of protein to synthetic polyriboadenylic acid. Polyribosomal RNPs were prepared from Ehrlich ascites tumor cells and eluted from the fiberglass filters in 0.2 ml of 10 mM Tris-HCl (pH 7.6) (see Methods) and incubated in this buffer with 200 μl of Sepharose beads containing immobilized pancreatic ribonuclease at 37 °C for 30 min, prepared as described under Methods. Following removal of the beads by centrifugation, the supernatant was adjusted to pH 2.8 with 0.01 M HCl, and to 150 mM KCl, and 1 μg of [^{3}H]polyriboadenylic acid (0.2 Ci/mmol) was added. Following a 10-min incubation at 37 °C, the pH was adjusted to 7.6 with 100 mM Tris-HCl (pH 9.0), and incubation was continued at 37 °C for 10 min and at 4 °C for 4 h. The solution was then applied to preformed cesium chloride gradients, sedimented, and collected as described under Methods. [^{3}H]Poly(A) alone and ribonuclease-digested mRNPs alone were subjected to identical procedure. Trichloroacetic acid precipitable radioactivity was determined on aliquots of every sample and buoyant density on every fifth sample. (Panel A) mRNPs + [^{3}H]poly(A); (panel B) [^{3}H]poly(A) alone. (○—○) Buoyant density; (●—●) cpm of trichloroacetic acid precipitable [^{3}H]polyriboadenylic acid.

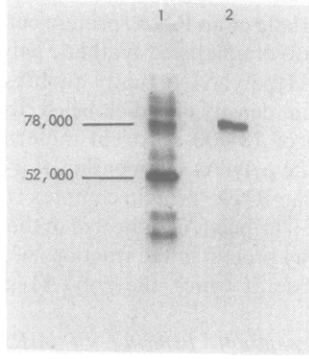

FIGURE 5: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the poly(A)-associated protein. Polyribosomal RNPs prepared from Ehrlich ascites tumor cells were either iodinated and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis immediately or following the procedures described in the legend to Figure 3. Fractions 8 and 9 of panel A (Figure 4) and fractions 8–10 from panel B (Figure 4) and fractions of comparable buoyant densities from cesium chloride gradients of RNase-treated mRNPs alone were dialyzed extensively against 0.1% sodium dodecyl sulfate, iodinated, and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Bovine serum albumin, ovalbumin, and soybean trypsin inhibitor were used as molecular weight standards. (1) RNPs alone; (2) iodinated protein associated with [^{3}H]poly(A) (fractions 8 and 9 of Figure 4, panel A). No iodinated proteins were observed in comparable fractions of gradients of poly(A) alone (fractions 8–10 of Figure 4, panel B), or in which the RNA was degraded by RNase digestion.

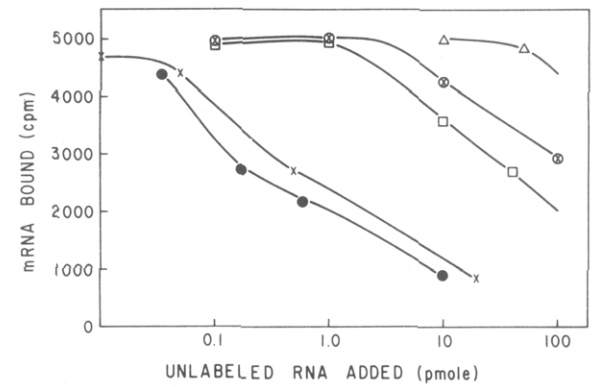

FIGURE 6: Competition of various RNAs for mRNP protein dependent mRNA binding. Ehrlich ascites tumor polyribosomal mRNPs (0.47 μg of protein) were added to 50 μl of assay buffer containing 0.4 μg of Ehrlich ascites tumor, [^{3}H]poly(A)-rich mRNA (35 000 cpm/μg RNA) and the indicated quantity incubated, applied to nitrocellulose filters, and analyzed as described under Methods. Where indicated, various concentrations of indicated synthetic and natural RNAs were added with the radiolabeled poly(A)-rich RNA prior to incubation. The [^{3}H]RNA retained in the absence of added mRNP (30 cpm) was subtracted from each determination; results are the average of duplicate determinations differing by less than 5%. Unlabeled RNAs added were Ehrlich ascites tumor poly(A)-rich mRNA (○—○), poly(G)·poly(C) (X—X), tRNA (—), poly(A) (⊗—⊗) and poly(C) (△—△). Approximate molecular weights assumed to calculate picomoles of RNA added were poly(A)-rich mRNA, 4.5×10^5 daltons; poly(G)·poly(C), 4.0×10^5 daltons; poly(A), 7×10^4 daltons; poly(A)·poly(U), 1.5×10^5 daltons; tRNA, 3×10^4 daltons; poly(C), 8×10^4 daltons.

poly(G) by mRNPs since [^{3}H]poly(G) was retained on Millipore filters in the presence of mRNPs. The mRNA binding properties of mRNPs were found to be similar to those of one of the initiation factors, the GTP-dependent Met-tRNA^{fMet}-binding protein. This factor, purified by phosphocellulose and *DEAE-cellulose chromatography and isoelectric focusing* (Rosenfeld, M. G., and Barrieux, A., submitted for publication), contained two subunits of 52 000 and 39 000 apparent molecular weights, in agreement with the data of Safer et al. (1975). As shown in Table II, the ability of various species of RNA to compete with [^{3}H]mRNA for binding was similar for *GTP-dependent Met-tRNA^{fMet}-binding protein and mRNPs*. The binding of mRNA to either fraction was found to be GTP

TABLE II: Relative Ability of Natural and Synthetic RNAs to Compete for mRNA Binding by mRNPs and the GTP-Dependent Met-tRNA^{fMet}-Binding Protein.[a]

Unlabeled RNA Added	Unlabeled RNA (μg) Producing 50% Inhibition of Binding to: GTP-Dependent, Met-tRNA^{fMet}-Binding Protein	mRNPs
mRNA	0.9	1
18S RNA	90	100
Poly(A)	180	130
Poly(C)	250	210
Poly(G)	0.1	0.2
Poly(G)/poly(C)	1	3

[a] Purified GTP-dependent Met-tRNA^{fMet}-binding protein (1 μg) or polyribosomal mRNP protein (2.5 μg) and various concentrations of the RNAs listed above were added to an assay mix containing 0.4 μg of [^{3}H]poly(A)-rich mRNA (35 000 cpm/μg RNA) and incubated and analyzed as described in the legend to Figure 5. The amount of unlabeled RNA (μg) required to inhibit by 50% the binding of [^{3}H]mRNA is shown for each species.

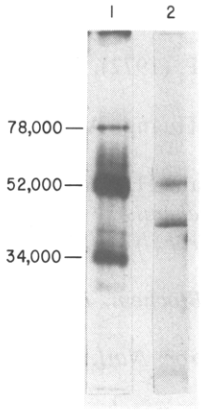

FIGURE 7: Comparison of reticulocyte RNPs and GTP-dependent, Met-tRNA^{fMet}-binding protein. Rabbit reticulocyte polyribosomal mRNPs and the GTP-dependent Met-tRNA^{fMet}-binding protein were prepared, iodinated, reduced in 1% sodium dodecyl sulfate, and subjected to polyacrylamide gel electrophoresis. In these preparations the major RNP proteins (1) were calculated to have 75 000, 52 000, and 34 000 apparent molecular weights; and the putative subunits of the GTP-dependent, Met-tRNA^{fMet}-binding protein (2) were calculated to have molecular weights of 52 000 and 39 000 based upon migration of bovine serum albumin, ovalbumin, and soybean trypsin inhibitor standards.

independent. When the two fractions were analyzed on sodium dodecyl sulfate–polyacrylamide gels, the 52 000 molecular weight subunit of the Met-tRNA^{fMet}-binding protein comigrated with the mRNP protein of similar molecular weight (Figure 7). When the two fractions were mixed before electrophoresis, a single protein band of 52 000 molecular weight was observed.

Discussion

Although mRNPs particles were first described in 1965, the specificity of mRNA–protein interaction is still questioned. The RNA:protein content of mRNPs, based upon the buoyant densities of glutaraldehyde-fixed mRNPs, has been variable, with a broad distribution, at 1.35–1.5 g cm^{-3} (Perry and Kelley, 1968; Henshaw, 1968; Burny et al., 1969; Olsnes, 1970) to 1.59–1.7 g cm^{-3} for mRNPs washed in buffers containing high salt. Quantitation of the RNA and protein content of polyribosomal mRNPs which were extensively washed in buffers containing 0.5 M KCl revealed an RNA:protein ratio (μg:μg) of approximately 2:1. The average molecular weight of mRNA used in these studies was 4.5×10^5 M and the two prominent proteins had apparent molecular weights of 78 000 and 52 000; therefore, the stoichiometry suggested that each mRNP molecule consisted of one mRNA molecule and one molecule of each of the major two proteins. In addition at least some of the mRNPs molecules could accommodate one or several minor proteins. These data were consistent with similar conclusions of Blobel (1973) and Chen et al. (1976) based upon mRNP buoyant density in CsCl. In contrast, subribosomal mRNPs contained eight- to tenfold more protein per μg of RNA. This could reflect (i) contamination by cytoplasmic proteins during preparation, (ii) the presence of the minor protein bands in all mRNPs which are not actively involved in translation, (iii) the different protein content of subribosomal RNPs, as previously suggested (Gander et al., 1973), or (iv) the presence of multiple identical subunits of some proteins, most of which dissociate at the time of initiation of translation. Gel analysis revealing additional proteins as well as increased relative intensity of minor proteins does not support the last hypothesis.

The poly(A) tract of mRNA was first suggested to be associated with a 78 000 protein by Kwan and Brawerman (1972). Following limited ribonuclease treatment of reticulocyte, hepatic, and L-cell polyribosomes, Blobel (1973) isolated RNA–protein particles containing the poly(A) tract and the 78 000 protein, although significant amounts of other proteins were also present. The stability of unfixed poly(A)–protein complex through CsCl centrifugation further confirms that the 78 000 protein is indeed the only protein associated with the poly(A) tract. Proteins of 78 000 (Firtel and Pederson, 1975), 74 000 and 86 000 (Kish and Pederson, 1975), and 40 000–50 000 (Schweiger and Mazur, 1975) molecular weights have been reported to be associated with the poly(A) tract of heterogeneous nuclear RNPs in various tissues, suggesting that packaging of mRNA into mRNPs is initiated in the nucleus. Evidence that other proteins present in mRNPs have a high affinity for mRNA regions other than the poly(A) tract was supported by the observations that neither poly(A) nor natural RNAs were able to compete with natural mRNA for binding. However, both reticulocyte mRNA, where globin mRNAs represent >90% of RNA, and Ehrlich ascites tumor mRNA were equally effective competitors; thus a region common to most RNAs appeared to be involved in binding. Since poly(G) was even more effective than mRNA at competing for binding, a poly(G)-rich region may be involved. No poly(G) region has been reported in eukaryotic mRNAs, although one of the putative loops at the 3′ terminus proximal to the poly(A) tract of globin mRNA involves an oligo(G)·oligo(C) hybrid (Proudfoot and Brownlee, 1974).

Hellerman and Shafritz (1975) suggested that the GTP-dependent, Met-tRNA^{fMet}-binding protein was present in purified mRNPs. The comigration of the 52 000 molecular weight RNP protein with the larger subunit of the GTP-dependent, Met-tRNA^{fMet}-binding protein and the similarity of mRNA binding competition by various natural and synthetic RNAs supported the possibility that the 52 000 molecular weight protein of mRNPs could represent one subunit of the GTP-dependent, Met-tRNA^{fMet}-binding protein. Further evidence will be required to rigorously document this postulate.

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