Studied on the Mechanism of Action of a Eukaryotic Codon-dependent Factor Specific for Initiator Met-tRNA<sub>f</sub> and Ribosomal 40 S Subunits*

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A putative eukaryotic initiation factor purified from rat liver cytosol promotes the ApUpGp- and Mg<sup>2+</sup>-dependent, GTP-independent binding of initiator Met-tRNA<sub>f</sub> to ribosomal 40 S subunits. The isotopically-labeled factor binds specifically to 40 S subunits to form a binary complex which can then bind Met-tRNA<sub>f</sub> in the presence of ApUpGp. When 60 S subunits are added, an 80 S complex is formed which contains Met-tRNA<sub>f</sub>, but not the factor. The Met-tRNA<sub>f</sub> bound to 80 S ribosomes reacts quantitatively with puromycin. Purified 40 S subunits exist in the form of monomers and dimers. The factor converts monomers, which appear to contain one molecule of tRNA, to a form that does not contain tRNA and dimerizes readily. Deacylated tRNA inhibits dimerization and, in the presence of the factor, converts dimers to the monomeric form; thus, the factor appears to act in a reversible manner. This protein factor may play a role in the removal of tRNA from 40 S subunits, which could be generated as a consequence of chain termination, a prerequisite to the binding of Met-tRNA<sub>f</sub>.

- eIF-2<sup>c</sup> catalyzes the binding of the initiator molecule Met-tRNA<sub>f</sub> to ribosomal 40 S subunits, an essential intermediary reaction in the initiation of protein synthesis (1–17). This factor has been demonstrated in a variety of cells including reticulocytes, wheat germ, liver, and several cultured cells. The eIF-2 forms a ternary complex with Met-tRNA<sub>f</sub> and GTP, which reacts with 40 S subunits in the absence of template to form a 40 S preinitiation complex. Subsequently, mRNA is bound to this complex, 60 S ribosomal subunits are joined, and an 80 S initiation complex is produced. The 80 S initiation complex, containing Met-tRNA<sub>f</sub> at the P site and mRNA, is free of initiation factors including eIF-2. Several ancillary protein factors, designated as Co-eIF-2A, Co-eIF-2B, Co-eIF-2C, sRF, etc., that may affect efficient formation and proper utilization of the eIF-2 ternary complex, have been described (18–24).

Another protein factor or factors designated as cIF-1, IF-M1, or cIF-2B have also been shown to catalyze the binding of Met-tRNA<sub>f</sub> (and the initiator tRNA analog, acetylphenylalanyl-tRNA) to ribosomal 40 S subunits (15, 24–38). The presence of such factors has been demonstrated in a number of cells such as Artemia salina (26, 29), liver (15, 27, 28, 31, 34), reticulocytes (30, 32, 33), human tonsils (35), hen oviduct (36, 38), wheat germ (24, 37), etc. Factor cIF-1 promotes the template-dependent, GTP-independent binding of Met-tRNA<sub>f</sub> with ApUpGp and of acetylphenylalanyl-tRNA with poly(U). The 40 S "preinitiation" complex reacts with 60 S ribosomal subunits to form as 80 S "initiation" complex containing Met-tRNA<sub>f</sub> at the P site.

Although there is no evidence that cIF-1 plays a direct role in the binding of Met-tRNA<sub>f</sub> in polypeptide chain initiation, a number of observations with the rat liver factor (15, 34) suggest that it would be important to study its mechanism of action and the way in which it interacts with other components in chain initiation. For example, cIF-1 is specific for Met-tRNA<sub>f</sub> and acetylphenylalanyl-tRNA (and to some extent Phe-tRNA), but does not appear to react with other aminoacyl-tRNAs; it is specific for 40 S subunits and does not react with 60 S subunits or 80 S ribosomes. It binds initiator tRNA to ribosomal subunits in a site that subsequently becomes the ribosomal P site capable of reacting with puromycin or participating as the NH<sub>2</sub>-terminal amino acid in a polypeptide chain; it is both found in the cytosol and associated with ribosomal subunits; further, it has been detected in practically all of the eukaryotic cells in which it has been investigated.

EXPERIMENTAL PROCEDURES

The purification of cIF-1 from the postribosomal fraction of rat liver homogenates was carried out using ammonium sulfate fractionation and chromatography on calcium phosphate, DEAE-cellulose, CM-cellulose, and cellulose phosphate, as described previously (15, 34). The purity of the factor after chromatography on cellulose phosphate columns was routinely greater than 85% as judged by polyacrylamide gel electrophoresis under nondenaturing conditions; the specific biological activity of preparations used in these studies was routinely about 1000 pmol of Met-tRNA<sub>f</sub> bound to derived 40 S ribosomal subunits, per mg of cIF-1 protein, when assayed as described below. Radioactive cIF-1 was prepared by reductive methylation (39, 40) with [¹⁴C]formaldehyde and sodium borohydride in sodium borate buffer at pH 10.0. Analysis of the [¹⁴C]cIF-1 prepared indicated the following: (a) the specific radioactivity was about 500 cpm of [¹⁴C]cIF-1; (b) the relative specific biological activity of radioactive cIF-1 was only 30% lower than that of untreated cIF-1; (c) both the 28,500- and the 18,500-molecular weight polypeptides (15) were radiolabeled and their relative specific radioactivities were within 40% of each other; and (d) between 5 and 10 lysine residues per molecule were modified by methylation. Initiation factor eIF-2 was purified from high salt extracts of native ribosomal subunits from rat liver (15); preparations used were completely resolved from the ribosome-associated activity analogous to cIF-1.

Derived ribosomal 40 S and 60 S subunits were prepared from rat liver polysomes and ribosomes purified by extraction with deoxycholate and NH<sub>4</sub>Cl, then stripped of endogenous peptidyl-tRNA with puromycin, dissociated into subunits with 0.08 M KCl and 12.5 mM MgCl<sub>2</sub>, and resolved by zonal centrifugation into 40 S and 60 S subunits (41). Incubation of the subunits individually and together, in the poly(U)-dependent polyphenylalanine-synthesizing system (41), as well as gel electrophoresis of the RNA extracted from the particles, indicated that the 40 S and the 60 S subunits were complementary.
but were well resolved from each other. The ribosomal 40 S subunit monomers (40S<sub>m</sub>) and dimers (40S<sub>d</sub>) were prepared by centrifuging the 40 S preparation through a linear 10–30% sucrose gradient containing 50 mM Tris-HCl buffer (pH 7.6), 4 mM MgCl<sub>2</sub>, 50 mM KCl, and 1 mM dithiothreitol. After 11 h at 95,000 × g (SW 27 rotor, Spinco) at 2 °C, the monomer (40S) and dimer (55S)-containing fractions were collected and the particles were sedimented from the apparently pooled gradient fractions. Isolated monomer and dimer subunit preparations actively carried out the poly(U)-dependent synthesis of polyphenylalanine in the presence of 60 S subunits, elongated by chromatography on BD-cellulose. The specific radioactivity of the tRNA-bound methionine was approximately 2800 cpm/pg of tRNA; the specific radioactivity of the tRNA-bound methionine was approximately 3600 cpm/pg.

Radioactive Met-tRNA<sub>f</sub> was prepared by incubating [14C]methionine and ATP<sub>d</sub> with decapsidated rat liver tRNA and an aminoacyl-tRNA synthetase fraction resolved from tRNA by chromatography of rat liver "pH 5 enzymes" on DEAE-cellulose (42); after extraction of the aminoacyl-tRNAs and uncharged tRNA from the incubation, the isotopically labeled Met-tRNA<sub>f</sub> and Met-tRNA<sub>a</sub> were separated by chromatography on BD-cellulose. The specific radioactivity of the tRNA-bound methionine was approximately 3600 cpm/pg of tRNA; the specific radioactivity of the tRNA-bound methionine was approximately 3600 cpm/pg/mmol.

The cIF-1-dependent binding of Met-tRNA<sub>f</sub> to 40 S ribosomal subunits was assayed by incubating 16 pmol of derived 40 S subunits, 10 pmol of ApUpGp, and varying concentrations of purified cIF-1 or [14C]cIF-1 in a buffered salts solution containing 30 mM 4-morpholinepropanesulfonic acid-KOH buffer (pH 7.2), 6 mM MgCl<sub>2</sub>, 60 mM NH<sub>4</sub>Cl, and 2 mM dithiothreitol. The total volume was 0.1 ml. After 40 min of incubation at 10 °C, 3 ml of 10° by 10° dithiothreitol was added, and the solution was filtered through Millipore membranes, washed, dried, and counted in a scintillation counter. When incubations were analyzed by gradient centrifugation, the reaction mixtures were layered on and centrifuged through 11.5 ml of a 10–30% sucrose gradient containing the same buffered salts as in the incubation, at 43,500 × g for 16 h (2 °C), in an SW 41 (Spinco) rotor. Some samples received formaldehyde (2 °C, 0.1–0.4% final concentration) prior to centrifugation; however, formaldehyde fixation was not necessary to demonstrate complexes when they were formed in the reaction. The gradients were analyzed automatically at 254 nm with a continuous recording spectrophotometer. When radioactive incubations were analyzed, 0.4-mI gradient fractions were collected and radioactivity determined with a scintillation counter. For double-labeling experiments, settings were used for maximum resolution of 1<sup>4</sup>C and 1<sup>3</sup>H; tritium counts were corrected for any spillover of radiocarbon.

The cIF-2-dependent binding of Met-tRNA<sub>f</sub> to 40 S subunits was assayed in a two-step incubation procedure; the first phase allowed the formation of the ternary complex and the second phase allowed the reaction between the ternary complex and 40 S subunits to form the preinitiation complex. Purified cIF-2 (16–20 pg) was incubated with 40 S subunits, all of the reaction mixtures were used for maximum resolution of 1<sup>4</sup>C and 1<sup>3</sup>H; tritium counts were corrected for any spillover of radiocarbon.

The cIF-1-dependent dimerization of 40 S ribosomal subunits was assayed by incubating about 11–12 pmol of resolved 40 S monomers and varying concentrations of cIF-1 with 30 mM 4-morpholinepropanesulfonic acid-KOH buffer (pH 7.2), 6 mM MgCl<sub>2</sub>, 70 mM monovalent cation, and 2–3 mM dithiothreitol; the incubations in a total volume of 0.05 ml were at 37 °C for 10 min. After incubation, the reaction mixtures were analyzed by centrifugation on 10–30% sucrose gradients, and the amount of subunits present as 55 S dimers was determined from the sedimentation patterns.

RESULTS

When the binding factor cIF-1 was incubated with initiator tRNA, ribosomal 40 S subunits, and the initiation codon ApUpGp, Met-tRNA<sub>a</sub> was bound to the subunits (15, 34). To determine whether intermediary complexes were formed between cIF-1 and other components, the effects of the other binding constituents on the rate of thermal inactivation of the factor were assayed. Purified cIF-1 was incubated at 53 °C by itself or with radioactive Met-tRNA<sub>f</sub>, ApUpGp, or 40 S subunits, individually. After varying periods of time, the temperature of the incubation was lowered to 10 °C, the components omitted from the first incubation at 53 °C were added, and the incubation was continued at 10 °C for 40 min. The amount of radioactive Met-tRNA<sub>f</sub> bound to 40 S particles measured the binding activity of the cIF-1 remaining after the 53 °C treatment. As shown in Fig. 1, ApUpGp and Met-tRNA<sub>f</sub>, individually (or together, not shown here) had no effect on the rate of inactivation of cIF-1 which had a half-life of about 4 min under these conditions; in the presence of derived 40 S ribosomal subunits, however, cIF-1 was more stable, suggesting that a binary complex was formed between cIF-1 and 40 S subunits which protected the binding factor against inactivation.

In order to study in more detail the interaction between cIF-1 and 40 S subunits, radioactive cIF-1 was prepared and used in these reactions. When [14C]cIF-1 was incubated with purified 40 S ribosomal subunits (Fig. 2A) composed of 40 S monomers and 55 S dimers as described previously (41, 43–46), 60 S ribosomal subunits (Fig. 2B) composed of 60 S monomers and 90 S dimers, or 80 S ribosomes (Fig. 2C) and analyzed by sucrose gradient centrifugation, cIF-1 was recovered only with 40 S monomers (Fig. 2A). Analysis of the particle-bound cIF-1 by gel electrophoresis indicated that both polypeptides in the factor were associated with the ribosomal subunit in essentially the same proportion (within 30%) as present in the starting material.

![Fig. 1. The effect of time of incubation at 53 °C and of various initiation components on the activity of cIF-1. Purified cIF-1 (0.5 μg) was maintained at 53 °C in 0.05 ml of buffered salts incubate with radioactive Met-tRNA<sub>f</sub> (open circles) or in the presence of 40 pmol of derived 40 S subunits (closed circles), 0.1 A<sub>260</sub> units of ApUpGp (squares), or 10 μg of [14C]Met-tRNA<sub>a</sub> (triangles). After varying periods of time as noted, samples received the components that were omitted from the incubation of 53 °C, all of the reaction mixtures containing cIF-1, 3<sup>14</sup>C Met-tRNA<sub>f</sub>, ApUpGp, and derived 40 S ribosomal subunits (0.1 ml) were then incubated for 40 min at 10 °C. At the end of the second incubation, the amount of radioactive Met-tRNA<sub>f</sub> bound to 40 S subunits was determined by measuring the amount of radioactivity retained on Millipore filters. The 100% value obtained from incubations that were not treated at 53 °C, represents approximately 4400 cpm of 40 S-bound [14C]Met-tRNA<sub>f</sub>.](http://www.jbc.org)
When [\(^{14}\text{C}\)]cIF-1, [\(^{3}\text{H}\)]Met-tRNA, and ApUpGp were incubated with 40 S subunit preparations containing 40 S monomers and 55 S dimers (Fig. 3A), both cIF-1 and Met-tRNA interacted only with 40 S monomers, and essentially equivalent quantities of the factor and the aminoacyl-tRNA were recovered in the 40 S peak. The [\(^{3}\text{H}\)]Met-tRNA did not bind to ribosomal particles in the absence of cIF-1 or ApUpGp; the addition of Met-tRNA, or ApUpGp did not significantly affect the binding of [\(^{14}\text{C}\)]cIF-1; and derived 60 S ribosomal subunits in these incubations markedly inhibited the binding of both cIF-1 and Met-tRNA. The latter finding was due to the fact that derived 40 S and 60 S subunits react rapidly and spontaneously to form 80 S ribosomes (46) which do not react with cIF-1 or Met-tRNA. Fig. 3B shows the effect of 60 S subunits on the 40 S complex containing cIF-1 and Met-tRNA. A similar reaction mixture containing all of the components described above received derived 60 S subunits after the first incubation period. The incubation was continued for 10 min, then gradient centrifugation analysis was carried out. Whereas [\(^{3}\text{H}\)]Met-tRNA was recovered with the 80 S ribosomes formed, none of the [\(^{14}\text{C}\)]cIF-1 was transferred to the 80 S complex. With higher concentrations of 60 S ribosomal subunits in the second step of the incubation, all of the radioactive Met-tRNA could be transferred from the 40 S peak to the 80 S material, and all of the cIF-1 was released from the particles and was recovered in the free form toward the top of the gradient.

The nature of the Met-tRNA bound to 80 S ribosomes, prepared in a 2-step incubation as described above, was investigated by incubating the 80 S Met-tRNA complexes with puromycin (42). Samples containing 80 S-bound [\(^{3}\text{H}\)]Met-tRNA were quantitatively converted to [\(^{3}\text{H}\)]methionyl-puromycin. Incubation with GTP and elongation factor EF-2 prior to the addition of puromycin had no effect on the peptidyl-transferase reaction; therefore, translocation was not required.

The addition of cIF-1 to a derived 40 S ribosomal subunit population shifted the proportion of particles toward the 55 S dimer species. When purified 40 S monomers (Fig. 4A) were incubated with cIF-1 at 37 °C (Fig. 4B), then analyzed by gradient centrifugation, the conversion of the monomeric subunits to dimers was proportional to the concentration of cIF-1 in the incubation; in some cases, up to 50% of the 40 S subunits were converted to the 55 S form with less than 0.5 μg of cIF-1. This cIF-1-catalyzed reaction was more extensive at 37 °C than at 10 °C; however, at both incubation temperatures radioactive cIF-1 was recovered only with the remaining 40 S monomers, and none was associated with dimers.

Wettenhall et al. (45) suggested that 1 mol of deacylated tRNA was associated with each monomeric 40 S subunit, and that dimers lacked tRNA; thus, conversion of subunits from one form to another could involve the addition or removal of tRNA from the particles. To determine the extent to which tRNA was associated with subunits in these studies, particles were resolved, total tRNA was extracted (47), high molecular

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**Fig. 2.** Binding of radioactive cIF-1 to ribosomal ribonucleoprotein particles. Approximately 44 pmol of [\(^{14}\text{C}\)]cIF-1 were incubated in 0.4 ml of buffered salts incubation solution with about 100 pmol of derived 40 S subunits (A), 100 pmol of 60 S subunits (B), or 100 pmol each of 40 S and 60 S subunits that had been preincubated for 5 min at 37 °C to form 80 S ribosomes (C). After 40 min at 10 °C, the reaction mixtures were analyzed on sucrose gradients.

**Fig. 3.** Release of cIF-1 during the formation of 80 S complex. Incubations as described above contained 100 pmol of derived 40 S subunits, 38 pmol of [\(^{3}\text{H}\)]Met-tRNA, 44 pmol of [\(^{14}\text{C}\)]cIF-1, and 0.4 A\(_{260}\) units of ApUpGp in 0.4 ml of buffered salts incubation solution. After 40 min at 10 °C, one of the reaction mixtures was analyzed by sucrose gradient centrifugation (A). The other reaction mixture received 100 pmol of derived 60 S subunits and the incubation at 10 °C was allowed to continue for an additional 10 min prior to gradient centrifugation.

**Fig. 4.** The effect of cIF-1 on ribosomal 40 S monomers. Approximately 20 pmol of resolved 40 S subunit monomers were incubated for 10 min at 37 °C as such (A) or with 0.5 μg of purified cIF-1 (B) in a total volume of 0.075 ml of buffered salts incubation solution. The reaction mixtures were then analyzed by gradient centrifugation.
weight RNA was removed by precipitation with 1 M NaCl (48), and the remaining RNA was quantitatively analyzed for 4S RNA on sucrose gradients. The total amount of RNA isolated from equal amounts of monomers and dimers was similar, but the amount of 4S RNA recovered from the 40S monomers was about 3 times greater than that from the 55S dimers.

When Met-tRNA and ApUpGp were present along with cIF-1, the shift in the ribosomal subunit population to 55S dimers was not observed and, in fact, the proportion of 40S monomers appeared to be greater. To determine whether the interconversion between monomer and dimer subunits was affected by Met-tRNA itself or by tRNA in the Met-tRNA preparation, 55S dimers were incubated with deacylated tRNAs in the presence and absence of cIF-1 (Fig. 5). Purified 55S dimers (Fig. 5A) were only slightly converted to 40S monomers in the presence of a mixed population of deacylated tRNAs (Fig. 5B); however, they were extensively converted to the monomeric form when cIF-1 was also present (Fig. 5C). Thus, the shift of subunits from the 55S to the 40S form in experiments with radioactive Met-tRNA was probably due to the presence of a mixture of deacylated tRNAs in the ['H] Met-tRNA preparation. The shift from 55S dimers to 40S monomers was also obtained when the incubations contained cIF-1 and purified tRNA\textsuperscript{M} but not with rat liver 5S RNA; preparations containing a number of deacylated tRNAs resolved from tRNA\textsuperscript{M} were also effective. The results suggested that the reaction was specific for tRNA but not for a specific isoacceptor species.

The effects of deacylated tRNA on the cIF-1-catalyzed conversion of 40S monomers to 55S dimers and on the cIF-1-dependent binding of Met-tRNA\textsubscript{f} are shown in Fig. 6. When resolved 40S monomeric subunits were incubated with cIF-1, ApUpGp, and [\textsuperscript{3}H]Met-tRNA\textsubscript{f} containing about 10 µg of deacylated tRNA, approximately 18% of the subunits were recovered in the 55S dimer form and about 4500 cpm of radioactive Met-tRNA\textsubscript{f} were bound to 40S particles (100% values). As the concentration of mixed deacylated tRNAs was increased, the amount of dimers formed and the amount of Met-tRNA\textsubscript{f} bound were concomitantly decreased, proportional to the amount of tRNA added.

The formation of a 40S-Met-tRNA\textsubscript{f} complex using purified 55S dimers was studied by incubating with [\textsuperscript{3}H] Met-tRNA\textsubscript{f}, in the presence and absence of cIF-1 or ApUpGp. Although a considerable amount (35-40%) of the 55S dimers was converted to 40S monomers in the presence of Met-tRNA\textsubscript{f} and cIF-1, binding of [\textsuperscript{3}H] Met-tRNA\textsubscript{f} without ApUpGp was negligible. If cIF-1 was omitted, a small amount of 40S monomers was formed due to the tRNA in the [\textsuperscript{3}H] Met-tRNA\textsubscript{f} preparation. When all of the components were present, conversion to 40S monomers was extensive (40-45% of the 55S subunits) and significant amounts of [\textsuperscript{3}H] Met-tRNA\textsubscript{f} were bound to them. The cIF-1 stimulated, to the same extent, both the conversion of 55S dimers to 40S monomers if tRNA was present, and the binding of [\textsuperscript{3}H] Met-tRNA\textsubscript{f} to 40S subunits if ApUpGp was present.

The interaction of 40S subunits with eIF-2, the initiator

![Fig. 5. The effect of cIF-1 and tRNA on ribosomal 55S dimers. Approximately 15 pmol of resolved 55S dimers were incubated in 0.05 ml of buffered salts incubation solution as such (A), with 50 µg of deacylated tRNAs (B), or with 50 µg of deacylated tRNAs plus 0.8 µg of purified cIF-1 (C). After 2 min at 37 °C, the reaction mixtures were analyzed by sucrose gradient centrifugation. The deacetylated tRNA was prepared from the mixture of tRNAs in the fraction obtained from BD-cellulose column that was resolved of Met-tRNA (see under "Experimental Procedures"), by incubation for 2 h at 53 °C in 30 mM 4-morpholinepropanesulfonic acid buffer, pH 7.2.](http://www.jbc.org/)

![Fig. 6. The effect of varying concentrations of deacylated tRNA on the cIF-1-directed binding of Met-tRNA\textsubscript{f} to 40S subunits and on the conversion of 40S monomers to 55S dimers. Incubations contained approximately 44 pmol of resolved 40S ribosomal subunit monomers, 17 µg of [\textsuperscript{3}H] Met-tRNA\textsubscript{f}, 1.4 µg of purified cIF-1, and varying amounts of deacylated tRNA as noted. The reaction mixtures, in a total volume of 0.175 ml, were maintained for 10 min at 37 °C, then 0.075 ml were removed from each and analyzed on sucrose gradients to determine the amount of 40S subunits present as 55S dimers. The remaining 0.1 ml received 0.1 A\textsubscript{260} unit of ApUpGp, and incubations were continued for 40 min at 10 °C. The amount of radioactive Met-tRNA\textsubscript{f} bound to 40S subunits was determined in the incubations containing ApUpGp by the Millipore filtration procedure. The 100% values (4480 cpm of particle-bound [\textsuperscript{3}H] Met-tRNA\textsubscript{f} and 18% of 55S dimers in the 40S subunit preparation) represent control incubations in which approximately 10 µg of deacylated tRNA, contributed by the [\textsuperscript{3}H] Met-tRNA\textsubscript{f} preparation, were present.](http://www.jbc.org/)
analyses were as described under "Experimental Procedures." Ternary complex formed on incubation of eIF-2, [3H]Met-tRNA$_A$, and GTP associated only with the monomeric form of 40 S subunits. When incubations were carried out with purified 55 S dimers, some 40 S monomers were formed due to the tRNA present in the [3H]Met-tRNA$_A$ preparations, and ternary complex interacted with them; ternary complex was not bound to the 55 S dimers.

Attempts were made to determine whether the reversible formation of 55 S dimers from 40 S monomers with cIF-1 had any effect on the subsequent interaction of 40 S monomers with ternary complex containing eIF-2, Met-tRNA$_A$, and GTP (Fig. 7). When resolved 40 S monomers were preincubated with cIF-1, a considerable amount of 55 S dimers was formed (Fig. 7A); without cIF-1, over 90% of the subunit population was in the monomeric form. When [3H]Met-tRNA$_A$ and eIF-2 were incubated without GTP so that the ternary complex could not be formed, and then were added to 40 S monomers, radioactive methionine was not recovered with ribosomal subunits (Fig. 7B). When ternary complex, allowed to form by incubation of eIF-2 with [3H]Met-tRNA$_A$ and GTP, was added to 40 S monomers (Fig. 7C), radioactive methionine was found associated with the 55 S dimeric peak. When the ternary complex was added to 40 S subunits that had been preincubated with cIF-1 in order to form a large amount of dimers, most of the 40 S population had reverted back to the monomer form and radioactive methionine was bound to it (Fig. 7D). The conversion of 55 S dimers to 40 S monomers which occurred in this incubation was brought about by cIF-1 and the tRNA in the [3H]Met-tRNA$_A$ preparation; however, the amount of 40 S-bound Met-tRNA$_A$ was exactly the same with untreated 40 S monomers or with 40 S monomers treated with cIF-1. The possibility exists that eIF-2 or ternary complex may dissociate or displace bound deacylated tRNA, or that it may bind at a different site on the 40 S monomer.

The studies presented here characterize a series of reactions for the formation of an alternate 80 S “initiation” complex. In this sequence, a protein factor cIF-1, distinct from eIF-2, catalyzes the binding of Met-tRNA$_A$ to 40 S ribosomal subunits when a template (ApUpGp) and Mg$^{2+}$ (4-8 mm) are present, but does not require GTP. The first step appears to be the formation of a binary complex between cIF-1 and 40 S subunits, prior to the interaction with template and initiator tRNA; this complex, described here with rat liver preparations, has also been detected in human tonsil cells (35) and in wheat germ (24), but not in A. salina (26, 29). Results presented above do not distinguish between the subsequent ordered addition of either ApUpGp or Met-tRNA$_A$ and a simultaneous concerted reaction between all three reactants.

Experiments with radioactive cIF-1 and Met-tRNA$_A$ indicate that although both are bound to 40 S subunits, factor cIF-1 is released in the course of the 60 S joining reaction, and the 80 S “initiation” complex which is formed contains Met-tRNA$_A$ but not cIF-1. The interaction of 60 S subunits with the 40 S “preinitiation” complex does not appear to require GTP or any additional protein factors. All of the Met-tRNA$_A$ in the 80 S complex is bound to the ribosomal P site as shown by its ability to react quantitatively with puromycin in the peptidyltransferase reaction.

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The reaction of sequences described above with cIF-1 differs in several respects from those postulated to occur with eIF-2 in protein synthesis. For example, isotopically-labeled rabbit reticulocyte eIF-2 does not form a binary complex with 40 S subunits (13, 49); eIF-2 forms a ternary complex with Met-tRNA$_A$ and GTP (1-17, 50), and formation of the complex is an obligatory intermediate in the subsequent reaction with 40 S subunits, although evidence for a binary complex between eIF-2 and Met-tRNA$_A$ was also obtained in the absence of GTP (50). Some laboratories have reported that synthesis of [eIF-2-Met-tRNA$_A$, GTP] ternary complex occurs at low (less than 1 mm) concentrations of magnesium ions, and that the ancillary factor Co-eIF-2A is required for ternary complex formation with rabbit reticulocyte preparations at physiological Mg$^{2+}$ concentrations (19, 23). Complex formation with cIF-1 occurs optimally between 4 and 8 mm Mg$^{2+}$ and no additional factors appear to be required. Synthesis of the 80 S initiation complex from the 40 S preinitiation complex generated by eIF-2 requires additional factors, for example eIF-4C, eIF-4D, eIF-5, etc., as well as GTP hydrolysis (4, 6, 51-53). Finally, no additional requirements for protein factors or energy were detected in the conversion of the 40 S “preinitiation” complex generate with cIF-1 to an 80 S “initiation” complex.

In some respects, the reactions of eukaryotic cIF-1 are similar to those catalyzed by the prokaryotic factor IF-2. For example, both form binary complexes with the small ribosomal subunit, and they require template to stabilize the binding of initiator tRNA; eukaryotic cIF-1 preparations from rat liver and A. salina catalyze the binding of Escherichia coli fMet-tRNA$_A$ to eukaryotic 40 S ribosomal subunits (26, 27, 29) and to E. coli ribosomes (54). It is also of interest that crude cIF-1 preparations from rabbit reticulocytes were shown to stimulate translation of endogenous mRNA by hen oviduct polyosomes (55). Attempts to carry out the binding of Met-tRNA$_A$ with cIF-1 and natural tRNA, with rat liver preparations, have been unsuccessful; the results could indicate that, as with eIF-2, additional factors may be required for the interaction of mRNA in contrast to ApUpGp.

The generation of 40 S subunits containing tRNA, which exist in a conformational state incapable of dimerizing, could result as the consequence of a termination event in protein...
interaction of a Met-tRNA, Binding Factor with 40S Subunits

synthesis in which the completed polypeptide chain is hydrolyzed from the terminal peptidyl-tRNA and the polypeptide and ribosomes or subunits are released from polysomes; the fate of the terminal tRNA remains unknown. It would be tempting to postulate that cIF-1 plays a role in the sequence of reactions involved in the ribosome cycle, between termination and initiation of protein synthesis. For example, if after termination deacylated tRNA remains associated with 40S subunits which are inactive in protein synthesis, interaction with cIF-1 could result in the removal of tRNA and the production of a more active subunit form, which favors dimerization. In the presence of other initiation components, formation of initiation complexes, rather than dimerization, would be favored. Also, initiation factor eIF-3 has been shown to dissociate 5S dimers into 40S monomers (56), making it unlikely that dimers would exist in vivo. However, the presence of dimers in these studies could be considered an important reflection of a change in ribosomal subunit conformation.

The function of cIF-1 does not appear to be limited to the production of more active (dimer) subunits, since binding of Met-tRNA to purified dimers also requires cIF-1. Although the factor appears to catalyze dimer formation in a reaction distinct from Met-tRNA binding, it forms binary complexes with monomers as a consequence of both reactions.

These studies on the mechanism of action of factor cIF-1 do not establish the function of this factor which is present in relatively high concentrations in most eukaryotic cells. The reason why eukaryotic cells possess more than one protein factor capable of binding initiator Met-tRNA to 40S subunits, and the specific role of cIF-1 in protein synthesis, if any, remain to be established. One possibility raised by these studies is that cIF-1 may function by participating in various interactions at the level of the ribosomal subunit pool in the ribosome cycle.

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