Interaction of Animal Mitochondrial EF-Tu•EF-Ts with Aminoacyl-tRNA, Guanine Nucleotides, and Ribosomes*

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Caryl J. Schwartzbach and Linda L. Spremulli‡

From the Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290

The mammalian mitochondrial complex consisting of elongation factors EF-Tu and EF-Ts (EF-Tu·Tsmt) is capable of efficiently binding aminoacyl-tRNA to the ribosome in the presence and absence of guanine nucleotides. In the presence of GTP the binding reaction is catalytic. In the absence of guanine nucleotides, or in the presence of a non-hydrolyzable GTP analog, only one round of ribosome binding occurs. EF-Tu·Tsmt is capable of forming a ternary complex with GTP and Escherichia coli Phe-tRNA as demonstrated by gel filtration chromatography, nitrocellulose filter binding, and by protection of the aminoacyl-tRNA bond from hydrolysis. GDP and the non-hydrolyzable GTP analog guanyl-5′-yl imidodiphosphate are also capable of facilitating ternary complex formation with EF-Tu·Tsmt, but are less effective. No kinetic advantages result from the formation of this ternary complex prior to ribosome binding, and EF-Tu·Tsmt may actually bind aminoacyl-tRNA directly to the ribosome prior to binding GTP. These results suggest that a variation of the prokaryotic elongation cycle is occurring in animal mitochondria. N-Ethylmaleimide inhibits the activity of EF-Tu·Tsmt in polymerization and in ribosome binding. However, the activity of the EF-Tu·Tsmt which can be measured independently, is not altered.

Prokaryotic elongation factor Tu (EF1-Tu) and the corresponding cytoplasmic factor (EF-1α) play a central role in the elongation cycle of protein biosynthesis by promoting the binding of aminoacyl-tRNA to the A-site of the ribosome. Escherichia coli EF Tu has been shown to require GTP for the formation of a ternary complex (GTP-EF-Tu-aminoacyl-tRNA) prior to ribosome binding (1). The ternary complex binds to the A-site of the 70 S ribosome in a message-dependent manner. GTP is then hydrolyzed and an EF-Tu-GDP complex is released from the ribosome. A second elongation factor, EF-Ts, displaces the GDP and is, in turn, displaced by GTP, thereby catalyzing the guanine nucleotide exchange reaction. The resulting EF-Tu-GTP complex binds aminoacyl-tRNA reforming the ternary complex (2). The eukaryotic factor EF-1γ, like E. coli EF-Ts, plays a role in the guanine nucleotide recycling reaction in the cytoplasmic system of higher cells (3, 4). The GTP-dependent mechanism for aminoacyl-tRNA binding to the ribosome and the subsequent release of the elongation factor upon the hydrolysis of GTP appears to be conserved among prokaryotes and eukaryotes.

We have recently reported the purification of the EF-Tu·Tsmt complex from bovine liver mitochondria (5). The apparent molecular weights for EF-Tu, EF-Ts, and EF-Tu·Tsmt are similar to the values which have been obtained for E. coli EF-Tu and EF-Ts. The mitochondrial factors are active on prokaryotic ribosomes as well as on mitochondrial ribosomes but have no activity on cytoplasmic ribosomes (5). EF-Tu, EF-Ts, and EF-Tu·Tsmt are present in a tightly associated complex (EF-Tu·Tsmt) which cannot be dissociated by GDP or GTP. No guanine nucleotide binding to the EF-Tu·Tsmt complex can be detected in a standard nitrocellulose filter binding assay. In these respects EF-Tu·Tsmt is distinct from the corresponding prokaryotic factor.

In the present work we have examined the interaction of EF-Tu·Tsmt with guanine nucleotides, aminoacyl-tRNA, and ribosomes and have investigated the possible role of the ternary complex in the elongation cycle in animal mitochondria.

EXPERIMENTAL PROCEDURES

Materials

GTP and GDP were obtained from P-L Biochemicals. Poly(U), Guanyl-5′-yl imidodiphosphate (GMP-PNP) and E. coli tRNA were from Boehringer Mannheim. Sephadex G-75, N-ethylmaleimide, bovine serum albumin (BSA), phospho(enol)pyruvate (PEP), glycerol, and pyruvate kinase were from Sigma. Nitrocellulose membrane filter paper Type HA (0.45-μm pore size) was from Millipore Corporation. Pure nitrocellulose membrane filter paper was purchased from Schleicher & Schuell. Scintiverse E was obtained from Fisher. [14C]Phenylalanine, [3H]GDP, [3H]GTP, and [35S]methionine were obtained from Du Pont-New England Nuclear. [3H]Phenylalanine was prepared with E. coli tRNA as described by Ravel and Shorey (6) using synthetases prepared by the method of Muench and Berg (7). [35S]Formylmethionyl-tRNAsat was prepared from yeast tRNA as described (8).

Buffers

Buffer A consists of 20 mM Hepes-KOH, pH 7.0, 40 mM KCl, 1 mM MgCl2, 0.1 mM EDTA, 6 mM β-mercaptoethanol, 10% glycerol, and 1.0 mM GDP. Buffer B consists of 20 mM Tris-HCl, pH 7.6, 60 mM KCl, and 6.5 mM MgCl2. Binding Buffer consists of 75 mM Hepes-KOH, pH 7.0, 75 mM KCl, 15 mM MgCl2, and 7.5 mM dithiothreitol.

Preparation of Elongation Factors and Ribosomes

Purified EF-Tu·Tsmt was prepared as described (5) except that GDP was omitted from the preparation buffers for all the high performance liquid chromatography steps. E. coli ribosomes were prepared as described previously (9). Elongation factors EF-G, EF-Tu, EF-Ts, and an EF-Tu·Ts complex were isolated from E. coli (1) and further purified as described (10).

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‡ To whom correspondence should be addressed.

The abbreviations used are: EF, elongation factor; GMP-PNP, guanyl-5′-yl imidodiphosphate; BSA, bovine serum albumin; PEP, phospho(enol)pyruvate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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**Assays**

**Elongation Factor Assays—**EF-Tu-Ts₃₉ was detected by its ability to replace the corresponding E. coli factor in poly(U)-directed polymerization of phenylalanine on E. coli ribosomes as described (5). EF-Ts₃₉ activity was measured as described (5). The ability of EF-Tu-Ts₃₉ to bind guanine nucleotides in a nucleotide-free filter assay was carried out as described previously (5).

**Termary Complex Formation—**Reaction mixtures contained 75 mM Hepes-KOH, pH 7.0, 75 mM KCl, 15 mM MgCl₂, 7.5 mM dithiothreitol, 3.5% glycerol, 30 mg/ml BSA, and EF-Tu-Ts₃₉ as indicated, either [¹⁴C]Phe-tRNA or [³²P]Met-tRNA, and guanine nucleotides as specified. Samples were incubated at 25 °C for 10 min. The complexes were then analyzed by one of the following methods.

**Nitrocellulose Filter Binding—**Reaction mixtures (10 μl) contained 10 pM of EF-Tu-Ts₃₉, 15 pM of E. coli [¹⁴C]Phe-tRNA, 5 μM [³²P]GTP, or unlabeled GTP, 2.25 mM PEP, and 0.5 μg of pyruvate kinase in the ionic conditions described above. Following incubation, the samples were placed on ice and diluted to 1.5 ml with Binding Buffer. Samples were filtered through pre-soaked, pure nitrocellulose membranes, and the tubes and filters were rinsed three times with the sample cold buffer, dried, and counted as described previously (5).

**Gel Filtration—**Formation of the ternary complex was carried out as described above with 1.5 μM EF-Tu-Ts₃₉, 1.4 μM [¹⁴C]Phe-tRNA without GTP or with either 0.1 μM [³²P]GTP or unlabeled GTP in the presence of 2.25 mM PEP and 15 μg of pyruvate kinase. The samples (300 × 7 mm) Sephadex G-75 gel filtration column at a flow rate of 0.35 ml/min. Column fractions (180 μl) were analyzed for the presence of radioactive and protein. The amount of label present was determined by counting aliquots (150 μl) of various fractions in a scintillation mixture consisting of 10 ml of Scintiverse E and 1 ml of H₂O. The amount of protein present was determined basically as described by Smedsak and Grossberg (11) with modifications by Bearden (12).

**Hydrolysis Protection—**Reaction mixtures (30 μl) were incubated for 90 min under the conditions described above with 1.5 μM EF-Tu-Ts₃₉, 1.4 μM [¹⁴C]Phe-tRNA and either 0.1 mM GTP in the presence of 2.25 mM PEP and 6.0 μg of pyruvate kinase or with the nucleotide indicated in the appropriate figure legend. Aliquots (5 μl) were removed at the indicated times, and the hydrolysis of the aminocyl-trNA bond was monitored by cold 5% trichloroacetic acid precipitation (5).

When testing for the ability of EF-Tu-Ts₃₉ to protect the ester bond on [³²P]Met-tRNA," in the hydrolysis protection assay, samples (30 μl) were incubated for the indicated times under the conditions described above with 0.12 μM EF-Tu-Ts₃₉, 0.11 μM [³²P]Met-tRNA, and 0.1 mM GTP. Aliquots (5 μl) were removed and precipitated with cold 5% trichloroacetic acid.

**Ribosome Binding Assay**

EF-Tu-Ts₃₉ was tested for its ability to catalyze the binding of [¹⁴C]Phe-tRNA to E. coli 70 S ribosomes. Reaction mixtures (100 μl) contained 50 mM Tris-HCl, pH 7.8, 60 mM KCl, 6.5 mM MgCl₂, 0.1 mM spermine, 1 mM dithiothreitol, 12.5 μg of poly(U), 50 μg of deacetylated E. coli tRNA, 35 nmol of [¹⁴C]Phe-tRNA, 100 μg of E. coli ribosomes, and the indicated concentrations of guanine nucleotides. Following incubation for 10 min at either 27 or 37 °C, samples were filtered through nitrocellulose membranes (Millipore Type HA) and washed with Buffer B. A regenerating system of PEP (2.5 mM) and pyruvate kinase (0.17 units) was added to the designated samples containing GTP. Under these assay conditions the amount of ternary complex formed was negligible (<0.1 pmol) and did not interfere with the quantitation of ribosome binding. Blanks arising from the non-enzymatic binding of [¹⁴C]Phe-tRNA to the ribosomes (varying from 0.1 to 0.8 pmol, depending on the guanine nucleotide present), have been subtracted from each value.

**Effect of N-Ethylmaleimide on EF-Tu-Ts₃₉**

Reaction mixtures (20 μl) contained 65 pmol of EF-Tu-Ts₃₉ in Buffer A without β-mercaptoethanol and final N-ethylmaleimide concentrations of 0, 10⁻³, or 10⁻⁴ M. Samples were incubated for 10 min at 0 °C. The reaction was terminated by the addition of β-mercaptoethanol to a final concentration of 96 mM. The N-ethylmaleimide-treated samples were then analyzed for their activity in poly(U)-directed polymerization, ribosome binding, and EF-Ts₃₉ activity as described above.

**RESULTS**

**EF-Tu-Ts₃₉-dependent Binding of Aminoacyl-tRNA to Ribosomes—**In the prokaryotic model for the elongation cycle, a preformed ternary complex consisting of EF-Tu, GTP, and aminocyl-tRNA is responsible for the binding of aminocyl-tRNA to the ribosomal A-site. The ability of EF-Tu-Ts₃₉ to bind [¹⁴C]Phe-tRNA to poly(U)-programmed E. coli ribosomes was analyzed in the presence and absence of GTP. In these experiments, approximately 1.5 pmol of EF-Tu-Ts₃₉ was incubated with excess [¹⁴C]Phe-tRNA and about 8 pmol of active ribosomes in the presence and absence of GTP. The amount of Phe-tRNA binding to the ribosome was then determined (Fig. 1). In the presence of GTP, EF-Tu-Ts₃₉ functioned catalytically, and each picomole of the factor promoted the binding of approximately 8 pmol of Phe-tRNA over the time course of the incubation (Fig. 1). When the non-hydrolyzable GTP analog was tested, only one round of binding was observed as expected (data not shown). However, surprisingly, in the absence of guanine nucleotides, EF-Tu-Ts₃₉ was able to promote the binding of a stoichiometric amount of the aminocyl-tRNA to the ribosome (Fig. 1). The addition of GDP to reaction mixtures had no effect on the amount of binding observed in the absence of GTP (data not shown). These observations suggest that EF-Tu-Ts₃₉ alone, in the absence of guanine nucleotides, is capable of facilitating the binding of aminocyl-tRNA to ribosomes. But, in the absence of GTP, the mitochondrial factor remains associated with the ribosome following Phe-tRNA binding and cannot function catalytically.

**Ternary Complex Formation—**The observations described above suggest that the details of the elongation cycle promoted by EF-Tu-Ts₃₉ may differ from those observed in the prokaryotic system. Furthermore, EF-Tu-Ts₃₉, unlike the corresponding bacterial complex, cannot be dissociated in the presence of guanine nucleotides (5). This complex shows no nucleotide binding activity using the normal nitrocellulose filter binding assay employed for comparable factors from other systems. These observations prompted us to examine the interaction of EF-Tu-Ts₃₉ with aminocyl-tRNA and guanine nucleotides in more detail.

The ability of EF-Tu-Ts₃₉ to form a ternary complex with GTP and Phe-tRNA was analyzed by gel filtration chromatography (Fig. 2). When [¹⁴C]Phe-tRNA and EF-Tu-Ts₃₉ were incubated in the absence of GTP and then subjected to chromatography on Sephadex G-75 (Fig. 2A) two peaks of

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**FIG. 1.** The effect of guanine nucleotides on aminocyl-tRNA binding to ribosomes in the presence of EF-Tu-Ts₃₉. The ribosome binding assay was performed as described under “Experimental Procedures.” The samples were incubated without guanine nucleotides (○) or in the presence of 0.1 mM GTP (□) or 0.1 mM GDP (O), and the amount of [¹⁴C]Phe-tRNA bound to the ribosome was determined at the indicated times.
amount of 14C and 3H present in the ternary complex indicates that there is a 1:1 stoichiometry of Phe-tRNA and GTP present in the ternary complex.

We have tried to determine whether formation of the ternary complex results in the dissociation of the two mitochondrial elongation factors by looking for the appearance of free EF-Tsmt from the gel filtration column using both Sephadex G-75 and G-100 resins under a variety of conditions (data not shown). EF-Tsmt can be detected independently of EF-Tu by its ability to stimulate guanine nucleotide exchange with E. coli EF-Tu and, therefore, to stimulate the activity of this factor in polymerization (5). However, no free factor could be detected under any of the conditions tried. This result leaves open the possibility that the "ternary" complex detected here represents a complex containing both EF-Tu and EF-Tsmt and is actually a quaternary complex.

One of the classical procedures used to detect the interaction of E. coli EF-Tu and aminoacyl-tRNA is the hydrolysis protection assay in which the interaction between the protein and Phe-tRNA results in a reduction in the rate of hydrolysis of the ester bond between the amino acid and the tRNA (18). We have used this protection assay to confirm the formation of the ternary complex and to examine the specificity of the interaction between EF-Tu-Tsmt and elongator tRNAs. As indicated in Fig. 3A, the Phe-tRNA bond is rather labile to hydrolysis at elevated temperatures (25 °C) and most of the aminoacyl-tRNA has been deacylated within 2 to 3 h at this temperature. However, EF-Tu-Tsmt was very effective in protecting Phe-tRNA from hydrolysis (Fig. 3A), and most of the Phe-tRNA remained intact even after 3 h in the presence of the mitochondrial factor and GTP. No protection was observed in the absence of GTP (data not shown). These results agree with that obtained by gel filtration chromatography and indicate that a ternary complex was formed between EF-Tu-Tsmt, GTP, and aminoacyl-tRNA. This ternary complex could also be detected by a nitrocellulose filter binding assay (Table I). The complex appeared to be quite labile or poorly retained by the filter, and only about 10% of the complex predicted to be present could be measured by this procedure. The use of [14C]Phe and [3H]Phe-tRNA in this assay again indicated that some Phe-tRNA was present in the complex at a 1:1 ratio. Studies on the amount of GTP required to confer protection of Phe-tRNA from hydrolysis indicated that stoichiometric levels of radioactivity could be observed. The first represented [14C]Phe-tRNA while the second represented free [14C]Phe resulting from the deacylation of the Phe-tRNA. No detectable [14C]Phe-tRNA eluted with the EF-Tu-Tsmt complex whose position was monitored by the elution position of an internal BSA marker (5). However, when GTP was added to the incubation (Fig. 2B), a significant amount of [14C]Phe-tRNA was found associated with the mitochondrial elongation factor complex. This observation indicates that EF-Tu-Tsmt is capable of interacting with aminoacyl-tRNA and that this interaction requires the presence of GTP. In this respect, the mitochondrial factor appears to be equivalent to E. coli EF-Tu. When [3H]GTP was incubated with [14C]Phe-tRNA and EF-Tu-Tsmt, [3H]GTP was found associated with the [14C]Phe-tRNA-EF-Tu-Tsmt complex (Fig. 2C). An analysis of the amount of 14C and 3H present in the ternary complex indicates

FIG. 2. Gel filtration analysis of aminoacyl-tRNA binding to EF-Tu-Tsmt. Panel A, incubation mixtures containing EF-Tu-Tsmt and E. coli [14C]Phe-tRNA were prepared without GTP as described under "Experimental Procedures" and were analyzed by G-75 gel filtration chromatography. The profile for BSA was used to indicate the elution position of EF-Tu-Tsmt and was determined by the Coomassie dye absorbance profile (---- - - ). The presence of [14C]Phe and [14C]Phe-tRNA (●) was determined by scintillation counting. Panel B, incubation mixtures contained EF-Tu-Tsmt, [3H]Phe-tRNA, and GTP and were analyzed as indicated under "Experimental Procedures." The profile for BSA which indicates the elution position of EF-Tu-Tsmt was determined by the Coomassie dye absorbance profile (−−−−), and the presence of [14C]Phe and [14C]Phe-tRNA (●) was determined by scintillation counting. Panel C, reaction mixtures containing EF-Tu-Tsmt, E. coli [14C]Phe-tRNA, and [3H]GTP were incubated to allow ternary complex formation and then subjected to chromatography on Sephadex G-75. The profile for BSA which indicates the elution position of EF-Tu-Tsmt was determined by the Coomassie dye absorbance profile (−−−−) and the presence of [14C]Phe and [14C]Phe-tRNA (●) was determined by scintillation counting.

FIG. 3. Selective ability of EF-Tu-Tsmt to protect elongator aminoacyl-tRNA from hydrolysis. The hydrolysis protection analysis was conducted as described under "Experimental Procedures." Panel A, E. coli [14C]Phe-tRNA and GTP are incubated in the absence (○) or presence (●) of EF-Tu-EF-Tsmt. Aliquots (5 μl) were removed at the indicated times and the amount of [14C]Phe-tRNA remaining determined. Panel B, yeast [35S]Met-tRNA Met and GTP were incubated in the absence (○) or presence (●) of EF-Tu-Tsmt. Aliquots (5 μl) were removed at the indicated times and the amount of charged tRNA determined as indicated under "Experimental Procedures."
the nucleotide were sufficient to provide nearly maximal protection (data not shown). This observation suggests that the ternary complex formed quite readily under the conditions used.

The interaction of E. coli EF-Tu with aminoacyl-tRNA requires the presence of GTP and the EF-Tu·GDP form of the factor is inactive in this interaction. Surprisingly, we have observed that EF-Tu·Ts, could interact with Phe-tRNA in the presence of high concentrations of GDP as indicated by the hydrolysis protection assay (Fig. 4A). However, the amount of protection observed was less than that obtained with GTP, and the level of GDP required appeared to be at least two orders of magnitude greater than the amount of GTP required. The non-hydrolyzable GTP analog, GMP-PNP, also appeared to be able to promote the interaction between EF-Tu·Ts, and Phe-tRNA (Fig. 4B). The formation of this complex required high levels of the nucleotide analog. One explanation for these observations may be that the ternary complexes formed in the presence of GDP or GMP-PNP are significantly less stable than that formed with GTP and require high nucleotide concentrations to promote their formation. Alternatively, the aminoacyl-tRNA present in the GDP or GMP-PNP complexes could remain somewhat susceptible to attack by solvent resulting in less protection of the aminoacyl-tRNA bond by EF-Tu·Ts, under these conditions (14).

E. coli EF-Tu is specific for elongator tRNAs and shows a significantly lower affinity for fMet-tRNA (14). We have tested the ability of EF-Tu·Ts, to differentiate the initiator tRNA from elongator tRNAs by examining the ability of this factor to protect fMet-tRNA from nonenzymatic hydrolysis. As indicated in Fig. 3B, no protection of fMet-tRNA from hydrolysis could be detected. The results obtained by the hydrolysis protection assay were also observed using the nitrocellulose filter binding assay for ternary complex formation (Table I) and again indicated that no ternary complex could be detected when fMet-tRNA was used in place of Phe-tRNA. These results indicate that the mitochondrial elongation factor, like bacterial EF-Tu, has the ability to discriminate against fMet-tRNA.

### Table I

**Detection of ternary complex formation by nitrocellulose filter binding**

| Aminoacyl-tRNA | (GTP·EF-Tu·Ts,·aa-tRNA) | [3H]GTP |
|----------------|--------------------------|---------|
| [3H]Phe-tRNA (E. coli) | 1.3 pmol | 1.1 pmol |
| [35S]fMet-tRNA<sup>Met</sup> (yeast) | 0.02 pmol | ND<sup>a</sup> |

<sup>a</sup> Value was not determined.

**Fig. 4.** The effects of GDP and GMP-PNP on the protection of Phe-tRNA from hydrolysis. All experiments were conducted as described under “Experimental Procedures.” Panel A, samples were incubated in the absence of any guanine nucleotides (●) or in the presence of 0.1 mM GDP (■), 1.0 mM GDP (▲), or 0.1 mM GTP (○). Panel B, samples were incubated in the absence of guanine nucleotides (●), or in the presence of either 0.2 mM GMP-PNP (■), or 0.1 mM GTP (○).
In order to determine whether the formation of the ternary complex confers a kinetic advantage for ribosome binding, we have tested the rate of ribosome binding in the presence and absence of the preformed ternary complex. The ternary complex was formed by a 5-min incubation using the conditions described under "Experimental Procedures." Ribosome binding was then carried out at 27°C for 10 min using the preformed ternary complex or without allowing the complex to form prior to the ribosome binding reaction.

**DISCUSSION**

Previous studies with EF-Tu-Ts have shown that this organellar complex is not readily dissociated by guanine nucleotides. Furthermore, in the absence of aminocyl-tRNA, no guanine nucleotide binding to the mitochondrial complex can be detected. These results indicate that there are distinct differences in the intermediates that can be observed in the elongation cycles found in mammalian mitochondria and in the prokaryotic and eukaryotic cytoplasmic systems. Elaboration of the "roles" of the intermediate complexes formed during the elongation cycle in mammalian mitochondria and the sequence of intermediates formed will be required for a clear understanding of this translational system.

The role of E. coli EF-Tu in translation is to facilitate aminocyl-tRNA binding to the ribosomal A-site. This binding occurs in a guanine nucleotide-dependent manner and is

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**Fig. 5.** The effect of GTP addition following Phe-tRNA binding to ribosomes by EF-Tu-Ts. Ribosome binding in the presence of EF-Tu-Ts was carried out in the absence of guanine nucleotides (○) or in the presence of 400 μM GMP-PNP (●) for the indicated times, and the amount of [14C]Phe-tRNA bound to the ribosome was determined. GTP (400 μM) was added after 10 min as indicated by the arrow on the figure, and the incubation continued for the indicated times followed by an analysis of the amount of Phe-tRNA bound to the ribosome.

**Fig. 6.** Inhibition of the catalytic use of EF-Tu-Ts in ribosome binding by GDP. Reaction mixtures (100 μl) contained 1.0 pmol of EF-Tu-Ts, 33 μg of E. coli ribosomes, 11 μg of deacylated E. coli tRNA, 1.0 μM GTP, and increasing levels of GDP as indicated. Incubation was for 10 min at 37°C. No pyruvate kinase or PEP were present in the reaction mixtures.

**Fig. 7.** Kinetics of ribosome binding in the presence and absence of a preformed ternary complex. The kinetics of ribosome binding were examined with and without the use of a performed ternary complex. The ternary complex was formed by a 5-min incubation using the conditions described under "Experimental Procedures." Ribosome binding was then carried out at 27°C for 10 min using the preformed ternary complex (○) or without allowing the complex to form prior to the ribosome binding reaction (●).
catalytic in the presence of GTP (15). Generally, it has been thought that the cytoplasmic factor EF-1 functions in a manner analogous to that of the prokaryotic factor forming a ternary complex which then promotes aminoacyl-tRNA binding to the A-site (16). However, there are indications that calf brain EF-1α can promote the partial binding of aminoacyl-tRNA to the ribosome in the absence of guanine nucleotides (17). Interestingly, it appears that the initial binding of aminoacyl-tRNA to ribosomes facilitated by the mitochondrial complex is not dependent on the presence of guanine nucleotides and that EF-Tu.Tsmt alone is capable of promoting one round of aminoacyl-tRNA binding to the ribosomal A-site. However, in the absence of GTP, the binding activity is not catalytic indicating that EF-Tu.Tsmt will bind aminoacyl-tRNA to the ribosome but will not recycle in the absence of GTP. The lack of a guanine nucleotide dependence for a single binding event distinguishes EF-Tu.Tsmt from the corresponding prokaryotic factor.

The ternary complex is a well-established intermediate in the prokaryotic translational system (2). It appears that this complex can also be formed by the eukaryotic cytoplasmic system (16). However, it has been difficult to determine whether it actually plays a role in the elongation cycle in eukaryotes (18). In the present work, we have shown that EF-Tu.Tsmt can participate in ternary complex formation using a variety of techniques including gel filtration chromatography and hydrolysis protection assays. In this respect the mitochondrial factor appears to be similar to E. coli EF-Tu.

The role of EF-Tsmt in the formation of the ternary complex is unclear. It is possible that EF-Tsmt is present in this complex, since no free EF-Tsmt could be detected by gel filtration chromatography following GTP and [3H]Phe-tRNA binding to the factor (data not shown). Attempts to radiolabel the EF-Ts component so that its presence could be analyzed directly have not yet been successful, due to the lability of the mitochondrial elongation factors (5). As with the eukaryotic cytoplasmic system, it is difficult to determine whether the ternary complex is an intermediate in the elongation cycle in animal mitochondria. The presence of the ternary complex does not confer any kinetic advantage and may actually reduce the rate of the first round of ribosome binding observed (Fig. 7).

These observations lead us to suggest that a variation of the prokaryotic elongation cycle may be occurring in animal mitochondria (Fig. 9A). In our suggested model, EF-Tu.Tsmt first interacts with the ribosome (Step 1) where it promotes the binding of a stoichiometric amount of aminoacyl-tRNA (Step 2). In the absence of GTP, the EF-Tu.Tsmt remains ribosome-associated. However, upon GTP binding (Step 3), EF-Tu catalyzes GTP hydrolysis, and the EF-Tu.Tsmt complex is released from the ribosome (Step 4). Direct nucleotide
binding to this complex is weak (presumably because of the strong interaction between the EF-Tu and EF-Ts components), and the complex is free of bound GDP at the time of, or shortly after, its release from the ribosome. In the admittedly speculative model presented in Fig. 9A, the ternary complex does not play an actual role in the elongation cycle. Rather, it represents a side reaction that can occur with either GTP or GDP and is not a direct part of the elongation pathway in the mitochondrial system. This idea is in agreement with the lack of a kinetic advantage in ribosome binding for aminoacyl-tRNA present in the mitochondrial ternary complex (Fig. 7).

It is also reasonable to suggest that the mitochondrial system has two acceptable routes for aminoacyl-tRNA binding to the A-site. In the first route (Fig. 9A), EF-Tu-Ts

may bind to the ribosome prior to GTP binding and carry out the sequence of events indicated in Steps 1 through 4 described above. In the alternative route (Fig. 9B), the ternary complex may interact directly with the ribosome in a manner analogous to that observed in the bacterial system. However, it should be noted that no intermediates equivalent to EF-Tu-GDP or EF-Tu-GTP can be observed in this system. The presence or absence of EF-Ts in the various complexes indicated here will require further study.

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REFERENCES
1. Ravel, J., Shorey, R., Froehner, S., and Shive, W. (1968) Arch. Biochem. Biophys. 125, 514–526
2. Miller, D., and Weissbach, H. (1977) in Molecular Mechanisms of Protein Biosynthesis (Weissbach, H., and Pestka, S., eds) pp. 324–373, Academic Press, New York
3. Motoyoshi, K., Iwasaki, K., and Kaziro, Y. (1977) J. Biochem. (Tokyo) 82, 145–155
4. Nagata, S., Motoyoshi, K., and Iwasaki, K. (1978) J. Biochem. (Tokyo) 83, 423–429
5. Schwartzbach, C. J., and Spremulli, L. L. (1989) J. Biol. Chem. 264, 19125–19131
6. Ravel, J., and Shorey, R. (1971) Methods Enzymol. 20C, 306–316
7. Muench, K., and Berg, P. (1966) in Procedures in Nucleic Acid Research (Cantoni, G., and Davies, D., eds) p. 375, Harper and Row, New York
8. Graves, M., and Spremulli, L. (1983) Arch. Biochem. Biophys. 222, 192–199
9. Graves, M., Breitenberger, C., and Spremulli, L. (1980) Arch. Biochem. Biophys. 204, 444–454
10. Eberly, S., Locklear, V., and Spremulli, L. (1985) J. Biol. Chem. 260, 8721–8725
11. Sedmak, J., and Grossberg, S. (1977) Anal. Biochem. 79, 544–552
12. Bearden, J. C., Jr. (1978) Biochim. Biophys. Acta. 533, 525–529
13. Pingoud, A., Urbaneke, C., Krauss, G., Peters, F., and Maass, G. (1977) Eur. J. Biochem. 78, 403–409
14. Janiak, F., Dell, A., Abrahamson, J., Watson, B., Miller, D., and Johnson, A. (1990) Biochemistry 29, 4268–4277
15. Hershey, J. (1987) in Escherichia coli and Salmonella typhimurium (Neidhardt, F., ed) pp. 613–647, American Society for Microbiology, Washington, D. C.
16. Moldave, K. (1984) Annu. Rev. Biochem. 54, 1109–1149
17. Crechet, J-B., and Parmeggiani, A. (1986) Eur. J. Biochem. 161, 647–653
18. Carvalho, M., Carvalho, J., and Merrick, W. (1984) Arch. Biochem. Biophys. 234, 603–611