Ribosome Binding to Mitochondria Is Regulated by GTP and the Transit Peptide*

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The association between ribosomes and the pore proteins at the endoplasmic reticulum membrane is important to co-translational translocation. To determine if a similar association occurs between the ribosome and mitochondrial membrane protein(s) during protein import in higher eukaryotes, we examined ribosome-mitochondria binding. By using spectral measurements, analysis of mitochondrial associated RNA, and electron microscopy, we demonstrated that ribosomes stably bind to purified rat liver mitochondria in vitro. Binding of ribosomes to mitochondria was markedly reduced by GTP and nearly abolished by the non-hydrolyzable GTP analogue, guanosine-5'-[thio]-triphosphate (GTP\(\gamma\)S), but was only modestly reduced by GDP or ATP and unaffected by GTP. The initial rate of GTP hydrolysis by mitochondria was increased by ribosomes, whereas the rate of ATP hydrolysis by mitochondria was not affected. Ribosomes programmed with mRNA for 92 amino acids of the N terminus of mitochondrial malate dehydrogenase bound to mitochondria, but unlike unprogrammed rat liver ribosomes, neither GTP nor GDP disrupted binding; however, GTP\(\gamma\)S did. These data show that receptors specific for ribosomes are present on the mitochondrial membrane, and a GTP-dependent process mediates this binding. The presence of a nascent chain alters these binding characteristics. These findings support the hypothesis that a co-translational translocation pathway exists for import of proteins into mitochondria.

Proteins can cross membranes by either post-translational or co-translational translocation. Based on in vitro observations, proteins targeted to the mitochondrion are thought to be completely synthesized in the cytosol and cross the mitochondrial membrane(s) post-translationally (1). However, evidence consistent with a co-translational translocation pathway for mitochondrial protein import has been reported. For example, 1) the surface of mitochondria isolated from cycloheximide-treated yeast cells is observed to be studded with polysomes (2); 2) the number of bound polysomes is dependent on the metabolic state of the cells from which the mitochondria are isolated (3); 3) ribosomes are bound to the mitochondria at the contact sites (4); and 4) the mRNA of polysomes that co-isolate with mitochondria is enriched in messages for mitochondrial proteins (5, 6). In addition, both in vitro and in vivo, mitochondrial protein import can be instantaneously disrupted by cycloheximide-induced translation arrest indicating that no detectable pool of full-length precursor exists in the cytosol and that translation and import are tightly coupled (7–9). Furthermore, methotrexate, which inhibits post-translational import of cytochrome oxidase subunit IV-dihydrofolate reductase by preventing its unfolding, does not inhibit cytochrome oxidase subunit IV-dihydrofolate reductase import in vivo (9). This indicates that co-translational translocation may eliminate the need for precursor proteins to be maintained in a translocation-competent state by chaperones in the cytoplasm.

Almost all mitochondrial matrix and inner membrane proteins are synthesized with an N-terminal presequence and then imported into the mitochondria (10–12). The presequence-containing precursor protein is presumably held in a translocation-competent conformation in the cytoplasm by hsp70 chaperonins probably in concert with the presequence-specific chaperonin, e.g. “targeting factor” or mitochondrial stimulating factor, and the precursor is recognized and imported by a multisubunit translocation complex in the mitochondrial membranes (13–18). Since these early events in targeting and translocation clearly and specifically involve the N-terminal presequence, it is entirely possible that mitochondrial protein import can be initiated long before translation is complete.

To examine the hypothesis that, in vivo, proteins import into the mitochondria by a co-translational translocation pathway, and to determine whether receptor(s) for the translation machinery at the outer membrane play a role in targeting an incompletely synthesized nascent polypeptide chain to the mitochondrial import site, we examined the interaction of the ribosome with the mitochondrion. Specifically, ribosome binding to mitochondria was shown using three independent methods, and the ability of ribosome-bound nascent polypeptide chains to target the ribosome to the mitochondria was determined. Finally, because recent studies have shown that GTP-binding proteins are not only involved in initiating protein translocation at the membrane of the endoplasmic reticulum (ER), but are also involved in protein import into chloroplast (19), we determined the effect of ATP and GTP on ribosome binding to mitochondria and the early events in protein translocation.

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1 The abbreviations used are: ER, endoplasmic reticulum; RER, rough endoplasmic reticulum; GTP\(\gamma\)S, guanosine-5'-[thio]-triphosphate; EKMT, EDTA, high salt-washed mitochondria; EKMPMT, EDTA/high salt, metrizamide/Percoll-purified mitochondria; mMDH, mitochondrial malate dehydrogenase; SRP, signal recognition particle; GNRP, guanine nucleotide release or exchange protein.
To verify that absorbance changes of the resuspended mitochondrial pellets reflected differential ribosomal binding, 400–450 μg of mitochondria were incubated with approximately 75 μg of ribosomes in 200 μl of RB/MIB (1:3) for 5 min at room temperature. The ribosome–mitochondria complexes were divided into 30-μl aliquots, sedimented (10,000 × g, 5 min, 4°C), and resuspended in 30 μl of RB/MIB (1:3) containing either 0.1 or 5 mM ATP, GDP, GTP, GTP-S, CTP, or no nucleotide. Samples were again incubated at room temperature for 5 min and sedimented through an equal volume of 1.0 M sucrose cushion (10,000 × g, 5 min, 4°C). Pellets and supernatants were extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and the RNA ethanol-precipitated. The RNA in the resuspended RNA was subjected to both agarose gel electrophoresis and dot blot analysis, using a [32P]dCTP-radiolabeled 300-nucleotide cDNA probe that had been amplified by polymerase chain reaction using cytoplasmic 18 S rRNA as a template (GenBankTM accession number V01270). Sense primer, 5'-TACATGCGAGGGCGC-GCTGACC-3'; antisense primer, 5'-CCTGTCGCCCTTCCTGATG-3'. The 18 S probe was tested by Northern blot analysis to confirm that the RNA visualized by ethidium bromide staining corresponded to cytosolic 18 S rRNA and not mitochondrial RNA.

Electron Microscopy—Samples were prepared for electron microscopy by adding 100 μl of mitochondria or ribosome-mitochondria complexes (approximately 7.5 μg of mitochondrial protein/μl) to 1 ml of fixative containing 4% glutaraldehyde, 100 mM sucrose, 100 mM cacodylate, pH 7.4 (glutaraldehyde/sucrose/cacodylate buffer). Fixed samples were washed with 100 μl sucrose, 100 mM cacodylate buffer, pH 7.4, postfixed in 1% OsO4, and dehydrated in graded ethanols. Samples were embedded in Spurr’s resin (29), thin sectioned, and examined using a Philips 400 TEM operating at 80 kV (Micromed, Wake Forest University School of Medicine). Ten randomly selected fields for mitochondria under each condition were photographed at a magnification of ×15,200, and the number of ribosomes per field was counted by hand. To ensure that each field had the same amount of mitochondrial surface area, point count stereology was used to quantify the relative surface areas of end face versus cross-sectional views of mitochondria (30). Between all fields in all conditions, this ratio was very constant at 0.31 to 0.33, and thus, absolute numbers of ribosomes are reported for comparison between fields. For each condition, the fields with the highest and lowest counts of ribosomes were discarded, and the average and standard deviation of the remaining eight fields are reported.

RESULTS

Ribosomes Bind to Mitochondria—Rat liver mitochondria prepared by differential centrifugation have been shown to contain other organelles including a significant amount of rough endoplasmic reticulum-derived microsomes (26). In order to examine only the ribosome–mitochondria interaction, crude mitochondrial preparations were either subjected to EDTA and high salt treatment (24), purification by density gradient centrifugation (23), or both. All preparations were analyzed for activity of the RER-specific enzymes, glucose-6-phosphatase, and arylesterase. Based on enzyme activity, either density gradient centrifugation (MPMT) or EDTA/high salt treatment of mitochondria (EKMT) reduced the amount of RER contamination by about 75%, whereas density gradient centrifugation followed by EDTA/high salt treatment (EK-MPMT) essentially eliminated RER contamination (Fig. 1).

Prior to conducting ribosome-mitochondria binding experiments, cytosolic rat liver ribosomes were washed in high salt to remove endogenous GTPase activity and were tested for inorganic phosphate production from GTP or ATP. The initial rate of GTP hydrolysis by ribosomes was reduced approximately 80% by high salt treatment (Table I). Ribosomes preparations were also analyzed for RER enzyme markers and were shown to be essentially free of RER contamination (Fig. 1).

High salt-washed ribosomes were incubated with EDTA/high salt-washed, density gradient purified mitochondria (EK-MPMT); the ribosome-mitochondria complexes were separated from free ribosomes by sedimentation, and the A260 of resuspended complexes and/or the free ribosomes remaining in the supernatant were measured. The difference in the A260 between resuspended mitochondrial incubated without ribosomes
and mitochondria incubated with ribosomes was taken to represent ribosome binding. As shown in Fig. 2, ribosomes bind to both freshly isolated and previously frozen mitochondria. The number of ribosomes bound per mg of mitochondria is much higher in the previously frozen samples, presumably because the mitochondrial contents leak out of the mitochondria upon

**Figure 1. Enzyme activity.** Glucose-6-phosphatase (B) and arylerase (A) activities were determined on preparations of endoplasmic reticulum, ribosomes, and mitochondria. Each solid bar represents the mean of at least three separate experiments. Error bars represent the standard deviation. MT, mitochondria from differential centrifugation; MPMT, metrizamide/Percoll-purified mitochondria; EKMT, EDTA/high potassium salt washed mitochondria; EKMPMT, EDTA/high-salt, metrizamide/Percoll-purified mitochondria; RER, rough endoplasmic reticulum.

**Table 1.** Phosphate production

Each value listed represents the mean ± S.D. from at least three experiments. Each assay included 150 μg of EDTA/high salt-washed mitochondria, 5 μg of ribosomes, and 1.2 mM nucleotides.

| Sample                      | 5 min | 10 min | $A_{260}/A_{230}^a$ |
|-----------------------------|-------|--------|-------------------|
| MT                          |       |        |                   |
| MPMT                        |       |        |                   |
| EKMT                        |       |        |                   |
| EKMPMT                      |       |        |                   |
| RER                         |       |        |                   |
| Ribosomes                    |       |        |                   |
| High salt-washed ribosomes  | 4.4 ± 0.7 | 6.3 ± 0.7 | 1.76 ± 0.04 |
| Mitochondria                | 2.9 ± 0.4 | 3.7 ± 1.1 |                   |
| High salt-washed ribosomes + mitochondria | 11.7 ± 2.0 | 15.9 ± 0.9 | 1.09 ± 0.02 |
| Ribosomes + ATP             | 14.0 ± 0.3 | 17.7 ± 0.8 | 1.15 ± 0.02 |
| High salt-washed ribosomes + ATP | 3.3 ± 0.9 | 4.9 ± 1.1 |                   |
| Mitochondria + ATP          | 0.7 ± 0.2 | 0.9 ± 1.1 |                   |
| High salt-washed ribosomes + mitochondria + ATP | 9.6 ± 0.6 | 15.6 ± 1.3 | 1.08 ± 0.02 |
| Ribosomes + GTP             | 18.9 ± 1.0 | 20.9 ± 0.9 | 1.09 ± 0.02 |
| High salt-washed ribosomes + GTP | 0.5 ± 0.5 | 1.3 ± 0.3 |                   |
| Mitochondria + GTP$_5$S     | 3.5 ± 0.5 | 3.5 ± 0.5 |                   |
| High salt-washed ribosomes + mitochondria + GTP$_5$S |        |        |                   |

$^a$ Measurements were taken for samples incubated at room temperature for 10 min.
freeze-thawing. Freshly isolated mitochondria will bind approximately one ribosome per 1.7 fg of mitochondrial protein when saturated with ribosomes (Fig. 2).

Because ATP is required for post-translational import of proteins into the mitochondria and because GTP is required for co-translational translocation of protein into the ER, we hypothesized that nucleotides would alter ribosome binding to mitochondria. Formation of ribosome-mitochondria complexes was measured as before except samples were initially incubated with or without ATP, GTP, GDP, GTP-γS, or CTP. A differential increase in the A260/A280 ratio (Table I) was observed when resuspended mitochondria had been incubated with ribosomes compared to when resuspended mitochondria had been incubated alone. This indicates that the ribonucleic acid-containing ribosomes associate with mitochondria, and the increase in the A260 was arbitrarily set to represent 100% ribosome binding (Fig. 3, lane MR). Similar increases in the A260 and A280 were observed when 1.2 mM ATP was included in binding assay (Fig. 3, lane MRATP). However, when 1.2 mM GTP or GTP-γS was included, only minimal increases in absorbance were observed (Fig. 3, lanes MRGTP and MRGTPS). Inclusion of GDP or ATP in the binding assay resulted in A260 increases that were somewhat lower than values obtained in the absence of nucleotide but were also significantly higher than GTP and GTP-γS. This indicates that the association between ribosomes and mitochondria can be blocked by GTP or GTP-γS but is only mildly affected by ATP or GDP. Under the conditions used here, it is unclear if the effect of ATP on binding is direct or a result of conversion of ATP to GTP from existing GDP. However, the dramatic effects of GTP or GTP-γS on complex formation was statistically different from complex formation under all other conditions tested indicating that GTP plays a dominant role in controlling the ribosome-mitochondria association.

To confirm that the increases in the A260 and the A280 for mitochondria incubated with ribosomes were due to ribosome-mitochondria binding, ribosome-mitochondria complexes were pre-formed by incubation of EKMPMT with high salt-washed ribosomes in the absence of nucleotides. The pre-formed complexes were isolated by sedimentation, and the pellets were resuspended in RB/MIB containing either no nucleotide, GTP, GDP, GTP-γS, or ATP at a concentration of either 0.1 or 5 mM.

Complexes were re-isolated by sedimentation, and to assess whether ribosomes remained bound under each condition, the relative amount of rRNA in the supernatants and pellets was determined by subjecting the extracted samples to agarose gel electrophoresis followed by ethidium bromide staining or by dot blot analysis (Fig. 4). Ribosomal RNA extracted from the pellet represents the stably bound ribosomes, whereas rRNA in the supernatant represents ribosomes that were released from the mitochondria.

When no nucleotide was added to the pre-formed complexes, nearly all rRNA re-sedimented with the mitochondria (Fig. 4, A and B, lane –NTP). Again, this indicates that the ribosomes remained stably bound to mitochondria in the absence of nucleotide. As expected, pellets obtained from pre-formed ribosome-mitochondria complexes incubated with GTP, GDP, or GTP-γS contained less rRNA demonstrating that guanine nucleotides selectively disrupt binding (Fig. 4A, lanes GTP, GDP, and GTP-γS). ATP did not significantly disrupt binding under these conditions, and the effect of GDP on binding was considerably less than GTP. These differences were observed even at low concentration (0.1 mM) of GTP (data not shown). Samples containing mitochondria alone were treated exactly as the ribosome-mitochondria complexes and subjected to dot blot analysis in parallel, but no radioactivity was detectable for these samples. In agreement with spectral data, GTP and GTP-γS have the greatest effect on ribosome-mitochondria complex stability. These data show that 1) the spectroscopic absorbance changes correlate well with changes in ribosome binding, and 2) the ribosome-mitochondria interaction is not the result of nonspecific binding since, when ribosome-mitochondria complexes are pre-formed, this interaction can be specifically reversed by GTP or GTP-γS.

Finally, to confirm that the spectral data and dot blot analysis measured ribosome-mitochondria binding and not another
Ribosomes Bind to Mitochondria

**Electron micrographs of mitochondria and ribosome-mitochondria complexes.** Panel A, endogenous rat liver ribosomes (arrow) remain bound to mitochondria after freezing and thawing. Panel B, however, mitochondria are essentially free of ribosomes after EDTA/high-salt treatment (EKMT). Panel C shows that when total ribosomes, isolated from rat liver, are added back to EKMT, the ribosomes (arrow) are again seen on the mitochondrial membranes. Finally, in panel D, addition of GTP to these complexes eliminates almost all ribosomes from the mitochondrial surface. The bar represents 1 μm. B, mitochondria isolated by differential centrifugation (lane A); EKMT, mitochondria that have been EDTA/high-salt-washed (lane B); R-EKM, ribosomes added back to EKMT (lane C); R-EKM-G, GTP added to R-EKM (lane D).

**Fig. 5.** GTP destabilizes ribosome-mitochondrial binding. EDTA/high salt-washed mitochondria (EKMT) (approximately 450 μg) were incubated alone or with ribosomes (75 μg) to form mitochondria-ribosome complexes. The 30 μl of mitochondria and mitochondria-ribosome complexes were sedimented and resuspended in RB/MIB (50 mM Tris, pH 7.4, 100 mM KOAc, 10 mM MgCl2, 10 mM Tris, pH 7.4, 70 mM mannitol, and 250 mM sucrose; 1:3) alone, or RB/MIB containing GTP, GDP, GTPγS, or ATP. After 5 min at room temperature, mitochondria and complexes were re-sedimented, and the rRNA was extracted from supernatants and pellets and was subjected to either 1% agarose gel electrophoresis or dot blot analysis. A, the rRNAs were visualized by ethidium bromide staining. Shown are the 28 S and 18 S rRNA remaining with mitochondrial pellets when no nucleotide was incubated (−NTP) or when 5 mM GTP, GDP, GTPγS, or ATP, respectively, was incubated with pre-formed mitochondria-ribosome complexes for 5 min. B, supernatants from samples corresponding to those in A were subjected to RNA dot blot analysis to quantify and confirm that differences in mitochondrial-associated rRNA were the result of ribosome release and not degradation. The dot blot was probed with [32P]dCTP-labeled cDNA for a portion of the 18 S rRNA. Dots were quantified using an AMBIS radiometric counter, and the cpm for each condition were plotted.

Panels B–D show the number of ribosomes bound expressed as a percentage of the number of ribosomes added and observed differences in ribosome binding, we counted the number of ribosomes binding to mitochondria from 10 random fields of all four conditions. Fig. 5B, lanes A and B, demonstrates that the observed differences in ribosome binding from Fig. 5A is real and that there is a statistically significant difference between panels C and D. Thus, these data show that ribosomes co-isolating with mitochondria remain bound to the mitochondrial surface even after freeze-thawing but that these ribosomes can be completely removed by EDTA/high salt treatment. EDTA/high-salt treatment, however, does not cause mitochondria to lose their ability to bind ribosomes (panel C). Finally, GTP is very effective at removing ribosomes from the mitochondrial surface and clearly disrupts the ribosome-mitochondrial interaction (panel D).

In summary, we have used three different experimental approaches to independently prove that ribosomes specifically bind to mitochondria. These data also show that the interaction is affected by GTP and is reversible, providing strong evidence...
that this interaction is physiologically significant.

**The Ribosome-Mitochondria Interaction Affects GTP Hydrolysis**—Because the presence of nucleotide had a marked effect on ribosome-mitochondria binding, we hypothesized that nucleotide hydrolysis was associated with this binding. The amount of inorganic phosphate (P$_i$) produced on the cytosolic side of isolated mitochondria was quantified in the absence and presence of ribosomes and nucleotides (Table I). Following sedimentation of the mitochondria, essentially no P$_i$ was detected in the supernatants from EKMT, high salt-washed ribosomes, or ribosomes and EKMT together, when incubated without a trinucleotide. In addition, very little P$_i$ was produced when EKMT, ribosomes, or ribosomes and EKMT together were incubated with GTP$_3$S (Table I). In contrast, Fig. 6 shows the curve for P$_i$ produced from GTP by EKMPMT, in the absence and presence of ribosomes. The initial rate of hydrolysis of GTP by mitochondria alone was 20 nmol/min/mg but was much higher at 85 nmol/min/mg for mitochondria and ribosomes together. Since ribosomes had no GTPase activity, acceleration of P$_i$ production by mitochondria in the presence of ribosomes is due to the interaction between mitochondria and ribosomes increasing the rate of GTP hydrolysis. The rate of P$_i$ production in the presence of ATP and, therefore, the rate of ATP hydrolysis by mitochondria in the presence of ribosomes, is approximately equal to the sum of the individual rates of P$_i$ production by mitochondria and ribosomes alone indicating that the ribosome-mitochondria interaction does not affect the rate of ATP hydrolysis (Table I). Thus, the ribosome-mitochondrial interaction stimulates GTP hydrolysis which suggests this is a specific receptor-ligand interaction that is controlled by a GTP-dependent step.

**Interaction of Programmed Ribosomes with Mitochondria**—The presence of ribosome-binding sites on the mitochondria suggested that ribosome-mitochondrial binding might be functionally important in co-translational translocation. At physiologic concentrations of GTP, however, ribosomes isolated from the post-mitochondrial cytosol did not stably bind to mitochondria. Since the transit peptide is responsible for targeting a mitochondrial protein in the post-translational import pathway, we proposed that a nascent chain containing a mitochondrial transit peptide would be capable of directing the ribosome to the mitochondria and stabilize binding in the presence of GTP, whereas ribosomes carrying non-mitochondrial nascent chains would behave like unprogrammed ribosomes in the presence of GTP.

Ribosomes protect about 40 of the most recently added C-terminal amino acids of the growing polypeptide chain from macromolecules of the cytosol but allow exposure of the N-terminal end of the nascent polypeptide to the cytosol prior to complete translation (31). A truncated mRNA lacking a stop codon and containing the coding region of the N-terminal end of precursor mMDH was designed to minimize exposure of the mature portion of mMDH while allowing complete exposure of the transit peptide to the cytosol (32). The mRNA for this 92-amino acid polypeptide was translated using $[^{35}S]$methionine in rabbit reticulocyte lysate, and the ribosome-nascent chain complexes were isolated by sedimentation, and the complexes were assayed for EKMT binding activity in the absence or presence of di- or trinucleotides. An 86-amino acid nascent luciferase polypeptide was used as a non-mitochondrial, targeted protein control and was produced similarly to the mMDH precursor.

The results of these experiments are summarized in Fig. 7 and show that when the ribosomes carry a mitochondrial transit peptide-containing nascent chain, the presence of GTP does not significantly reduce the amount of ribosome-nascent chain complexes bound to the mitochondria compared with the amount of ribosome-nascent chain complexes bound when no nucleotide is present (Fig. 7A). Similarly, when GDP was included, the number of ribosome-nascent chain complexes bound to mitochondria was not significantly different from the number of complexes bound in the absence of nucleotide. However, the presence of GTP$_3$S greatly reduced the formation of stable ribosome-mitochondria complexes. These results differ from our earlier findings using unprogrammed ribosomes that showed that GTP caused a significant reduction in stable ribosome-mitochondria complex formation. In addition, the ribosomes carrying the non-mitochondrial luciferase nascent chains behave like unprogrammed ribosomes (Fig. 7B) and do not stably bind to mitochondria in the presence of either GTP or GTP$_3$S. Thus, the presence of the transit peptide blocks GTP-dependent release of the ribosome from the mitochondria and allows stable formation of a ribosome-nascent chain-mitochondrial complex.

These experiments show that the transit peptide causes selective stabilization of ribosome-mitochondria binding in the presence of GTP and suggest a mechanism for selective import in the presence of a nonselective ribosome receptor; unprogrammed ribosomes and ribosomes programmed with non-mitochondrial proteins are not stably bound to the mitochondrial membrane. Additionally, it is clear that in the presence of ribosomes, the transit peptide alone is not capable of directing the nascent polypeptide to the mitochondria since the non-hydrolyzable GTP$_3$S destabilizes this binding. A GTP-dependent process, therefore, must function in ribosome binding to mitochondria.

**DISCUSSION**

In this report we have demonstrated that ribosomes bind specifically to rat liver mitochondria and that both GTP and the nature of the nascent chain attached to the ribosome affect binding. Specifically, GTP significantly attenuated stable ribosome-mitochondria binding unless mitochondrial transit peptide-containing translation intermediates were complexed with the ribosomes. In addition, a nonhydrolyzable analog of GTP almost completely abolished the formation of stable ribosome-mitochondria complexes whether or not ribosomes carried presequence-containing nascent chains. We also demonstrated that whereas isolated intact mitochondria hydrolyze GTP, hydrolysis of GTP was greatly accelerated when GTPase-depleted
ribosomes were added to the reaction.

This report supports, but does not prove, the hypothesis of co-translational mitochondrial import by establishing a central element in this model, namely ribosomes selectively and stably bind to mitochondria. Our data suggest that the interaction of ribosomes with the mitochondria could use components and proceed by a pathway, similar to those used to initiate co-translational mitochondrial import by establishing a central element in this model, namely ribosomes selectively and stably bind to mitochondria. The first 92 amino acids of mMDH and the first 86 amino acids of luciferase gene were translated in the presence of \([35S]\)methionine. The first 92 amino acids of mMDH and the first 86 amino acids of luciferase ribosome-nascent chain complexes to mitochondrial ribosome-nascent chain complexes to mitochondrial translocon, but under physiological conditions, a G-protein of the binding apparatus is free of nucleotide or loaded with GDP. Since most G-proteins tightly bind GDP, ribosomes probably bind to mitochondria when a G-protein of the binding apparatus is GDP-bound. In general, it is the release of GDP by the action of guanine nucleotide release or exchange protein (GNRP) that allows for G-proteins to bind GTP and the action of a third protein, G activation protein, that induces hydrolysis of GTP by G-proteins (35). Because GTP\(\gamma\)S destabilizes ribosome-mitochondrial interaction, we know that hydrolysis of GTP is not required to destabilize binding. This implies that it is the action of a GNRP upon ribosome-mitochondrial binding that destabilizes the interaction. Since both stimulation of GNRP activity and G activation protein activity result in accelerated GTP hydrolysis, this is consistent with the observation that the rate of GTP hydrolysis by mitochondria increases in the presence of ribosomes. Therefore, we propose that the ribosome-mitochondria interaction stimulates GNRP activity, and that GTP binding to an unidentified G-protein destabilizes binding unless a transit peptide is present. We also suggest that a GNRP is located either on the ribosome, or the mitochondria, and that the G-protein is located on the other so that GTP binding essentially lowers the affinity of the ribosome for the mitochondria. The ribosome-mitochondria interaction behaves similarly to the ribosome-ER interaction except that the action of an SRP-like component that recognizes the transit peptide occurs at the mitochondrial membrane.

These data clearly show a direct link between the cytosolic translation machinery and the mitochondria, as well as a ribosomal influence on mitochondrial GTP hydrolysis. These findings are strongly supportive of the hypothesis that in vivo mitochondrial proteins are imported by co-translational translocation and indicate the potential importance of GTP in the mitochondrial protein import system (7). The current view is that precursor proteins are translated in the cytosol, accompanied by chaperones to the mitochondrial surface, and imported proteins affect the interactions between components of the targeting apparatus including the SRP-SRP receptor interaction, the ribosome-membrane attachment to accomplish correct protein trafficking.

A cytosolic mitochondrial precursor-specific chaperonin, mitochondrial stimulating factor, has been functionally compared with SRP; however, it is an ATP-dependent protein and has only been shown to function post-translationally having no known interaction with the ribosome (13–16). In our experiments, ribosome-mitochondria binding was only modestly affected by ATP, and the ribosome-mitochondria interaction did not have any effect on the rate of ATP hydrolysis. Whether ATP has a direct effect on binding or simply provides a source for generating GTP is unclear. What is clear, however, is that GTP\(\gamma\)S almost completely disrupted the ribosome-mitochondria binding showing that GTP hydrolysis has an important role in controlling this interaction.

Our results demonstrate that, similar to what occurs at the ER membrane, ribosomes bind tightly to the mitochondrial membrane surface and that at least one GTP-binding factor, and the nature of the nascent chain bound to the ribosome, affect the stability of this interaction. These analogies to protein targeting to the ER have led us to propose that higher eukaryotic mitochondria contain ribosome receptors, possibly similar to the sec61p complex, which are active in initiating co-translational translocation.

We observed that ribosome-mitochondria binding is actively destabilized by GTP unless the ribosome carries a transit peptide-containing nascent chain. That binding is stable in the absence of nucleotide and only mildly disturbed by GDP indicates that ribosomes interact with mitochondria when a G-protein of the binding apparatus is free of nucleotide or loaded with GDP. Since most G-proteins tightly bind GDP, ribosomes probably bind to mitochondria when a G-protein of the binding apparatus is GDP-bound. In general, it is the release of GDP by the action of guanine nucleotide release or exchange protein (GNRP) that allows G-proteins to bind GTP and the action of a third protein, G activation protein, that induces hydrolysis of GTP by G-proteins (35). Because GTP\(\gamma\)S destabilizes ribosome-mitochondria binding, we know that hydrolysis of GTP is not required to destabilize binding. This implies that it is the action of a GNRP upon ribosome-mitochondrial binding that destabilizes the interaction. Since both stimulation of GNRP activity and G activation protein activity result in accelerated GTP hydrolysis, this is consistent with the observation that the rate of GTP hydrolysis by mitochondria increases in the presence of ribosomes. Therefore, we propose that the ribosome-mitochondria interaction stimulates GNRP activity, and that GTP binding to an unidentified G-protein destabilizes binding unless a transit peptide is present. We also suggest that a GNRP is located either on the ribosome, or the mitochondria, and that the G-protein is located on the other so that GTP binding essentially lowers the affinity of the ribosome for the mitochondria. The ribosome-mitochondria interaction behaves similarly to the ribosome-ER interaction except that the action of an SRP-like component that recognizes the transit peptide occurs at the mitochondrial membrane.

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We observed that ribosome-mitochondria binding is actively destabilized by GTP unless the ribosome carries a transit peptide-containing nascent chain. That binding is stable in the absence of nucleotide and only mildly disturbed by GDP indicates that ribosomes interact with mitochondria when a G-protein of the binding apparatus is free of nucleotide or loaded with GDP. Since most G-proteins tightly bind GDP, ribosomes probably bind to mitochondria when a G-protein of the binding apparatus is GDP-bound. In general, it is the release of GDP by the action of guanine nucleotide release or exchange protein (GNRP) that allows G-proteins to bind GTP and the action of a third protein, G activation protein, that induces hydrolysis of GTP by G-proteins (35). Because GTP\(\gamma\)S destabilizes ribosome-mitochondria binding, we know that hydrolysis of GTP is not required to destabilize binding. This implies that it is the action of a GNRP upon ribosome-mitochondrial binding that destabilizes the interaction. Since both stimulation of GNRP activity and G activation protein activity result in accelerated GTP hydrolysis, this is consistent with the observation that the rate of GTP hydrolysis by mitochondria increases in the presence of ribosomes. Therefore, we propose that the ribosome-mitochondria interaction stimulates GNRP activity, and that GTP binding to an unidentified G-protein destabilizes binding unless a transit peptide is present. We also suggest that a GNRP is located either on the ribosome, or the mitochondria, and that the G-protein is located on the other so that GTP binding essentially lowers the affinity of the ribosome for the mitochondria. The ribosome-mitochondria interaction behaves similarly to the ribosome-ER interaction except that the action of an SRP-like component that recognizes the transit peptide occurs at the mitochondrial membrane.

These data clearly show a direct link between the cytosolic translation machinery and the mitochondria, as well as a ribosomal influence on mitochondrial GTP hydrolysis. These findings are strongly supportive of the hypothesis that in vivo mitochondrial proteins are imported by co-translational translocation and indicate the potential importance of GTP in the mitochondrial protein import system (7). The current view is that precursor proteins are translated in the cytosol, accompanied by chaperones to the mitochondrial surface, and imported
by a specific, multi-subunit import apparatus. However, the experimental observations on which this model is based is biased for several reasons. First, nearly all of the studies defining mitochondrial protein import have used in vitro systems in which the precursor proteins are first translated and then the translated product is added to mitochondria for import. This system works well in defining the import apparatus, but mechanisms for co-translational import are completely missed by this approach. Second, most of the work defining protein import has been accomplished in yeast and Neurospora. However, there are significant differences in transmembrane protein transfer at the ER between yeast and higher eukaryotic cells, showing that yeast is not always representative of more complex cellular processes such as those found in mammalian mitochondria (36). Finally, it is clear that the interior of a eukaryotic cell is highly structured so that even soluble cytoplasmic proteins do not diffuse freely but rather are recruited in specific cytoplasmic domains (37). The current assumption that precursor proteins are translated in the cytosol and then reach their target by chemical diffusion is inconsistent with the current view of a highly structured cytosol and could potentially be rate-limiting for cells with high rates of metabolism. While at least some completely synthesized mitochondrial proteins import post-translationally into isolated mitochondria, the fact that most mitochondrial matrix and inner membrane proteins contain an N-terminal transit peptide suggests that recruiting begins long before protein synthesis is complete.

Further work is necessary to establish the relationship between mitochondria, ribosomes, the nascent polypeptide, and GTP. It is intriguing, however, that other investigators have found G-proteins in the membrane and contact sites of mitochondria of higher eukaryotes (38–40), and it has been recently reported that GTP hydrolysis is required for post-translational protein import into yeast mitochondria (41). Their identity and function remain to be evaluated.

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