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Interaction of Mammalian Mitochondrial Ribosomes with the Inner Membrane*

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Meiqin Liu and Linda Spremulli‡

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

All of the products of mitochondrial protein biosynthesis in animals are hydrophobic proteins that are localized in the inner membrane. Hence, it is possible that the synthesis of these proteins could occur on ribosomes associated with the inner membrane. To examine this possibility, inner membrane and matrix fractions of bovine mitochondria were examined for the presence of ribosomes using probes for the rRNAs. Between 40 and 50% of the ribosomes were found to fractionate with the inner membrane. About half of the ribosomes associated with the inner membrane could be released by high salt treatment, indicating that they interact with the membrane largely through electrostatic forces. No release of the ribosome was observed upon treatment with puromycin, suggesting that the association observed is not due to insertion of a nascent polypeptide chain into the membrane. A fraction of the ribosomes remained with residual portions of the membranes that cannot be solubilized in the presence of Triton X-100. These ribosomes may be associated with large oligomeric complexes in the membrane.

Animal mitochondrial DNA contains the genetic information for 13 discrete polypeptides that are synthesized by the mitochondrial protein biosynthetic system (1–3). All of these proteins are components of the respiratory complexes located in the inner membrane. The assembly of these complexes requires proteins synthesized within the mitochondria and additional subunits synthesized in the cell cytoplasm and subsequently imported into this organelle. Much remains to be learned about the assembly of these multisubunit complexes.

Although all of the polypeptides synthesized by the mitochondrial translational system are localized in the inner membrane, it is not yet clear whether they are actually synthesized on membrane bound ribosomes. Further, there is limited information on whether the insertion of these polypeptides is a co-translational or post-translational process. In yeast, a considerable body of evidence suggests that mitochondrial ribosomes are associated with the membrane and that translational initiation involves an interaction of the ribosome with the inner membrane. Early electron microscopic studies showed an association of ribosomes with the inner membrane in yeast (4). Furthermore, a combination of high salt and detergents was required to purify yeast mitochondrial ribosomes free of membrane contamination (5). In addition, genetic evidence indicates that the expression of antibiotic resistance by mutant ribosomes in yeast is affected by changes in the mitochondrial membrane (6, 7).

Yeast mitochondrial mRNAs have long 5′-untranslated regions, and translational initiation in yeast mitochondria requires nuclearly encoded mRNA-specific translational activator proteins that appear to interact with the 5′-untranslated regions (8, 9). This translational activator complex binds to the inner membrane and recognizes both the 5′-untranslated regions of a specific mRNA and mitochondrial ribosomes. The translation of these mRNAs then occurs at the surface of the inner membrane, allowing their synthesis to occur near sites of their assembly into the multisubunit respiratory complexes. It should be noted, however, that the insertion of the mitochondrially encoded polypeptides does not absolutely require a co-translational process. Herrmann *et al.* (10) have shown that the correct insertion of cytochrome oxidase subunit II into the inner membrane is not obligatorily coupled to its synthesis. The relationship between the translation of mitochondrial mRNAs and the assembly of the respiratory chain complexes remains to be clarified.

Unlike the yeast mitochondrial mRNAs, animal mitochondrial mRNAs lack significant 5′- and 3′-untranslated nucleotides. The start codon is generally located within 3 nucleotides of the 5′ end of the mRNA (11, 12). The stop codon is generally followed immediately by a poly(A) tail added post-transcriptionally. The ribosomes present in mammalian mitochondria are 55–60 S particles and are composed of 28 and 39 S subunits. The rRNAs in mammalian mitochondrial ribosomes are significantly smaller than those of other ribosomes (12 S in the small subunit and 16 S in the large subunit). These ribosomes have 85–90 proteins (13, 14). This number is significantly higher than that observed in bacterial ribosomes and may exceed the number of proteins present in eukaryotic cytoplasmic ribosomes (15, 16). Limited information is available on the identity of these proteins, although rapid progress is being made on their identification (17–20). In the current paper, we demonstrate that a significant fraction of bovine mitochondrial ribosomes are associated with the inner membrane and examine the nature of the forces governing this association.

MATERIALS AND METHODS

Preparation of Inner Membrane and Matrix Fractions—Mitochondria from 4 kg of bovine liver were prepared as described previously, and the outer membrane was removed by treatment with 0.12 mg of digitonin/mg of mitochondrial protein (13, 21, 22). The large scale preparation of mitochondria required about 10 h. The indicated amounts of mitoplasts were resuspended in 0.6 volumes of buffer A (20 mM Hepes-KOH, pH 7.6, 40 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol) using a Dounce homogenizer. Samples were sonicated at 4 °C for a total of 3 min using 18 bursts of 10 s each. The mixture was transferred to centrifuge tubes using buffer A and was centrifuged in a Sorvall SS34 rotor at 2,500 rpm for 45 min at 4 °C. The resulting supernatant containing fragments of the inner membrane, and the matrix of the

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‡ To whom correspondence should be addressed: Dept. of Chemistry, Campus Box 3290, University of North Carolina, Chapel Hill, NC 27599-3290. Tel.: 919-966-1567; Fax: 919-966-3675; E-mail: Linda_Spremulli@unc.edu.

mitochondria (23) was centrifuged at 40,800 rpm in a Beckman Type 50 rotor for 12 min at 4 °C. The pellet from this step was used as the inner membrane fraction, and the supernatant was used as the matrix fraction.

Free mitochondrial ribosomes were prepared as described previously (13, 21). To ensure that free mitochondrial ribosomes would not sediment under the conditions used to prepare the inner membrane, 2.5 A_{260} of bovine mitochondrial ribosomes were diluted in 2 ml of buffer A and treated as described above.

Effects of Various Treatments on the Association of the Small Subunit with the Inner Membrane—To assess the effects of various conditions on the association of the 28 S subunit with the inner membrane, mitoplasts (about 20 g) were resuspended in 10 ml of buffer A and sonicated as described above. The volume of the sample was adjusted to 34 ml by the addition of buffer A and subjected to low speed centrifugation. Following this step, the supernatant (30 ml) was divided into aliquots of 3.5 ml each. The aliquots were treated as follows: 1) nothing was added, 2) KCl was added to a final concentration of 90 mM, 3) KCl was added to a concentration of 280 mM, 4) the concentration of KCl was adjusted to 520 mM, 5) puromycin was added to a final concentration of 1 mM, 6) Triton X-100 was added to a final concentration of 1%, and 7) Triton X-100 was added to a final concentration of 1.6%. In separate experiments, both 1 mM puromycin and KCl (final concentration, 280 mM) were added sequentially to the same sample. Finally, samples were prepared in the presence of 1% Triton X-100 and 280 mM KCl. All samples were incubated on ice for 30 min and then separated into matrix and inner membrane fractions by high speed centrifugation as described above. The inner membrane fraction in each case was resuspended in 0.5 ml of buffer A. The concentration of protein was determined as described (24).

Extraction of RNA—Nucleic acids in aliquots (0.5 ml containing 17 A_{260} from the inner membrane or 1 ml containing 1.8 A_{260} from the matrix) were extracted using the SDS-phenol/chloroform method (25). For this analysis, samples were adjusted to 1.5 volumes with buffer B (0.3 M sodium acetate, pH 5.2, 5 mM EDTA, and 0.5% SDS) and extracted with an equal volume of a 1:1 mixture of phenol and chloroform. Nucleic acid was precipitated by the addition of 0.6 volumes of isopropyl alcohol at −20 °C overnight. The precipitate was collected by centrifugation and resuspended in 50 μl of TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). DNA was removed by digestion with 0.01 mg/ml DNase as described (25) followed by phenol/chloroform extraction and ethanol precipitation. Samples were dissolved in water and stored at −70 °C. RNA was extracted from mitochondrial ribosomes (48 A_{260}) and wheat germ cytoplasmic ribosomes (150 A_{260}) as described above.

Determination of rRNA Levels by Dot Blot Analysis—Biotinylated (Bt)[1] probes for the 12 S rRNA (residues 370–391, 5′-GGCTATTGTAGGGTCACTTTCG-Bt), for the 16 S rRNA (residues 236–255, GGAATGCTGGAGGTGATGTT-Bt or residues 849–868 GTCTGGTTTCGGGCTGCTTA-Bt) of the mitochondrial rRNAs, and for the 18 S cytoplasmic rRNA (residues 1371–1390, GTCTCGTTCGTTATCGGAAT-Bt) were prepared in the Lineberger Cancer Research Center at the University of North Carolina at Chapel Hill. RNA samples (0.001–0.01 A_{260} in 10 μl) were denatured, immobilized on Zeta-probe nylon membranes by UV cross-linking, and probed with the biotinylated rRNA probes (26). For detection, blots were blocked with TBST (100 mM Tris-HCl, pH 7.5, 0.9% NaCl, and 0.1% Tween 20) for 1 h at room temperature. Streptavidin-alkaline phosphatase was added for 1 h, and blots were washed three times with TBST. Colorimetric detection with the BCIP/NBT color substrate was performed according to manufacturer's recommendations (Amersham Pharmacia Biotech). Signal intensities were determined by transferring blots of colorimetric reactions into a PC using a HP ScanJet 4P and analyzing images for relative intensities via the CorelDraw graphics software (27) or the Scion Image Beta 3b software.

Determination of COXIV and Hsp60 by ELISA—The procedure used was basically as described previously (28). Samples were diluted as needed in 100 mM Hepes-KOH, pH 7.6, and 150 mM NaCl, with or without 0.02% SDS and immobilized in microtiter plates at 4 °C overnight. The primary antibodies used were anti-bovine COXIV, mouse monoclonal 20E8-C12 (Molecular Probes, Inc.) and anti-Hsp60, mouse monoclonal IgG1, LK-1 (StressGen Biotechnologies Corp.). Color development was carried out using 2,2′-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) following the manufacturer's directions and monitored on a SpectraMax 250 plate reader (Molecular Devices) at 405 nm. All samples were assayed in triplicate. Background absorbency (well blocked and treated as described except for the addition of sample) was subtracted from each value.

[1] The abbreviations used are: Bt, biotinylated; ELISA, enzyme-linked immunosorbent assay; COXIV, subunit IV of cytochrome oxidase.

RESULTS

Association of Mammalian Mitochondrial Ribosomes with the Inner Membrane—To assess the possibility that mammalian mitochondrial ribosomes were associated with the inner membrane, it was essential to obtain reasonably clean preparations of the inner membrane and of the soluble interior portion of the mitochondrion (the matrix). One of the standard protocols for this fractionation uses the detergent lubrol to create fragments and vesicles of the inner membrane that can be separated from the matrix by a high speed centrifugation step (22). This method allows the preparation of inner membrane and matrix with little cross-contamination. However, when ribosomes alone were taken through this procedure, they pelleted during the high speed centrifugation step used to sediment the membranes. Hence, it was not feasible to use this procedure for the analysis of an association between mitochondrial ribosomes and the membrane.

As an alternative, a sonication method was developed to allow the preparation of inner membrane and matrix fractions reasonably free of cross-contamination. In this procedure, membrane fragments were created by a brief sonication step. The resulting membrane fragments and the matrix were separated by very brief high speed centrifugation under conditions in which free ribosomes would not sediment with the inner membrane. The inner membrane and matrix fractions thus obtained were examined for cross-contamination using antibodies for marker proteins (COXIV for the inner membrane and Hsp60 for the matrix fraction). Quantitative ELISAs were used to determine the amount of COXIV and Hsp60 in the fractions obtained. As indicated in Fig. 1, over 85% of the COXIV was found associated with the inner membrane fraction as expected (Fig. 1, *A* and *C*). About 15% was located in the matrix fraction. This percentage reflects the short centrifugation time used to prevent sedimentation of the ribosomes during the fractionation process. The inner membrane is largely free of contamination from the matrix as indicated by the very small amounts of Hsp60 detectable in the preparations (Fig. 1, *B* and *C*). To ensure that free ribosomes would not sediment during the brief high speed centrifugation step used to separate the matrix and inner membrane fractions, free ribosomes were carried through this procedure. No free ribosomes could be detected sedimenting under the conditions used.

To assess the presence of mitochondrial ribosomes on the inner membrane, it was essential to have a quantitative method to carry out this analysis. For this work, a biotinylated oligonucleotide probe for the small subunit rRNA (12 S rRNA) was prepared. As indicated in Fig. 2*A*, this probe gave an increasing signal on dot blots when used to detect increasing amounts of rRNA extracted from isolated mitochondrial ribosomes. The signal obtained was reasonably linear with the amount of rRNA used in the dot blot allowing the quantitation of the amount of the small ribosomal subunit present in the initial preparations (Fig. 2*B*).

The specificity of the probe for mitochondrial rRNA was examined by hybridization to wheat germ cytoplasmic rRNA containing the 18 S rRNA from the 40 S subunit. As indicated in Fig. 2*C*, no signal was detected from this rRNA. Wheat germ rRNA was used as a control because 18 S rRNA is reasonably well conserved and preparations of cytoplasmic ribosomes from bovine liver are generally contaminated with small amounts of mitochondrial ribosomes because of organelle breakage during the preparation of the cytoplasmic fraction.

To examine matrix and inner membrane preparations for the

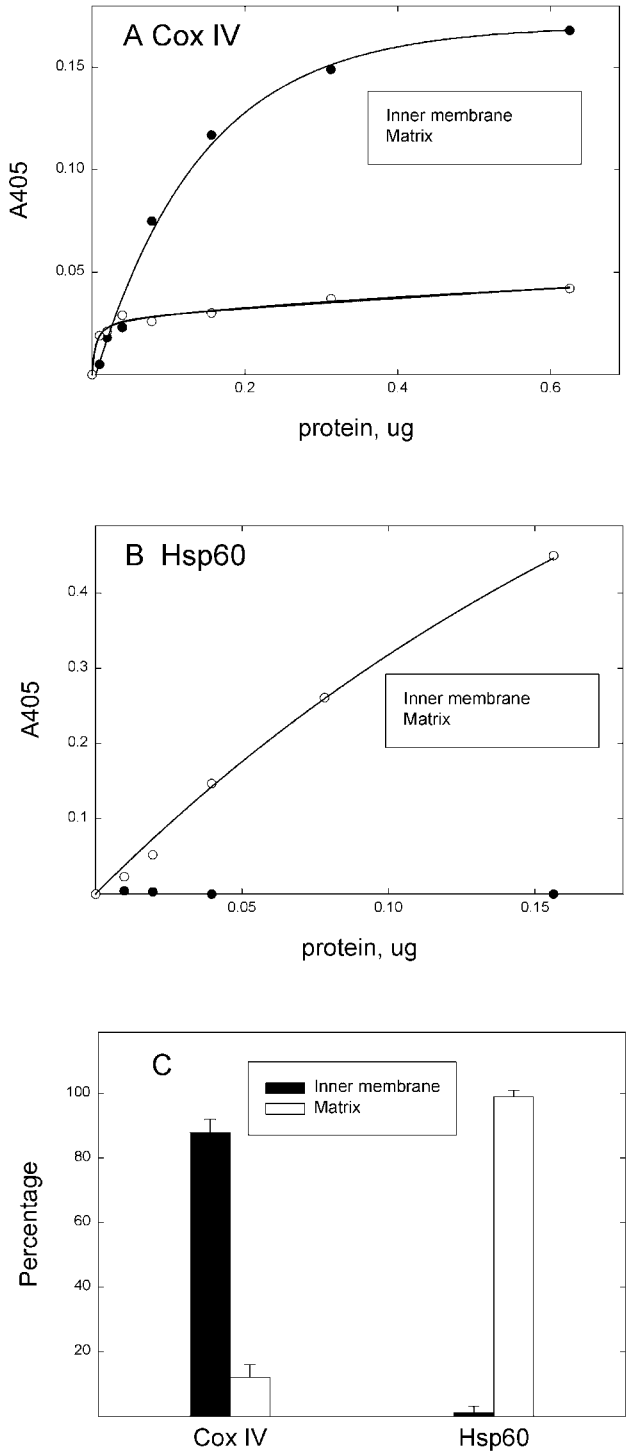

FIG. 1. **Examination of the purity of the inner membrane and matrix preparations.** *A*, quantitation of the amount of membrane marker, COXIV, in the inner membrane and matrix fractions using the ELISA method described under "Materials and Methods." *B*, quantitation of the amount of the matrix-marker, Hsp60, in the inner membrane and matrix preparations using an ELISA. Determinations in each assay were done in triplicate, and each ELISA was repeated six times. Background absorbance from a sample containing no protein was subtracted from each experimental value. *C*, percentage of COXIV and Hsp60 in the inner membrane and matrix fractions. The linear portions of the dose response curves in *A* and *B* and the total amount of protein in each preparation were used to determine the distribution of the marker proteins.

presence of 28 S ribosomal subunits, the nucleic acid in these fractions was extracted. Treatment with DNase was used to remove mitochondrial DNA. The amount of the 12 S rRNA was determined using the dot blot assay. The signal from the biotinylated probe for the 12 S rRNA and the amount of material recovered in the inner membrane and matrix fractions were used to calculate the relative amount of small subunit rRNA associated with each faction. This analysis indicates that about 40% of the small subunits are located in the inner membrane fraction, suggesting that there is a significant association between the mammalian mitochondrial ribosome and the inner membrane (Fig. 2*D*).

Nature of the Interaction between the Small Ribosomal Subunit and the Inner Membrane—Mitochondrial ribosomes could be associated with the inner membrane through a number of different types of interactions including 1) an electrostatic interaction between the polar surface of the membrane and charged/polar groups on the ribosome; 2) the nascent chain as it is being targeted to assemble into the large oligomeric complexes in which mitochondrial translational products are localized; 3) interaction with one or more specific proteins in the membrane (a putative ribosome receptor); 4) partial insertion of a portion of the ribosome into the membrane allowing strong hydrophobic interactions to occur; and 5) a combination of several of these interactions. It should also be kept in mind that there may be different populations of ribosomes associated with membranes in different ways reflecting the different stages of protein synthesis.

The importance of electrostatic interactions on the binding of mitochondrial 28 S subunits to the inner membrane was assessed by examining the effects of increasing concentrations of salt. The preparations described above were carried out in 40 mM KCl. When the salt concentration is raised to 90 mM, the percentage of the subunits that fractionate with the inner membrane decreases from about 40% to about 30% (Fig. 3). As the concentration of salt is increased still further (to 280 and 520 mM), the fraction of ribosomal subunits associated with the membrane decreases further until about 15% of the subunits remain membrane-associated. These observations indicate that a little over half of the inner membrane-associated ribosomes are released by salt treatment, suggesting that electrostatic interactions play an important role in their ability to bind to membranes. The remainder of the subunits appear to remain associated with the membrane even at elevated salt concentrations, suggesting that factors other than simple electrostatic interactions are important for their association with the membrane. At the higher concentrations of salt, there is a decrease in the total recovery of 28 S subunits in the inner membrane and matrix fractions, suggesting that high levels of KCl cause some damage to these particles (data not shown). This observation is in agreement with previous data (29) indicating that mitochondrial ribosomes lose activity when treated at elevated concentrations of salt.

The role that the nascent chain plays in the interaction of the small subunit with the membrane was assessed by examining the treatment of the ribosome-membrane complexes with puromycin. Transfer of the nascent chain to puromycin triggers the release of the nascent chain from the ribosome. The nascent chain normally emerges from the ribosome through the exit tunnel in the large subunit. The release of the nascent chain could reduce interaction of the large (39 S) subunit with the membrane, indirectly triggering the release of its associated 28 S subunit. For these experiments, mitochondrial extracts were incubated with puromycin and then fractionated into the matrix and inner membrane, which were probed for the 12 S rRNA. Treatment with puromycin did not cause a significant reduction in the fraction of the small subunits associated with the inner membrane (Fig. 4). This observation indicates that the interaction of the 28 S subunit with the membrane is not

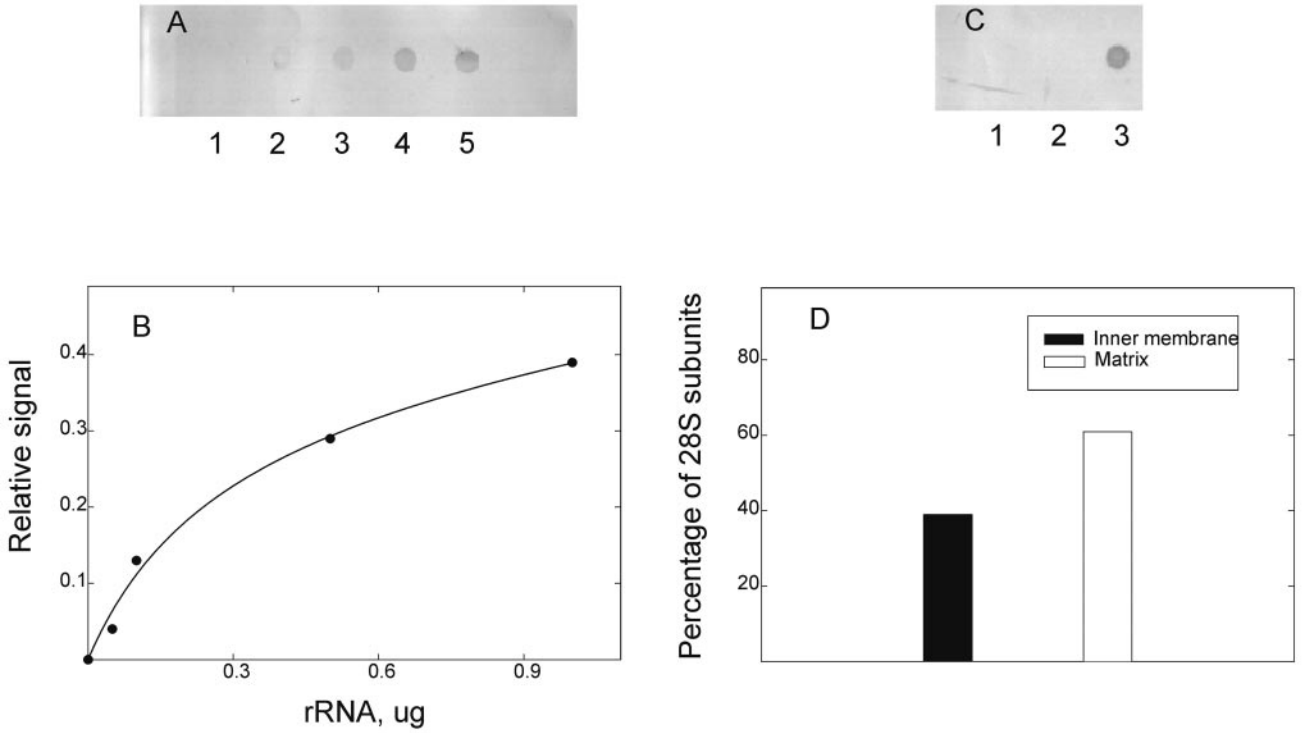

FIG. 2. **Determination of the amount of the 28 S subunit in inner membranes and matrix fractions through dot blot analysis.** *A*, response of the dot blot method to the amount of rRNA tested. Total rRNA from mitochondrial ribosomes was probed for 12 S rRNA as described under "Materials and Methods." *Lane 1*, no rRNA; *lane 2*, 0.05 μg of rRNA; *lane 3*, 0.1 μg of rRNA; *lane 4*, 0.5 μg of rRNA; *lane 5*, 1 μg of rRNA. *B*, the relative signal from the dot blot was determined using the Scion Image for Windows software program and plotted as a function of the amount of rRNA probed. *C*, control showing that cytoplasmic rRNA is not detected by the 12 S rRNA probe. *Lane 1*, no RNA; *lane 2*, 2.5 μg of rRNA from 80 S ribosomes; *lane 3*, 2.5 μg of mitochondrial rRNA. *D*, percentages of the 28 S subunit in inner membrane and matrix fractions. RNA isolated from the matrix and inner membrane was probed for the presence of the 12 S rRNA and quantitated using a dot blot as in *B*. The relative amount of the 12 S rRNA was quantitated using the Scion Image software package, and the distribution of the rRNA between these two fractions was calculated from this value and the total amount of RNA in each preparation.

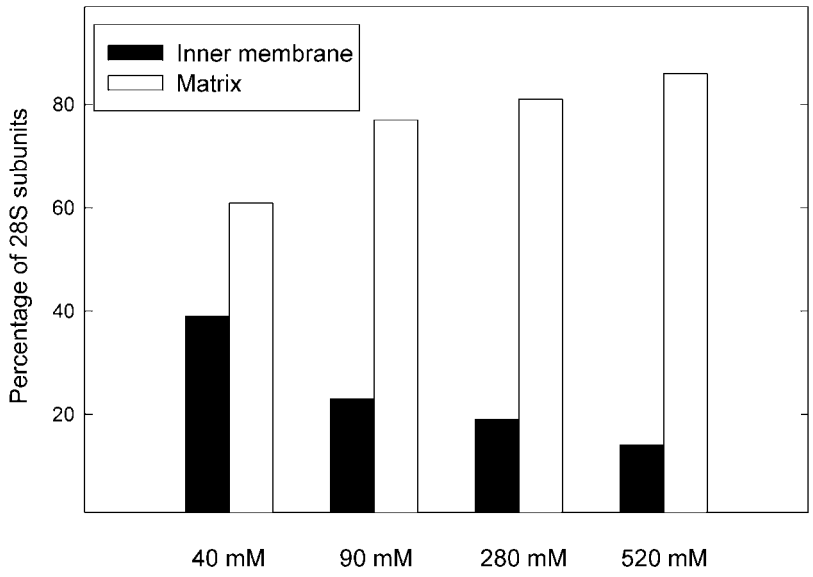

FIG. 3. **Effect of the concentration of salt on the association of the 28 S subunit with the inner membrane.** RNA was prepared from matrix and inner membrane fractions treated with the indicated concentration of KCl. The distribution of the small subunit between the two fractions was determined as described in the legend to Fig. 2 and under "Materials and Methods."

indirectly mediated through the nascent chain emerging from the large ribosomal subunit. A combination of high salt and puromycin on the 28 S subunit-membrane association was examined and indicated that puromycin did not increase the release of small subunit mediated by high salt concentrations (Fig. 4).

The data provided above suggest that electrostatic forces play a significant role in the interactions of at least half of the 28 S subunits associated with the membrane. Additional interactions must be important for at least a portion of the small subunits. One of the standard methods to determine whether a peripheral or intrinsic membrane protein plays an important role in mediating an interaction with the membrane is to examine the effect of alkaline Na_2CO_3, which strips peripheral membrane proteins leaving intrinsic membrane proteins associated with the lipid bilayer. Unfortunately, alkaline Na_2CO_3 (pH 11) damages ribosomes significantly and cannot be used to examine the role of a peripheral membrane protein in the observed association.

As suggested above, it is conceivable that the ribosome could

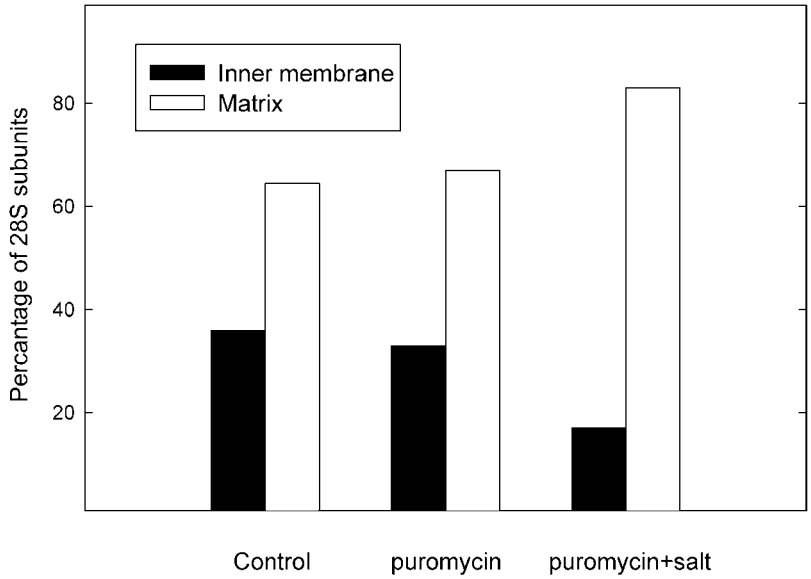

FIG. 4. **Effect of puromycin and high salt on the interaction of the 28 S subunit with the inner membrane.** The matrix and inner membrane fractions were treated with 1 mM puromycin alone and with 1 mM puromycin followed by 280 mM KCl as described under "Materials and Methods." The amount of 12 S rRNA was quantitated to determine the distribution of the ribosomes in the matrix and inner membrane.

be associated with the inner membrane through an integral membrane protein or through a direct interaction with the lipid bilayer. To gain some insight into this possibility, preparations were made in the presence of 1% and 1.6% Triton X-100. This detergent solubilizes membranes with the exception of regions that are enriched in sphingolipids and cholesterol (regions forming lipid rafts) (30). As indicated in Fig. 5, treatment of extracts with 1% Triton releases more than half of the small subunits associated with the inner membrane. However, 15–20% of the mitochondrial 28 S subunits remain associated with the Triton-insoluble portion of the inner membrane. Treatment at a higher concentration of Triton (1.6%) resulted in about the same percentage of mitochondrial ribosomes associated with the membrane. When mitoplast extracts were treated with a combination of high salt (280 mM) and 1% Triton X-100, close to 15% of the ribosomes still remain associated with the inner membrane. This observation suggests that some fraction of the small subunits have a rather strong association with the inner membrane, possibly with regions containing large oligomeric complexes or regions forming lipid rafts.

Association of the 39 S Subunit with the Inner Membrane—The experiments described above focused on the association of the small ribosomal subunit with the inner membrane. A similar approach was used to determine whether the large (39 S) subunit was also located on the membrane. Initial experiments indicated that the total recovery of the large subunit in the inner membrane and matrix was lower than that of the 28 S subunit for unknown reasons. Two different probes for the large subunit rRNA (16 S rRNA) gave similar responses, suggesting that the lower apparent yield was not related to difficulties with the hybridization protocol used. It is possible that the 39 S subunit becomes damaged under some of the conditions used as has been observed with the large subunit of cytoplasmic ribosomes (31).

Despite some difficulties with the overall recovery, examination of the percentage of the large subunit present in the membrane and matrix fraction indicated that about 40% of the large subunit was associated with the membrane (Fig. 6). This value is essentially the same as that observed with the small subunit. Treatment of extracts with puromycin had only a small effect on the association of the 39 S subunit with the membrane, suggesting that the interaction is not mediated by the nascent chain. Treatment with elevated concentrations of salt in addition to puromycin removed about half of the large subunits from the inner membrane. These observations suggest that a significant fraction of the 39 S subunit associates with membrane via forces in addition to an electrostatic interaction.

DISCUSSION

The data reported here demonstrate that about half of the ribosomes in bovine liver mitochondria are associated with the inner membrane. This number probably represents an underestimate of the actual degree of association because the time required for the large scale preparation of bovine mitoplasts might result in the release of some of the ribosomes from the inner membrane. Ribosomes are associated with cellular membranes in a number of cases. Ribosomes associated with the endoplasmic reticulum are involved in the synthesis of proteins for several membrane systems in the cell, in the synthesis of exported proteins, and in the synthesis of proteins localized in certain subcellular organelles such as lysosomes (32). Cytoplasmic ribosomes are also found associated with the outer membrane of mitochondria (33).

The synthesis of organellar membrane proteins and their assembly into large oligomeric complexes is poorly understood. A considerable body of evidence in the yeast mitochondrial system suggests that the ribosomes are associated with the inner membrane. Further, the synthesis of thylakoid membrane proteins encoded in the chloroplast genome is thought to occur on membrane-associated ribosomes. No signal sequences are observed at the N termini of the mitochondrially synthesized proteins in mammalian cells, and the mechanism by which they are inserted into the inner membrane and assembled into the respiratory chain complexes is not well understood.

The association of bovine mitochondrial ribosomes with the inner membrane has both similarities and differences with the association of ribosomes with other cellular membranes such as the endoplasmic reticulum membrane. Only about half of the mitochondrial ribosomes are released by treatment with high salt, and puromycin has no significant effect on their association with the membranes. In contrast, cytoplasmic ribosomes associated with the endoplasmic reticulum can generally be released from the membranes by a combination of high salt and puromycin (34). These observations suggest that they interact with the translocon (Sec61p system) through electrostatic interactions and through the nascent chain. Chloroplast ribosomes associated with thylakoid membranes are also largely released by the combination of high salt and puromycin (35).

It is possible that mitochondrial ribosomes associate with

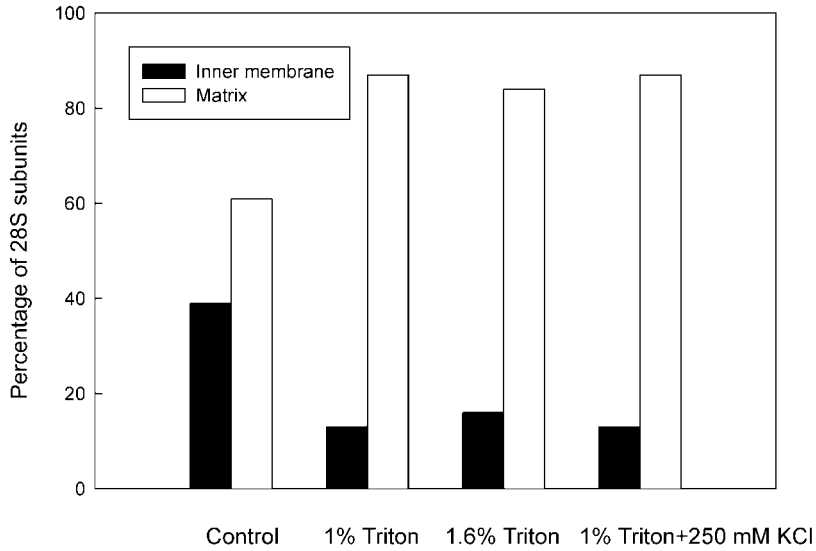

FIG. 5. **Effect of salt and detergent extraction on the presence of 28 S subunits in the inner membrane.** Matrix and inner membrane fractions were prepared in the presence of the indicated concentration of Triton X-100 (1 or 1.6%) at low salt concentrations (40 mM KCl) or in 1% Triton X-100 at high salt concentrations (280 mM). The distribution of the 12 S rRNA was quantitated in the residual membrane fraction and in the soluble fraction as described under "Materials and Methods."

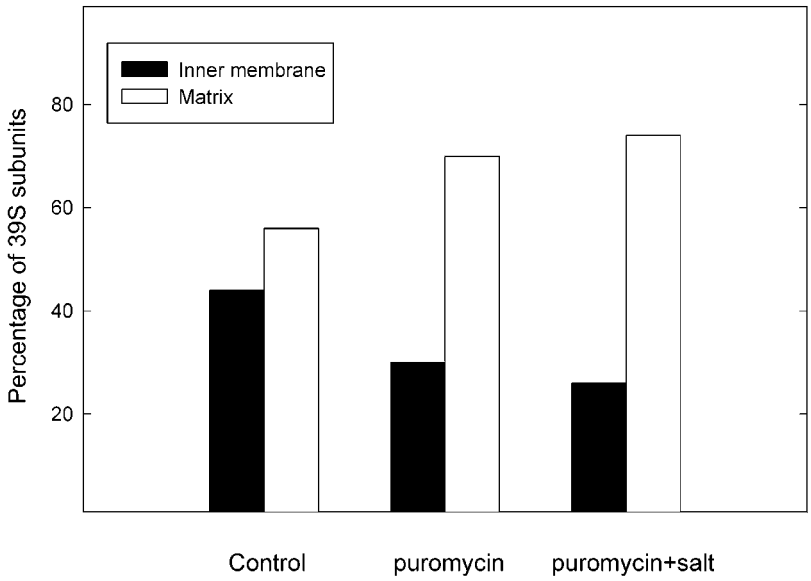

FIG. 6. **Presence of the large mitochondrial ribosomal subunit in inner membrane fractions.** The presence of the 16 S rRNA from the 39 S subunit of mitochondrial ribosomes was determined by dot blot analysis in preparations obtained under normal conditions and in preparations treated with puromycin or puromycin followed by high salt. The samples were quantitated as described under "Materials and Methods."

membranes through different interactions at discrete stages of the translation cycle. Endoplasmic reticulum-associated cytoplasmic ribosomes are essentially all in the stage of polypeptide chain elongation. Their interaction with the endoplasmic reticulum membrane is governed primarily by the association of the large subunit with the translocon complex. During synthesis the nascent chain passes through the exit tunnel in the large subunit directly into the translocon pore (32). In the current models, the small ribosomal subunit does not contact the endoplasmic reticulum membrane directly. Work in the yeast mitochondrial system suggests that polypeptide chain initiation as well as chain elongation will occur in association with the inner membrane (8, 36–38). During initiation, the small subunit of the ribosome should be in contact with the membrane. This association would allow the small subunit to contact mRNA-specific binding proteins that have been shown to be associated with the inner membrane (8, 37–39). However, during chain elongation, any co-translational insertion of the nascent chain into the membrane would require contact between the large subunit of the ribosome and the membrane because the nascent chain emerges from the back of this subunit.

Somewhat surprisingly, about 15% of the mitochondrial ribosomes remain associated with the membrane after Triton X-100 extraction. Extraction of membranes can yield detergent-insoluble lipids (lipid rafts) that appear to be enriched in cholesterol and sphingolipids (30). The inner membrane of liver mitochondria does not contain high levels of these lipids. Rather it is enriched in phosphatidylcholine, phosphatidylethanolamine, and cardiolipin (40), which tend to be solubilized by Triton (30). Previous work has shown that the lipids tend to be more effectively solubilized than the proteins when the inner membrane is treated with Triton X-100 (40). The inner membrane is very protein-rich (about 75% protein) compared with most membranes (about 50% protein) (41). Thus, it seems likely that the mitochondrial ribosomes not solubilized with Triton X-100 are associated with one or more large protein complexes in the membrane rather than primarily associated with lipids. Current efforts are underway to identify membrane components involved in this interaction.

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