Another Function for the Mitochondrial Ribosomal RNA: Protein Folding†

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ABSTRACT: Specialized proteins known as molecular chaperones bind transiently to non-native conformational states of proteins and protein complexes to promote transition to a biologically active conformation. Recently, it was demonstrated in vitro that proteins do not uniquely possess this activity. We show that mitochondrial 12S and 16S ribosomal RNA can fold chemically denatured proteins and reactivate heat-induced aggregated proteins in vitro. This chaperone action is ATP-independent. The specific secondary structure of the mitochondrial rRNA is critical to its folding activity. Furthermore, mutant mitochondrial 16S rRNA from aged cardiac muscle cells lacked this activity. We propose that mitochondrial 12S and 16S ribosomal RNA may play an important role in protein folding in mitochondria.

The term "molecular chaperone" is generally used to describe a group of proteins that aid in the assembly of other proteins. But recently it was found that other molecules such as the *Escherichia coli* 23S rRNA[1] (*1*, *2*) and phosphatidylethanolamine (*3*) also possess protein-folding activity. The 23S rRNA but not the bacterial 16S rRNA can reactivate enzymatic activity of some denatured proteins in dilute solutions in vitro. The folding activity has been traced to the central loop of domain V of 23S rRNA (*4*). Antibiotics that bind to domain V and block ribosomal peptidyl transferase activity also inhibit enzymatic reactivation of some denatured pig muscle lactate dehydrogenase and fungal glucose-6-phosphate dehydrogenase completely (*4*). At present, the mechanism of refolding is not known.

The chaperone function of molecules other than proteins has not yet been demonstrated in vivo, but protein chaperone-independent folding was demonstrated in yeast mitochondria (*5*). It is known that most of the proteins imported from the cytosol into the mitochondria cross the mitochondrial membranes in an unfolded conformation and then fold in the matrix with the ATP-dependent chaperone action of the hsp60 (*19*). Recently, it was demonstrated that, in mitochondria from a mutant yeast strain with inactivated hsp60, the mitochondrial protein cyclophilin Cpr3p as well as the matrix-targeted variants of dihydrofolate reductase and barnase folded efficiently without hsp60. Only rhodanese required hsp60 for folding (*5*). Thus, the mitochondrial heat shock protein chaperone system is not essential for the folding of all matrix proteins. These observations raise the possibility that many other nonprotein chaperones have yet to be discovered.

Here we report a new activity of the mitochondrial rRNA. Our results demonstrate that both 12S and 16S mitochondrial rRNAs can promote refolding and recovery of enzymatic activity of denatured proteins from prokaryotic and eukaryotic origins. In addition, we demonstrate that the presence of single point mutations in the mitochondrial 16S rRNA result in reduced protein refolding activity.

Ribosomal RNA has already been shown to possess a myriad of other "protein-like" functions including ribonuclease activity (*6*), RNA polymerase activity (*7*), and directing the synthesis of peptide bonds (*8*). For these reasons, it is believed that RNA preceded both DNA and protein in the evolution of the first self-replicating system.

MATERIALS AND METHODS

Mouse and human mitochondrial 12S and 16S rRNAs were prepared by in vitro transcription (Maxiscript kit, Ambion) from mouse and human mitochondrial DNA templates containing the SP6 or T7 promoter sequence at the 5′ end. The template for mouse antisense 16S rRNA was constructed with an SP6 polymerase promoter at the 3′ end. After transcription the DNA template was degraded with DNase I, and RNA was purified by LiCl precipitation according to the Ambion protocol. The mutant mouse mitochondrial 16S rRNA was selected from a cardiac muscle cell cDNA library prepared from hearts of 2-year-old mice (Sulijoadikusumo and Usheva, to be published). The mutations in the clone, which were used in the experiments, are A295G, A365G, G462C, and U528C. The numbering of the mutations reflects the nucleotide position in the mouse mitochondrial 16S rRNA. RNase-negative yeast tRNA (Boehringer) was further purified by phenol−chloroform extraction.

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[1] Abbreviations: rRNA, ribosomal RNA; *E. coli*, *Escherichia coli*; ATP, adenosine 5′-triphosphate; hsp, heat shock protein; BSA, bovine serum albumin; DTT, DL-dithiothreitol; MDH, malate dehydrogenase.

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RNA secondary structure calculations for the mouse mitochondrial wild-type 16S, antisense 16S, and mutant 16S were performed using the respective RNA sequences and "RNAdraw", an integrated program for RNA secondary structure calculation and analysis under 32-bit Microsoft Windows (*9*).

*Denaturation and Refolding of EcoRI. Eco*RI (Boehringer) was denatured in 2 M guanidine hydrochloride as described in ref *10* for 2 h. Refolding of denatured *Eco*RI was initiated by dilution to an enzyme concentration of 1 μM in restriction buffer with or without rRNA. Chloramphenicol and 16S rRNA were mixed and preincubated before being added to the refolding reaction (*12*). After incubation at 25 °C the refolding was terminated by incubation of the reaction mixtures with 10 units of RNase A (Boehringer) for 2 min at 37 °C. Recovery of activity was next assayed by adding plasmid DNA with a 0.7 kb insert flanked by *Eco*RI sites on both the 5′ and 3′ ends. Recovery of activity was reproducible in each of five to seven independent experiments and at least nine independent RNA syntheses. The intensities of the 0.7 kb DNA bands were estimated by applying the NIH Image 1.59 program after scanning of the agarose gels, previously stained with ethidium bromide for visualization of DNA.

Intrinsic Fluorescence Measurements. Measurements were made at 25 °C on an SLM 8000 spectrofluorometer and 5 nm excitation and emission band-passes were used. The emission spectra are reproducible in five independent experiments.

Denaturation and Refolding of T7 RNA Polymerase. T7 RNA polymerase (Ambion) was denatured in 6 M guanidine hydrochloride for 60 min at 22 °C. Refolding was initiated by dilution to a final protein concentration of 3 nM in transcription reaction buffer [50 mM Tris-HCl, pH 7.5, 6 mM magnesium chloride, 10 mg/mL acetylated, RNase-free BSA (New England Biolabs), 10 mg/mL sonicated salmon sperm DNA (Stratagene), and 2 mM DTT] with or without 5 nM RNA. Transcription reactions were assembled, and the transcription runoff RNA products were analyzed on 6% acrylamide sequencing gels as recommended by Ambion. pSP72 plasmid linearized with *Xho*I was used as a transcription template. The size of the specific transcript is 97 nt. The results in Figure 3B are the average of four independent experiments.

Denaturation and Refolding of Firefly Luciferase. Aggregation of firefly luciferase (Sigma) was induced by incubation in PBS at 42 °C for 30 min at a protein concentration of 1 μM as described (*11*). Renaturation of aggregated luciferase was initiated at 22 °C by dilution in PBS containing 10 mg/mL acetylated BSA with or without RNA. Luciferase activity was measured with the Promega luciferase assay system as suggested by the manufacturer and a Bio-Orbit luminometer. All experiments were performed in triplicate and in six independent experiments, with three different preparations of rRNA.

Denaturation and Refolding of Mitochondrial Malate Dehydrogenase. Pig heart mitochondrial malate dehydrogenase (Worthington) at a concentration of 8.6 μM with respect to monomer was denatured with 6 M guanidine hydrochloride at 25 °C. Refolding was initiated by diluting the enzyme 80-fold in 50 mM sodium phosphate, pH 7.6, 200 mM NaCl, 5 mM 2-mercaptoethanol, and 4 mM magnesium acetate and incubating at 25 °C in the presence or absence of RNA (*12*) at the concentrations indicated in the figure legends. All reactions were conducted under macromolecular crowding conditions in the presence of 10 mg/mL acetylated BSA and 30% Ficoll 70 (Sigma) (*13*, *14*). In some experiments RNase-free ATP (Amersham Pharmacia Biotech) and chloramphenicol (Sigma) were included in the refolding reactions as indicated in the figure legends. Refolding in the pesence of chloramphenicol was conducted as described in ref *12*. The enzyme concentration during refolding was 107 nM with respect to monomer. Recovery of enzymatic activity was assayed by measuring of the rate of decrease in A_{340} at 25 °C as described in ref *12*.

All figures were assembled in Adobe PhotoShop 5.5.

RESULTS

We first tested for the folding activity of mouse mitochondrial 16S and 12S rRNAs on chemically denatured bacterial endonuclease *Eco*RI. More than 60% recovery of *Eco*RI activity was observed upon incubation of the refolding reaction with either mitochondrial 12S or 16S rRNA (Figure 1A, lanes 2−7). Release of a 0.7 kb *Eco*RI fragment from the plasmid was used as a signature for recovered activity. In the absence of rRNA, refolding led to only 5% of *Eco*RI activity (Figure 1A, lane 1). The folding activity was enhanced to more than 60% in the presence of increasing rRNA concentrations, reaching a plateau at molar ratios of 1:8 *Eco*RI to 16S rRNA, and 1:16 for 12S rRNAs (Figure 1A). Both human mitochondrial 16S and 12S rRNAs also exhibited protein-folding activity, suggesting that this activity is not unique to the mouse mitochondrial rRNA (Figure 1C, lanes 3−6). Neither tRNA (Figure 1B, lanes 4−6) nor mRNA possessed this activity (data not shown). We also conducted experiments in the presence of ATP and the antibiotic chloramphenicol. It is known that the mitochondrial heat shock protein chaperones fold proteins in an ATP-dependent fashion. However, we did not observe any change in effects on rRNA protein-folding activity in the presence of ATP (Figure 1D, lanes 2−5). It was demonstrated that in the presence of chloramphenicol the enzymatic 23S rRNA-dependent reactivation of denatured pig muscle lactate dehydrogenase and fungal glucose-6-phosphate dehydrogenase was inhibited. We did not observe any change in effects on rRNA protein-folding activity in the presence of chloramphenicol (Figure 1D, lanes 6−8).

We next investigated the role of mitochondrial rRNA structure in its protein-folding activity. The secondary structure of rRNA is largely determined by internal base pairing. To investigate the relationship between the RNA structure and the protein-folding activity of these rRNAs, we examined the activity of the RNA strand that is complementary to the 16S rRNA strand. Although the sequences of the sense and antisense mitochondrial 16S rRNA strands share little similarity, the internal base pairing and hence the energy and secondary structure of the molecule should be conserved. The folding activity of the complementary RNA strand should be similar. Energy-based calculations (*9*) yielded nearly identical structures for both sense and antisense mitochondrial 16S rRNAs (data not shown). We found that the efficiency of refolding in the presence of either the sense or antisense rRNA was similar

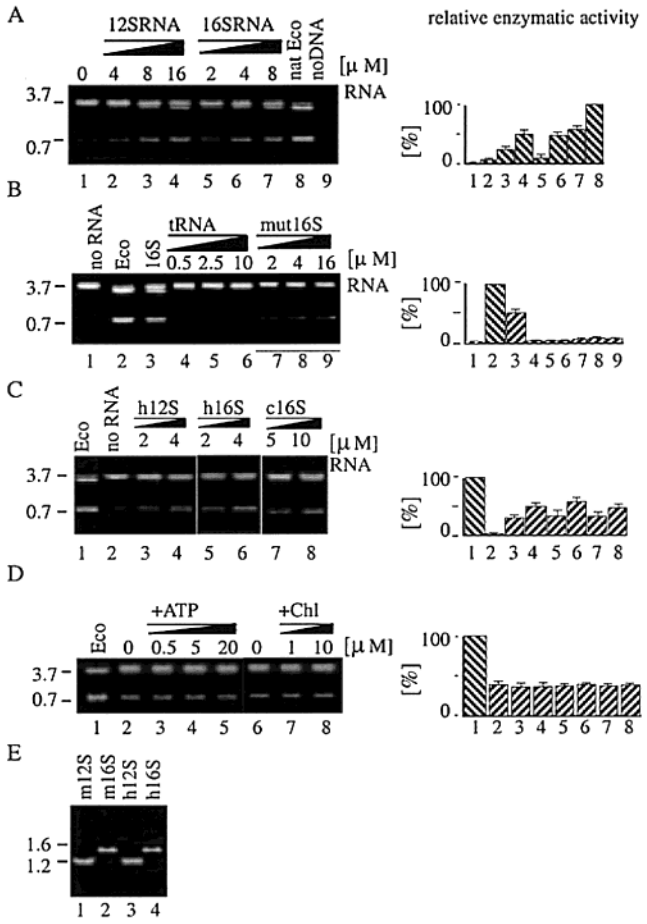

FIGURE 1: (A) Mitochondrial 16S and 12S rRNA can refold *Eco*RI. *Eco*RI endonuclease was denatured in 2 M guanidine hydrochloride, and refolding was initiated with either no rRNA (lane 1) or with different concentrations of mitochondrial 12S (lanes 2−4) and 16S rRNA (lanes 5−7) as indicated at the top of each lane. RNase A was added to stop rRNA-mediated *Eco*RI folding by digesting the rRNA. Recovery of activity was assayed by adding a plasmid substrate for *Eco*RI with a 0.7 kb insert. Observation of the 0.7 kb fragment using agarose gel electrophoresis and ethidium bromide staining revealed *Eco*RI activity. Lane 8: endonuclease activity of native *Eco*RI. The reaction in lane 9 is the same as the reaction in lane 4 but lacked substrate plasmid DNA. (B) Neither mutant 16S mitochondrial rRNA nor tRNA refolds *Eco*RI. The concentrations of mutant 16S rRNA or tRNA in the refolding reactions with a constant amount of *Eco*RI are indicated at the top of the lanes. Lane 1: Endonuclease activity of denatured *Eco*RI alone. Lane 2: Activity of native enzyme. Lane 3: Denatured *Eco*RI folded in the presence of 16S rRNA. (C) Human mitochondrial rRNA and mouse antisense 16S rRNA can efficiently refold *Eco*RI in a concentration-dependent manner. Folding reactions were assembled with 1 μM denatured *Eco*RI in the presence of different concentrations of mitochondrial rRNA, as indicated at the top of the lanes: lanes 3 and 4, human 12S rRNA (h12S); lanes 5 and 6, human 16S rRNA (h16S); lanes 7 and 8, mouse 16S antisense rRNA (c16S). (D) Folding reactions were assembled with 0.5 μM denatured *Eco*RI and 1 μM mitochondrial rRNA in the presence of different concentrations of ATP (+ATP, lanes 3−5) and chloramphenicol (+CHL, lanes 7 and 8) as indicated. (E) Agarose gel electrophoresis of mouse (m16S, m12S) and human (h12S, h16S) mitochondrial rRNAs was used to confirm their sizes and purity: lane 1, mouse 12S rRNA; lane 2, mouse 16S; lane 3, human 12S rRNA; lane 4, human 16S. The migration of a DNA size marker is indicated on the left of the panels. The relative enzymatic activity in the refolding reactions is shown with the diagrams on the right site of the panels. The enzymatic activity of nondenatured enzyme was used as a base (100%). The calculations were conducted in the Microsoft Exel 98 program. Data were collected from five independent experiments.

(Figure 1A, lanes 6 and 7, and Figure 1C, lanes 7 and 8), strongly suggesting that the secondary structure of the molecule is a major determinant of its protein-folding activity.

We also investigated the effect of mutations, which alter the structure of the rRNA molecule, on its activity. We chose to examine a mutant mitochondrial 16S rRNA with four single base substitutions (A295G, A365G, G462C, and U528C) that we identified in mouse cardiac muscle cell cDNA library prepared from 2-year-old mouse hearts. 16S rRNA transcribed from the mutant templates revealed significantly reduced protein-folding activity (Figure 1B, lanes 7−9). Energy-based calculations (*9*) indicated the complete alteration of the wild-type structure by the mutations (data not shown). This strongly suggests that the secondary structure of the molecule most likely is a major determinant of its activity.

We directly monitored changes in protein conformation upon refolding by measuring the *Eco*RI intrinsic tyrosine fluorescence. Upon refolding with a molar ratio of 1:4 *Eco*RI to 16S rRNA, the intensity of the tyrosine fluorescence emission decreased (Figure 2A, panel c, and Figure 2B). Furthermore, after 10 min the value of the 300 nm emission maximum was the same as that of the native *Eco*RI (Figure 2A, panel d), providing further evidence that the enzyme was refolded in the presence of the 16S rRNA (Figure 2A, panels c and d). An aliquot of the refolding reaction with RNA, taken after the last scan, was assayed to confirm the recovery of enzymatic activity (Figure 2C). In general, a decrease in tyrosine emission intensity indicates the increased exposure of the tyrosine residues, subjecting it to quenching mechanisms through the solvent. The decrease in emission we observed upon refolding could be explained by the fact that, in the native state, four tyrosines, Tyr 27, Tyr 101, Tyr 193, and Tyr 209 are solvent-exposed, as revealed by the crystal structure of *Eco*RI (*15*).

The tyrosine emission signal changed less significantly upon incubation with the mutant mitochondrial 16S rRNA (Figure 2A, panel b, and Figure 2B). This suggests that there was less change in conformation from the denatured state upon folding with the mutant. The quenching of tyrosine emission could be partly due to interactions with the mitochondrial 16S rRNA. Furthermore, differences in the ability of the wild-type and mutant mitochondrial 16S rRNA to interact with *Eco*RI may also cause differences in tyrosine emission spectra. The exact mechanism of interaction remains to be solved.

Mitochondrial rRNA was also able to refold chemically denatured bacteriophage T7 RNA polymerase (Figure 3). The T7 polymerase is a single-subunit enzyme with a molecular mass of 98 849 kDa and is the closest homologue to the mitochondrial RNA polymerase (*16*). We observed that, after incubation with either 12S or 16S rRNA, more than 40% of the T7 polymerase activity was recovered after guanidine hydrochloride denaturation, demonstrated by transcription from a DNA template with a T7 promoter (Figure 3A). When tRNA or no rRNA was added to the folding reaction, less than 10% of the enzymatic activity was recovered (Figure 3B).

The ability of mitochondrial rRNA to reactivate heat-aggregated proteins was established using heat-aggregated firefly luciferase. Incubation with either 12S or 16S rRNA

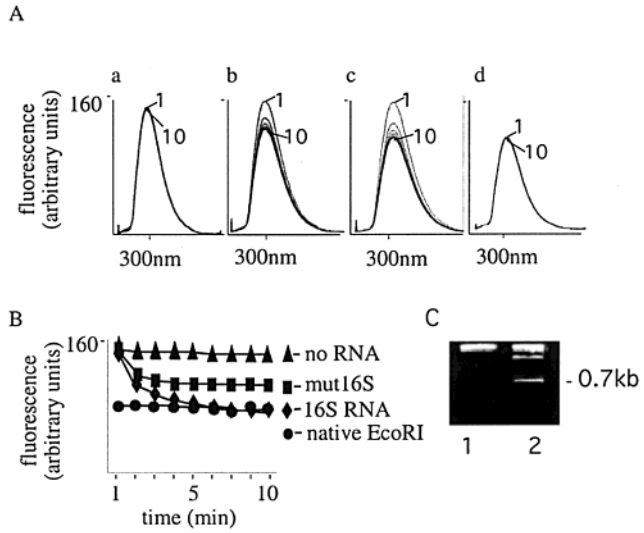

FIGURE 2: Tyrosine fluorescence emission during *Eco*RI refolding in the presence of mitochondrial 16S rRNA. *Eco*RI was denatured and refolding initiated as described above in the presence of either mitochondrial 16S rRNA, mutant 16S rRNA at concentration of 4 μM, or no RNA. The concentration of denatured *Eco*RI in the reactions was 1 μM. A sample containing the native *Eco*RI at the same dilution was also analyzed. Emission wavelengths 290−350 nm were scanned with excitation at 275 nm. The intensities of the major emission peak at 300 nm, which is characteristic of tyrosine, were compared. Ten sequential scans were performed at a rate of 0.95 s/nm, giving 60 s per scan (8). (A) Emission spectra of (a) refolding of denatured *Eco*RI alone, (b) *Eco*RI refolding in the presence of the mutant 16S rRNA, and (c) refolding in the presence of the wild-type 16S rRNA, and (d) the emission spectrum for the native enzyme. Emission curve 1 corresponds to the scan completed 60 s after initiation of folding and curve 10 after 10 min. (B) The emission values at 300 nm from panel A are plotted as a function of time for native *Eco*RI and refolding of denatured *Eco*RI in the presence of either 16S rRNA, mutant 16S rRNA, or no RNA. RNA was visualized by ethidium bromide staining after separation on a 1.2% agarose gel. (C) Lane 1: *Eco*RI digestion with an aliquot of the refolding reaction in panel A, b, after the last scan. Lane 2: *Eco*RI digestion of an aliquot of the refolding reaction in panel A, c, after the last scan. In each reaction DNA plasmid substrate was added and analyzed as described in Materials and Methods. The position of the specifically cleaved product is shown on the right of the panel.

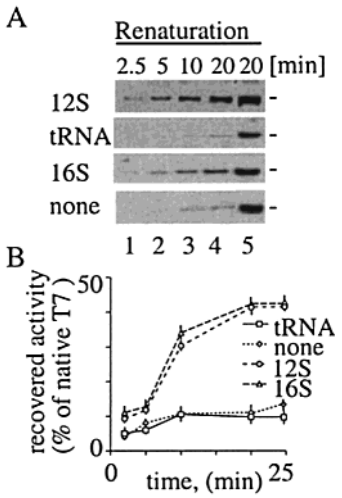

FIGURE 3: Mitochondrial 16S and 12S rRNA refold chemically denatured T7 RNA polymerase. Refolding of T7 polymerase denatured with 6 M guanidine hydrochloride was initiated with no RNA, 12S rRNA, 16S rRNA, or tRNA. (A) Refolding was assayed by runoff transcription assays. Bands of [^{32}P]UTP-labeled specific transcript, separated in 7 M urea−polyacrylamide gels, were quantitated using phosphorimager analysis. Lanes 1−4 include runoff transcripts with denatured T7 polymerase after 2.5, 5, 10, and 20 min of refolding in the presence or absence of rRNA as indicated on the left site of the panel; lane 5 includes the transcription reaction with native polymerase. (B) Percent recovery of native enzymatic activity is plotted as a function of time for refolding reactions with 12S, 16S, tRNA, and no RNA. The yield of refolded protein increases with time until 20 min.

reactivated more than 35% of heat-aggregated luciferase (Figure 4A) in a rRNA concentration-dependent manner (Figure 4B). In contrast, incubation with mutant 16S rRNA yielded less than 16% recovery of enzymatic activity (Figure 4A). Furthermore, with either tRNA or no RNA not more than 5% of enzymatic activity was recovered (Figure 4A).

Mitochondrial rRNA was also able to refold chemically denatured pig heart mitochondrial MDH. The mitochondrial matrix is the most crowded cellular compartment due to the protein density, which may be as high as 270−560 mg/mL (*17*, *18*). It has been proposed that such a high concentration of molecules results in alterations of the protein-refolding processes (*14*). To mimic more closely the conditions relevant to the folding in the mitochondrial matrix in vivo, we have analyzed the effect of rRNA on the denatured MDH refolding under macromolecular crowding conditions by including 10 mg/mL BSA and 30% Ficoll 70 in the refolding reactions (*13*, *14*). After denaturation with 6 M guanidine hydrochloride, approximately 29% of the native enzymatic activity was recovered upon initiation of refolding with mitochondrial 12S, 16S, and c16S rRNA (Figure 5A). In the absence of rRNA, or in the presence of tRNA, less than 3% enzymatic activity was recovered. In the presence of mutant 16S rRNA less than 8% of the MDH activity was recovered. No change in the 16S rRNA folding activity was recorded in the presence of ATP (Figure 5B) or chloramphenicol (Figure 5C).

DISCUSSION

Our data show that the 12S and 16S mitochondrial rRNAs can each reactivate chemically denatured and heat-aggregated proteins. The versatility of the mitochondrial rRNAs was quite surprising. The proteins used were from both prokaryotic and eukaryotic origins. In addition, the eukaryotic proteins were both cytoplasmic and mitochondrial.

Furthermore, the secondary structure of the rRNA molecule is most likely an important determinant of its protein-folding activity. Mutant 16S rRNA isolated from cardiomyocytes of aged mouse hearts shows diminished activity. It is known that mutations accumulate in the mitochondrial genome including the region coding for the 16S rRNA gene with aging. By changing the primary structure of the 16S rRNA, such mutations may also introduce changes in the secondary rRNA structure and therefore alteration in protein-folding activity. Further studies need to be performed to address the prevalence of the accumulation of somatic mutations in the mitochondrial 16S rRNA gene with aging and their possible effects on the protein-folding capacity.

A similar protein-folding activity was previously reported for the *E. coli* 23S rRNA (*1*, *2*). In *E. coli* the rRNA from the small subunit lacked this activity. Yet the mitochondrial rRNAs from both the small and large ribosomal subunits, the 12S and 16S rRNAs, respectively, were able to promote

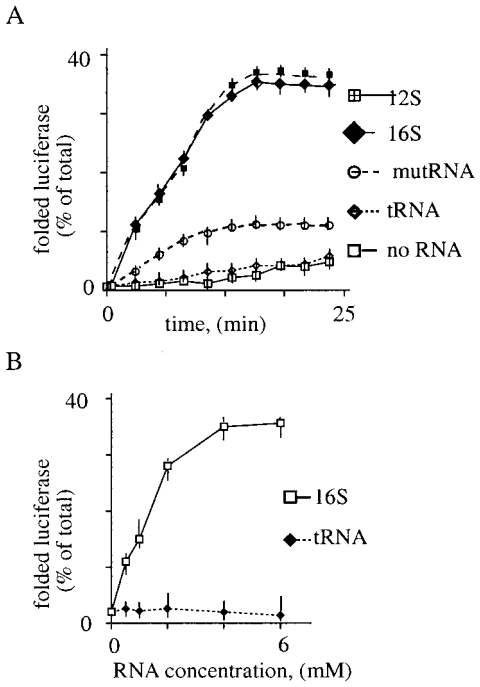

FIGURE 4: Mitochondrial 12S and 16S rRNA can refold heat-denatured firefly luciferase. Refolding was initiated at 22 °C by dilution in the presence of 16S, 12S, mutant 16S, tRNA, or no RNA. Folding of luciferase was assayed as described in Materials and Methods. (A) The percent recovery of enzymatic activity produced by denatured firefly luciferase is plotted as a function of incubation time with RNA prior to activity assay. (B) Mitochondrial 16S rRNA folds luciferase in a concentration-dependent manner. The percent recovery of luciferase activity is plotted as a function of the concentration of rRNA in refolding reactions with a constant volume for 20 min. Refolding was initiated with 0.5, 2, 4, and 6 μM 16S rRNA.

folding. Furthermore, while the antibiotic chloramphenicol inhibited the activity of the *E. coli* 23S rRNA (*12*), it had no effect on the protein-folding activity of the mitochondrial rRNAs. This suggests that different domains may be critical for the activities of the *E. coli* 23S rRNA and the mitochondrial rRNAs.

At present it is not clear how the mitochondrial rRNA promotes protein folding. Like the protein chaperones, it is highly possible that the mitochondrial rRNA has no information about the specific folding pathways of the protein. Unlike hsp60- and hsp70-mediated protein folding (*19*), we found that the process is not ATP-dependent, making it a very economical means to fold proteins. As is the case with the *E. coli* 23S rRNA (*1*, *2*), the exact mechanism of mitochondrial rRNA-mediated protein folding remains to be elucidated.

An important question that arises is whether the mitochondrial rRNA folds proteins in vivo. It has been shown that the chaperonin system is not required for the folding of all mitochondrial matrix proteins (*5*). Furthermore, it has been shown that only a subset of proteins can be efficiently folded by protein chaperones (*5*). These two observations suggest the possible existence of a protein chaperone-independent mechanism for protein folding in the mitochondrial matrix. The number of rRNA transcripts in the mitochondria is much higher than is needed for the formation of ribosomes (*20*). The function of these "ribosome-free" transcripts has not been elucidated. We observed that rRNA supports refolding

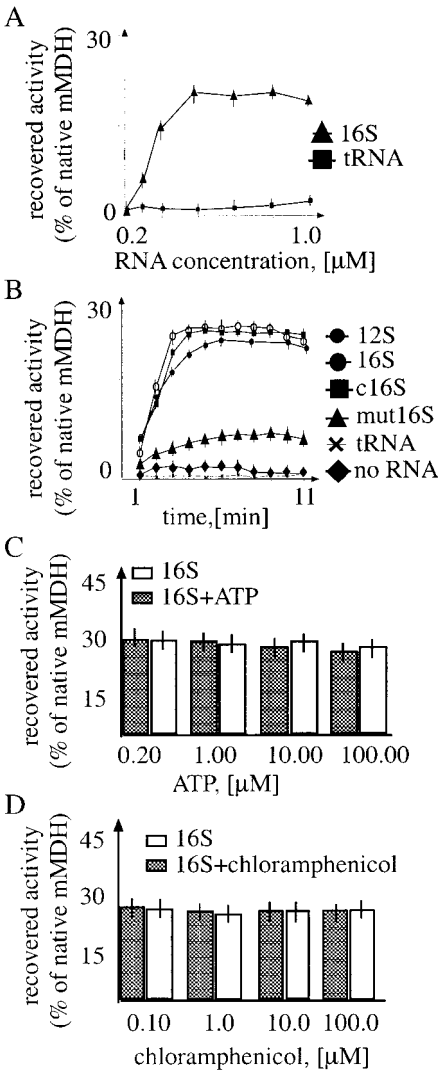

FIGURE 5: Mitochondrial 16S rRNA folds chemically denatured mitochondrial MDH. (A) Mitochondrial 16S rRNA but not tRNA folds MDH in a concentration-dependent manner. The recovered MDH activity is plotted as a function of the concentration of rRNA in refolding reactions with a constant amount of MDH (107 nM) for 3 min. Refolding was initiated with 0.1, 0.2, 0.4, 0.6, and 1 μM 16S rRNA (16S) or tRNA as indicated on the right site of the panel. The deviations in the values of each point in four independent measurements are indicated with bars. (B) The percent recovery of MDH activity is plotted as a function of the time of refolding in reactions assembled with a constant concentration of rRNAs (400 nM) and MDH (107 nM with respect to monomer). Refolding was initiated with addition of denatured MDH to the reaction mixtures assembled with 30% Ficoll 70, 10 mg/mL acetylated BSA, and 10 mg/mL tRNA in the presence of 12S rRNA (12S), 16S rRNA (16S), antisense 16S rRNA (c16S), and mutant 16S rRNA (mut16S) as indicated on the right site of the diagram. The deviations in the values of each point in four independent measurements are indicated with bars. (C) Mitochondrial 16S rRNA folds mitochondrial MDH in an ATP-independent manner. The percent recovery of MDH activity is plotted as a function of the ATP concentration in refolding reactions with a constant amount of MDH (107 nM) and 16S rRNA (400 nM) for 3 min in the presence of 30% Ficoll 70 and 10 mg/mL acetylated BSA. The height of the columns reflects the recovery of the MDH activity. ATP was added to the reactions together with the rRNA. (D) The recovered MDH activity is plotted as a function of the chloramphenicol concentration in the refolding reactions. Reactions were assembled as described in (C). Nonpatterned columns (16S): no ATP or chloramphenicol. Patterned columns (16S + ATP/chloramphenicol): reactions in the presence of ATP or chloramphenicol. The deviations in the values of each column in four independent measurements are indicated with bars.

of denatured proteins under macromolecular crowding conditions that partially mimic the conditions in the mitochondria. These observations, combined with the very low concentration of heat shock proteins in the mitochondrial matrix during times of no stress (Bodyak and Usheva unpublished), suggest that rRNA may act as the major chaperone during no-stress conditions, in an energy-conserving, ATP-independent fashion. When the cells are exposed to stresses such as higher temperatures resulting in heat shock, the ribosome-free rRNA may degrade or assume an inactive conformation, necessitating the expression of the highly specialized heat shock proteins.

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