Chronic ethanol feeding causes depression of mitochondrial elongation factor Tu in the rat liver: implications for the mitochondrial ribosome

Brian Weiser,1 Gregory Gonye,1 Peter Sykora,2 Sara Crumm,1 and Alan Cahill1

1Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania; and 2School of Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey

Submitted 12 March 2010; accepted in final form 13 February 2011

Weiser B, Gonye G, Sykora P, Crumm S, Cahill A. Chronic ethanol feeding causes depression of mitochondrial elongation factor Tu in the rat liver: implications for the mitochondrial ribosome. Am J Physiol Gastrointest Liver Physiol 300: G815–G822, 2011. First published February 24, 2011; doi:10.1152/ajpgi.00108.2010.—Chronic ethanol feeding is known to negatively impact hepatic energy metabolism. Previous studies have indicated that the underlying lesion responsible for this may lie at the level of the mitoribosome. The aim of this study was to characterize the structure of the hepatic mitoribosome in alcoholic male rats and their isocalorically paired controls. Our experiments revealed that chronic ethanol feeding resulted in a significant depletion of both structural (death-associated protein 3) and functional [elongation factor thermo unstable (EF-Tu)] mitoribosomal proteins. In addition, significant increases were found in nucleotide elongation factor thermo stable (EF-Ts) and structural mitochondrial ribosomal protein L12 (MRPL12). The increase in MRPL12 was found to correlate with an increase in the levels of the 39S large mitoribosomal subunit. These changes were accompanied by decreased levels of nuclear- and mitochrondrially encoded respiratory subunits, decreased amounts of intact respiratory complexes, decreased hepatic ATP levels, and depressed mitochondrial translation. Mathematical modeling of ethanol-mediated changes in EF-Tu and EF-Ts using prederived kinetic data predicted that the ethanol-mediated decrease in EF-Tu levels could completely account for the impaired mitochondrial protein synthesis. In conclusion, chronic ethanol feeding results in a depletion of mitochondrial EF-Tu levels within the liver that is mathematically predicted to be responsible for the impaired mitochondrial protein synthesis seen in alcoholic animals.

EXPERIMENTAL PROCEDURES

Reagents and chemicals. All reagents were of molecular biology grade and obtained from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) except where otherwise stated.

Animal experimentation. Male Sprague-Dawley rats (250 g) were maintained on a nutritionally sufficient liquid diet (15) with ethanol constituting 36% of the calories for 12 mo. Control animals were pair fed an identical diet but with maltose dextrin isocalorically substituted for ethanol. Animals received humane care according to criteria outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985).

Preparation of mitochondria and protein determination. Mitochondria were prepared from liver homogenates by differential centrifugation (5). Protein levels were determined using a Bio-Rad detergent-compatible protein assay (Bio-Rad Laboratories, Hercules, CA) with BSA as the protein standard.

Isolation of mitoribosomes and assay of activity. Purified mitoribosomes were isolated from mitochondria through 10–30% sucrose density gradients as previously described (2). The polyU-directed phenylalanine polymerization assay and isolation of mitochondrial translation factors were performed as previously described.

Blue-native gel electrophoresis of respiratory complexes and in-gel assay of complex I activity. Blue-native gel electrophoresis and the in-gel assay of NADH dehydrogenase were performed as previously described (18).

Western blot analysis. Mitochondrial protein (20 µg) or sucrose density gradient fractions (25 µl) were separated by SDS-PAGE in precast NuPage 10% polyacrylamide bis-Tris gels (Invitrogen, Carlsbad, CA). Proteins were transferred for 1.25 h at 300 mA in NuPage transfer buffer plus methanol [12.5% (vol/vol)] onto nitrocellulose using the Bio-Rad Mini Trans-Blot apparatus (Bio-Rad Laboratories). After being blocked with Bio-Rad nonfat dry milk dissolved in

THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN is a series of five protein respiratory complexes, located in the inner mitochondrial membrane, that collectively take part in the transference of electrons from donors such as NADH to an electron acceptor, i.e., oxygen. This is coupled to the pumping of H+ out across the inner membrane, with the gradient that ensues being used by ATP synthase to generate ATP. The majority of the protein components of the electron transport chain are nuclear encoded, whereas the remaining 13 components are encoded for by the mitochondrial genome and then translated on mitochondrial ribosomes (mitoribosomes). Mammalian 55S mitoribosomes consist of 28S small subunits and 39S large subunits that are assembled from nuclear-encoded proteins and mitochondrial-encoded 12S rRNA (28S, small subunit) and 16S rRNA (39S, large subunit), respectively. Our previous study (18) and those of Cunningham and colleagues (6, 16, 18) have demonstrated that 4–6 wk of chronic ethanol feeding results in a decrease in the number of functionally active mitoribosomes and depressed rates of mitochondrial translation. Mitoribosomes isolated from alcoholic livers were found to have altered protein composition (3) and physicochemical properties, e.g., sedimentation coefficients and hydrodynamic volumes (16). In this study, we demonstrate that 12 mo of ethanol feeding results in a significant decrease in cellular ATP levels, a marked depression in the levels of intact respiratory complexes and their subunits, and significant alterations in the levels of proteins involved in both the structure [i.e., death-associated protein 3 (DAP3) and mitochondrial ribosomal protein L12 (MRPL12)] and function [i.e., elongation factor thermo unstable (EF-Tu) and elongation factor thermo stable (EF-Ts)] of the mitochondrial translational machinery. In addition, we mathematically modeled mitochondrial EF-Tu and EF-Ts and predicted how an ethanol-induced change in their steady-state levels can explain the decreased mitochondrial protein synthesis that occurs in alcoholic animals.
Tris-buffered saline-Tween 20 [5% (wt/vol)], membranes were hybridized with the primary antibody overnight at 4°C followed by a 1-h incubation with the appropriate horseradish peroxidase-conjugated secondary antibody and developed with SuperSignal West Femto ECL substrate (Thermo Scientific, Rockford, IL). Chemiluminescence was visualized and bands were quantified using the Kodak Image Station 440 CF and accompanying Kodak Digital Science 1D software (Eastman Kodak, Rochester, NY). The primary antibodies and dilutions used were as follows: voltage-gated anion channel (VDAC), 1:1,000 (Calbiochem, San Diego, CA); EF-Tu and EF-Ts, 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA); MRPL12, 1:1,000 (Abcam, Cambridge, MA); DAP3, 1:1,000 (BD Biosciences, San Jose, CA); cytochrome c oxidase I (COX I) and NADH dehydrogenase (ubiquinone)-1β subcomplex subunit 8 (NDUFB8), 1:1,000 (MitoSciences, Eugene, OR); and COX Vlc, 1:1,000 (Molecular Probes, Eugene, OR).

**Adenine nucleotide determination.** Adenine nucleotide levels were measured spectrophotometrically using standard enzymatic analyses as described by Trautschold et al. [ATP (20)] and Jaworek and Welsch [ADP and AMP (9)].

**Mathematical modeling of steady-state Tu.GTP levels.** Steady-state Tu.GTP levels were predicted using a mass action kinetic model implemented in Systems Biology Markup Language (SBML) using CellDesigner 4.0.1 (12) with the interaction kinetic constants determined in Ref. 17 (see Supplemental Material, Supplemental Table S2). Initial concentrations for GDP, GTP, EF-Tu, and EF-Ts were obtained from Ref. 4 (see Supplemental Table S3). Rate laws representing reversible reactions were generated using the SMBlSqueezer plug-in for CellDesigner, and simulations were run using the SOSlib ODE solver.

**RESULTS**

**Effects of long-term chronic ethanol feeding on adenine nucleotide levels, rates of respiration, levels of respiratory complexes, and levels of respiratory complex subunits.** Figure 1 shows that chronic ethanol feeding produced a 17% decrease in ATP, a 21% increase in ADP, and a 58% increase in AMP levels. No changes in total adenine nucleotide levels were detected. These data show that long-term ethanol feeding detrimentally impacts hepatic energy levels. This interference in energy metabolism is likely to enhance the susceptibility of the liver to further alcohol-mediated damage.

Figure 2 shows blue-native gel analyses of respiratory complexes I, III, and V isolated from mitochondria. Ethanol feeding resulted in significant depletion of all three complexes. In addition, Fig. 2B shows that the decrease in complex I levels (52%) was directly responsible for the depressed complex I activity (48%), whereas the 28% decrease in complex V levels (Fig. 2D) is a potential mechanism underlying the decrease in ATP levels (Fig. 1) seen in alcoholic livers. To further understand the mechanism(s) responsible for the depressed levels of respiratory complexes, Western blot analyses were used to monitor the levels of respiratory complex subunits. Figure 3, A and C, shows that chronic ethanol feeding resulted in the depletion of complex I subunits NDUFB8 and NDUF A9. The functions of these proteins are not fully understood, although recent evidence has implicated NDUFB8 in nitric oxide (NO)-mediated necrosis (7). In addition, Figs. 3, B–E, shows that chronic ethanol feeding resulted in a marked depletion of both nuclear-encoded (i.e., COX IV and COX Vlc) and mitochondrial encoded (i.e., COX I) subunits of complex IV. No changes were seen in the mitochondrial marker protein VDAC (Fig. 3, A and B). Taken together, these data strongly suggest that chronic alcohol consumption causes a general depression in the level of respiratory complexes by decreasing the availability of complex subunits. Ethanol-mediated changes in respiratory complex subunits were accompanied by decreased mitochondrial respiration (Supplemental Table S1). Ethanol feeding resulted in significant depressions in state 3 respiration and, consequently, respiratory control ratios when substrates of complexes I and II were used.

**Effect of long-term chronic ethanol feeding on the structure and function of the mitochondrial ribosome.** To further understand the mechanism by which chronic ethanol feeding impairs the synthesis of mitochondrial respiratory subunits, purified mitoribosomes were prepared from the livers of control and alcoholic animals, and their sedimentation profiles were examined. Alcohol feeding caused a 34% decrease in the intensity of the 55S monomeric mitoribosomal peak and a 51% increase in the intensity of the free 39S mitoribosomal subunit (Fig. 4, A and B). No corresponding increase in free 28S subunits was detected.

Figure 5 shows immunoblot analyses of a number of protein components, both structural and functional, of the mitochondrial translation system. Ethanol feeding was found to produce a significant 32% decrease in DAP3 (Fig. 5A), a component of the 28S mitoribosomal subunit. In contrast, levels of MRPL12, a protein component of the 39S large subunit, were elevated twofold (Fig. 5D). EF-Tu, a mitochondrial protein that binds aminoacylated tRNA molecules and guides them into the mitoribosome, was depressed by 31% (Fig. 5B). The decrease in EF-Tu levels was accompanied by a 75% increase in the level of the 28S monomeric mitoribosomal peak and a 51% increase in the intensity of the free 39S mitoribosomal subunit (Fig. 4, A and B). No corresponding increase in free 28S subunits was detected.

Figure 4, C–F, shows representative sucrose density gradient analyses of control and alcoholic mitoribosomes along with the relative distribution along the gradients of EF-Tu, MRPL12, and DAP3. DAP3 can clearly be seen to associate primarily with the 28S subunit and the 55S monosome, whereas EF-Tu was found exclusively to be associated with the 28S small subunit. As expected, MRPL12 is associated with the 39S large subunit and the 55S monomer and is

---

1 Supplemental Material for this article is available at the American Journal of Physiology-Gastrointestinal and Liver Physiology website.
present in the gradients prepared from alcoholic animals in significantly larger levels than that of their paired controls, indicative of the increased levels of 39S subunits seen in these animals (Fig. 4B).

To investigate the effects of the ethanol-mediated changes in protein composition on mitoribosomal activity, a poly(U)-directed phenylalanine polymerization assay was used on sucrose cushion-purified, total mitoribosomal particles. Chronic ethanol feeding was found to decrease mitoribosomal activity by \( \frac{10}{11} \times 10^{-1} \times 10^{-1} \) (14.8 vs. 9.2 pmol \(^{1}\)H\text{Phe}·mg RNA\(^{-1}\)·pmol ribosomes\(^{-1}\) in 20 min for control vs. ethanol feeding, \( n = 4, P < 0.05 \)).

Fig. 2. Effects of chronic ethanol feeding on levels of mitochondrial respiratory complexes in male rat livers. Levels of respiratory complexes were determined using blue-native gel electrophoresis from 100 µg mitochondrial protein, and complex I activities were determined in gel as described in EXPERIMENTAL PROCEDURES. A: complex I. B: complex I activity. C: complex III. D: complex V. C, control; E, ethanol feeding. Results are means \( \pm \) SE; \( n = 4 \). Statistical significance of differences was determined by a paired \( t \)-test (\( **P < 0.01; ***P < 0.001 \)).

Fig. 3. Effects of chronic ethanol feeding on levels of respiratory complex subunits in male rat livers. Levels of complex subunits were determined by immunoblot analysis as described in EXPERIMENTAL PROCEDURES. Protein levels were normalized for levels of voltage-gated anion channel (VDAC). A: NADH dehydrogenase (ubiquinone)-1β subcomplex subunit 8 (NDUFB8). B: cytochrome c oxidase (COX) I. C: NADH dehydrogenase (ubiquinone)-1α subcomplex subunit 9 (NDUFA9). D: COX IV. E: COX Vlc. Results in A and B are means \( \pm \) SE; \( n = 4 \). Statistical significance of differences was determined by a paired \( t \)-test (\( *P < 0.05, **P < 0.01 \)).
Simulated effects of ethanol-induced changes in EF-tu and EF-ts levels on mitochondrial Tu.GTP levels. EF-Tu functions by binding aminoacylated (charged) tRNA molecules and mediating their entry into the ribosomal A site. Binding of EF-Tu to tRNAs requires the “activation” of EF-Tu, which is accomplished by replacing a bound GDP with GTP. Alterations in levels of Tu.GTP may be expected to have a critical impact on mitochondrial translation. Our observations that long-term chronic ethanol feeding resulted in significant changes to the levels of EF-Tu and EF-Ts led us to model the effects of these changes on the predicted levels of Tu.GTP in alcoholic animals with the aim of eliciting a mechanistic explanation for the decreased mitochondrial protein synthesis. Figure 6A shows the EF-Tu/EF-Ts cycle that was modeled as reversible reactions and parameterized using the reaction rate constants shown in Supplemental Table S2 (17). Simulations were run until a steady state was reached (Fig. 6) from initial conditions representing both a range of concentrations bracketing published control values for EF-Tu and EF-Ts levels as well as using the experimentally determined ethanol-elicited alterations reported here (Fig. 5, B–E, and Supplemental Table S3). The model was first used to investigate the relative dependence of Tu.GTP steady-state levels on changes in EF-Tu and EF-Ts levels. Initial concentrations were investigated over two orders of magnitude (0.01–1.0 μM) for each species (Fig. 6B). Simulation results revealed a linear dependence associated with EF-Tu levels when EF-Ts levels were kept constant at 0.3 μM (slope = 0.71, \( r^2 = 0.9998 \)) and a nonlinear inverse dependence on EF-Ts levels when EF-Tu levels were fixed at 0.3 μM (fitted to a second-order polynomial, \( r^2 = 0.9999 \)). Dependence on total EF-Tu concentration was very strong as free EF-Tu was predicted to be essentially nonexistent (not shown) and therefore limiting. The model predicted a 35% reduction in Tu.GTP concentration given the combined ethanol-mediated and experimentally determined alterations in both total EF-Tu and EF-Ts levels (Fig. 5, B–E). The contribution of the ethanol-induced 75% increase in EF-Ts (see Fig. 5C) on Tu.GTP concentration was determined to be an additional 11% from the difference in simulation results between the EF-Tu change alone (initial concentration of EF-Tu = 0.21 μM, see Supple-

Fig. 4. Representative sucrose density gradients of mitoribosomes isolated from mitochondria prepared from control and alcoholic male rat livers. A: control. B: ethanol feeding. C and D: fractions from the gradients in A and B, respectively, blotted for mitoribosomal proteins. E and F: relative quantification of the results in C and D, respectively. A260nm, absorbance at 260 nm; EF-Tu, elongation factor (EF) thermo unstable; MRPL12, mitochondrial ribosomal protein L12; DAP3, death-associated protein 3.
mental Table S3; with initial concentration of EF-Ts = 0.3 μM, see Supplemental Table S3 and Ref. 17) and the combined changes of a decrease of EF-Tu and an increase of EF-Ts (initial EF-Ts concentration = 0.53 μM, see Supplemental Table S3). Thus, we predict that the changes in mitochondrial protein synthesis that have been shown to occur in alcoholic animals arise from a synergy of the changes that chronic ethanol feeding imparts on EF-Tu and EF-Ts levels.

DISCUSSION

Chronic ethanol feeding has been shown to impair hepatic energy metabolism. Here, we show that long-term ethanol feeding for longer than 12 mo results in significant depletions of ATP (Fig. 1), respiratory complexes (Fig. 2), and both nuclear- and mitochondrially encoded respiratory complex subunits (Fig. 3). One of the complex I subunits, i.e., NDUFB8, was found to be depleted by >80% within the mitochondria. A recent study (7) has suggested that modification of NDUFB8 by NO causes necrosis in a Ras-interacting protein (RIP)1- and RIP3-mediated fashion. Downregulation of NDUFB8 by alcohol in long-term, chronically fed animals may therefore result in the elimination of this NO sensor and lead to the survival of dysfunctional mitochondria. NDUFB8 may also have a role to play in formation of respiratory supercomplexes (7). How respiratory complexes coordinate the incorporation of both nuclear- and mitochondrially encoded subunits into their structures is not clear, but the fact that depressions in mitochondrial protein synthesis result in decreased levels of nuclear-encoded subunits rather than a compensatory upregulation implies a complex regulatory mechanism. It may be that the ethanol-elicited decreases in mitochondrially encoded subunits results in the presence of fewer binding sites within the complex for the nuclear-encoded subunits and leads to their downregulation and/or degradation. Alternatively, it may be that the overall product of the respiratory chain, e.g., O2 consumption, ATP production, and elec-
tron leakage, are themselves signaling mediators of nuclear responses. Of the two theories, the first seems the more likely as it would allow for both increased and decreased production of nuclear-derived components. Decreased levels of respiratory complexes, due to impaired mitochondrial protein synthesis, may give rise initially to the upregulation of nuclear-encoded subunits, but if there are no binding sites for these proteins, then they are targeted for degradation or, alternatively, down-regulated. Those proteins that are able to bind within the complexes remain induced. The consequence of this mechanism would be a respiratory chain in alcoholic animals that is significantly altered in structure compared with their paired controls.

Previous studies from our group have suggested that the protein composition of the mitoribosomes was altered in alcoholic animals (3) and that may account for the changes seen in a number of their physiochemical properties (16). At the time, the identity of the proteins found to be altered was unknown. Our experiments have now identified novel alterations in a number of mitochondrial proteins, both structural and functional, linked to the translational machinery in alcoholic animals. Ethanol feeding resulted in significant depression of DAP3 (Fig. 5A), a component of the 28S small ribosomal subunit (Fig. 4, C–F) that is also capable of participating in the pathways of apoptosis triggered by TNF-α (10), interferon-γ (11), and the Fas ligand (10). Additionally, DAP3 has been shown to be involved in a staurosporine-induced apoptotic pathway via interactions with respiratory complex I and human NO-associated protein 1 (19). As with NDUFB8, a depression in DAP3 levels may allow the survival of dysfunctional mitochondria in alcoholic animals, this time by decreasing their ability to undergo apoptosis. The precise role that DAP3 plays within the 28S subunit has yet to be determined, but it is known to bind GTP and have a number of phosphorylation sites clustered around the GTP-binding site (15), suggesting that it may have a functional role that involves its phosphorylation. In contrast to the depression of DAP3, ethanol feeding caused an induction of MRPL12 (Fig. 5D), a component of the 39S large ribosomal subunit (Fig. 4, C–F). As well as being involved in mitoribosomal structure, MRPL12 has also been suggested to modulate mitochondrial gene expression by directly binding to mitochondrial RNA polymerase (22) and to be involved in cell growth (8). In our study, the induction of MRPL12 appeared to be solely as a consequence of increased levels of 39S subunit (Fig. 4, C–F). Immunoprecipitation of MRPL12 in samples of mitochondrial matrix that had first been clarified to remove...
mitoribosomes revealed no free MRPL12 (A. Cahill, unpublished observations). All the MRPL12 was found associated with the pelleted mitoribosomes. In addition, no mitochondrial RNA polymerase was found to be associated with MRPL12 upon immunoprecipitation (A. Cahill, unpublished observations). We conclude that the increased level of 39S mitoribosomal subunits reported here is a compensatory response to either the depressed mitochondrial protein synthesis or to impaired assembly of the 28S subunit. Increased levels of 39S are not due to the dissociation of intact 55S monomers. Support for this theory comes from a recent study (14) by Larsson and colleagues, in which it was shown that methylation of 12S rRNA was essential for the stability of the 28S mitoribosomal subunit and that, in its absence, the 28S subunits destabilized, whereas the 39S subunit was upregulated.

Mitochondrial EF-Tu is a GTPase that delivers aminoacylated tRNA molecules to the mitoribosome during the process of mitochondrial translation. It is associated with the 28S small mitoribosomal subunit (Fig. 4, C–F), where it interacts with ERAL1, a protein involved in 28S subunit assembly and mitochondrial translation (21). Along with its guanine nucleotide exchange factor EF-Ts, it represents an essential component of the mitochondrial protein synthetic machinery. In an effort to identify a molecular mechanism that could explain the depression in mitochondrial protein synthesis seen in alcoholic animals, we adopted a mathematical modeling strategy. Using previously published kinetic data (Supplemental Table S2) (17), we modeled the EF-Tu/EF-Ts cycle and determined the effects of changes in EF-Tu and EF-Ts levels on the steady-state levels of Tu.GTP, i.e., the specific EF-Tu species that is responsible for directing charged tRNAs into the mitoribosome and therefore the initiator of protein synthesis. Our in silico data show that the steady-state levels of Tu.GTP are strongly related to the levels of EF-Tu and, albeit to a much lesser extent, inversely related to the levels of EF-Ts (Fig. 6B). Thus, decreased levels of EF-Tu and/or increased levels of EF-Ts would be predicted to have a profound effect on mitochondrial protein synthesis. When the levels of EF-Tu and EF-Ts were measured in alcoholic animals, it was found that the relative depression in EF-Tu (Fig. 5B) and upregulation of EF-Ts (Fig. 5C) was sufficient to result in a 35% decrease in the steady-state levels of Tu.GTP (Fig. 6C), as predicted by our EF-Tu/EF-Ts model (Fig. 6A and Supplemental Tables S2 and S3). Such a decrease in Tu.GTP would be expected to result in depressed levels of charged tRNA molecules being directed into the mitoribosome and decreased rates of protein synthesis. Measurements of mitochondrial translation, as assessed by the poly(U)-directed phenylalanine polymerization assay, support the modeling predictions with a 38% decrease in translation activity being detected in alcoholic animals (see RESULTS). Taken together, our modeling and experimental data suggest that the decrease in mitochondrial protein synthesis seen in alcoholic animals arises as a consequence of changes in elongation factor activation. Other factors that may have an impact on mitochondrial protein synthesis are transcript abundance and the levels of mitochondrial initiation factors. At present, it is unclear how ethanol feeding impacts these parameters, and it may be that they do play some role in ethanol-mediated lesion development. Our study, however, clearly demonstrates that impaired mitochondrial protein synthesis in alcoholic rat livers can be completely accounted for solely by changes in elongation factor activation. Any process that results in depressed levels of Tu.GTP is likely to result in decreased mitochondrial translation. This has recently been demonstrated in a study by Spremulli and colleagues (1), in which a mutation in EF-Ts was shown to inhibit its binding to EF-Tu and consequentially depress nucleotide exchange. This led to decreased rates of mitochondrial translation, most likely as a consequence of depressed Tu.GTP levels. When these data are compared with previous work from our laboratory in which ethanol was fed to male rats for only 3–5 wk, it is clear that hepatic mitoribosomal dysfunction is a progressive lesion that gradually intensifies as alcohol feeding times are extended. Long-term (12 mo) chronic ethanol feeding resulted in greater decreases in the levels of 55S mitoribosomes, larger increases in the levels of 39S subunits, and a more severe depression in translational activity (3, 16, 18) compared with the results from the short-term chronic feeding model. Consequentially, these advanced lesions produced more severe depressions in both the levels and activities of respiratory complexes along with significant decreases in ATP levels.

In conclusion, we present here the novel concept that alcohol-mediated inhibition of mitochondrial protein synthesis involves a mechanism involving impaired elongation factor activation within the mitochondrion (Fig. 7). We have supported this idea with both experimental data and mathematical modeling analyses. The specific mechanism underlying the depression of EF-Tu in alcoholic animals is unclear and warrants further investigation. It is not clear how EF-Tu expression is regulated. It may be that ethanol feeding impacts upstream transcription factors involved in the control of EF-Tu expression. Alternatively, it may be that ethanol feeding specifically targets the levels of, or assembly of, the 28S mitoribosomal subunit. It is interesting that both DAP3 and EF-Tu, two proteins that are associated with the small subunit, were both found to be depressed. Additionally, it has been shown that 28S subunits from alcoholic animals sediment at a lower sedimentation coefficient than those isolated from paired controls and are present in lower amounts (16). In contrast, ethanol feeding was not found to affect the sedimentation properties of the 39S subunit. Failure of the 28S to assemble properly or its decreased presence may result in the availability of fewer binding sites for EF-Tu and its resulting downregulation. Studies investigating the assembly of the 28S mitoribosomal subunit are ongoing.

GRANTS

This work was funded by National Institute on Alcohol Abuse and Alcoholism Grants AA-012225, AA-014151, and AA-014986.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES