Translation rates of isolated liver mitochondria under conditions of hepatic mitochondrial proliferation

Eric P. BRASS

Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, U.S.A.

The hepatic mitochondrial content is increased in rats by treatment with the hypolipidaemic drug clofibrate and by administration of the cobalamin analogue hydroxycobalamin[c-lactam] (HCCL), an inhibitor of hepatic L-methylmalonyl-CoA mutase activity. As a first step in defining the mechanisms regulating liver mitochondrial contents in these models, the current studies were designed to test the hypothesis that hepatic mitochondrial proliferation is associated with enhanced translation rates of mitochondrial DNA gene products. Incorporation of [³⁵S]methionine and [³H]leucine into protein was quantified in mitochondria isolated from control, clofibrate- and HCCL-treated rats. Use of multiple amino acid substrate concentrations permitted the maximal rate of translation ($V_{\max}$) to be determined independent of endogenous amino acid concentrations. The $V_{\max}$ for methionine incorporation was not different in the models evaluated (0.062, 0.057 and 0.061 pmol/min per mg of mitochondrial protein in control, clofibrate- and HCCL-treated rats respectively). Similar results were obtained for leucine incorporation when absolute fractional radiolabel incorporation rates were analysed and when conventional Lineweaver–Burk analysis was employed. These results demonstrate no change in the intrinsic capacity of mitochondrial translation in these two models of hepatic mitochondrial proliferation.

INTRODUCTION

Mitochondria are the site of cellular oxidative phosphorylation, and are the major source of ATP in most cell types. The cellular content of mitochondria is regulated to match energy demands and generating capacities. For example, the increased energy demands associated with aerobic exercise training or liver regeneration are associated with increased mitochondrial biogenesis in skeletal muscle [1] and liver [2] respectively. Similarly, thyroid hormone, a major regulator of the basal metabolic rate, can directly modulate cellular mitochondrial content [3]. More recently, it has been observed that mitochondrial proliferation is associated with a number of metabolic diseases [4,5], and may represent an important compensatory response under these conditions.

Mitochondrial content is primarily regulated by rates of mitochondrial biogenesis [6]. Mitochondrial biogenesis is a complex process, dependent on the expression and cellular sorting of hundreds of nuclear gene products and the expression of 13 polypeptides by the mitochondrial genome [6,7]. Translation of the mitochondrial DNA (mtDNA) products can be directly studied in isolated mitochondria [8,9]. The molecular mechanisms responsible for the physiological regulation of cellular mitochondrial expression are unknown. An important aspect of this problem is the concordant change in nuclear and mitochondrial genome expression. As the majority of mitochondrial proteins are nuclear-encoded, expression of mitochondrial-encoded proteins may be independently regulated or constitutively generated. Decreases in mitochondrial biogenesis due to hypothyroidism [10,11] and severe hypoxia [12] are clearly associated with decreased rates of transcription and/or translation from mtDNA. However, data concerning changes in mtDNA expression associated with increased mitochondrial content above normal cellular levels are ambiguous. For example, translation rates by isolated liver mitochondria are low in organelles isolated from hypothyroid rats compared with control rats, and these translation rates are increased by thyroid

replacement [13,14]. However, hyperthyroidism, while increasing mitochondrial content [3], failed to significantly increase amino acid incorporation by isolated mitochondria [13].

The current studies were designed to test the hypothesis that hepatic mitochondrial proliferation is associated with enhanced rates of translation of mtDNA gene products. Two models of mitochondrial proliferation were studied. Administration of the hypolipidaemic drug clofibrate is associated with increased contents of liver mitochondria and peroxisomes, via unknown mechanisms [15]. Recently we have described hepatic mitochondrial proliferation in an animal model of methylmalonic aciduria induced by administration of the cobalamin analogue hydroxycobalamin[c-lactam] (HCCL) [16,17]. Translation rates were quantified in liver mitochondria isolated from control, clofibrate- and HCCL-treated rats to determine if the treatments modified mitochondrial protein expression. Kinetic techniques were employed to allow determination of intrinsic maximal translation rates independent of any changes in endogenous amino acid metabolism associated with the models.

EXPERIMENTAL

Animals

Male, fed Sprague–Dawley rats were used in all experiments. At the time of mitochondrial isolation rats weighed 410 ± 20 g ($n = 16$). Clofibrate-treated rats were maintained on a standard rat chow (Ralston Purina Rat Chow, St. Louis, MO, U.S.A.) supplemented with 0.5% (w/w) clofibrate. Clofibrate-fed rats were housed in individual wire-bottomed cages with *ad libitum* access to the clofibrate-supplemented chow and water for a period of 8–10 days. Control rats were handled identically to clofibrate-fed rats, except they were fed on control chow. An osmotic minipump (Alza Corporation, Palo Alto, CA, U.S.A.; model 2001) containing HCCL was subcutaneously implanted under ether anaesthesia into HCCL-treated rats. HCCL was delivered at a rate of 2 μ g/h; this drug results in severe inhibition of hepatic L-methylmalonyl-CoA mutase activity and produces

methylmalonic aciduria after 4–7 days [18]. The HCCL-treated rats were studied 14 days after pump implantation. Previous experiments utilizing rats implanted with saline-containing pumps as a control group for HCCL treatment demonstrated no differences between the saline-pump controls and non-pump-implanted controls from other studies (results not shown). Therefore the single non-pump control group was used for comparisons with clofibrate- and HCCL-treated rats. As previously described [18,19], rats tolerate the clofibrate or HCCL treatments without difficulty and gain weight at rates equivalent to control rats, and the responses to drug treatment have been extensively characterized.

Measurement of mitochondrial translation

Liver mitochondria were isolated by differential centrifugation as detailed by Hoppel *et al.* [20]. Final mitochondrial preparations were suspended in 220 mM-mannitol, 70 mM-sucrose, 5 mM-Mops, pH 7.4. The conditions for mitochondrial translation were modified from those described by Mills *et al.* [9] and Beattie [8]. Incubations were conducted in a total volume of 400 μ l and contained mitochondria at concentrations noted in individual experiments, plus 80 mM-KCl, 5 mM- K_2HPO_4 , 50 mM-Mops, 2 mM-EDTA, 10 mM-MgCl₂, 0.1 mg of defatted BSA/ml, 5 mM-ATP, 5 mM-phosphoenolpyruvate, 10 units of pyruvate kinase/ml and amino acids at 60 μ g/ml, exclusive of the specific amino acid used as a radiolabelled substrate (amino acids included alanine, arginine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, isoleucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan and valine). Incubations were conducted at pH 7.4 and 30 °C. Unlabelled amino acid corresponding to the radiolabelled substrate was included in the incubation at concentrations indicated in specific experiments. The incubation vial was pre-incubated for 5 min and [³⁵S]methionine or [³H]leucine was added at time zero (approx. 200 μ Ci/ml for ³⁵S or 20 μ Ci/ml for ³H, with actual radioactivity being quantified for each incubation vial). At time points of interest, aliquots (10 μ l) were removed in duplicate from the incubation and spotted on to filter paper (Whitman #541; approx. 3 cm $\times$ 3 cm). The filters were dried and washed three times in 10% (w/v) trichloroacetic acid (3 min per wash). After washing, the filters were dried and placed in glass liquid scintillation vials for quantification of retained radioactivity.

Kinetic analysis of mitochondrial translation rates

Rates of radioactivity incorporated into trichloroacetic acid-insoluble material were calculated as the fractional incorporation/min. For incubations from which samples at multiple time points were obtained (incubations with 50 ng of exogenous radiolabelled amino acid/ml were used for the full time curve with each mitochondrial preparation), the slope of the d.p.m./10 μ l of incubation versus time curve was determined over the first 20 min by least-squares regression (time points at 0, 5, 10, 15 and 20 min in duplicate). This slope was then divided by the total radioactivity added and the result multiplied by 10⁶ for convenience of expression. Incubations sampled at only 0 and 20 min were analysed in the same manner, except that radioactivity incorporated per 20 min was calculated by subtraction.

Rates of radioactivity incorporation cannot be readily converted to rates of amino acid incorporation or used for comparison between different mitochondrial preparations, as the true specific radioactivity is not known (due to a contribution of endogenous amino acids), and because sub-saturating concentrations of labelled amino acids are used to facilitate incorporation of quantifiable radioactivity. To permit more direct estimates of actual amino acid incorporation rates, fractional radioactivity incorporation rates were determined at three

to five concentrations of exogenous radiolabelled amino acid for each preparation. To avoid assumptions about the endogenous amino acid concentration, a Michaelis–Menten kinetic model was used with [substrate] = [exogenous substrate] + [endogenous substrate], and the velocity expressed as the fractional incorporation rate. Transformation of this expression yields:

$$\frac{1}{V_{\max.}}[\text{Exogenous substrate}] + \frac{1}{V_{\max.}}([\text{Endogenous substrate}] + K_m) = \frac{1}{\text{Rate}}$$

This expression, analogous to one developed by Dowse *et al.* [21] for other applications, permits a single reciprocal plot of [exogenous substrate] versus 1/rate to be constructed which yields $V_{\max.}$ without explicit knowledge of [endogenous substrate]. Similarly, the sum [endogenous substrate] + K_m can be explicitly calculated. This single reciprocal plot was used to determine $V_{\max.}$ for each individual mitochondrial preparation, and to calculate the $V_{\max.}$ based on the mean fractional incorporation rates at each [exogenous substrate] for each treatment group.

If [endogenous substrate] is much less than [exogenous substrate], a traditional Lineweaver–Burk double-reciprocal plot of 1/[exogenous substrate] versus 1/rate can be used to define $V_{\max.}$ The double-reciprocal plots were generated from the mean fractional incorporation rates at each [exogenous substrate] for each of the treatment groups.

Other assays

Radiolabelled products of mitochondrial protein syntheses were identified by SDS/PAGE and fluorography. An aliquot of mitochondrial incubation (250 μ l) was removed at 30 min and centrifuged at 10000 g for 10 min at 4 °C. The supernatant was removed and the pellet resuspended in SDS buffer for electrophoresis on a 12% polyacrylamide gel [22]. Bands were visualized after treatment of the gel with 1 M-salicylate [23] or Enhance Spray (New England Nuclear Research Products, Boston, MA, U.S.A.) by exposure to photographic film (X-OMAT AR; Eastman Kodak Company, Rochester, NY, U.S.A.). Bands were quantified by scanning densitometry.

Protein concentrations were determined by the biuret method [24].

Materials

[³⁵S]Methionine and [³H]leucine were purchased from American Radiolabelled Chemicals, Inc. (St. Louis, MO, U.S.A.). Pyruvate kinase, ATP, phosphoenolpyruvate, clofibrate and defatted BSA were obtained from Sigma (St. Louis, MO, U.S.A.). HCCL was synthesized by the method of Bonnett *et al.* [25] as previously described [26].

Statistics

All data are presented as means $\pm$ S.E.M., with n referring to the number of distinct mitochondrial preparations used for the analysis. Effects of treatments were determined by Student's unpaired t test, with $P < 0.05$ considered significant.

RESULTS

Incorporation of amino acids into trichloroacetic acid-insoluble protein was linear with respect to time for at least 20 min for both [³⁵S]methionine (Fig. 1) and [³H]leucine (results not shown) in isolated liver mitochondria from control rats. Translation was inhibited 90% by chloramphenicol (100 μ g/ml), but not by cycloheximide (results not shown). Additionally, amino acid incorporation was linearly dependent on

Fig. 1. Incorporation of [^{35}S]methionine by isolated mitochondria

Liver mitochondria were isolated from control (●) or HCCL-treated (○) rats as described in the text. Mitochondria (0.4 mg/400 μl total volume) were incubated with [^{35}S]methionine at 50 ng of amino acid/ml in the translation incubation mixture. Radiolabel incorporated into trichloroacetic acid-insoluble material was quantified at the times indicated (expressed as d.p.m. incorporated/10 μl divided by the total radioactivity/10 μl , multiplied by 10^6 for convenience). Values are means $\pm$ S.E.M. from three to five mitochondrial preparations per point. Actual regression equations for 0–20 min are $y = 88.7x - 26$ for control ($r = 0.998$) and $y = 77.3x - 28$ for HCCL treatment ($r = 0.997$).

Fig. 2. Relationship between exogenous substrate concentration and fractional amino acid incorporation by isolated mitochondria

Liver mitochondria (0.4 mg/400 μl incubation) from control rats were isolated and incubated with [^{35}S]methionine to determine fractional ^{35}S incorporation. Exogenous [^{35}S]methionine was varied from 50–550 ng/ml. The reciprocal of the fractional incorporation rate was plotted as function of exogenous substrate concentration. Values are means $\pm$ S.E.M. with n in parentheses. The actual regression equation was $y = 108x + 13900$ ($r = 0.9722$).

Table 1. Rates of amino acid incorporation by isolated liver mitochondria

Liver mitochondria were isolated from control, clofibrate- and HCCL-treated rats. Fractional incorporation rates for [^3H]leucine and [^{35}S]methionine were determined for each amino acid (exogenous amino acid concentration, 50 ng/ml; 0.4 mg of mitochondrial protein/incubation) based on 0, 5, 10, 15 and 20 min time points. Data are expressed as the means $\pm$ S.E.M. of the incorporation rates from (n) individual mitochondrial preparations.

Rats	Amino acid incorporation rate ($10^6 \times$ fractional incorporation/min)	
	[^{35}S]Methionine	[^3H]Leucine
Control	77.2 $\pm$ 10.5 (5)	70.0 $\pm$ 11.8 (3)
Clofibrate-treated	84.5 $\pm$ 14.5 (5)	43.0 $\pm$ 3.2 (2)
HCCL-treated	77.3 $\pm$ 21.0 (4)	38.3 $\pm$ 7.2 (3)

mitochondrial protein content of the incubation (results not shown).

Rates of [^{35}S]methionine and [^3H]leucine incorporation by liver mitochondria isolated from control, clofibrate- and HCCL-treated rats were initially determined at a fixed exogenous amino acid concentration (50 ng/ml). Incorporation rates, determined for each mitochondrial preparation by linear regression of the time course over 0–20 min, were normalized to total radioactivity to yield fractional incorporation rates. No significant differences in amino acid incorporation rates were observed between mitochondria from control versus HCCL-treated rats (Fig. 1, Table 1) or from control versus clofibrate-treated rats (Table 1). Homogenate citrate synthase activities, a marker of mitochondrial proliferation, were 8.5 ± 0.8 , 9.3 ± 0.8 and 10.0 ± 0.7 $\mu\text{mol/min per g}$ of liver for control ($n = 3$), clofibrate-treated ($n = 2$) and HCCL-treated ($n = 3$) rats respectively in a non-selected subset of the livers. This pattern of increase is similar to that observed for the clofibrate- and HCCL-treated rat

models in previous studies with a similar duration of treatment [16,19,27].

Interpretation of radiolabelled amino acid incorporation rates is potentially complicated by changes in endogenous amino acid content in the isolated mitochondria following the treatments of interest. This concern is of particular importance as both clofibrate and HCCL treatment might be anticipated to alter amino acid metabolism [26,28]. Additionally, as sub-saturating concentrations of labelled amino acid are used, the true V_{max} of the system is not obtained from the data in Table 1. To circumvent these problems, amino acid incorporation rates were determined at a minimum of three concentrations of exogenous labelled amino acid. A transformation of the Michaelis–Menten equation to yield a plot of [exogenous substrate] versus $1/\text{rate}$ provides a straight line of slope $1/V_{\text{max}}$, independent of endogenous amino acid concentration (single-reciprocal plot; see the Experimental section). To assess the appropriateness of this model, [^{35}S]methionine fractional incorporation rates at five exogenous methionine concentrations were averaged from a total of five liver mitochondrial preparations from control rats. A single-reciprocal plot was generated from these mean data (Fig. 2). The experimental data fit the model well ($r = 0.972$). Using this approach, V_{max} was calculated for each mitochondrial preparation (Table 2a). This analysis demonstrated no significant effects of clofibrate or HCCL treatment on V_{max} values for mitochondrial incorporation of either [^3H]leucine or [^{35}S]methionine. As indicated in Fig. 2, the experimental variation in the measurement of fractional incorporation rate increases as the exogenous substrate concentration increases, secondary to a decrease in the absolute radioactivity incorporated. To partially circumvent the variability in V_{max} estimates due to this non-constant error, the mean fractional incorporation rates at each exogenous amino acid concentration were calculated and used to estimate V_{max} (as illustrated in Fig. 2). This analysis confirmed the lack of difference in V_{max} between the experimental groups (Table 2b). The single-reciprocal plots also permitted calculation of [endogenous amino acid] + K_m (Table 3; note the relatively large standard errors, as the values require the use of the slopes and intercepts from the single-reciprocal plot, with the resultant

Table 2. Kinetic analysis of amino acid incorporation by isolated liver mitochondria

Fractional incorporation rates for [^3H]leucine and [^{35}S]methionine were determined in liver mitochondria isolated from control, clofibrate- and HCCL-treated rats. Each mitochondrial preparation was studied at a minimum of three exogenous amino acid concentrations over the range 50–550 ng/ml and contained 0.4 mg of mitochondrial protein/incubation. Incorporation V_{max} was calculated as detailed in the text using the single-reciprocal plot from each experiment (a , presented as means $\pm$ S.E.M. with n in parentheses), a single-reciprocal plot generated from the mean fractional incorporation rate at each exogenous amino acid concentration (b) or the double-reciprocal plot analysis of the mean data (c). N.D., not determined.

	V_{max} (pmol/min per mg of mitochondrial protein)	
	[^{35}S]Methionine	[^3H]Leucine
(a) Single-reciprocal plot: individual experiments		
Control	0.054 $\pm$ 0.014 (5)	0.076 $\pm$ 0.014 (3)
Clofibrate-treated	0.090 $\pm$ 0.014 (5)	N.D.
HCCL-treated	0.097 $\pm$ 0.035 (3)	0.070 $\pm$ 0.014 (3)
(b) Single-reciprocal plot: mean data		
Control	0.062 $\pm$ 0.009	0.050 $\pm$ 0.016
Clofibrate-treated	0.057 $\pm$ 0.013	0.050 $\pm$ 0.016
HCCL-treated	0.061 $\pm$ 0.019	0.066 $\pm$ 0.035
(c) Double-reciprocal plot: mean data		
Control	0.047 $\pm$ 0.010	0.043 $\pm$ 0.008
Clofibrate-treated	0.053 $\pm$ 0.015	0.061 $\pm$ 0.016
HCCL-treated	0.060 $\pm$ 0.025	0.089 $\pm$ 0.038

Table 3. Calculation of [endogenous amino acid] + K_m from single-reciprocal plots

Radiolabel incorporation rates were determined as in Table 2. The sum [endogenous amino acid] + K_m was calculated as detailed in the text using the single-reciprocal plot from each experiment (a , presented as means $\pm$ S.E.M. with n in parentheses) or using a single-reciprocal plot generated from the mean fractional incorporation rate at each exogenous amino acid concentration (b). N.D., not determined.

	[Endogenous amino acid] + K_m (ng/ml)	
	Methionine	Leucine
(a) Individual experiments		
Control	197 $\pm$ 131 (5)	116 $\pm$ 30 (3)
Clofibrate-treated	136 $\pm$ 37 (5)	N.D.
HCCL-treated	90 $\pm$ 53 (3)	189 $\pm$ 36 (3)
(b) Mean data		
Control	129 $\pm$ 56	40 $\pm$ 22
Clofibrate-treated	76 $\pm$ 94	87 $\pm$ 23
HCCL-treated	67 $\pm$ 79	188 $\pm$ 38

accumulation of variability). The values of [endogenous amino acid] + K_m for [^{35}S]methionine incorporation were similar for the three treatment groups. In contrast, the sum for [^3H]leucine was increased in the clofibrate- and HCCL-treated groups, correlated with the relative decrease in absolute fractional incorporation rates for [^3H]leucine observed in these groups (Table 1). If the contribution of endogenous amino acid is ignored, the incor-

Fig. 3. Incorporation of [^{35}S]methionine into mitochondrial translation products

Liver mitochondria was isolated from control (lane 1), clofibrate-treated (lane 2) and HCCL-treated (lane 3) rats and incubated (0.4 mg of mitochondria/400 μl incubation) with [^{35}S]methionine (50 ng/ml) for 30 min as detailed in the text. Protein was then separated by SDS/PAGE and radioactivity localized by fluorography. The positions, based on the molecular mass, of mitochondrial translation products noted are shown on the left (ND, NADH dehydrogenase, followed by subunit number; Cyt. b , cytochrome b ; CO, cytochrome oxidase, followed by subunit number; ATPase, ATPase subunits).

poration data can be analysed by conventional Lineweaver–Burk analysis (Table 2c). Unlike the previous methods of analysis, the double-reciprocal plot suggests an increase in the rate of [^3H]leucine incorporation. This variance from the absolute incorporation rates and the single-reciprocal analysis is consistent with the increased [endogenous amino acid] + K_m sum observed (Table 3).

The mitochondrial genome codes for 13 polypeptides [6,7]. To verify that [^{35}S]methionine was incorporated into mature mitochondrial polypeptide, the mitochondrial protein was size-fractionated by SDS/PAGE at the conclusion of 30 min of incubation (Fig. 3). Approx. 10–15 major polypeptide products were observed, of which 11 corresponded to known mitochondrial gene products based on electrophoretic mobility. No differences in the distribution of ^{35}S among translation products was observed in incubations from the three treatment groups (Fig. 3, representative of at least two mitochondrial incubations analysed for each treatment group).

DISCUSSION

Mitochondrial biogenesis is dependent on the expression of both nuclear and mitochondrial DNA. The mitochondrial genome codes for 13 polypeptides that are critical for normal oxidative phosphorylation [6,7]. Increased mitochondrial biogenesis in response to metabolic stress requires incorporation of both nuclear and mitochondrial polypeptide products to yield mature, functional mitochondria. The current studies demonstrate in two models of hepatic mitochondria proliferation that maximal mitochondrial translation rates (per mg of mitochondrial protein) are not increased during the proliferative response.

Interpretation of tracer amino acid incorporation data for kinetic questions is problematic. To ensure valid quantitative comparisons between the treatment groups, several important factors were incorporated into the current experimental design.

First, amino acid incorporation was linear with respect to time and protein concentration, resulting in optimal quantification of fractional incorporation rates. Second, by varying the exogenous amino acid concentration, translation $V_{\max}$ could be determined without assumptions concerning endogenous amino acid concentrations or sub-saturating substrate concentrations. Previous estimates of mitochondrial endogenous amino acid pool sizes have ranged from negligible [29] to 100 μM [30]. However, most previous methods have not distinguished between differences in true endogenous amino acid concentrations and the effects of sub-saturating substrate concentrations. The current data suggest a sum of [endogenous substrate] + K_m of 129 ng/ml, or 0.86 μM . The possible changes in endogenous leucine content (or leucine K_m) with clofibrate and HCCL treatments would explain the seemingly paradoxical previously reported observation of decreased mitochondrial incorporation of [^3H]leucine, but not [^{35}S]methionine, after clofibrate treatment [15]. Third, two distinct amino acids were used to ensure concordant changes (if any) in translation rates, thus excluding a change in amino acid handling. Amino acids, including methionine and leucine, enter mitochondria via carrier-mediated mechanisms [31,32] as well as by diffusion [33]. Based on published values, rates of amino acid uptake should not be limiting for mitochondrial protein synthesis under the conditions studied [31]. Fourth, SDS/PAGE and fluorography was used to confirm the formation of mitochondrial translation products. The formation of mature mitochondrial polypeptides, dependence on mitochondrial protein content and inhibition of chloramphenicol (an inhibitor of mitochondrial, but not cytosolic, translation [34]) all document that mitochondrial translation rates were being quantified.

The measured mitochondrial translation rates represent a single time point in the course of the proliferative responses to HCCL or clofibrate. Time points for each treatment were selected based on previous studies to ensure that mitochondrial proliferation had been initiated, but that a new steady-state maximal mitochondrial content had not been reached. HCCL-induced mitochondrial proliferation is seen after 2 weeks of treatment, but is progressive for at least 6 weeks [16], while clofibrate-induced proliferation is progressive for 15–20 days [15]. In the current studies, 8–10 days of clofibrate treatment or 14 days of HCCL treatment resulted in mean citrate synthase activities (per g of liver) of 9.5% and 17.6% respectively above control, a magnitude of change similar to that observed in previous studies [16,19,27] and consistent with the initiation of the mitochondrial proliferative response. These responses are also sub-maximal based on previous studies, and thus support the assumption that the animals used in the current studies were responding to treatment in a manner similar to those previously characterized.

The lack of increase in mitochondrial translation rates in clofibrate- and HCCL-induced mitochondrial proliferation provides insights into the cellular regulation of mitochondrial content. The majority of mitochondrial proteins are coded by the nuclear genome and translated in the cytosol. Thus cellular mitochondrial protein content can be increased without enhanced mitochondrial translation, but the result would be apparent dilution of the mitochondrial gene products. Mitochondria from HCCL-treated rats have a low cytochrome *b* content, and decreased cytochrome oxidase and ubiquinol:ferricytochrome *c* oxidoreductase activities per mg of mitochondrial protein [35], which might arise from this mechanism. Alternatively, as the nuclear-encoded proteins are transported to the mitochondria, mitochondrial gene expression may increase proportionally as important regulatory factors, included in the spectrum of nuclear-encoded proteins, accumulate. Thus, although mitochondrial translation per mg of mitochondrial protein remained unchanged, as total mitochondrial protein increased, then

mitochondrial translation per cell would be increased. The current studies do not exclude increased mitochondrial translation *in vivo* if the increase in mitochondrial translation was dependent on an unstable factor, on an aspect of the *in vivo* cellular environment not reproduced *in vitro*, or due to regulatory changes not associated with a change in translation $V_{\max}$.

The normal hepatocyte mitochondrial content is remarkably constant (approx. 60 mg of mitochondrial protein/g of liver [16]) and may reflect a mitochondrial translation capacity intrinsic to the liver mitochondria. Thus, while decreases in the normal hepatic mitochondrial content are associated with decreases in mitochondrial translation, increases from the normal as a result of hyperthyroidism [13] or HCCL or clofibrate treatment are not. These results suggest that the major regulation of mitochondrial content to supra-normal levels occurs in the nucleus.

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