CONTROL OF YEAST AND MAMMALIAN MITOCHONDRIAL PROTEIN SYNTHESIS BY CYTOPLASMIC FACTORS

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1. Introduction

The biogenesis of the mitochondrial inner membrane requires the synthesis of proteins at 2 intracellular sites: 1 cytoplasmic; 1 mitochondrial. A mechanism must exist to coordinate the synthesis of proteins at the 2 distinct intracellular sites such that mitochondrial formation occurs in an orderly manner. Previous studies in yeast using selective inhibitors of cytoplasmic and mitochondrial protein synthesis have suggested that cytoplasmically synthesized proteins may control mitochondrial protein synthesis both in vivo [1–4] and in vitro [3] and that this control may occur by either stimulation of chain initiation or increased formation of mitochondrial mRNAs [5]. In [6] protein synthesis by yeast mitochondria in vitro was reported stimulated by addition of a high-speed cytoplasmic supernatant from yeast [6]. This effect is not species-specific, as protein synthesis in vitro by isolated yeast mitochondria could be stimulated markedly by post-polysomal supernatants from either *E. coli*, rat liver or yeast [7]. However, stimulation of yeast mitochondrial protein synthesis by all of these high speed supernatants may be due to the presence of non-dialyzable GMP which is converted to GDP (or GTP) during the incubation in the protein-synthesizing medium [8]. We have confirmed that GTP stimulates yeast mitochondrial protein synthesis in vitro, but addition of a post-polysomal supernatant from yeast doubles the rate of mitochondrial protein synthesis in the presence of GTP [9]. These data indicate that high-speed supernatants from rat skeletal muscle and liver also stimulate yeast mitochondrial protein synthesis in the presence of GTP. In addition, evidence has been obtained to suggest the control of protein synthesis in liver mitochondria by cytoplasmic proteins.

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; TLCK, *N*-α-*p*-tosyllysine chloromethyl ketone

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2. Experimental

2.1. *Preparation of mitochondria*

Yeast mitochondria were prepared as in [9]. Skeletal muscle mitochondria were prepared as in [10] or from mechanically homogenized muscles [11]. Rat liver mitochondria were prepared in 0.2 M mannitol, 0.07 M sucrose, 10 mM Hepes (pH 7.7) and 2 mM EDTA as in [12].

2.2. *In vitro mitochondrial protein synthesis*

The incubation mixture for yeast mitochondrial protein synthesis in vitro was modified [6] to contain 20 nmol/ml of L-leucine, 1 mg/ml of bovine serum albumin and, where indicated, 0.5 mM GTP. Protein synthesis was initiated by addition of mitochondria and determined by the incorporation of L-[4,5-^{3}H]-leucine (0.04 mCi/ml, 55.9 Ci/mmol) into trichloroacetic acid-precipitable protein [13]. Amino acid incorporation by either skeletal muscle or liver mitochondria was carried out as in [11]. Where indicated, cycloheximide (0.5 mg/ml in 0.85 g% NaCl) was administered intraperitoneally from 2–24 h before sacrifice.

2.3. *Preparation of cytosolic post-polysomal supernatants (S-140)*

Rat skeletal muscles were excised and homogenized

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with a Brinkmann Polytron homogenizer in buffer D (10 mM potassium phosphate (pH 7.4), 1 mM EDTA, 10% glycerol, 1 mM PMSF and 1 mM TLCK). Immediately after sacrifice, rat livers were perfused via the vena cava with ice-cold 10 mM potassium phosphate (pH 7.4), 130 mM NaCl, 1 mM EDTA to remove erythrocytes, followed by homogenization in buffer D. The post-mitochondrial supernatant from the homogenates was then centrifuged for 90 min at 140 000 × *g*. The upper 60–70% of the supernatant was dialyzed 15 h in Spectrapore tubing (M_r-cutoff 3500) against 10 mM potassium phosphate (pH 7.4), 10% glycerol. The dialyzed S-140 was concentrated by treating the intact dialysis bag with Sephadex G-50, then frozen with liquid nitrogen, and stored in aliquots at −70°C. Yeast S-140 fractions were prepared as in [9].

3. Results and discussion

The effect of exogenous GTP on the rate of protein synthesis in vitro was compared in yeast and mammalian mitochondria (table 1). Addition of 10–1000 μM GTP to the incubation medium stimulated yeast mitochondrial protein synthesis >3-fold. A marked effect was observed at 10 μM of GTP with the maximum stimulation observed at 100 μM. By contrast, addition of these same concentrations of GTP had almost no effect on the rate of protein synthesis by either rat liver or skeletal muscle mitochondria. At most, a marginally significant increase was observed. Perhaps, mammalian mitochondria have a larger endogenous pool of guanine nucleotides as compared to yeast mitochondria. Alternatively, yeast mitochondria may suffer a greater loss of nucleotides during preparation.

In [7] the stimulation of yeast mitochondrial protein synthesis by addition of post-polysomal supernatant fractions isolated from *E. coli*, rat liver and yeast was observed in a medium lacking exogenous GTP. As shown in table 2, addition of an S-140 from either rat skeletal muscle, rat liver or yeast significantly stimulated yeast mitochondrial protein synthesis in the presence of 0.5 mM GTP. S-140 from rat liver increased the incorporation rate in vitro more effectively than the S-140 from yeast. Protein synthesis in the presence of added S-140 fractions was 97% chloramphenicol-sensitive, indicating its mitochondrial origin.

While most efforts have focused on the cytoplasmic control of yeast mitochondrial protein synthesis, some studies have suggested a similar control of protein synthesis in rat liver mitochondria. Therefore, we investigated the effect of addition of cytoplasmic S-140 fractions on the rate of mammalian mitochondrial protein synthesis. In contrast to the above data, addition of S-140 fraction from either *E. coli*, yeast, rat skeletal muscle or rat liver failed to stimulate rat liver mitochondrial protein synthesis in vitro (not shown). Similarly, addition of an S-140 fraction isolated from regenerating rat liver in which the rate of mitochondrial protein synthesis is increased [14], was without effect. All attempts to stimulate rat skeletal muscle mitochondrial protein synthesis by addition of S-140 fractions were unsuccessful.

The effects of cycloheximide administration to rats upon RNA and protein synthesis provided another approach to the cytoplasmic control of mammalian mitochondrial protein synthesis [15–20]. After administration of cycloheximide, the synthesis of total mitochondrial protein, >90% of which is cytoplasmically synthesized, was inhibited at 2 h followed

Table 1
Effect of GTP on protein synthesis in vitro by yeast and mammalian mitochondria

Source of mitochondria	Protein synthesis[a]			
	Control	10 μM GTP	100 μM GTP	1 mM GTP
Yeast	58	181	227	192
Rat skeletal muscle	5.5	–	6.1	–
Rat liver	10.0	10.2	11.2	12.5

[a] Incorporation is pmol leucine incorp./mg mitochondrial protein^{-1} . incubation^{-1}

Protein synthesis in isolated mitochondria was determined as in section 2

Table 2
Effect of cytoplasmic S-140 fractions from yeast, rat liver and skeletal muscle on protein synthesis in vitro by yeast mitochondria

Addition to mitochondria	Incorporation rate[a]					
	Expt 1		Expt 2		Expt 3	
	Activity	% Stim.	Activity	% Stim.	Activity	% Stim.
GTP (control)	192 ± 10.9		141 ± 6.4		91.5 ± 2.6	
GTP + S-140 (yeast)	379 ± 33.2	97	204 ± 21.6	45	147 ± 3.8	61
GTP + S-140 (skeletal muscle)	265 ± 14.8	38	–		–	
GTP + S-140 (rat liver)	–		243 ± 7.4	72	223 ± 9.0	144

[a] Incorporation is pmol leucine incorporated . mg mitochondrial protein^{-1} . incubation^{-1}. Values shown are the means ± SE for each experiment

Protein synthesis in isolated yeast mitochondria was determined as in section 2. Where indicated, 1.0 mg of S-140 fractions were added to the incubation

by a recovery phase during which an actual stimulation was observed at 24 h. Here, the rates of liver mitochondrial protein synthesis in vitro were measured at several time intervals after the administration of cycloheximide in vivo (fig.1). A 30–40% decline in

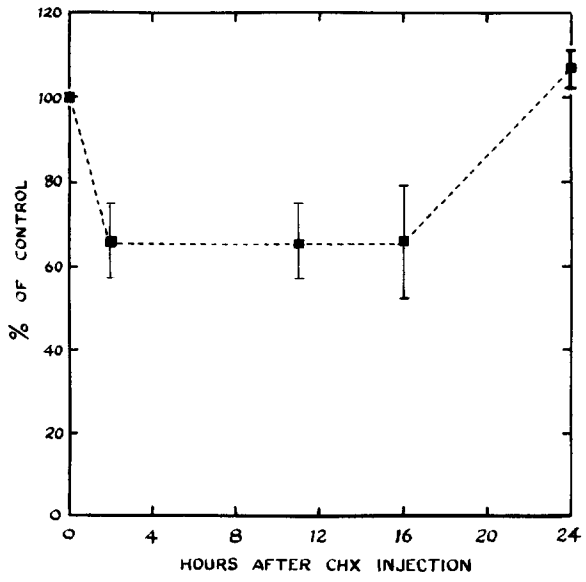

Fig.1. Effect of cycloheximide administration in vivo upon liver mitochondrial protein synthesis in vitro. Cycloheximide (2.5 mg/kg) was injected intraperitoneally at the indicated times prior to sacrifice. Protein synthesis in vitro by rat liver mitochondria was determined as in section 2. The data shown represent the mean values and ranges of 2 separate experiments.

the incorporation rate was evident 2 h after injection and was still observed after 16 h; however, by 24 h after cycloheximide treatment, the ability of liver mitochondria to synthesize protein in vitro had increased slightly above the control level. These results suggest that inhibition of cytoplasmic translation may lead to a decrease in the rate of mitochondrial protein synthesis. Restoration of the rate of cytoplasmic protein synthesis after the metabolism and excretion of cycloheximide may then be followed by return of the rate of mitochondrial protein synthesis to control levels or higher. The effect of cycloheximide administration in vivo was dose-dependent when the rate of liver mitochondrial protein synthesis in vitro was examined 2 h and 18 h after injection (table 3). A maximum inhibition of 30–40% was observed at either time.

The above experiments with cycloheximide suggest a control of rat liver mitochondrial protein synthesis by cytoplasmically synthesized proteins; however, no direct stimulation of mammalian mitochondrial protein synthesis could be demonstrated after addition of S-140 fractions. Thus it was of interest to determine the stimulatory effect of high-speed supernatants from cycloheximide-treated rats on the rate of protein synthesis in vitro by isolated yeast mitochondria. The S-140 fractions were prepared from rat livers after intraperitoneal injection of cycloheximide at the times indicated in fig.1 and tested for their ability to stimulate protein synthesis (table 4). All the S-140 fractions tested increased the incorporation rate in

Table 3
Effect of dosage level of cycloheximide administered in vivo upon liver mitochondrial protein synthesis in vitro

Cycloheximide (mg/kg)	Hours after injection	Incorporation rate[a]	% of control
0	2	26.7 ± 1.1	100.0
2.0	2	22.0 ± 0.8	82.3
10.0	2	21.3 ± 2.1	79.6
100.0	2	15.6 ± 1.6	58.3
0	18	30.4 ± 3.5	100
1.25	18	26.1 ± 0.8	85.8
2.50	18	21.3 ± 1.3	70.1

[a] Incorporation rate is pmol leucine incorporated . mg mitochondrial protein^{-1} . incubation^{-1}. Values shown are the means ± SE of a representative experiment which was repeated 2 times

The indicated dose of cycloheximide dissolved in physiological saline was injected intraperitoneally at 2 or 18 h prior to sacrifice. Protein synthesis in vitro by isolated liver mitochondria was measured as in section 2

Table 4
Effect of liver cytoplasmic S-140 fractions from control and cycloheximide-treated rats upon protein synthesis in vitro by yeast mitochondria

Treatment of yeast mitochondria	Incorp. rate[a]	% Stimulatory activity
No addition	91.5 ± 2.2	–
Plus S-140	223 ± 7.6	100
Plus S-140 2 h after cycloheximide	185 ± 6.1	70
Plus S-140 16 h after cycloheximide	185 ± 8.8	70
Plus S-140 24 h after cycloheximide	168 ± 5.5	57

[a] Incorporation is pmol leucine incorporated . mg mitochondrial protein^{-1} . incubation^{-1}

Protein synthesis in vitro by isolated yeast mitochondria was determined as in section 2. Rat liver S-140 fractions (1.0 mg) were prepared from animals sacrificed at the stated time after intraperitoneal administration of cycloheximide (2.5 mg/kg). Values shown are the means ± SE of a representative experiment which has been repeated 3 times

isolated yeast mitochondria; however, the stimulatory ability of S-140 fractions isolated from rats 2–24 h after cycloheximide treatment was decreased compared with the control. These results suggest that the inhibition of cytoplasmic translation in rat liver by cycloheximide may result in a decreased leveling of the factors present in the cytosol which stimulate yeast mitochondrial protein synthesis in vitro.

These results indicate that high-speed supernatant fractions obtained from both rat liver and skeletal muscle can significantly stimulate protein synthesis by isolated yeast mitochondria in the presence of optimal concentrations of GTP. Furthermore, these results do not support the conclusion that stimulation of protein synthesis in isolated yeast mitochondria by high speed supernatant from different organisms is solely due to guanine nucleotides [8]. Moreover, cycloheximide administration in vivo resulted in decreased rate of protein synthesis in vitro by rat liver mitochondria. This decrease could be correlated with a diminution in the ability of the supernatant fractions to stimulate yeast mitochondrial protein synthesis. Despite these indications of a cytoplasmic regulation of mammalian mitochondrial protein synthesis, no stimulation of the latter by addition of S-140 fractions was observed. Perhaps, isolated liver mitochondria contains a sufficient pool of the stimulatory factor(s) and hence do not require additional stimulatory factor(s) during the course of the incubation. In support of this, rat liver mitochondria synthesize proteins at a linear rate for 40–60 min in vitro [21]; while protein synthesis by yeast mitochondria in vitro ceases after 20–30 min [6,7,9].

We have purified a low-M_r fraction from yeast that stimulates protein synthesis by isolated yeast mitochondria [9]. The possibility that a similar stimulatory factor(s) is present in rat liver supernatants is under investigation.

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