Biochimica et Biophysica Acta, 936 (1988) 372–376
Elsevier

BBA 42890

Inhibition of mitochondrial protein synthesis in regenerating rat liver stimulates mitochondrial transcription

Stefan Kuzela *, Ann Mutvei and B. Dean Nelson

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm (Sweden)

(Received 1 March 1988)

Key words: Mitochondrial transcription; Transcription; Protein synthesis; (Regenerating rat liver)

Partially hepatectomized rats were treated in vivo with thiamphenicol for 3 days to block mitochondrial protein synthesis. Protein synthesis, RNA synthesis and the steady-state levels of individual transcripts were measured in mitochondria in vitro in the absence of thiamphenicol. Incorporation of [^{35}S]methionine and [^{3}H]UTP into protein and RNA, respectively, was increased 2–3-fold in isolated mitochondria from thiamphenicol-treated animals, indicating increased rates of synthesis of both. Electrophoretic analysis of transcripts labelled with [^{32}P]UTP suggests that synthesis of all the transcripts is increased. The steady-state concentrations of mitochondrial transcripts, measured by Northern blotting using nick-translated cloned *Eco*RI fragments of rat liver mtDNA, were also elevated 2–4-fold in thiamphenicol-treated animals. The data suggest that mitochondrial transcription is under control of a mitochondrial factor which, in turn, is dependent upon mitochondrial protein synthesis.

Introduction

Biogenesis of mitochondria requires the participation of two genetic systems. Interactions between these systems are, however, poorly understood. Nuclear genes encode all proteins involved in mitochondrial nucleic acid and protein synthesis in mammals. The role of these genes in regulating the expression of the mammalian mitochondrial genome is evident.

In contrast, less is known about the influence of mitochondrial gene products on the expression of nuclear or mitochondrial genes. Mitochondrial gene products are present outside the mitochondrion [1] and, at least in lower eucaryotes, appear to influence the expression of specific nuclear genes [2–6].

In order to determine whether mammalian mitochondrial transcription and translation are dependent on mitochondrial gene products, studies were conducted on partially hepatectomized rats treated in vivo with thiamphenicol [7]. Our findings show that the steady-state levels of mitochondrial transcripts, as measured by Northern blots, as well as the rates of mRNA and protein synthesis, were elevated in mitochondria from thiamphenicol-treated rats. Mechanisms by which blocked mitochondrial protein synthesis might influence transcription of the mitochondrial genome are discussed.

Methods and Materials

Partially hepatectomized [7] male, Sprague-Dawley rats (200 g) were treated daily for 3 days

* Permanent address: Cancer Research Institute, Slovak Academy of Science, Bratislava, Czechoslovakia.

Correspondence: B.D. Nelson, Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106, 91 Stockholm, Sweden.

with thiamphenicol (TAP) (400 mg/kg body weight) by i.p. injection [8]. Mitochondria were isolated as described earlier [9] in a medium comprising 220 mM mannitol/70 mM sucrose/5 mM Hepes (pH 7.4). The final pellet was suspended in isolation medium to give 80–100 mg protein/ml, and the suspension was incubated for 3 min at 0 °C with 20 μg digitonin/mg protein in the presence of 0.1% bovine serum albumin. The suspension was diluted and washed twice in isolation medium. This treatment removes more than 90% of the contaminating microsomal enzymes (Kuzela, S. and Luciakova, K., unpublished data).

Mitochondrial RNA synthesis was measured in 100 μl of an incubation mixture containing: 3 mg mitochondrial protein/ml, 50 mM Bicine, 5 mM KH_2PO_4, 90 mM KCl, 1 mM EGTA, 10 mM $MgCl_2$, 0.5 mM each of ATP, GTP and CTP, 0.1% fatty acid free bovine serum albumin, 2.5 mM phosph*enol*pyruvate, pyruvate kinase (20 μg/ml) and radiolabelled UTP. Samples were incubated for 30 min at 30 °C. For transcript analysis, the RNA was labelled with [α-^{32}P]UTP (53 Ci/mmol, 365 μCi/ml incubation mix). For kinetic studies, RNA was labelled with [5,6-^{3}H]UTP (11 Ci/mmol, 10 μCi/ml incubation mix), and the incorporation of radioactivity into the trichloroacetic-acid-insoluble fraction was monitored.

Labeled RNA was extracted by addition of 6 vol. of 4 M guanidine-SCN [10]. RNA was precipitated by adding 0.025 vol. of 1 M acetic acid and 0.70 vol. ethanol to the lysate. The precipitate which formed overnight at −20 °C was dissolved in 400 μl of 1% SDS/50 mM sodium acetate/and 10 mM EDTA (pH 5.1). It was sonicated for 5 min in a Branson sonicator bath and then heated for 1 min at 68 °C. $NaClO_4$ (0.5 M final concn.) was added and the dissolved RNA was extracted with an equal volume of chloroform/isoamyl alcohol (24 : 1). The mixture was vortexed and allowed to stand on ice for 15 min. After centrifugation, the water phase was reextracted with chloroform/isoamyl alcohol (24 : 1) and RNA was precipitated at −20 °C after adding 0.1 vol. of 2 M NaCl and 2 vol. of ethanol. RNA used in Northern blot experiments was isolated in the same manner.

Purified RNA was resolved on 2% agarose-formaldehyde gels [12] and the gels were either dried for subsequent autoradiographic analysis of the labelled transcripts or the RNA was transferred to Biodyne membranes for hybridization to nick-translated mitochondrial DNA. The nick-translated probes used in these latter experiments are the *Eco*RI A and C fragments of rat liver mitochondrial DNA which is cloned into pUN [12].

In vitro mitochondrial protein synthesis and analysis of the radiolabelled translation products were carried out as in Ref. 13.

Results

Thiamphenicol, the methylsulfonyl analogue of chloramphenicol, is an efficient inhibitor of mitochondrial protein synthesis in regenerating rat liver [8]. Decreases in cytochrome oxidase activity and heme aa_3 are reliable measures of the efficiency of this in vivo inhibition [14,15]. Under the conditions used in the present study, thiamphenicol treatment of partially hepatectomized rats decreased liver mitochondrial cytochrome oxidase activity and heme aa_3 of approx. 50% (not shown).

Protein synthesis and RNA synthesis were also measured in liver mitochondria isolated from regenerating livers of rats treated with thiamphenicol. Since thiamphenicol, like chloramphenicol [16], is washed from the mitochondria during isolation, radiolabelling experiments are carried out

TABLE I

STIMULATION OF RNA AND PROTEIN SYNTHESIS IN ISOLATED MITOCHONDRIA UPON IN VIVO TREATMENT WITH THIAMPHENICOL

Hepatectomized rats were treated with thiamphenicol for 3 days as detailed in Methods and Materials. RNA and protein synthesis were measured by incorporation of labeled precursors into isolated mitochondria in the absence of thiamphenicol as described in Methods and Materials. The incorporation of both precursors was linear within the time interval shown. The values are the means ± S.E. ($n = 3$).

Precursor	cpm × 10^{-3} per mg protein per 20 min		
	−TAP	+TAP	+TAP/−TAP
^{3}H-UTP	2.53 ± 0.54	5.40 ± 0.96	2.2 ± 0.1
^{35}S-methionine	139 ± 14	321 ± 27	2.4 ± 0.2

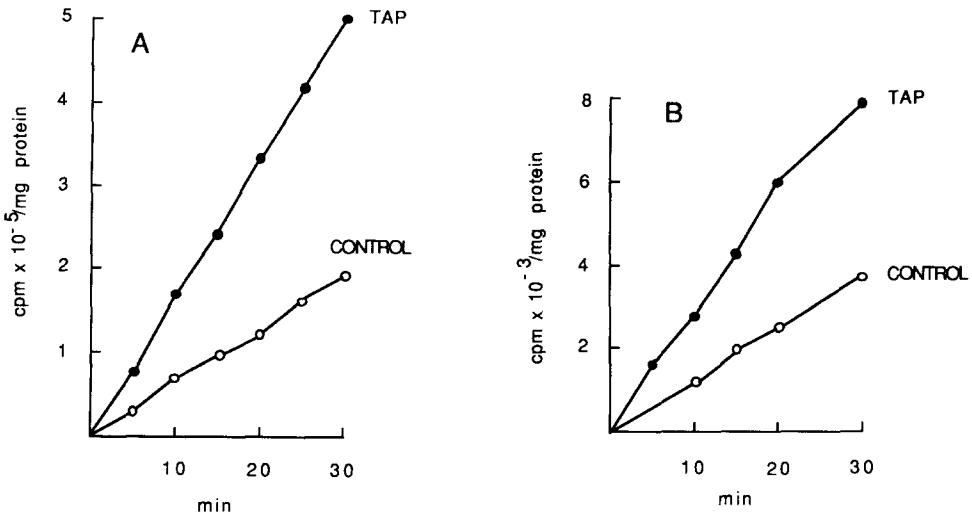

Fig. 1. In vivo treatment with thiamphenicol enhances mitochondrial protein synthesis and RNA synthesis in vitro. Partially hepatectomized rats were treated with thiamphenicol for 3 days as described in Methods and Materials. Liver mitochondria were isolated, and protein synthesis (A) and RNA synthesis (B) were measured in vitro in the absence of thiamphenicol as described in Methods and Materials.

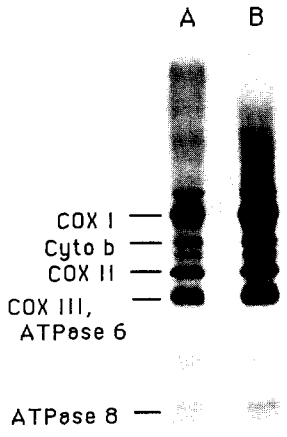

Fig. 2. Analysis of the translation products synthesized in vitro by mitochondria isolated from thiamphenicol-treated rats. Mitochondria were isolated from the regenerating livers of (A) control rats and (B) rats treated in vivo for 3 days with thiamphenicol. Equal amounts of both types of mitochondria were labelled in vitro for 30 min with [^{35}S]methionine in the absence of thiamphenicol, and the labelled translation products were separated on SDS-polyacrylamide gels. A fluorograph of the gel is shown. COX I, II and III are subunits of cytochrome oxidase; Cyto b, cytochrome *b*.

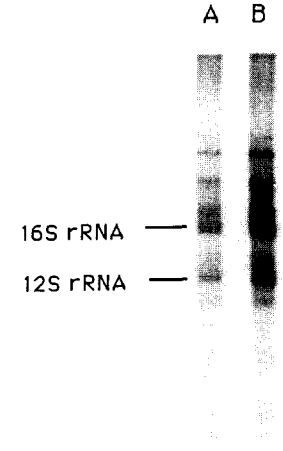

Fig. 3. Analysis of the RNA species synthesized in mitochondria isolated from regenerating liver of thiamphenicol-treated rats. Mitochondria were isolated from the regenerating livers of: (A) control rats and (B) rats treated in vivo for 3 days with thiamphenicol. Mitochondria were labelled in vitro with [^{32}P]UTP. RNA was extracted, resolved on 2% agarose-formaldehyde gels, blotted to Biodyne membranes and exposed to film.

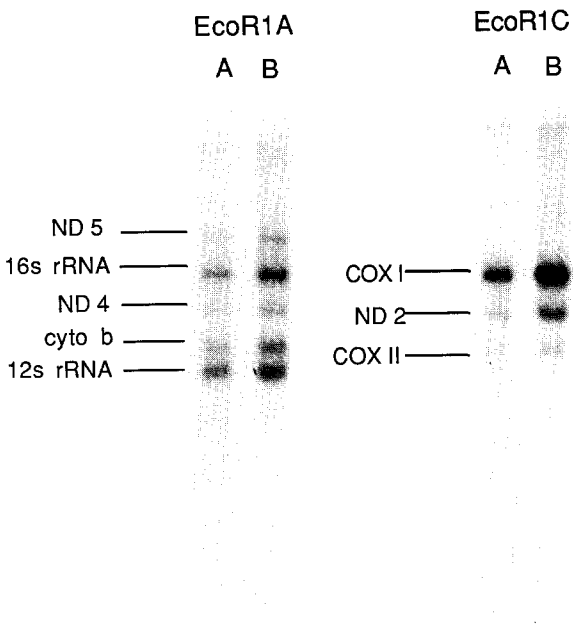

Fig. 4. Northern blot analysis of RNA in mitochondria isolated from regenerating livers of thiamphenicol-treated rats. RNA isolated from 0.1 mg of liver mitochondria from control (A) and thiamphenicol-treated (B) rats was separated on agarose-formaldehyde gels as in Fig. 3. The RNA was blotted onto Biodyne membranes and then probed with nick-translated plasmids (pUN) carrying the *Eco*RI fragments A or C of rat liver mitochondrial DNA [12]. COX I and II, cytochrome oxidase subunits; ND 2, 4 and 5, subunit of the NADH–ubiquinone reductase; cyto b, cytochrome *b*.

essentially in the absence of the inhibitor. The results are given in Fig. 1 and Table I. In vitro labelling of RNA and proteins was increased 2–2.5-fold by thiamphenicol treatment.

All mitochondrial translation products were more highly labelled (2–3-fold as judged from fluorographs) after thiamphenicol treatment (Fig. 2), and the increase is commensurate with the incorporation data (Fig. 1). Thus, in vivo treatment with thiamphenicol leads to a general increase in mitochondrial protein synthesis upon removal of the drug.

The increased rate of RNA synthesis observed in mitochondria from rats treated with thiamphenicol (Fig. 1) suggests that the overall expression of the mitochondrial genome is enhanced. This is supported by analysis of mitochondrial transcript synthesis (Fig. 3), which shows that incorporation of [^{32}P]UTP is enhanced 2–4-fold in mitochondria from thiamphenicol-treated rats. In view of the mechanism of transcription of mammalian mtDNA and the stoichiometry of transcript synthesis (see Ref. 17 for review), the observed labelling pattern probably results from enhanced transcription of the genome.

To test the effect of thiamphenicol on the steady-state levels of individual mitochondrial transcripts, the transcripts were measured by Northern blotting. Fig. 4 shows northern blots in which total mitochondrial RNA from 0.1 mg mitochondria was hybridized with nick-translated *Eco*RI fragments A and C from rat liver mtDNA. The two fragments together contain the genes for cytochrome oxidase I and II, ND-2, -4, -5 and -6, cytochrome *b* and the 16 S and 12 S rRNAs. The steady-state concentrations of transcripts for all genes were elevated 2–4 fold as judged from the autoradiographs. Transcripts of the ND 6 gene (L-strand) are too weak to visualize.

Discussion

In vitro mitochondrial RNA and protein synthesis are increased in mitochondria isolated from regenerating livers of rats treated with thiamphenicol for 3 days. Steady-state levels of mitochondrial transcripts are also increased, as shown by Northern blot experiments. Increases both in the rate of radiolabelling of RNA and in the steady-state concentrations of mitochondrial transcripts strongly suggests that transcription is activated by treatment with thiamphenicol. Although increased steady-state concentrations of transcripts could result from their enhanced stabilities in the presence of thiamphenicol, this could not account for increased rates of transcript synthesis. One should be aware, however, that labelling of protein and RNA could, theoretically, be affected by precursor pool sizes, although it seems unlikely that differences in pool sizes established in vivo would survive the processes of mitochondrial isolation and in vitro incubation. Thus, a more probable explanation is that a TAP-dependent increase in all three parameters (protein synthesis, RNA synthesis and steady-state concentrations of transcripts) reflect regulation at the transcriptional level.

An enhancement of mitochondrial transcription upon blockage of mitochondrial translation could be explained in several ways. The most direct explanation is that a block in mitochondrial translation eliminates a factor which influences mitochondrial transcription. This factor could be a translation product acting as a repressor, or a small-molecular-weight substance, such as ATP, which is required for mitochondrial transcription [18].

A mitochondrial translation-dependent factor could also act on nuclear genes which, in turn, control mitochondrial transcription. Indeed, recent findings in yeast suggest that a mitochondrial gene product regulates the levels of transcripts produced from specific nuclear genes coding for mitochondrial proteins [6]. Thus, our results could also be explained if some of the enzymes in mitochondrial RNA metabolism in rat liver are under similar control. This would resemble, for example, the situation reported for *Neurospora crassa* in which inhibition of mitochondrial protein synthesis increased mitochondrial RNA polymerase [2,3]. Whatever the detailed mechanism, the present data show that transcription in rat liver mitochondria can be modulated by a factor or factors which are dependent upon mitochondrial translation.

Elevated mitochondrial protein synthesis observed in thiamphenicol-treated rats is in accord similar studies with inhibitors of mitochondrial protein synthesis carried out on lower eucaryotic cells [19,20]. However, prolonged treatment of HeLa cells with chloramphenicol did not increase synthesis of mitochondrial protein [21] or RNA [22]. The reasons for this apparent discrepancy are not clear, but might be related to differences in the relative kinetics of the expression of the nuclear and mitochondrial genomes in cultured, transformed cells and in non-transformed cells in vivo.

Acknowledgment

This study was supported by funds from the Swedish Natural Science Research Council (B.D.N.).

References

1 Rodgers, J.R., Smith, R., Huston, M.M. and Rich, R.R. (1986) Adv. Immunol. 38, 313–359.
2 Barath, Z. and Küntzel, H. (1972) Proc. Natl. Acad. Sci. USA 69, 1371–1374.
3 Küntzel, H. and Barath, Z. (1972) Nature New Biol. 69, 195.
4 Rouslin, W. (1976) Biochim. Biophys. Acta 73, 364–368.
5 Berendes, H.D. (1975) Proc. 10th FEBS Mett. 10, 159–174.
6 Parikh, V.S., Morgan, M.M., Scott, R., Clements, L.S. and Butow, R.A. (1987) Science 235, 576–580.
7 Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–211.
8 DeJong, L., Holtrop, M. and Kroon, A.M. (1979) Biochim. Biophys. Acta 548, 48–62.
9 Kuzela, S., Kolarov, J. and Krempasky, V. (1973) Neoplasma 21, 623–630.
10 Chirwing, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
11 Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, pp. 545, Cold Spring Harbor Press.
12 Nelson, B.D., Mutvei, A., Joste, V., Wielburski, A. and Kuzela, S. (1987) in the 66th Nobel Symposium, Membrane Proteins: Structure, Function, Assembly (Rydström, J., ed.), Vol. 27B, pp. 239–244. Chemica Scripta, Cambridge University Press.
13 Kuzela, S., Luciakova, K. and Lakota, J. (1980) FEBS Lett. 14, 197–201.
14 Firkin, F.C. and Linnane, A.W. (1969) Exp. Cell Res. 55, 68–67.
15 DeVries, H. and Kroon, A.M. (1970) Biochim. Biophys. Acta 204, 531–541.
16 Davey, P.J., Yu, R. and Linnane, A.W. (1969) Biochem. Biophys. Res. Commun. 36, 30–34.
17 Attardi, G. (1985) Int. Rev. Cytol. 93–145.
18 Gaines, G., Rossi, C. and Attardi, G. (1987) J. Biol. Chem. 262, 1907–1915.
19 Tzagoloff, A. (1971) J. Biol. Chem. 246, 3050–3056.
20 Sebald, W., Machleidt, W. and Otto, J. (1973) Eur. J. Biochem. 38, 311–324.
21 Costantino, P. and Attardi, G. (1977) J. Biol. Chem. 252, 1702–1711.
22 Storrie, B. and Attardi, G. (1972) J. Mol. Biol. 71, 177–199.