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Low translational efficiency of the F_1-ATPase β-subunit mRNA largely accounts for the decreased ATPase content in brown adipose tissue mitochondria

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The half-life of the F_1-ATPase β-subunit (F_1-β) mRNA in ATPase-poor brown adipose tissue (BAT) ($t_{1/2}$ = 9.5 h) was found to be 3–7-fold shorter than in liver ($t_{1/2}$ = 27 h) and heart ($t_{1/2}$ = 63 h) of mice. When translated in reticulocyte lysate, a 2–3-fold lower efficiency appeared with F_1-β mRNA from BAT than from other tissues. The in vitro synthesized F_1-β protein precursors of BAT, liver and heart origin were imported and processed by mouse liver mitochondria with equal efficiency. The results indicate that the pool of abundant F_1-β mRNA in BAT is not fully translatable, most likely due to its low metabolic stability.

Brown adipose tissue; ATPase; mRNA half-life; Translational efficiency

1. INTRODUCTION

The physiological role of specialized energy conversion in brown adipose tissue (BAT) is to produce heat instead of ATP. Consequently, the exceptionally low content of ATPase [1–4] and presence of the tissue-specific uncoupling protein (UCP), thermogenin [5], are the most characteristic features of BAT mitochondria. While the UCP synthesis is subtly controlled at the transcriptional level, predominantly through β-adrenergic receptors [6–8], the transcription of nucleus-encoded and mitochondrially encoded genes for ATPase subunits is maintained paradoxically high as shown by the levels of F_1-ATPase β-subunit (F_1-β) and ATPase 6 mRNAs both in BAT and in cultured brown adipocytes [4]. The F_1-β mRNA level in BAT is also insensitive to cold exposure of animals and to norepinephrine treatment of cultured cells [4], although the hormonal control of the F_1-β gene expression is possible, as documented in the liver of young rats [9] or in rat cardiomyocytes [10].

Therefore, a post-transcriptional down-regulating event must be involved in the regulation of ATPase biosynthesis in BAT and we attempted to identify its nature in the present report. We found that the F_1-β mRNA had a much faster turnover in BAT than in liver and heart. The translational efficiency of the BAT F_1-β mRNA was markedly lowered. The F_1-β protein precursor of BAT origin, however, was fully recognized by the importing machinery of the heterologous mouse liver mitochondria.

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Abbreviations: BAT, brown adipose tissue; ATPase, mitochondrial ATP synthase; F_1-β, β subunit of the catalytic F_1-part of mitochondrial ATP synthase; UCP, uncoupling protein.

2. MATERIALS AND METHODS

Four-week-old mice of the inbred strain Balb/c (Kolec, Czechoslovakia) were kept two per cage at 4°C for 3 days with free access to food. In order to block the transcription mice were injected i.p. with actinomycin D (0.4 mg per kg b.wt.) and killed 3, 6 and 12 h later. Mice treated for longer periods received additional half-doses of the inhibitor after 3 and 6 h. Animals were killed by cervical distortion and excised tissues were immediately frozen in liquid nitrogen.

Total RNA was isolated according to the acid-phenol method [11]. Heart, interscapular BAT and liver from each animal were homogenized and processed separately. To isolate poly(A)$^+$ RNA from 15 control mice was pooled yielding at least 1 mg from each tissue. The poly(A)$^+$ RNA fraction was purified using Hybond-mAP membrane (Amersham) following the protocol of the manufacturer. About 20 μg of poly(A)$^+$ RNA was obtained from 1 mg of total RNA.

For Northern blot analysis, 10 μg of total RNA or 1.5 μg of poly(A)$^+$ RNA was separated in 1.0% agarose formaldehyde gel, blotted onto Nylon membrane and hybridized with probes for F_1-β, COX I and UCP as described previously [4].

In vitro translation was done using the rabbit reticulocyte lysate (Promega) according to manufacturer's instructions. 400 μl of the optimized lysate was programmed with 12 μg of a specific poly(A)$^+$ RNA and incubated at 30°C in the presence of [^{35}S]methionine (Amersham, final radioactivity 1 mCi/ml). After 50 min the samples were chilled on ice and centrifuged for 1 h at 120,000 × g. For direct immunoprecipitation the samples were heated for 3 min at 100°C with a 0.2 vol. 20% SDS, diluted with 50 mM Tris-HCl, 1% Triton X-100, 0.3 M NaCl, 5 mM EDTA, pH 8.0, containing 250 U Trasylol/ml and centrifuged at 100,000 × g for 45 min. The supernatants were incu-

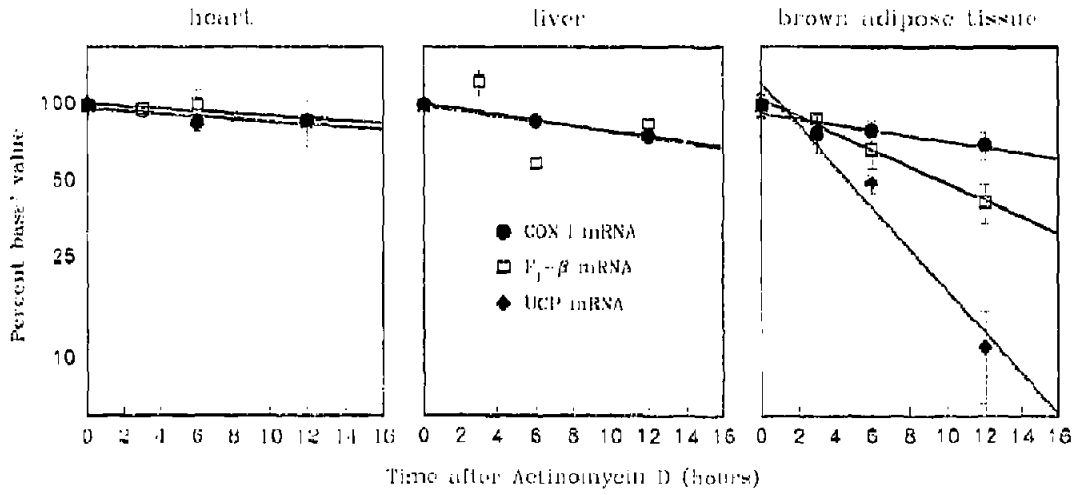

Fig. 1. Relative decrease of COX I, F_1-β and UCP mRNA levels in heart, liver and brown adipose tissue of mice after transcription block. Northern blot analysis of the mRNA content was performed 3, 6 and 12 h after injection of actinomycin D and the densitometric values were plotted in semilogarithmic graphs. Each point represents the mean ± S.E.M. of 2–3 mice.

bated with 30 μl of antiserum against F_1-β at 4°C overnight. The immune complexes were recovered using Sepharose-Protein-A in excess as described in [12].

Precursor processing by isolated mitochondria was carried out as in [12]. The labeled lysate was equilibrated with 50 mM HEPES, 90 mM KCl, 5 mM KH_2PO_4, 0.5 mM EGTA, pH 7.5, and then adjusted to 1 mM ATP, 1 mM ADP, 5 mM succinate, 5 mM $MgCl_2$ and 0.5% fatty acid-free BSA. Freshly isolated [13] mouse liver mitochondria were added (1 mg protein/ml) and the mixture was incubated for 30 min at 25°C. After incubation, the whole lysate including mitochondria was boiled with SDS and processed as detailed above.

The immunoadsorbed material was separated on 10% SDS-polyacrylamide gels [14] and the gels were fluorographed using salicylate [15]. Autoradiograms were scanned on Shimadzu TLC Scanner CS 930.

3. RESULTS AND DISCUSSION

Following gene transcription and splicing of the transcript, the first potential regulatory step of a protein biogenesis may occur at the level of mRNA degradation. In order to examine the stability of the F_1-β mRNA in different tissues we measured the half-life of this mRNA in heart, liver and BAT of mice after inhibition of the RNA synthesis. The samples obtained 3, 6 and 12 h after the transcription block were tested also for the content of the mitochondrially encoded COX I mRNA to evaluate the mRNA turnover inside mitochondria. In addition, the UCP mRNA was quantitated in BAT (Fig. 1).

As seen in Table I, the half-lives of ergastoplasmic F_1-β and mitochondrial COX I transcripts are very close in liver ($t_{1/2}$ = 27 h and $t_{1/2}$ = 29.5 h, respectively) as well as in heart ($t_{1/2}$ = 63 h and $t_{1/2}$ = 58 h, respectively) where the rate of anabolic reactions is generally lower. In BAT, the F_1-β mRNA half-life was only 9.5 h, which represents about 0.33 and 0.66 of the values found for the liver and heart, respectively. However, the COX I mRNA half-life in BAT was 27.5 h, suggesting that the decrease of β-ATPase mRNA $t_{1/2}$ in this tissue is selective. The UCP mRNA which had been shown to be markedly destabilized in the cold-adapted animals [16] exhibited a half-life as short as 3.7 h (Fig. 1).

The portion of the transcripts that can be actually translated is most probably different from their steady-state level estimated by Northern blot. Both 5′ and 3′ mRNA structures which greatly influence the translational efficiency of a mRNA [17,18] are necessarily more vulnerable to a nucleolytic attack if the mRNA turnover is fast. The specific mRNA pools which differ in their half-lives may, therefore, be translated with different efficiency.

To estimate the translational capability of the F_1-β mRNA pool in BAT we performed in vitro translation using poly(A)$^+$ RNA from the tissues tested. The content of the F_1-β mRNA was checked in aliquots of the purified mRNAs, as well as in the original total RNAs. As evident from Fig. 2, the relative abundance of the F_1-β transcripts has not been changed by the poly(A)$^+$ RNA preparation.

Fig. 3A shows the F_1-β precursors translated from

Table I

Relative steady-state levels and half-lives of COX I and F_1-β mRNAs in mouse tissues

Tissue	mRNA level (%)		$t_{1/2}$ mRNA	
	COX I	F_1-β	COX I	F_1-β
Heart	100	100	58	63
Liver	12.0	5.9	29.5	27.5
BAT	120	223	27.5	9.5

The mRNA levels are averages of densitometric values of two Northern blots expressed in relation to the heart levels. The mRNA half-lives were calculated from the graphs in Fig. 1.

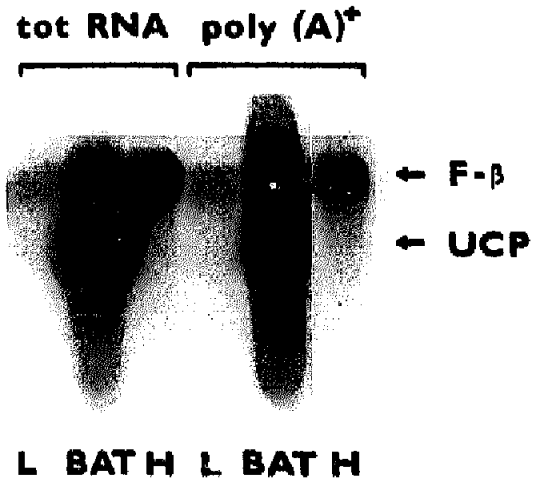

Fig. 2. Northern blot of total RNAs and their respective poly(A)$^+$ fractions isolated from liver (L), heart (H) and brown adipose tissue (BAT). Samples of 10 μg RNA or 1.5 μg poly(A)$^+$ were used. Hybridization was performed with probes for F_1-β and UCP mRNAs simultaneously.

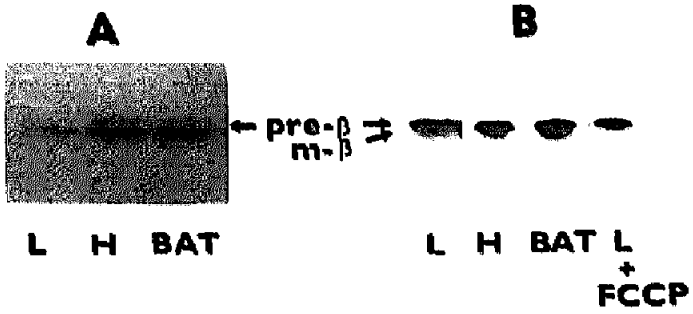

Fig. 3. In vitro translation (A) and processing (B) of F_1-β by mouse liver mitochondria. In A, aliquots of poly(A)$^+$ RNA from Fig. 2 were translated in vitro in the presence of [^{35}S]methionine, the F_1-β was immunoadsorbed, electrophoresed and autoradiographed. In B, the radiolabelled proteins prepared as described in A were incubated with isolated mouse liver mitochondria, and both the precursor (pre-β) and the mature form (m-β) of F_1-ATPase β-subunit were immunoadsorbed and electrophoresed. To compensate for the tissue-dependent differences between the intensity of the autoradiographic spots (see A), different exposures of individual lanes were combined in the figure. As a negative control, mitochondria uncoupled with 5 μM carbonylcyanide *m*-fluorophenyl-hydrazone (FCCP) were used. L, liver; H, heart; BAT, brown adipose tissue.

poly(A)$^+$ RNAs which were characterized in Fig. 2. The evaluated amounts of F_1-β mRNAs and their respective translational products in two independent experiments were used to calculate the translational efficiency (Table II). A several-times lower efficiency was found in both experiments with the BAT F_1-β mRNA as compared to the liver and heart F_1-β mRNA. This finding apparently reflects the mRNA integrity and provides further evidence that an elevated mRNA instability significantly down-regulates ATPase biosynthesis in BAT.

As depicted in Fig. 3B the precursor proteins derived from BAT, liver and heart are converted equally well into the mature form by mouse liver mitochondria, irrespective of the above described translational efficiencies. Roughly the same percentage of each pre-F_1-β was processed, giving rise to the mature F_1-β protein. The addition of an uncoupler completely prevented the processing (Fig. 3B).

Table II

Translational efficiency of F_1-β mRNA isolated from different mouse tissues

Tissue	F_1-β mRNA level	Immunoadsorbed F_1-β	Translational efficiency
Experiment I			
Heart	100	100	1
Liver	18.0	25.6	1.42
BAT	204.4	89.7	0.44
Experiment II			
Heart	100	100	1
BAT	244.4	80.2	0.33

Aliquots of poly(A)$^+$ RNA were used to program translation in rabbit reticulocyte lysate. The F_1-β was immunoadsorbed from the labelled lysate by specific antiserum, electrophoresed and autoradiographed. Densitometric values of the autoradiographic spots for heart were set to 100 and translational efficiency (F_1-β/F_1-β mRNA) was calculated.

This indicates that the translated pre-F_1-β proteins of BAT, liver and heart can be equally imported and processed by mitochondria. In accordance with the highly conserved and relatively non-specific character of the protein importing machinery processing a large number of different precursor proteins it would be difficult to envisage a tissue-specific block at the level of F_1-β precursor transport into BAT mitochondria.

The effect of mRNA stability on the protein concentration at steady state was investigated by mathematical modeling [19]. It strongly indicates that the protein level is directly proportional to the half-life of its mRNA. Thus, extrapolating from our data for the F_1-β subunit, the low ATPase content in BAT may be largely attributed to the short half-life of the corresponding mRNAs.

Since the observed decrease in translational efficiency of the F_1-β mRNA in BAT does not entirely account for the low content of ATPase, it is possible that other steps might also be involved in the control of ATPase biosynthesis. The content of the enzyme may be regulated at the level of the enzyme complex assembly via availability of other nucleus-encoded subunits, e.g. F_1-γ or F_1-α [20,21]. Interestingly, a clear difference between F_1-β and F_1-α mRNA levels was found in rat liver [9].

In addition, the regulation might occur at the protein degradation level. A fast turnover of newly synthesized but as yet unassembled UCP was observed in cultured brown adipocytes stimulated by norepinephrine [22], and a similar mechanism might operate also in the case of the F_1-ATPase β-subunit.

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