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Low content of mitochondrial ATPase in brown adipose tissue is the result of post-transcriptional regulation

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The mRNA levels of ATPase β, ATPase 6, cytochrome oxidase (COX) VIb and COX I subunits were found to be 2.4–13.8-fold higher in brown adipose tissue (BAT) than in heart, skeletal muscle, brain and liver of mice. The comparison with tissue contents of ATPase and COX revealed that the selective, 5–11-fold reduction of ATPase in BAT is not caused by decreased transcription of ATPase genes. Likewise, the ATPase β and COX VIb mRNA levels in cultured brown adipocytes were also not influenced by norepinephrine, which activated the expression of the UCP gene by two orders of magnitude. The results indicate that the biosynthesis of mitochondrial ATPase in BAT is post-transcriptionally regulated.

Brown adipose tissue; Mitochondrion; ATPase; Cytochrome oxidase; Transcription; Nuclear gene

1. INTRODUCTION

In brown adipose tissue (BAT), oxidative phosphorylation is physiologically uncoupled due to the presence of a tissue-specific mitochondrial uncoupling protein (UCP), and the chemical energy of oxidized substrates is fully converted into heat rather than into ATP formation [1]. The high oxidative capacity of BAT mitochondria is not paralelled by phosphorylating activity and, in contrast with other tissues, the content of mitochondrial ATP synthase (ATPase) is exceptionally low in BAT [2,3]. This concerns both the catalytic F_1 part and the membrane-bound F_0 part of the enzyme [2–4]. The mechanism of selective reduction of ATPase in BAT is not known. Mitochondrial ATPase is composed of 11 nucleus-encoded and 2 mitochondrially encoded subunits [5,6] and the low content of the enzyme may reflect its depressed synthesis due to a reduced expression of ATPase nuclear genes.

In the present study we compared the steady state mRNA levels of representative mitochondrial and nuclear genes encoding ATPase and cytochrome oxidase subunits in BAT and other tissues of mice. It was found that, in spite of the low content of ATPase in BAT, the expression of all the genes tested is highest in BAT, indicating that not transcriptional, but extra transcriptional events are responsible for the selective reduction of the ATPase content in BAT.

Abbreviations: BAT, brown adipose tissue; ATPase, mitochondrial ATP synthase; F_0, membrane part of ATPase; F_1, catalytic part of ATPase; UCP, uncoupling protein; COX, cytochrome *c* oxidase.

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2. MATERIALS AND METHODS

Four-week-old mice of Balb/c outbred strain were maintained at room temperature, on a 12 h light/12 h dark cycle. When indicated, animals were exposed to 4°C prior to use. The animals were killed by decapitation and tissue samples for RNA isolation were frozen in liquid nitrogen. For other assays, 5% homogenates (w/v) were prepared in 0.25 M sucrose, 10 mM Tris/HCl, 1 mM EDTA, pH 7.4, and filtered through a 250 μm nylon screen.

The stromal-vascular cells from BAT were isolated and cultivated as previously [7]. Experiments were performed with cells cultured for 7 days.

Total RNA was isolated as described in [8] and checked for purity and integrity. For Northern blot analysis, 20 μg RNA aliquots were denatured in the presence of glyoxal, separated by electrophoresis in 1.2% agarose gel and transferred to a nylon membrane (Hybond-N, Amersham) according to standard procedures [9]. Hybridization was carried out overnight at 68°C in 5× Denhardt's solution, 5× SSC, 50 mM sodium phosphate, pH 6.5, 0.1% SDS and 250 μg/ml of herring sperm DNA. Blots were washed in 2× SSC, 0.1 % SDS (2× 15 min, room temp.) and 0.1× SSC, 0.1% SDS (2× 15 min, 55°C). Following autoradiography the blots were stripped for 60 min at 95°C in 0.1% SDS and sequentially re-hybridized with several DNA probes.

The cDNA probes were generously provided by the following investigators: 1.26 kb *Eco*RI fragment of the rat ATPase β-subunit cDNA in pUC19 [10], by Prof. P.L. Pedersen (The Johns Hopkins University, Baltimore, USA); 0.4 kb *Eco*RI fragment of the bovine COX VIb subunit cDNA in pT_7T_3, by Prof. B. Kadenbach (Phillips University, Marburg, Germany); 1 kb *Pst*I fragment of the mouse UCP cDNA in pBR322 [11], by Dr. a. Jacobsson (Stockholm University, Sweden); 3 kb *Eco*RI fragment C of rat mtDNA (encoding COX I and II and ND 2) in pUN121 [12], by Prof B.D. Nelson (Stockholm University, Sweden); and 0.5 kb fragment of rat mtDNA [13] encoding ATPase subunits 6 and 8 in the Bluescribe vector by Dr V. Petruzella (University of Bari, Bari, Italy). The clones were linearized and labeled with [α-^{32}P]dCTP by random priming.

The content of COX and F_1-ATPase was measured by immunoblot-

Table I

Specific content of COX and ATPase in mouse tissues

Tissue	COX		ATPase	ATPase/COX (β/COX IV)
	Activity (μmoles/min/mg protein)	COX IV content (aU/mg protein)	β-subunit content (aU/mg protein)	
Liver	0.72 ± 0.12	37 ± 4	36 ± 2	0.97
Brown adipose	1.72 ± 0.42	100 ± 25	17 ± 2	0.17
Heart	1.22 ± 0.14	100 ± 17	100 ± 10	1.00
Muscle	0.26 ± 0.08	17 ± 4	32 ± 13	1.88
Brain	0.66 ± 0.18	26 ± 3	22 ± 4	0.85

COX activity was measured spectrophotometrically, the content of COX IV and of ATPase β-subunits was determined by Western blot analysis using tissue homogenates. Immune complexes of the respective signals were evaluated by densitometry, and specific content of subunits was expressed in arbitrary units (aU)/mg protein in relation to the values observed in heart. The values are means ± SD(n = 4–6).

ting as before [14] using rabbit antisera to rat heart cytochrome oxidase subunit IV [15] and bovine heart F_1-ATPase [16]. The protein content was determined as described [17] using BSA as standard. For densitometric scanning of Northern and Western blots Molecular Dynamics Densitometer and Shimadzu TLC Scanner CS 930 were used.

3. RESULTS AND DISCUSSION

With the aim to investigate the events responsible for the low content of ATPase in BAT, several mouse tissues were first analyzed for the content of ATPase and cytochrome oxidase. Since ATPase decreases in BAT with increasing thermogenic capacity [14], cold-exposed (3 days) animals were used. As shown in Table I, both the COX activity and COX content, measured immunochemically as the content of the COX IV subunit, were found to be highest in BAT, when compared with heart, liver, muscle and brain. Oligomycin-sensitive ATPase activity cannot be precisely measured in tissue homogenates: therefore, the enzyme was quantified as the content of the highly homologous ATPase β-subunit [6,18] using anti-F_1 antibodies. In line with previous reports [2,3] the content of ATPase was lowest in BAT, both absolutely and in relation to the COX content. The respective ATPase/COX ratio was thus 5–11-times lower in BAT.

In an attempt to determine whether the transcription of genes for ATPase subunits is suppressed in BAT, the total RNA isolated from different mouse tissues was analyzed by Northern blotting. The steady state levels of transcripts of nuclear genes for tissue-unspecific [19] COX VIb subunit and for the catalytic ATPase β-subunit, as well as of mitochondrial genes for ATPase 6 and COX I subunits, were measured (Fig. 1). The highest levels of COX VIb mRNA were found in BAT and were

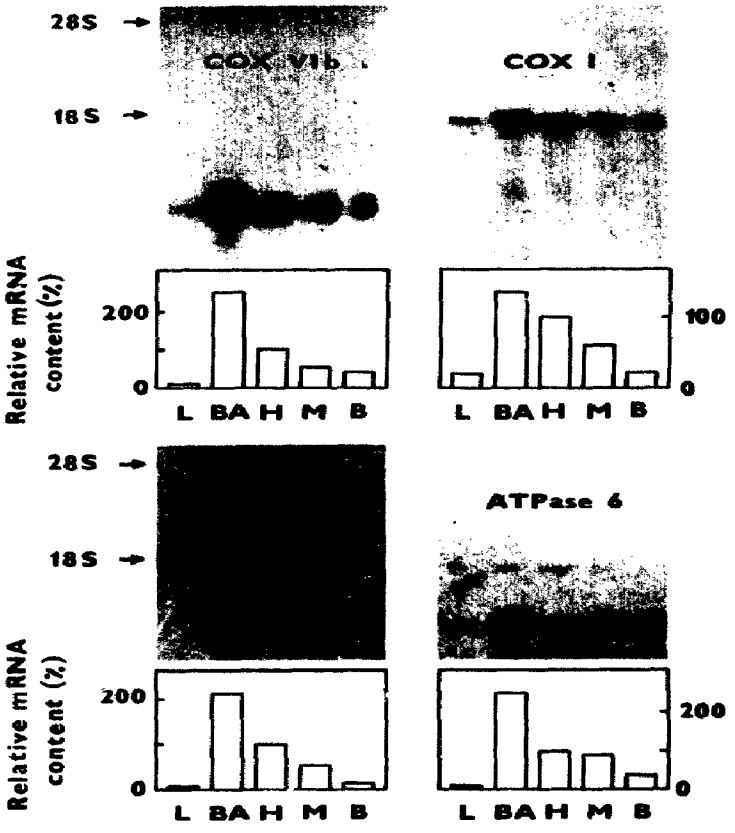

Fig. 1. Northern blot analysis of transcripts of two nuclear and two mitochondrial genes coding for subunits of ATPase and COX. 20 μg aliquots of total RNA from mouse liver (L), brown adipose tissue (BA), heart (H), skeletal muscle (M) and brain (B) were analyzed. Sequential hybridization was performed with appropriate ^{32}P-labeled cDNA probes for the COX VIb subunit, COX I subunit, ATPase β-subunit and ATPase 6 subunit. The position of 28 S and 18 S ribosomal RNA is indicated. The content of transcripts evaluated from autoradiographs by densitometry was expressed in % of value for heart.

lower in heart, muscle, brain and liver, and a similar pattern was obtained in the case of COX I mRNA. The total RNA from BAT also contained the highest levels of transcripts of ATPase subunits of both nuclear and mitochondrial origin. The observed profiles in different tissues were again very similar to those of mRNAs of COX subunits indicating that the levels of nuclear transcripts of ATPase and COX subunits are in close relation (the ATPase β-subunit mRNA/COX VIb mRNA arbitrary ratio was 1.26 in liver, 0.85 in BAT, 1 in heart, 1.1 in muscle and 0.87 in brain) and that the expression of nuclear and mitochondrial genes investigated is well coordinated in tissues that differ 2.4–13.8-fold (BAT vs. other tissues) in transcriptional activity (Table II). The results are in agreement with the differential expression of the ATPase β-subunit gene in human tissues [18] and

Table II

Relative levels of ATPase β-subunit and COX VIb mRNAs in mouse tissues

Tissue	β-Subunit mRNA		COX VIb mRNA		β-subunit mRNA / COX VIb mRNa
	(/mg tissue)	(/aU ATPase)	(/mg tissue)	(/aU COX)	
Liver	2.78	0.37	2.20	0.29	1.26
Brown adipose	25.87	7.84	30.27	1.54	0.86
Heart	10.75	0.70	10.75	0.70	1.00
Muscle	3.17	1.50	2.87	1.79	1.10
Brain	2.65	0.56	3.05	1.22	0.87

Densitometric data of β-subunit and COX VIb mRNAs were expressed in % of heart values. Relative levels of transcripts were calculated per mg wet weight and in relation to the content of ATPase and COX, respectively. The mean of mRNA values determined from 3 Northern blots, performed as in Fig. 1, and Western blotting data from Table I, were used for calculations.

also with previous findings of coordinated expression of COX III and COX VIc genes in rat tissues [20]. Most importantly, however, the results rule out the possibility that the low content of ATPase in BAT results from low levels of transcripts of the nucleus-encoded ATPase subunits due to transcriptional regulation.

The translational efficiency of mRNAs of ATPase subunits therefore appears to be extremely low in BAT. This is indicated in Table II where the amounts of ATPase β-subunit and COX VIb transcripts were calculated per mg wet weight and were also related to the content of corresponding enzyme in each tissue. The ATPase β-subunit mRNA/ATPase ratio in BAT was thus found to be 5-times higher than in muscle and 11–16-times higher than in other tissues. In contrast, the COX VIb mRNA/COX ratio in BAT was within the range of values found in other tissues, being almost the same as in muscle and brain.

Expression of several nuclear genes encoding mitochondrial proteins has been shown to be developmentally and hormonally regulated. This concerns, for example, COX subunits [21], acyl CoA dehydrogenase [22], malate dehydrogenase [22], ADN translocator [23,24] and also the ATPase β-subunit [25]. This subunit is one of the six proteins selectively stimulated in liver by thyroid hormones [25], and regulation is attained in different developmental states either at transcriptional or at translational levels [25]. In BAT numerous trophic processes are controlled and modulated by catecholamines (for review see [26]), including the biogenesis of mitochondria [7,27,28]. Activation of synthesis of mitochondrial UCP [7,28] or of lipoprotein lipase [29] are fine examples of transcriptional regulation of nuclear genes which is attained through the action of catecholamines liberated upon exposure of animals to cold [26]. As shown in Fig. 2 and Table III, the steady state level of UCP mRNA is highly stimulated in primary BAT cell

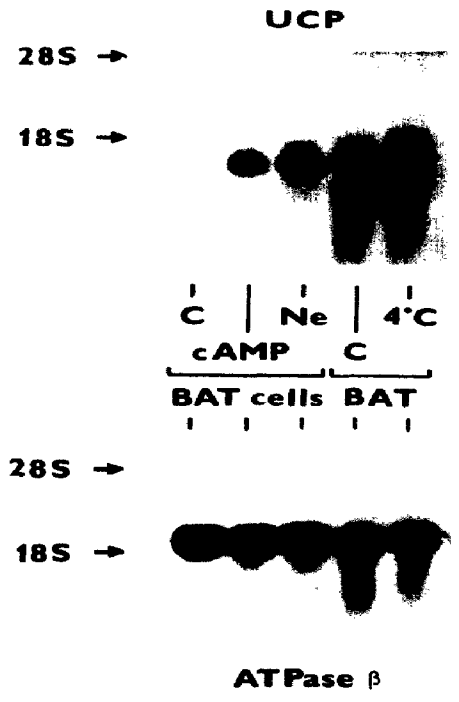

Fig. 2. Northern blot analysis of UCP and ATPase β-subunit mRNAs. 20 μg aliquots of total RNA isolated from cultured mouse BAT cells (C, control; Ne, 1 μM norepinephrine for 12 h), BAT of control (C) and cold-exposed (4°C; 24 h) mouse were analyzed using UCP (upper panel) and ATPase β-subunit (lower panel) cDNA probes. The position of 28 S and 18 S ribosomal RNA is indicated.

cultures when exposed at confluence to norepinephrine or to dibutyryl cAMP. The level of UCP mRNA is also further increased in mouse BAT when the animals are briefly exposed to cold (Fig. 2). Neither in cultured cells nor in BAT, however, are these changes accompanied by any change in the ATPase β-subunit mRNA levels. The expression of the ATPase β-subunit gene thus does not seem to be influenced in BAT by norepinephrine and changes in intracellular cAMP levels. The same seems to be true for the COX VIb gene (Table III). These observations are in agreement with Western blot data on the synthesis of UCP, F_1-ATPase and cyto-

Table III

Effect of norepinephrine on UCP, ATPase β-subunit and COX VIb mRNA levels in cultured BAT cells

mRNA	Relative change after norepinephrine stimulation
UCP	» 100
ATPase β	0.96 ± 0.22
COX VIb	0.88 ± 0.26

The levels of transcripts in cultured BAT cells supplemented for 12 h with 1 μM norepinephrine were related to the respective levels of transcripts in control cells. Data are the mean ± SD of 4 experiments.

chrome oxidase in cultured BAT cells [7,27] and suggest that the control of ATPase synthesis in BAT is attained at a post-transcriptional level.

Further experiments would be necessary to exactly determine which step in ATPase synthesis and assembly is rate-limiting in BAT and what are the putative regulatory factors involved. Preliminary data on the stability of mRNAs in BAT (P. Tvrdík and J. Houštěk, unpublished) are rather in favor of the control occurring at translational or post-translational levels.

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REFERENCES

[1] Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1–64.
[2] Houštěk, J., Kopecký, J. and Drahota, Z. (1978) Comp. Biochem. Physiol. 60B, 209–214.
[3] Cannon, B. and Vogel, G. (1977) FEBS Lett. 76, 284–289.
[4] Svoboda, P., Houštěk, J., Kopecký, J. and Drahota, Z. (1981) Biochim. Biophys. Acta 634, 321–330.
[5] Fearnley, I.M. and Walker, J.E. (1986) EMBO J. 5, 2003–2008.
[6] Walker, J.E., Cozens, A.L., Dyer, M.R., Fearnley, I.M., Powell, S.J. and Runswick, M.J. (1987) Chem. Scr. 27B, 97–105.
[7] Kopecký, J., Baudyšová, M., Zanotti, F., Janíková, D., Pavelka, S. and Houštěk, J. (1990) J. Biol. Chem. 265, 22204–22209.
[8] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
[10] Garboczi, D.N., Fox, A., Gerring, S.L. and Pedersen, P.L. (1988) Biochemistry 27, 553–560.
[11] Jacobsson, A., Stadler, U., Glotzer, M.A. and Kozak, L.P. (1985) J. Biol. Chem. 260, 16250–16254.
[12] Mutvei, A., Kužela, S. and Nelson, B.D. (1989) Eur. J. Biochem. 180, 235–240.
[13] Gadaleta, G., Pepe, G., De Candida, G., Quagliariello, C., Sbisa, E. and Saccone C. (1989) J. Mol. Evol. 28, 497–516.
[14] Houštěk, J., Janíková, D., Bednár, J., Kopecký, J., Šebestián, J. and Soukup, T. (1990) Biochim. Biophys. Acta 1015, 441–449.
[15] Kolarov, J., Wielbursky, A., Mendel-Hartwig, I. and Nelson, B.D. (1981) Biochim. Biophys Acta 652, 334–336.
[16] Kužela, S., Luciaková, K. and Lakota, J. (1980) FEBS Lett. 114, 197–201.
[17] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[18] Neckelmann, N., Warner, C.K., Chung, A., Kudoh, J., Minoshima, S., Fukuyama, R., Maekawa, M., Shimizu, Y., Shimizu, N., Liu, J.D. and Wallace, D.C. (1989) Genomics 5, 829–843.
[19] Taauman, J.W., Scharge, C., Ponne, N.J., Das, A.T., Bolhuis, P.A., de Vries, H. and Agsteribbe, E. (1990) Gene 93, 285–291.
[20] Hood, D.A. (1990) Biochem. J. 269, 503–506.
[21] Kadenbach, B., Schlerf, A., Mengel, T., Hengst, L., Cao, X., Suske, G., Eckerskorn, C. and Lottspeich, F. (1989) in: Organelles in Eukaryotic Cells (J.M. Tager, A. Azzi, S. Papa and F. Guerrieri eds.) pp. 143–156, Plenum Press, New York.
[22] Kelly, D.P., Gordon, J.I., Alpers, R. and Straus, A.W. (1989) J. Biol. Chem. 264, 18921–18925.
[23] Battini, R., Ferrari, S., Kaczmarek, K., Calabretta, B., Chen, S. and Baserga, R. (1987) J. Biol. Chem. 262, 4355–4359.
[24] Greenberg, M.E. and Ziff, E.B. (1984) Nature 311, 433–438.
[25] Izquierdo, J.M., Luis, A.M. and Cuezva, J.M. (1990) J. Biol. Chem. 265, 9090–9097.
[26] Himms-Hagen, J. (1986) in: Brown Adipose Tissue (P. Trayhurn and D.G. Nicholls eds.) pp. 214–268, Edward Arnold Ltd., London.
[27] Rehnmark, S., Nechad, M., Herron, D., Cannon, B. and Nedergaard, J. (1990) J. Biol. Chem. 265, 16464–16471.
[28] Houštěk, J., Kopecký, J., Baudyšová, M., Janíková, D., Pavelka, S. and Klement, P. (1990) Biochim. Biophys. Acta 1018, 243–247.
[29] Giralt, M., Martin, I., Vilaro, S., Villarroya, F., Mampel, T., Iglesias, R. and Vinas, O. (1990) Biochim. Biophys. Acta 1048, 270–273.