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EVIDENCE OF POST-TRANSCRIPTIONAL REGULATION IN MAMMALIAN MITOCHONDRIAL BIOGENESIS

J.M. Izquierdo and J.M. Cuezva[1]

Departamento de Biología Molecular, Centro de Biología Molecular
"Severo Ochoa" (C.S.I.C.-U.A.M.), Universidad
Autónoma de Madrid, 28049 Madrid, Spain

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SUMMARY: We have previously shown that the nuclear-encoded gene's expression of mitochondrial β-subunit of the F1-ATPase complex in rat liver is regulated at the translational level (Luis, A.M., Izquierdo, J.M., Ostronoff, L.K., Santarén, J., Salinas, M., and Cuezva, J.M. (1993) J. Biol. Chem. 268, 1868-1875). In this paper we report that the different steady-state levels of ATP synthase β subunit mRNA detected in rat tissues are not paralleled by a proportional content of immunodetectable β-F_1-ATPase protein. The results suggest that tissue-specific transcriptional and post-transcriptional mechanisms contribute to differential mitochondrial biogenesis in mammalian cells. On the other hand, steady-state mRNA levels of the mitochondrial encoded ATP synthase subunits (ATP 6+8) indicate that nuclear and mitochondrial-encoded transcripts for this complex are in close relation, that is, the expression of both nuclear and mitochondrial genes is coordinated in all tissues examined.

Mitochondrial oxidative phosphorylation represents a key step in the provision of ATP in most eukaryotic cells. H^+-ATP synthase is the multisubunit protein complex of the mitochondrial inner membrane that couples the proton gradient generated by the respiratory chain to the synthesis of ATP. H^+-ATP synthase is composed of two parts, a water soluble complex (F_1) in which the catalytic activity of ATP synthesis resides, and the membrane H^+-pore (F_o) (1-6). The mammalian F_1-ATPase is an oligomeric protein complex composed of five main subunits which are encoded in the nuclear genome (7). The catalytic activity of the F_1-complex is localized in the β subunit. The membrane F_o portion of ATP synthase is composed of three subunits, two of which (ATPase 6 and 8) are encoded by the mitochondrial genome (7). The dual genomic localization of the ATP synthase subunits in eukaryotic cells involves a complex set of regulatory pathways for its biogenesis. For instance, nuclear-encoded proteins are synthetized on cytosolic polyribosomes with cleavable presequences, imported into the organelle, and further sorted and assembled within the mitochondria with the other mitochondrial encoded subunits (7-9).

Regulation of the expression of mammalian nuclear-encoded mitochondrial proteins involved in the bioenergetic function of the organelle has been described mostly at the transcriptional level (10-14). However, recent findings have provided evidence that in liver, during a most active period of mitochondrial biogenesis (15-16), the expression of the β-subunit

[1]Corresponding author. Fax: 34-1-397-4799.

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of the F_1-ATPase complex is exerted at the translational level (11, 17). Besides, it has been documented that thyroid hormones regulate the basal expression of β-F_1-ATPase gene at the transcriptional level (11, 18). In order to elucidate further possible regulatory levels controlling mitochondrial biogenesis in mammalian cellular types we have studied the tissue-dependent expression pattern of β-F_1-ATPase mRNA, as well as that of the mitochondrial encoded subunits of ATP synthase complex (subunits 6-8). Steady-state levels of both transcripts have been analyzed together with parallel determination of steady-state amounts of β-F_1-ATPase protein in rat tissue homogenates, the latter determination used as an index of the relative mitochondrial abundance (11). The results obtained provided the following findings: (i) the relative nuclear-and mitochondrial-encoded transcript levels for the ATP synthase complex varied in parallel in the tissues examined and (ii) with the exception of heart tissue, transcript and protein levels in other rat tissues showed negligible correlation. Altogether, tissue-dependent expression of mitochondrial ATP synthase suggests that mitochondrial biogenesis in mammalian cells could be regulated at both transcriptional and post-transcriptional levels and that nuclear and mitochondrial genomes act in a coordinated manner.

METHODS

Animals and tissue sampling. Male albino Wistar rats (200-250 g) were fed standard laboratory chow and water *ad libitum*. Rats were killed by decapitation and a pool of samples from heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis were obtained for preparation of homogenates (11,17). The protein concentration in each sample was determined with Bradford reagent (Bio-Rad) using crystalline bovine serum albumin as standard.

"Western blot" analysis of β-F_1-ATPase. Fractionation of proteins (50 μg) from tissue samples by polyacrylamide gel electrophoresis, electrophoretic transfer of the proteins to polyvinylidene difluoride membranes (Millipore), Western blotting with affinity purified antibodies against rat liver mitochondrial β-F_1-ATPase and visualization of the immunoreactive protein with a goat anti-rabbit IgG peroxidase conjugate (GAR/IgG(H+L)/PO) (Nordic Immunology, Tilburg, The Netherlands) were carried out as previously reported in detail (11,17). Replicate gels were fixed, stained with 0.25 % Coomasie Brillant Blue R-250 and destained.

Nucleic acid hybridization analysis. A multiple tissue Northern blot containing 2 μg of purified poly A^+ RNA from different rat tissues (Clontech, Palo Alto, USA) was utilized. Conditions for labeling synthetic cDNA probes, hybridization and membrane washing were those previously described (11,17) for Northern blot analysis of rat liver β-subunit mRNA. The blot was stripped in water for 20 min at 95 °C and subsequently dried for 60 min at room temperature. The same filter was then hybridized with the following probes: (i) a 1676 bp cDNA for rat liver COIII+ATP 6-8, generously provided by Dr. P. Cantatore (University of Bari, Italy) and (ii) a full-length human β-actin cDNA probe (Clontech) (data not shown). The relative amount of β-F_1-ATPase and COIII+ATP 6-8 mRNAs were determined by densitometric scanning of the resulting x-ray films exposed to the membranes at -70 °C with an intensifying screen.

RESULTS AND DISCUSSION

It is widely accepted that the expression level of nuclear and mitochondrial genes encoding for mitochondrial proteins varies among rat tissues and that in general it reflects the tissue oxidative capacity (19). Fig. 1 (B and E) shows that the expression pattern of nuclear encoded β-F_1-ATPase mRNA varies much within the tissues examined: heart >> liver > brain >kidney > muscle > spleen >> lung > testis. Further, each tissue reveals a single hybridization signal of ~

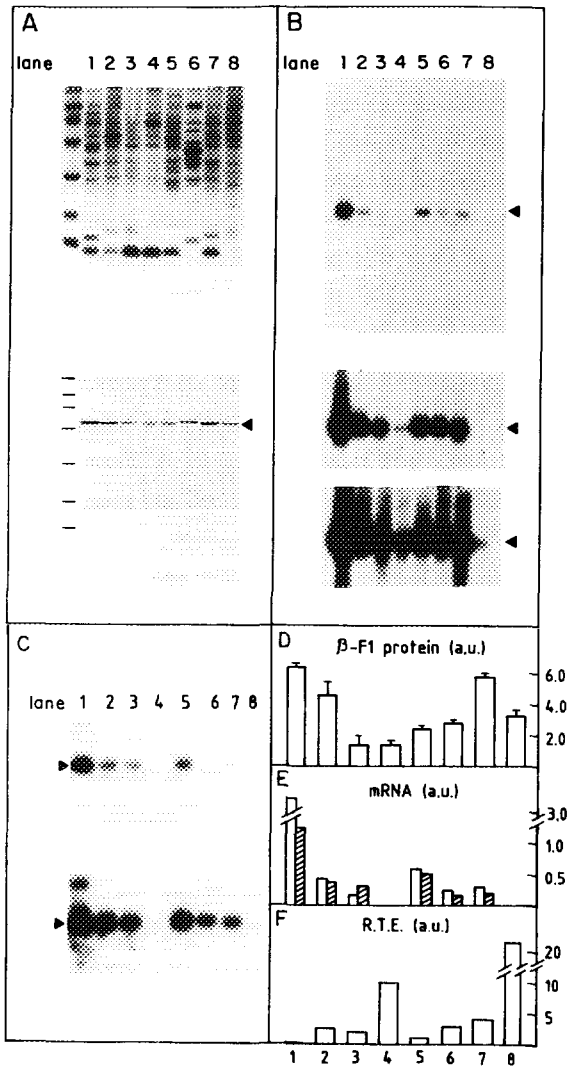

Figure 1. Expression of ATP synthase in rat tissues. 50 μg of protein (panel A) and 2 μg of poly (A)$^+$ RNA (panels B and C) from rat heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (lanes 1 to 8, respectively) were obtained and processed as described.(A) Upper panel, Coomassie blue stained gel of 50 μg of proteins from different tissues fractionated on 12% SDS-PAGE. Molecular weight markers are shown on the left. Lower panel shows a representative blot obtained from a parallel gel transferred to PVDF membranes and probed with affinity purified anti-β-F_1-ATPase antibodies. Migration of the β subunit of the F_1-ATPase complex is indicated by a closed arrowhead. (B) Autoradiograms showing the hybridization signal of β-F_1-ATPase mRNA in different rat tissues. The filter was exposed to films with an intensifying screen at -70 °C for 2h (upper panel), 14 h (middle panel) and 36 h (lower panel). Migration of β-F_1-ATPase mRNA is indicated by a closed arrowhead. (C) The same filter was subsequently hybridized with a rat liver COIII+ATP 6+8 cDNA probe and exposed for 4 days (upper panel) and 10 days (lower panel). (D, E) Quantitation was done by laser densitometric scanning of the immunoreactive bands or hybridization signals (D, arbitrary units/μg protein or E, arbitrary units/μg poli (A)$^+$ RNA) of the amount for protein and mRNA levels in rat tissues. From 1 to 8, for heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis, respectively. The values shown in panel D for β-F_1-ATPase protein content are means±S.E.M. of two different preparations. The relative translational efficiency (R.T.E.) was calculated by dividing the amount of β-F_1-ATPase protein present in homogenates into the amount of β-F_1-ATPase mRNA. These ratios were relatively compared with those found in liver, fixed arbitrarily to the value of 1.

1.8 Kb consistent with the expected length of β-F_1-ATPase mRNA from various mammalian tissues and origins (20-23). Consistent with the differential tissue expression pattern of β-F_1-ATPase mRNA, the expression of the mitochondrial encoded ATPase 6+8 mRNA follows a similar profile (Fig. 1 C and D), although lung and testis transcript levels were below detection limits (Fig. 1C). These results are in agreement with previous observations for tissue-specific expression of other rat nuclear-encoded genes implicated in mitochondrial respiration (19). However, our results reveal some differences when compared to previous reports of both transcripts in some human tissues (24) and tissues of the cold-adapted mouse (25), indicating most likely the differential expression among mammalian species and in cold-adaptation.

The β-F_1-ATPase protein profile (Fig. 1A and D) reveals that heart, kidney and brain are the tissues that contain the high relative amount of β-F_1-ATPase protein. Tissues such as testis, muscle, liver, spleen and lung, show much lower β-F1-ATPase protein (Fig. 1A and D) most likely illustrating their lower mitochondrial abundance and thus energy demand. Interestingly, among the three tissues that have the highest amount of β-F_1-ATPase protein or relative mitochondrial abundance (heart, kidney and brain) only one of them (heart) presented the highest mRNA levels for both mitochondrial transcripts (Fig. 1B, C and E). Even, and only in the case of heart, longer blot exposures reveal the presence of the unprocessed mitochondrial precursor for COIII+ATPase 6+8 mRNA (see, in lower panel of Fig. 1C, the hybridization signal above the closed arrowhead). Likewise, among the tissues showing a low relative mitochondrial abundance (testis, muscle, liver, lung and spleen) (Fig. 1D), no correlation is observed between β-F_1-ATPase protein and mitochondrial transcript levels (Fig. 1E). For instance, the testis shows negligible mRNA levels when compared to liver, while the amount of β-F_1-ATPase protein is higher. Conversely, spleen with higher transcript levels than testis shows much lower β-F_1-ATPase protein amount. A similar observation regarding the lack of correlation between β-F_1-ATPase transcript and protein levels has been recently documented in cold-adapted mouse brown adipose tissue (25). All together, these results suggest, with the clear exception of heart tissue, that the apparent lack of correlation existing between both mitochondrial transcript levels and β-F_1-ATPase protein results from post-transcriptional regulated events. This implies that nuclear and mitochondrial gene expression for biogenesis of mitochondria in most mammalian tissues is also regulated by specific tissue factors that could determine the half-life of nuclear encoded mitochondrial mRNAs, their translational efficiency, as well as mitochondrial protein turnover.

The recent finding that β-F_1-ATPase mRNA experiences a 3 fold increase in translational efficiency between two stages of liver development (17) strengthens the idea that cell- or tissue-specific regulation of mitochondrial biogenesis is much exerted at the translational level. In this regard, rough estimates of the relative translational efficiency (R.T.E.) (Fig. 1F) (estimated as the ratio of β-F_1-ATPase protein/mRNA levels) indicate that tissues such as testis and lung could provide a source for the isolation of some of these regulatory factors. On the other hand, the high abundance of mitochondrial transcripts and protein levels in the heart (Fig. 1A-E), suggests that regulation of mitochondrial biogenesis in this tissue is exerted at the transcriptional level, in agreement with the existence in this tissue of specific *trans-acting* factors that promote transcriptional activation of certain nuclear-encoded mitochondrial genes (26, 27).

The parallelism observed between the tissue levels of mRNAs for β-F1-ATPase (Fig. 1B and 1E, open bars) and ATP 6+8 (Fig. 1C and 1E, hatched bars) subunits indicates the existence of a conserved mechanism among mammalian cells, either at the transcriptional and/or post-transcriptional levels, for coordination of the expression of the two genomes involved in mitochondrial biogenesis. These findings suggest that basic mechanisms of nucleo-mitochondrial interactions for mitochondrial biogenesis are the same among mammalian and probably other eukaryotic cells.

According to the two proposed mechanisms (28) for regulation of nuclear-encoded genes involved in energy metabolism in mammalian cells: (i) differential expression of tissue-specific isoforms and (ii) variable expression of functional single-copy oxidative phosphorylation genes, our results indicate that the second mechanism operates for rat ATP synthase β-subunit gene. This might be due either to differential tissue-specific rates of transcription or to the differential stability of cytoplasmic β-F_1-ATPase mRNA. However, as previously pointed out, additional levels of regulation at the translational and/or post-translational levels may influence the expression of mitochondrial nuclear-encoded genes in response to tissue demands for cellular energy.

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