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Enhanced mitochondrial biogenesis is associated with increased expression of the mitochondrial ATP-dependent Lon protease

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Abstract Rats bearing the Zajdela hepatoma tumor and T3-treated hypothyroid rats were used to study the role of protein degradation in the process of mitochondrial biogenesis. It was shown that the activity, protein and mRNA levels of the ATP-dependent Lon protease increased in rapidly growing Zajdela hepatoma cells. The increase in the rate of mitochondrial biogenesis by thyroid hormone was similarly accompanied by enhanced expression of the Lon protease. The results imply that mitochondrial biogenesis in mammalian cells is, at least partially, regulated by the matrix Lon protease.

Key words: ATP-dependent protease; Intramitochondrial proteolysis; Mitochondrial biogenesis

1. Introduction

A number of physiological conditions alter the expression of the mitochondrial genome in mammalian cells [1–5]. However, these alterations are not always coordinated with changes in the levels of extramitochondrially synthesized subunits, suggesting that expression of nuclear and mitochondrial genes is not necessarily tightly coordinated. In such cases, a question arises concerning the fate of the unassembled proteins.

Several mammalian mitochondrial ATP-dependent proteases have been described. The ATP-dependent Lon protease, PIM1, was purified from the matrix fraction [6,7], and the gene for the human homologue has been cloned [8]. Recently, the inner membrane ATP-dependent, M-aaa, protease [9] and the mitochondrial intermembrane space ATP-dependent protease, MISP1 [10], were also characterized and purified. Although the physiological functions of these mammalian proteases are not well understood, bacterial and yeast mitochondrial ATP-dependent proteases appear to mediate not only proteolysis, but also the insertion of proteins into membranes and the disassembly or oligomerization of protein complexes (reviewed in [11,12]). Thus, the ATP-dependent proteases may have a role in ensuring protein integrity and the overall level of organellar biogenesis.

In view of the suggested, but poorly studied, role of protein degradation in regulation of mitochondrial biogenesis in mammals, we investigated the effect of altered mitochondrial biogenesis on the ATP-dependent proteolysis mediated by the matrix Lon protease in isolated mitochondria. As model systems, we have used mitochondria from rapidly growing Zajdela hepatoma cells and liver mitochondria from T3-treated hypothyroid rats. The mitochondrial content of Zajdela cells is severely reduced, possibly controlled by protease-induced degradation [13], whereas thyroid hormone increases the expression of the mitochondrial genome [14,15] and the rate of mitochondrial protein degradation [16,17]. Here we report that both the rapid growth of Zajdela cells and the injection of hypothyroid rats with T3 are associated with increased levels of the ATP-dependent Lon protease mRNA and protein. Our findings are consistent with the participation of the Lon protein in regulated organellar proteolysis.

2. Materials and methods

2.1. Animals, experimental tumor and hormone treatment

Male Wistar rats of 120–150 g were used for experiments with Zajdela hepatoma. Zajdela hepatoma ascites cells was maintained and propagated as described [18]. For hormone experiments male Sprague-Dawley rats were used. Rats of 80 g were made hypothyroid by providing Tapazol in their drinking water [5]. T3 (20 μg/100 g body mass) was injected daily for 3 days [5].

2.2. Isolation of mitochondria and mitochondrial matrix

Rat liver and hepatoma mitochondria were isolated as described [18]. Treatment of the organelles with a low concentration of digitonin, which removed most of the contaminating microsomes, was performed as detailed in [19]. Mitochondrial matrix was prepared by sonicating mitochondria (20 mg/ml) on ice for 2 min at maximum output followed by centrifugation for 30 min at $100\,000 \times g$.

2.3. cDNA clones

cDNA clones for the Lon protease [8] and cytochrome *c* oxidase subunit IV [20] were from human. cDNA for actin was from mouse.

2.4. RNA extraction and Northern analysis

Total RNA was extracted as described [21]. mRNA was prepared by oligo(dT)-cellulose (Pharmacia Biotech) according to manufacturer's instructions. The samples (5 μg) were separated in 1.2% formaldehyde/agarose gels, transferred to nylon membranes (Hybond N, Amersham) and hybridized with DNA probes according to standard procedures. The mRNA was hybridized to ^{32}P-labeled specific cDNA sequences which were labeled by random priming. Autoradiographs were analyzed using an LKB 2222-020 Laser Ultrascan.

2.5. Miscellaneous methods

SDS-polyacrylamide gel electrophoresis [22], immunoblotting [23], and hydrolysis of [^{14}C]methylcasein to acid-soluble products [24] were performed according to published procedures.

3. Results and discussion

ATP-dependent proteolytic activity was measured in isolated rat liver and Zajdela hepatoma mitochondria in the presence or absence of ATP (Table 1). Oligomycin and apyr-

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Abbreviations: T3, triiodothyronine

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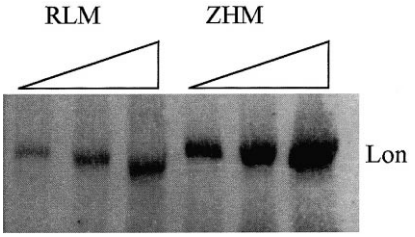

Fig. 1. The content of Lon protease in rat liver and Zajdela hepatoma mitochondria. Western blot analysis of the Lon protease was performed as specified in Section 2. Isolated mitochondria were first precipitated with 7% trichloroacetic acid, washed with cold water, sonicated for 2 min in suspension and solubilized with SDS. The amounts for both types of mitochondria were 12.5, 25 and 50 μg, respectively. RLM, rat liver mitochondria; ZHM, Zajdela hepatoma mitochondria.

ase were used to inhibit the mitochondrial ATPase and to hydrolyze the endogenous ATP and ADP, respectively [25]. The data, representing only the ATP-dependent activity, show an approximately 6-fold higher ATP-dependent hydrolysis of [^{14}C]methylcasein in Zajdela hepatoma mitochondria compared to normal rat liver. This value is even greater (8-fold) when the data are corrected for ATP-independent hydrolysis. Essentially the same results were obtained using the soluble matrix fraction only (Table 1). These results suggest that, under the experimental conditions used, the measured ATP-dependent proteolytic activity may be attributed to the Lon protease. To confirm this, we compared the levels of the ATP-dependent protease in rat liver and Zajdela hepatoma mitochondria by Western blotting using antibodies against the rat liver Lon protease (Fig. 1). In good agreement with activity measurements, the levels of the ATP-dependent protease were approximately 8-fold higher in Zajdela hepatoma mitochondria than in rat liver mitochondria.

Steady-state levels of mRNA encoding the Lon protease were also assayed. Fig. 2 shows a Northern blot analysis of rat liver and Zajdela hepatoma mitochondria probed with human Lon cDNA. Lon protease mRNA abundance in Zajdela hepatoma was increased 18-fold over that in normal rat liver (Fig. 3). COX IV mRNA on the other hand was only 2.5-fold more abundant in Zajdela hepatoma cells than in rat liver, in agreement with previous results [13]. Therefore, the content of the Lon mRNA in the tumor cells is approximately 4–6-fold higher than in their normal counterparts. These results extend our earlier observation of increased intramitochondrial proteolysis in tumor mitochondria [13] and suggest this to be due to the enhanced expression of the Lon protease.

To test whether the observed induction of the Lon protease

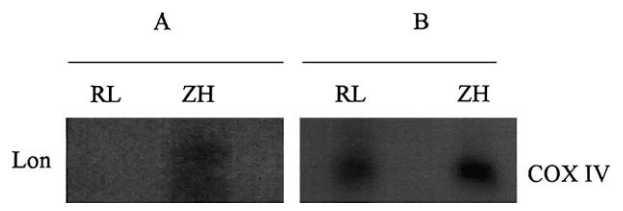

Fig. 2. Increased steady-state levels of the Lon protease in Zajdela hepatoma. mRNA from rat liver and Zajdela hepatoma (5 μg/lane) was electrophoresed, transferred to nylon membrane and assayed as described in Section 2. Lon, Lon protease; COX IV, cytochrome oxidase subunit IV; RL, rat liver; ZH, Zajdela hepatoma.

Table 1
ATP-dependent degradation of [^{14}C]methylcasein by mitochondrial and matrix fractions of rat liver and Zajdela hepatoma

Fraction	[^{14}C]Methylcasein hydrolyzed/mg/h		
	liver	hepatoma	fold increase hepatoma/liver
Mitochondria	0.50	3.14	6.3
Matrix	0.81	4.60	5.7
Mitochondria*	0.30	2.64	8.5
Matrix*	0.59	3.98	6.8

Experimental details are given in Section 2. Values represent results from three independent experiments and the standard error did not exceed 10%. Values marked with asterisks were corrected for ATP-independent activity (dependent−independent).

in Zajdela hepatoma mitochondria is seen only in tumor cells or is a more general reaction to changes in the state of mitochondrial biogenesis, experiments were performed with rat liver mitochondria from hypothyroid and T3-treated hypothyroid animals. Thyroid hormone increases mitochondrial mass [14,15] as well as the expression of mitochondrial genes [19] and some nuclear-encoded genes of the oxidative phosphorylation system [26]. Hyperthyroidism was induced in hypothyroid rats by daily injections of 20 μg T3/100 g body mass for 3 days. Lon protease activity in whole mitochondria and mitochondrial matrix fractions from hypothyroid and T3-treated hypothyroid animals are summarized in Table 2. An approximately 2-fold increase in the ATP-dependent hydrolysis of [^{14}C]methylcasein is observed in whole mitochondria and matrix fractions from T3-treated rats (Table 2). This correlates well with the 2-fold increase in Lon protein observed in these samples by Western blot analysis (Fig. 3) and a 3–4-fold increase in Lon mRNA (Fig. 4) in T3-treated rat livers. Thus the transition from the hypo- to the hyperthyroid state, which results in approximately a doubling of mitochondrial mass [14,15], is also associated with a similar increase in Lon protein and Lon activity.

In summary, our data show that increasing the rates of biogenesis of mammalian mitochondria either by neoplastic transformation (rapid cell growth and division) or by thyroid hormone is associated with enhanced expression of the mitochondrial Lon protease. The physiological role of the increased Lon protease in mitochondrial biogenesis remains to be firmly established. However, it is likely that both the rapid growth and division of the Zajdela cell and T3 treatment of rats induce a disproportionate expression of some OXPHOS proteins, resulting in accumulation of unassembled subunits. Unassembled, overexpressed subunits of mitochondrial proteins in yeast are subject to degradation by the Lon protease

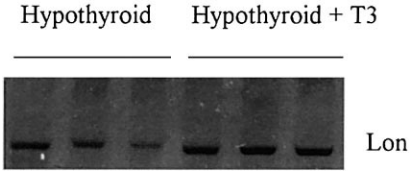

Fig. 3. The content of Lon protease in hypothyroid and T3-treated hypothyroid rats. Mitochondria were prepared from the livers of three hypothyroid rats and three hypothyroid rats treated with 20 μg T3/100 g body mass for 3 days. Western blot analysis and treatment of mitochondria prior to electrophoresis were done as in Fig. 1. Each lane represents 50 μg of mitochondria isolated from an individual animal. Lon, Lon protease.

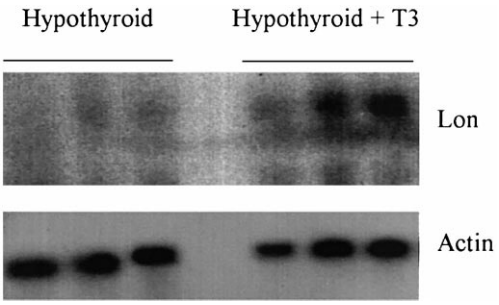

Fig. 4. Thyroid hormone increases the steady-state levels of the Lon transcript. mRNA was prepared from livers of three hypothyroid and three T3-treated hypothyroid rats. Details of the T3 treatment are given in Section 2. Each lane reperesents 5 μg of mRNA from an individual animal. Lon, Lon protease.

(see [12] and references therein). This scenario fits with the enhanced expression of the mitochondrial genome observed in rapidly growing neoplastic cells [13] and in T3-treated animals [19,26], and with the increased turnover of mitochondrial proteins in rapidly growing cells [13] and in rat liver after thyroid hormone injection [16,17]. Moreover, it has recently been demonstrated that the Lon protease is directly required for expression of certain mitochondrially encoded proteins in yeast [27].

However, the Lon protease is not the only protease involved in controlling the proper assembly of multimeric complexes. In yeast, the M-aaa complex is responsible for rapid degradation of unassembled cytochrome oxidase subunit 2 [28] and subunits of the mitochondrial ATP synthase [29–31]. Although we know little about the role of the M-aaa complex in mammalian mitochondria, the present data demonstrating nearly proportional increases in Lon protein and ATP-dependent protease activity in two different experimental systems suggest that the ATP-dependent Lon protease may have the major role in the regulation of mitochondrial biogenesis in mammalian cells. This notion is compatible with the growing body of evidence that ATP-dependent mitochondrial proteases play an indispensable role in the overall regulation of mitochondrial biogenesis.

Table 2
ATP-dependent degradation of [^{14}C]methylcasein by mitochondrial and matrix fractions of hypothyroid and T3-treated hypothyroid rats

Fraction	[^{14}C]Methylcasein hydrolyzed/mg/h		
	hypo	+T3	fold increase T3/hypo
Mitochondria*	0.154	0.365	2.4
Matrix*	0.263	0.583	2.2

Experimental details are given in Section 2. Values represent the mean of three independent experiments and were corrected for ATP-independent activity (dependent−independent). The standard error did not exceed 10%.

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