RENEWAL OF MITOCHONDRIAL PROTEINS IN RAT LIVER AT DIFFERENT AGES

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Processes of synthesis and catabolism of macromolecules and cell organelles are of great importance to the regulation of their intracellular concentration. Mitochondria, self-replicating organelles, are particularly interesting from this point of view because the synthesis of their proteins is controlled by both nuclear and mitochondrial genomes and is effected on cyto- and mitoribosomes. There is no general agreement in the literature on whether mitochondria are renewed as a single entity or whether their different components are renewed at different rates [5]. Moreover, the rate of catabolism of mitochondrial proteins synthesized in the cytoplasm and in the mitochondria themselves has not been investigated separately.

Since it has been shown that the number of mitochondria changes during aging [9] and that coordination between the two systems of mitochondrial protein synthesis is disturbed because of a fall in the level of protein synthesis on mitoribosomes [1], the investigation described below was carried out to study the renewal time of liver mitochondrial proteins in rats of different ages.

EXPERIMENTAL METHOD

Female Wistar rats, aged 7 (adult) and 28 (old) months, were given an intraperitoneal injection of a [^{14}C]-hydrolysate of *Chlorella* protein (Czechoslovakia) in a dose of 25 μCi/100 g body weight. The animals were decapitated on the 8th, 15th, and 22nd days after injection of the label. The washed liver (3 g) was squeezed through a press and homogenized in 20 ml of isolation medium (0.25 M sucrose; 0.001 M EDTA, 0.01 M Tris-HCl, pH 7.4) in a homogenizer of the proper type. Nuclei and undestroyed cells were removed by differential centrifugation (600g, 10 min) and fractions of heavy (6500g, 10 min) and light (10,000g, 10 min) mitochondria were isolated [6]. Each fraction was washed twice and resuspended in 10 ml of isolation medium. The suspension (0.3 ml) of mitochondria was added to 5 ml of 5% TCA in order to determine the radioactivity of the total mitochondrial proteins. The residual part of the suspension was kept overnight at −20°C. After thawing, the mitochondrial suspension was centrifuged at 30,000g (20 min). The residue was resuspended in 20 ml of 0.05 M Na-phosphate buffer (pH 11.5) and sedimented at 30,000g (20 min). This procedure was repeated. The final residue, insoluble in phosphate buffer, pH 11.5, consisted of proteins synthesized on mitoribosomes [7]. This residue was resuspended in 1 ml of the same buffer. To determine its radioactivity, 0.1 or 0.2 ml of the suspension of insoluble proteins of the fraction of heavy or light mitochondria respectively was added to 5 ml of 5% TCA. The TCA precipitates were filtered through Millipore filters of the "HA" type with a pore diameter of 0.45 μ (USA) and their radioactivity was measured on an Intertechnique SL-30 spectrometer (France), using ZhS-106 toluene scintillator (USSR). Protein was determined by a modified Lowry's method [8]. The half-life and regression coefficient of the proteins were calculated [2, 3] (in reality the apparent half-life and regression coefficient of the mitochondrial proteins were determined because possible reutilization of breakdown products cannot be ruled out).

EXPERIMENTAL RESULTS

The results show that liver mitochondrial proteins are renewed at different rates (Table 1). These differences are due both to the genesis of the proteins and the age of the animal. It will be noted that investigation of total mitochondrial proteins revealed virtually no differences in the rate of their renewal whether between animals of different ages or between

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TABLE 1. Breakdown Constants of Liver Mitochondrial Proteins from Rats of Different Ages

Preparation of mitochondrial proteins	Fraction of mitochondria	Age of animals, months	Coefficient of regression, log of specific radioactivity/day	Half-life, days	P
Total proteins	Total population of mitochondria	7	—0,0349±0,0033	8,6±0,8	
		28	—0,0402±0,0031	7,5±0,6	
Synthesized in cytoplasm		7	—0,0351±0,0037	8,5±0,9	
		28 (A)	—0,0421±0,0033	7,1±0,6	$P_{A-C}<0,001$
Synthesized in mitochondria		7 (B)	—0,0307±0,0019	9,8±0,6	$P_{B-C}<0,05$
		28 (C)	—0,0241±0,0026	12,4±1,3	
Total proteins	Heavy mitochondria	7	—0,0343±0,0034	8,7±0,9	
		28	—0,0397±0,0033	7,6±0,6	
Synthesized in cytoplasm		7	—0,0350±0,0039	8,6±1,0	$P_{D-F}<0,001$
		28 (D)	—0,0415±0,0036	7,2±0,6	
Synthesized in mitochondria		7 (E)	—0,0284±0,0021	10,6±0,8	$P_{E-I}<0,001$
		28 (F)	—0,0240±0,0026	12,5±1,4	
Total proteins	Light mitochondria	7	—0,0389±0,0027	7,7±0,5	
		28	—0,0420±0,0033	7,1±0,6	
Synthesized in cytoplasm		7 (G)	—0,0352±0,0038	8,5±0,9	$P_{G-I}<0,05$
		28 (H)	—0,0422±0,0040	7,1±0,7	$P_{H-J}<0,1$
Synthesized in mitochondria		7 (I)	—0,0451±0,0040	6,7±0,6	$P_{I-J}<0,05$
		28 (J)	—0,0313±0,0043	9,6±1,3	

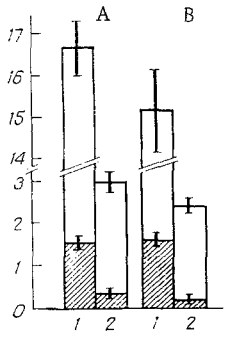

Fig. 1. Content of mitochondrial proteins in liver of rats of different ages. A) Adult rats, B) old rats; 1) heavy mitochondria, 2) light; whole columns denote total mitochondrial proteins, shaded part of column denotes mitochondrial proteins coded by mitochondrial genome. Vertical scale — protein content (in mg/g tissue).

different mitochondrial fractions within each age group. Meanwhile the use of a method enabling the rate of renewal of mitochondrial proteins coded by nuclear and mitochondrial genomes, and synthesized in the cytoplasm and mitochondria (soluble and insoluble in phosphate buffer, pH 11.5, respectively) separately revealed significant differences. For instance, the old animals differed from the adults primarily by the fact that their rate of renewal of proteins synthesized in the mitochondria was significantly lower (in the whole population and in the fraction of heavy mitochondria) or it had a tendency to decline (in the fraction of light mitochondria) compared with mitochondrial proteins synthesized in the cytoplasm. Meanwhile, differences in the adult animals were observed only in the fraction of light mitochondria and they were opposite in time to those observed in the old animals: the rate of renewal of proteins synthesized in the mitochondria was higher than that of mitochondrial proteins synthesized in the cytoplasm.

Whereas in the liver of the adult animals renewal of mitochondrial proteins synthesized on cyto- and mitoribosomes thus proceeded at the same rate (except the fraction of light mitochondria), in old rats the rate of renewal of mitochondrial translation products was slower.

Age differences also were found in the rate of renewal of mitochondrial translation products in adult and old rats: in the whole mitochondrial population and in their light fraction the rate of renewal was significantly lower in the old animals. In the fraction of light liver mitochondria from old rats the amount of proteins synthesized in the mitochondria was less than in adult rats (Fig. 1).

The results described above are in good agreement with those obtained by the study of the rate of synthesis of liver mitochondrial proteins during aging [1], which show that the rate of synthesis of proteins formed in the mitochondria themselves is reduced in the liver of old rats, whereas the rate of synthesis of total liver mitochondrial proteins does not change significantly during aging.

The fall in the rate of synthesis and metabolism of liver mitochondrial proteins synthesized on mitoribosomes in old rats and the fact that these parameters are unchanged in total mitochondrial proteins can evidently be explained primarily by the fact that proteins synthesized in mitochondria accounts for only about 10% of total mitochondrial proteins (Fig. 1).

The results thus demonstrate that the rate of metabolism of mitochondrial proteins synthesized on mitoribosomes, and incorporated into the coupling mitochondrial membrane decreases during aging. This decrease is observed in both mitochondrial fractions and it does not therefore characterize "aging" of the mitochondria (the light mitochondria are "young," the heavy are "old" [4]), but aging of the organism itself. Two conclusions can thus be drawn. First: the mitochondria are not renewed as a single entity, which is seen particularly clearly in the late stages of ontogeny. Second: the energy "insufficiency" of the old organism is due to changes in metabolism of proteins coded by the mitochondrial genome.

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