THE CHARACTERIZATION OF MITOCHONDRIAL TRANSLATION PRODUCTS IN RAT LIVER AND RAT HEPATOMA CELLS

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1. Introduction

The mitochondrial translation products of yeast and fungi are well documented (reviews [1,2]). Some of the proteins synthesized in mitochondria of higher eukaryotic cells have also been identified as components of mitochondrial ATPase complex [3–5] and cytochrome oxidase [3,6–10]. However, the use of various organisms and cells, different methods of labeling and identification of products of mitochondrial protein synthesis resulted in some confusion as to the number, molecular weights (or M_r) and functions of these products.

Here, we have undertaken a detailed investigation of the mitochondrial translation products labeled in in vitro isolated cells and mitochondria. Two cell types were used: the rat hepatocytes and Zajdela hepatoma derived from rats. Mitochondrial translation products of the cytochrome b–c_1 complex, cytochrome oxidase, and the ATPase complex were identified by immunological means. This study provides so far the most detailed analysis of mitochondrial translation products of higher eukaryotic cells.

2. Materials and methods

Rat hepatocytes were prepared from 150–180 g male Sprague-Dawley rats as in [11] with some modifications [12]. The maintenance, propagation and harvesting of Zajdela hepatoma cells and the isolation of mitochondria from the tumor and rat liver were done as in [13] with the exception that the preparation of mitochondria from small amounts of isolated cells were performed according to [12].

The labeling conditions for isolated hepatocytes and hepatoma cells were essentially identical. The cells were labeled in a medium [11] supplemented with unlabeled amino acid mixture and [^{35}S]methionine (>1000 Ci/mmol) as in [8,14]. In vitro incorporation of [^{35}S]methionine by isolated mitochondria was performed as in [4].

Beef heart cytochrome b–c_1 complex, rat liver cytochrome oxidase and rat liver F_1-ATPase were isolated and corresponding antisera raised as in [4,8,15].

The ATPase complex was solubilized and immunoprecipitated from labeled mitochondria by direct precipitation with immunoglobulin fraction from anti-F_1 sera [4,16].

For immunoadsorption of cytochrome oxidase and cytochrome b–c_1 complex the isolated mitochondria were solubilized (5 mg/ml of 2% Triton X-100, 1 M NaCl, 50 mM KP_i, 1 mM phenylmethyl sulfonylfluoride (PMSF), pH 8.0) and diluted with half volume of 2% Triton X-100, phosphate-buffered saline, 5 mM EDTA, 1 mM PMSF (pH 7.4). The insoluble material was removed by 30 min centrifugation at 120 000 × g. The supernatant was mixed with antiserum at an optimal antigen/antibody ratio, then incubated for 16 h at 4°C. The adsorption of antigen–IgG complex on Sepharose–protein A and the release of the adsorbed enzyme was performed as in [8].

SDS–polyacrylamide gel electrophoresis was performed on slab gels with a linear 12–20% acrylamide gradient [17] or in 12.5% acrylamide gels containing 8 M urea [18]. The samples were treated before electrophoresis for 1 h at room temperature with 2% SDS, 10 mM 2-mercaptoethanol, 10% glycerol, 6 M urea, 10 mM Tris–HCl (pH 6.8). Radioactivity distribution in the gels was detected by fluorography [19].

3. Results and discussion

3.1. *Total mitochondrial translation products in rat liver and hepatoma*

Fig.1 shows the mitochondrial translation products labeled in isolated rat hepatocytes (lanes1,5) and Zajdela hepatoma cells (lanes 2,7). For comparison, the translation products labeled in isolated rat liver mitochondria (lanes 3,6) and hepatoma mitochondria (lane 4) are also shown. The translation products appear identical in all 4 mitochondrial preparations when separated either on 12–20% acrylamide gradient gels (lanes 1–4) or 12.5% acrylamide gels containing 8 M urea (lanes 5–7). However, 2–3 additional products are observed when radioactivity on the gels is increased (lanes 6,7). Since the translation products are the same in rat hepatocytes and hepatoma cells as indicated by SDS-gel electrophoresis and immunological cross reactivity [20,21], future studies with hepatoma cells should prove advantageous due to their high rates of amino acid incorporation.

3.2. *Mitochondrial translation products associated with cytochrome* b–c_1 *complex*

The identification of an intramitochondrially synthesized protein associated with the cytochrome b–c_1 complex was performed using in vitro labeled rat liver mitochondria (fig.2, lanes 1,2) and in vitro labeled

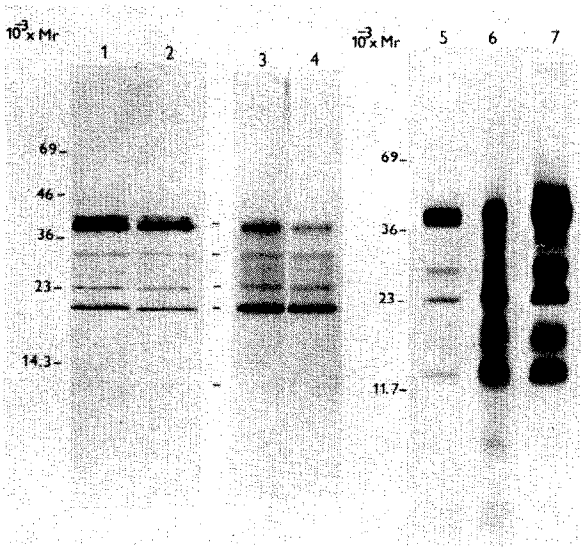

Fig.1. Products of mitochondrial protein synthesis in rat liver and Zajdela hepatoma. Mitochondrial translation products in whole cells were obtained after 2 h incubation of isolated rat hepatocytes or hepatoma cells in medium containing [^{35}S]-methionine (30–60 μCi/ml) and 0.5 mM cycloheximide. In vitro products were labeled by incubation of isolated mitochondria with [^{35}S]methionine (30–100 μCi/ml). Mitochondria isolated from labeled hepatocytes (lanes 1,5), labeled hepatoma cells (lanes 2,7) as well as in vitro labeled liver (lanes 3,6) and hepatoma (lane 4) mitochondria were separated on 12–20% gradient gels (lanes 1–4) or on 12.5% gels containing 8 M urea (lanes 5–7). Equal amounts of radioactivity (lanes 1–4) or equal amounts of protein (lanes 5–7) were applied on the gels.

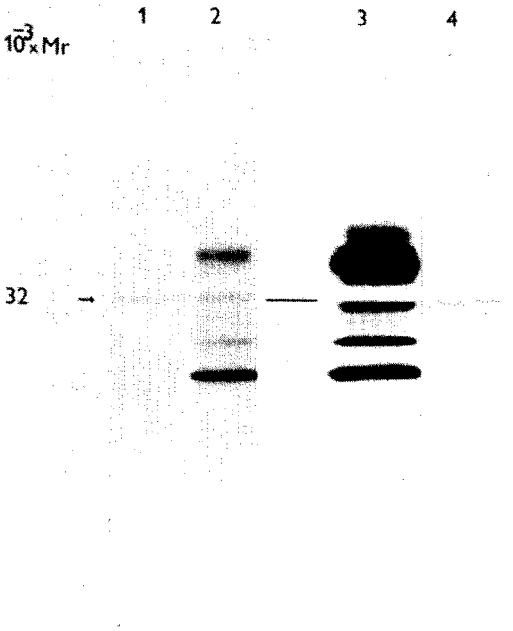

Fig.2. Immunoadsorption of 32 000 M_r component of mitochondrial translation products with antiserum to cytochrome b–c_1 complex. [^{35}S]Methionine-labeled mitochondria were solubilized and immunoadsorbed with an antiserum against beef heart cytochrome b–c_1 complex and Sepharose–protein A as in section 2. This antiserum has been shown to form immune complexes with the antigen from rat liver mitochondria [15]. Electrophoresis was performed on gradient gels; cytochrome b–c_1 complex immunoadsorbed from in vitro labeled rat liver mitochondria (lane 1) or from mitochondria labeled in whole hepatoma cells in the presence of cycloheximide (lane 4); total mitochondrial translation products from in vitro labeled rat liver mitochondria (lane 2), and from hepatoma mitochondria labeled in whole cells (lane 3).

hepatoma cells (lanes 3,4). A single labeled component with app. $M_r \sim 32\,000$ was immunoadsorbed from both types of mitochondria, with antiserum against beef heart cytochrome b–c_1 complex (fig.2). In accordance with the results obtained from yeast [22] and *Neurospora* [23], and the M_r reported for beef heart cytochrome b [24], we conclude that this mitochondrially translated peptide is cytochrome b apoprotein. Similar conclusions were drawn from studies in which rat liver cytochrome b–c_1 complex was chemically isolated from labeled hepatocytes P. Gellerfors, B. D. N., unpublished).

3.3. *Mitochondrial translation products associated with ATPase complex*

The immunoprecipitated ATPase complex from rat liver mitochondria was reported to contain two labeled components of M_r 21 500 and 9000 [4]. A similar result is also obtained with isolated hepatoma cells labeled in the presence of cycloheximide (fig.3, lanes 1,2). This observation is in partial variance with the conclusion that 21 000 and 25 000 M_r components of liver ATPase complex are synthesized in the mitochondria [5]. The results in fig.3 indicate that no proteins of the mitochondrial ATPase complex with $M_r > 21\,000$ are synthesized in liver or hepatoma mitochondria.

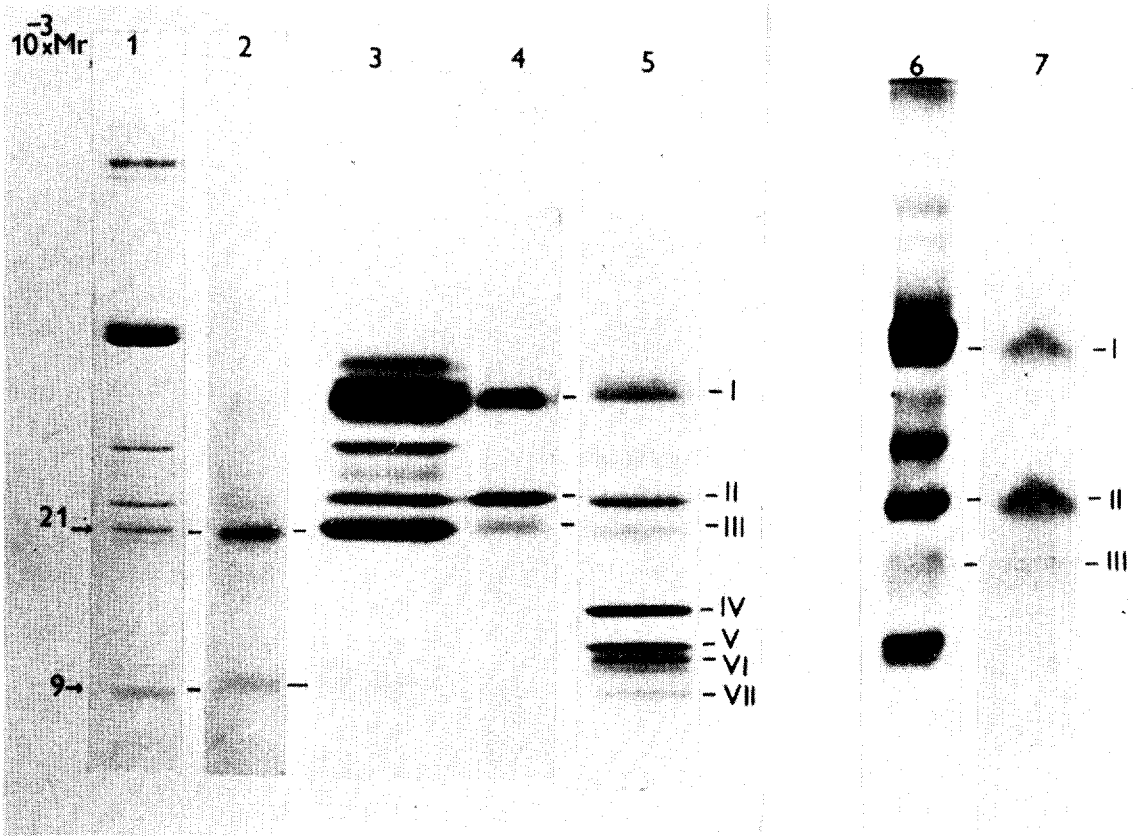

Fig.3. Intramitochondrially synthesized components of ATPase complex and cytochrome oxidase. After hepatoma cells were incubated 2 h with [^{35}S]methionine in the presence of cycloheximide the mitochondria were isolated, solubilized and aliquots were used to immunoprecipitate ATPase complex or to immunoadsorb cytochrome oxidase. Electrophoretic separation was carried out in 12–20% gradient gels (lanes 1–5) or on 12.5% gels containing 8 M urea (lanes 6,7): (1) stained ATPase complex immunoprecipitated from unlabeled mitochondria; (2) ATPase complex immunoprecipitated from mitochondria labeled in hepatoma cells; (3,6) total mitochondrial translation products in hepatoma cells; (4,7) cytochrome oxidase immunoadsorbed from mitochondria labeled in hepatoma cells; (5) stained isolated rat liver cytochrome oxidase. The roman numerals indicate the 7 cytochrome oxidase subunits.

3.4. *Mitochondrial translation products associated with cytochrome oxidase*

Fig.3 also demonstrates the synthesis of cytochrome oxidase in hepatoma cells labeled in vitro with [^{35}S]methionine in the presence of cycloheximide (lanes 3–7). Total mitochondrial translation products (lanes 3,6) and immunoadsorbed cytochrome oxidase (lanes 4,7) are compared with Coomassie blue-stained rat liver enzyme (lane 5). Three components of hepatoma cytochrome oxidase are translated on mitochondrial ribosomes (lanes 4,7). These correspond to the 3 largest subunits of the isolated rat liver enzyme (M_r 39 000, 23 000, 21 000).

Subunit III of cytochrome oxidase is weakly labeled in comparison to subunit I and II. This can be due to:

(i) A low methionine content of subunit III;
(ii) Unequal rates of formation and/or assembly of the subunits;
(iii) Substoichiometric amounts of subunit III in the immunoadsorbed enzyme [24,25]; or
(iv) The presence of antibodies directed specifically to subunit I and II resulting in the precipitation of these peptides even in the unassembled form.

The latter is the least likely since the antiserum used can not interact with subunit I (not shown). In any event, the low incorporation into subunit III explains our inability to detect its synthesis in isolated hepatocytes [8], which incorporate much less radioactivity than hepatoma cells.

3.5. *Comparison of the mitochondrial translation products in mammalian and yeast cells*

The mitochondrial translation products of *Saccharomyces cerevisiae* are well characterized [1,2]. For this reason, we have compared the translation products of yeast and hepatoma cells (fig.4). Their mobilities on SDS gels are surprisingly similar. The figure also summarizes the hepatoma translation products which have been identified to date. These products include 3 subunits of cytochrome oxidase (M_r 39 000, 23 000, 21 000), one subunit of the cytochrome b–c_1 complex (32 000), and two subunits of the ATPase complex (21 000 and 9000). As indicated above, this identification also applies to normal rat liver mitochondria. A minimum of two additional translation products remain unidentified, but they do not appear to participate in the complexes listed above. It may be speculated that at least one of them might have its counterpart in the yeast *var* proteins.

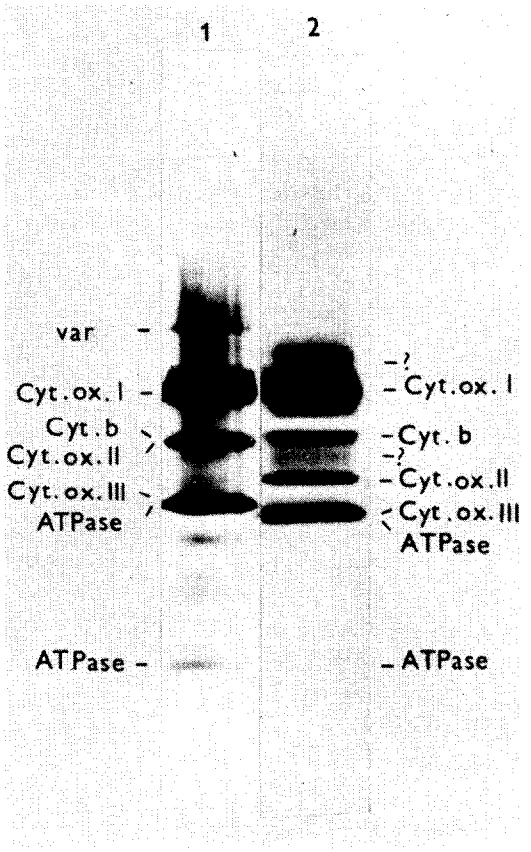

Fig.4. Mitochondrial translation products in yeast and mammalian cells. Mitochondria from yeast *Saccharomyces cerevisiae* (lane 1) and hepatoma (lane 2) cells labeled in the presence of cycloheximide were separated on 12–20% gradient gels. Yeast mitochondria specifically labeled with $^{35}SO_4^{2-}$ in the presence of cycloheximide according to [26] were generously provided by Dr J. Šubík of Food Res. Inst., Bratislava. The location of mitochondrially synthesized polypeptides is indicated.

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