Identification and characterization of mitochondrial translation products in various yeasts and in Prototheca zopfii

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Summary. Mitochondrial genomes of different eucaryotes are not all alike. We have examined mitochondrial translation products in a number of yeasts (Candida krusei, Hansenula saturnus, Rhodotorula glutinis, Rhodotorula rubra, Torulopsis glabrata and Saccharomyces cerevisiae) and in Prototheca zopfii, a colorless alga, in order to determine whether certain proteins are invariably synthesized within mitochondria, how different these proteins are, and what additional proteins, if any, might be synthesized by diverse mitochondria. Using a variety of techniques and criteria, including immunological analysis and peptide mapping, we show that all the yeasts studied, and probably P. zopfii as well, make versions of the 3 large subunits of cytochrome c oxidase. Not all of these oxidase subunits are equally closely related to their counterparts in S. cerevisiae, however. Mitochondria of some of the yeasts studied do not make, or make only small amounts of, a counterpart to Varl, a major mitochondrially made protein in S. cerevisiae. Mitochondria of P. zopfii possibly do not make an apocytochrome b. T. glabrata, H. saturnus and the two Rhodotorula species each make one or more proteins whose relationship, if any, to mitochondrial translation products of S. cerevisiae is not apparent. These results provide new information about mitochondrial diversity. Whereas mitochondria of all the organisms that we have studied devote the major part of their synthetic effort to making the three large subunits of cytochrome c oxidase, and probably make certain other proteins in common, they do not all synthesize a completely identical set of proteins.

Key words: Mitochondrial protein synthesis — Yeast mitochondria — Cytochrome oxidase — Prototheca zopfii

Introduction

Mitochondria are complex organelles that are present in nearly all eucaryotic cells. In those organisms that have been fairly well studied, the biosynthesis of mitochondria is dependent upon the operation and interaction of two physically separated genetic systems. Most of the hundreds of proteins that make up mitochondria are nuclearly encoded, cytoplasmically translated, and then imported into mitochondria (Schatz and Mason 1974; Schatz and Butow 1983). The remaining few proteins are encoded by mitochondrial DNA and translated on mitochondrial ribosomes (Borst and Grivell 1978; Dujon 1981; Tzagoloff 1982; Wilkie et al. 1983). These mitochondrial translation products commonly include versions of three large subunits of cytochrome oxidase, apocytochrome b, and at least one subunit of the ATP synthetase complex. In humans and in Saccharomyces cerevisiae, mitochondrial DNA also codes for two ribosomal RNAs and a set of 22 to 26 tRNAs (Anderson et al. 1981; Borst and Grivell 1978; Dujon 1981). A similar situation prevails in at least a few other mammals (Anderson et al. 1982; Bibb et al. 1981; Gortz and Feldman 1982), in Neurospora crassa (Burke and RajBhandary 1982; Macino 1980; Yin et al. 1982) and in Aspergillus nidulans (Brown et al. 1983; Macino et al. 1980).

To understand more fully the role of the mitochondrial genetic system in eucaryotic cells, we are examining mitochondrial gene expression in a number of fungi. Initially we have focused on mitochondrial protein synthesis because we believe it is important to establish what pro-
teins are made by different mitochondria. In related work we report the extent and nucleotide sequence of the mitochondrial subunit II gene of one of the yeasts, H. saturnus (Lawson and Deters 1985a, b).

Our results provide new information about mitochondrial species diversity. While mitochondria of the organisms that we have studied dedicate the major part of their total protein synthetic effort to the three large subunits of cytochrome c oxidase, they may not otherwise necessarily synthesize an identical set of proteins. Moreover, by immunological and partial peptide mapping criteria, it is apparent that the primary structure of the three large cytochrome c oxidase subunits can vary considerably even among the lower eucaryotes. The full extent of this diversity is presently unknown, however.

Materials and methods

Yeast strains used. Saccharomyces cerevisiae haploid strain D273–10B (ATCC 24657) is a defined laboratory strain frequently used in mitochondrial studies (Borst and Grivell 1978). S. cerevisiae diploid strain KM91 has been described (Claise et al. 1978). Candida krusei, Hansenula saturnus, Rhodotorula glutinis, Rhodotorula rubra and Torulopsis glabrata are from the fungal collection of the Microbiology Department of the University of Texas. Prototheca zopfii (UTEX 1438), an alga (Ahearn 1978), is from the Culture Collection of Algae, University of Texas at Austin.

Sodium [35S]-sulfate was obtained from Amersham. Cycloheximide (actidione) was from Sigma Chemical Co., St. Louis, Mo. Bactopeptone was from Difco. Staphylococcus aureus V8 Protease was from Miles Laboratories, Elkhart, Ind. Papain, type IV, was from Sigma. All other chemicals and reagents used were reagent grade or better, and were purchased from standard chemical suppliers.

YP is 1% yeast extract, 2% Bactopeptone; it is supplemented with indicated amounts of glucose, glycerol, or some other carbon source, to support growth.

Labeling of mitochondrial translation products and isolation of labeled mitochondria. Cells were grown at 30 °C for 18 h with vigorous shaking in YM media containing 0.5% glucose. In vivo labeling of mitochondria in the presence of cycloheximide was carried out in low sulfate media using [35S]-sulfate as described for S. cerevisiae (Douglas and Sutow 1976; Douglas et al. 1979).

After labeling, the cells were suspended in buffer containing 0.6 M mannitol, 0.02 M Tris-sulfate and 0.001 M disodium-EDTA, pH 7.4 (MTE), and broken by vigorous vortexing with glass beads. The extent of cell breakage was monitored by microscopic examination of the suspension. Typically, 4–5 min of vortexing produced greater than 90% breakage of the cells. Labeled mitochondria were isolated by differential centrifugation and suspended in MTE. Aliquots of this were analyzed immediately by SDS polyacrylamide gel electrophoresis or stored frozen at −80 °C until needed.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Mitochondria were dissociated in buffer containing 0.050 M Tris-chloride, 2% SDS, 1% betamercaptoethanol and 10% v/v glycerol (pH 6.8). Electrophoresis was carried out on high resolution exponential gradient (10% to 15%) polyacrylamide slab gels (Douglas et al. 1979). The cross-linker used in making the gel was usually N,N'-methylene-bis-acrylamide, used at a ratio of 1 g to 37.5 g acrylamide. However, for some experiments N,N'-diallyltartardiamide (DATD) (BioRad) was used as gel cross-linker, at a ratio of 1 g to 6.25 g acrylamide. After electrophoresis the gel was dried onto Whatman 3M filter paper and then exposed to Kodak SB-5 X-ray film. Exposure times typically were from 1 to 3 days.

Preparation of monospecific antisera to subunits I, II, and III of S. cerevisiae cytochrome c oxidase. Mitochondria were isolated on a large scale from commercial bakers' yeast as described earlier (Deters et al. 1976). Cytochrome c oxidase was isolated from these mitochondria by using a slight modification of the procedure described (Mason et al. 1973). Subunits I, II and III of cytochrome c oxidase were separated on 1.0 cm thick preparative SDS-12% polyacrylamide slab gels. The region of the slab gel containing the desired protein was cut from the gel and the protein electrophoresed into a dialysis bag by scaling up procedures that were previously described (Deters et al. 1975). The solution of the eluted protein was exhaustively dialyzed against 0.001 M Tris-chloride, pH 7.0, the protein was concentrated by evaporation of the excess water and the resulting protein analyzed for purity by analytical SDS-PAGE.

The isolated proteins (200 µg each) were mixed with complete Freund's adjuvant and injected into rabbits. After 1 month the rabbits were boosted intravenously with 100 µg of additional pure subunit. On the 9th, 11th and 13th days following the boost, 20 ml of blood were taken from the ear vein. After 1 month the rabbits were reboosted, and again 20 ml of blood taken from the ear vein on the 9th, 11th and 13th days following the boost. Serum was isolated from the blood by standard procedures as described (Nelson et al. 1973), and stored frozen at −20 °C.

Immunological analysis. Antiserum-antigen interactions were analyzed by incubating a thin, antiserum-containing, 1% agarose gel in contact with a conventional SDS-polyacrylamide slab gel which contained electrophoretically resolved, labeled, mitochondrial proteins (Cabral et al. 1977; Cabral et al. 1978). During the 16-h incubation period, proteins diffuse from the SDS gel into the antiserum-containing agarose gel. Those proteins recognized by the antibodies in the gel are precipitated in the agarose gel. Any other proteins are removed by subsequent washing. Control experiments, using relatively large amounts of purified cytochrome oxidase, showed that antibodies were always present in sufficient quantities to recognize available antigen. The labeled immunocomplexes were identified by autoradiography of the dried agarose slabs.

Peptide mapping. The mitochondrially synthesized proteins were labeled using [35S]-sulfate as described above. Mitochondria were isolated and the labeled proteins resolved on SDS-polyacrylamide gradient gels as described above, except that the thickness of the gels was reduced to 0.75 mm, and the sample was placed on the entire surface of the slab gel (i.e., a gel comb was not used). After electrophoresis the gel was stained briefly (5–10 min), then destained. A 1 cm vertical strip of gel was cut from the edge of the slab, dried onto filter paper and autoradiographed. By comparing the stained gel strip with its autoradiograph, the positions of the desired radioactive bands relative to the staining pattern on the gel were determined. The regions of the gel containing the desired radioactive bands were then cut from the main part of the gel. One-half centimeter wide pieces of the gel containing the labeled protein of interest were then excised.