SYNTHESIS OF PROTEOLIPID PROTEIN BY YEAST MITOCHONDRIA

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SUMMARY: Extracted proteolipid protein accounts for up to 3% of total yeast mitochondrial protein. The incorporation of label from ^{14}C-leucine into proteolipid by isolated mitochondria is inhibited by erythromycin and mikamycin, specific inhibitors of protein synthesis by mitochondrial ribosomes, and not by the cytoplasmic ribosomal inhibitor, cycloheximide; it appears completely stable to a puromycin chase which solubilizes about 40% of total mitochondrial label. The exact proportion of the label incorporated into proteolipid, ranging from 20 to 40% of that in completed polypeptides, is dependent upon the growth phase of the yeast cells. It is concluded that the protein of proteolipid is a major product of the mitochondrial system of protein synthesis in S. cerevisiae, but not the sole product.

INTRODUCTION

The protein components of the mitochondrion are formed largely by the cytoplasmic ribosomes, while the mitochondrial ribosomes contribute a small but essential group of proteins (for a review see 1). The incorporation of ^{14}C-labelled amino acids into membrane proteins by isolated mitochondria has been known for some time (2,3) but difficulties in dissociating mitochondrial membranes in such a way as to preserve the structure and the catalytic function of these proteins have prevented their definitive chemical identification.

One recent approach adopted for the isolation of specific membrane protein fractions has been to extract certain hydrophobic

proteins found in association with membrane lipids directly into 2/1 (v/v) chloroform: methanol (4,5,6). These fractions, called proteolipids, are of general occurrence in biological membranes (for reviews see 7,8) and those from ox heart mitochondria have been found to selectively bind dicyclohexylcarbodiimide (5) a known inhibitor of oxidative phosphorylation. Kadenbach (6) has reported that the proteolipid fraction obtained from rat liver mitochondria contains a single hydrophobic peptide of low molecular weight (about 2,000). This peptide was reported to be the sole product of liver mitochondrial protein synthesis *in vivo* (6). In this communication, we report evidence that the protein of yeast mitochondrial proteolipid is formed by the mitochondrial protein synthesizing system, but that it is not the sole product of this system.

METHODS

Saccharomyces cerevisiae haploid strain L-410 was grown aerobically at 28° with ethanol (1% v/v) as carbon source in a 1% (w/v) Difco yeast extract - salts medium (9). The isolation of mitochondria (10) and the assay for mitochondrial protein synthesis (11) were carried out as previously described, except that ^{14}C-leucine of specific activity 331 mCi/mmol (obtained from the Radiochemical Centre, Amersham) was used without dilution.

To study the labelling of proteolipid, the protein synthesis assay was scaled up to a volume of 10ml, with mitochondria corresponding to 14-20mg protein and cycloheximide included at 0.5mM to eliminate any possible contribution to the incorporation of

^{14}C-leucine by cytoplasmic ribosomes (1,11). At the conclusion of the incubation period (usually 20 mins at 30°) each sample was diluted 2-3 fold with cold resuspension buffer (0.6M sorbitol, 1mM EDTA, 15mM Tris HCl, pH 7.4) and the mitochondria were collected by centrifugation for 5 mins at 22,000g. The pellets were stored at -80° and thawed to room temperature just prior to extraction with 10-15ml of chloroform: methanol (2:1, v/v) for 40 mins. After filtering, the extracts were washed once with 5 ml of water and three times with 11 ml of chloroform: methanol: water (3:48:47 v/v), following Soto et al. (4).

Proteolipid protein was assayed by the Lowry method according to Hess and Lewin (12) with Bovine Serum Albumin as standard. The radioactivity of dried samples was measured by scintillation spectrometry.

RESULTS AND DISCUSSION

The isolated proteolipid accounted for between 1-3% of total mitochondrial protein and contained from 11 to 22% of the total label incorporated (Table 1), depending on the growth stage of the culture. During the transition from log phase to stationary phase, the proportion of total mitochondrial protein in proteolipid increased, while there was a marked decline in the overall rate of mitochondrial protein synthesis. The ratio of specific activities of proteolipid protein to total mitochondrial protein remained at 10 until late log phase and then fell to 4 in early stationary phase.

The effects of the mitochondrial protein synthesis inhibitors

TABLE 1. VARIATION IN ^{14}C-LEUCINE INCORPORATION INTO MITOCHONDRIAL PROTEOLIPID AS INFLUENCED BY THE GROWTH PHASE OF S. CEREVISIAE

	CELL GROWTH PHASE		
	LOG	LATE LOG	EARLY STATIONAR
Rate of mitochondrial protein synthesis	613	441	177
% of total mitochondrial protein in proteolipid	1.2	2.3	2.7
% of total radioactivity in proteolipid	12	22	11
Specific activity ratio (proteolipid protein: total mitochondrial protein)	10:1	9:1	4:1

The cells were grown on ethanol medium and mitochondria isolated and assayed for protein synthesis all as referred to in Methods. The rate of mitochondrial protein synthesis is expressed as cpm/mg protein/min.

erythromycin (11) and mikamycin (13) on the labelling of proteolipid were examined. Erythromycin at 0.005 mM inhibited both proteolipid labelling and total mitochondrial protein labelling by about 70%. Mikamycin at 0.025 mM resulted in over 90% inhibition of total mitochondrial protein labelling with 80% inhibition of th incorporation of label into proteolipid.

To exclude the possibility that the material extracted into chloroform: methanol might have included incomplete hydrophobic

TABLE 2. THE EFFECT OF SUBSEQUENT ADDITION OF PUROMYCIN ON THE INCORPORATION OF LABEL FROM ^{14}C-LEUCINE BY YEAST MITOCHONDRIA

DURATION OF PUROMYCIN CHASE (MINS)	TOTAL PROTEIN INCORPORATION		PROTEOLIPID INCORPORATION	
	CPM/mg PROTEIN	% CONTROL	CPM/mg PROTEIN	% CONTROL
0	4,025	100	15,040	100
10	2,350	58	-	-
20	2,290	57	16,730	111

The cells were grown on ethanol medium and mitochondria isolated and assayed for protein synthesis all as referred to in Methods. The concentration of puromycin was 0.07 mM (40 μg/ml) which completely inhibited protein synthesis when present throughout a 20 min incubation period.

polypeptides, puromycin was employed to release incomplete polypeptides from the ribosomes, making them susceptible to the action of endopeptidases (3). As shown in Table 2, treatment with puromycin resulted in the loss of about 40% of the label incorporated into total mitochondrial protein, but there was no reduction in the amount of label incorporated into the proteolipid fraction. It therefore appears that the measured incorporation of label into proteolipid fractions of yeast mitochondria represents an incorporation of ^{14}C-leucine into completed polypeptide chains which then apparently become associated with lipids. Since only 60% of the total mitochondrial incorporation is stable to puromycin and

represents completed polypeptides, it can be calculated that the proportion of completed polypeptides constituted by proteolipid protein ranges from 20 to 40% instead of the figure of 11-22% obtained from the total incorporation (Table 2).

The antibiotic sensitivity of the incorporation of label into proteolipid protein, together with its stability to a puromycin chase, establish the proteolipid protein as a product (or products) of the mitochondrial protein synthesizing system. The ratio of specific activities of proteolipid protein to total mitochondrial protein suggests that at least in the log phase of growth, the proteolipid protein may be made entirely within the mitochondrion. It has been estimated that mitochondria of derepressed yeast and mammalian tissues make about 10% of their total protein components *in vivo* (14,15,16). Thus the specific activity of an individual protein fraction made entirely by the mitochondrion would be of the order of 10 times the specific activity of the total mitochondrial protein and this is indeed the observed ratio *in vitro* for proteolipid protein for mitochondria isolated from cells in the log phase of growth (Table 1). The fall in this ratio for mitochondria isolated from cells at the onset of the stationary phase may indicate the selective slowing down of the synthesis of this fraction at that time, or the accumulation of the material from another source. The latter is less likely as the absolute proportion of proteolipid does not change significantly between late log and stationary phases.

In the present work, proteolipid protein accounted for 20-40% of the label incorporated from ^{14}C-leucine into completed

products of the yeast mitochondrial system of protein synthesis, so there are clearly other products of the mitochondrial system. This contrasts with the observation of Kadenbach (6) that a peptide of MW 2,000 in the proteolipid protein of rat liver mitochondria was the sole product of the mitochondrial system of protein synthesis *in vivo* following administration to the animal of ^{14}C-leucine in the presence of cycloheximide for 100 minutes, followed by a 25 minute chase of unlabelled leucine. Further evidence that the findings of Kadenbach may represent an oversimplification of the system is seen in the paper by Coote and Work (16), who used polyacrylamide gel electrophoresis to separate proteins from the pH 11.5 phosphate-insoluble fraction of rat and hamster liver mitochondria. About ten protein bands which ranged in MW from 14,000 to 50,000 were found to contain label incorated from radioactive amino acids by the mitochondrial system of protein synthesis, both *in vitro* and *in vivo*. The length of their labelling period *in vivo* was only 5 minutes, so that the possibility is raised that in Kadenbach's experiments the length of the labelling period in the presence of cycloheximide may have limited the number of products formed by the mitochondrial system of protein synthesis. It has been shown both in rat liver (17) and in yeast cells (14) *in vivo* that the rate of incorporation of labelled amino acids by the mitochondrial system of protein synthesis progressively decreases as the duration of exposure to cycloheximide increases. It may well be that this is a selective process, so that there are qualitative changes in the products of the mitochondrial system of protein syn-

thesis during prolonged exposure to conditions when the cytoplasmic components are not available.

Further work is proceeding on the characterization of the protein portion of yeast mitochondrial proteolipid.

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