mit⁻ MUTATIONS IN THE *oli2* REGION OF MITOCHONDRIAL DNA AFFECTING THE 20 000 DALTON SUBUNIT OF THE MITOCHONDRIAL ATPase IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Mitochondrial mutations affecting the function of the mtATPase in *Saccharomyces cerevisiae* have been shown to be located in two distinct regions of the mitochondrial genome [1–7], separated from each other by a large segment of the mitochondrial DNA (~12–15 kilobase pairs long) containing the structural gene for the apoprotein of cytochrome *b* [8–11]. One of these regions, which lies between 18 and 20 map units on the mitochondrial DNA [3], has been shown to contain the structural gene for the proteolipid (subunit 9) of the mtATPase [12–14]. However, the gene product of the other region has still not been identified. This region, called *oli2*, was initially defined by the loci of oligomycin-resistance mutations of the class C [3], such as the O_{II} locus [2], which has been mapped between 34 and 36 map units [7]. More recently, some *mit*⁻ mutations affecting the function of the mtATPase (*pho1* mutations) have been shown to be genetically closely linked to the O_{II} locus [4].

Here we describe three *mit*⁻ strains which carry mutations closely linked to the O_{II} locus. In these strains, the 20 000 dalton mitochondrially-synthesized protein subunit (subunit 6) of the mtATPase is either modified or absent, suggesting that the mutations are located in the structural gene for this subunit.

Abbreviations: mtATPase, mitochondrial oligomycin-sensitive ATP phosphohydrolase; SDS, sodium dodecyl sulphate

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2. Materials and methods

2.1. *Strains of yeast*

The three *mit*⁻ strains used in this study, Mal, Mb12 and Ma30, were derived from the wild-type strain J69-1B by mutagenesis with $MnCl_2$ [15], and were selected by their inability to grow oxidatively on ethanol as an energy source. The mutations were shown to map in the *oli2* region of the mitochondrial DNA by crossing the *mit*⁻ strains with a set of ρ^- tester strains which carried the O_{II} mutation but had sustained extensive deletions in other parts of the mitochondrial genome. The mutations were found to be closely linked to the O_{II} mutation (< 1.5% recombination).

2.2. *Analysis of mitochondrial translation products*

Cells were grown aerobically at 28°C with 2% glucose as the carbon source. The products of mitochondrial protein synthesis were labelled with [^{35}S]-sulphate in the presence of cycloheximide, and displayed by electrophoresis on SDS–polyacrylamide gels, as in [16]. In order to analyse the mitochondrially-synthesised proteins associated with the mitochondrial ATPase, mitochondria isolated from the labelled cells were treated with Triton X-100 [17] in the presence of protease inhibitors [18]. The ATPase was then immunoprecipitated from the Triton extracts using a specific antiserum raised against a purified oligomycin-sensitive ATPase complex [17]. The immunoprecipitates were repeatedly washed with

Tris–acetate buffer (5 mM, pH 7.5) containing 0.5% Triton X-100 and protease inhibitors, dissociated with SDS, and electrophoresed in polyacrylamide gels [16].

3. Results and discussion

The *mit*⁻ strains used in the present study are unable to carry out oxidative phosphorylation. The mutants do not grow on media with ethanol as the energy source, but the cells respire at rates comparable to those of the wild-type strain. Furthermore, mitochondria isolated from the mutant cells exhibit significant rates of electron transfer (table 1). However, the mitochondrial ATPase of the mutant strains has a lower specific activity than that of the wild-type strain, and is only inhibited by ~25% by oligomycin. Thus these mutant strains are quite similar to the *pho1* mutants reported in [4]. Moreover, the mitochondria from the mutants isolated in this laboratory do not catalyse ATP–P_i exchange, in contrast to the wild-type mitochondria. These results suggest that the mutations in the *mit*⁻ strains have produced a defect in the mitochondrial ATPase resulting in the loss of coupling of oxidative phosphorylation.

An analysis of the mitochondrial translation products of the mutant strains showed that these strains are deficient in the mitochondrially synthesised ATPase subunit which has mol. wt ~20 000. For this analysis, the mitochondrial translation products of the wild-type and mutant cells were labelled with [^{35}S]sulphate in the presence of cycloheximide, a specific inhibitor of cytoplasmic protein synthesis. The mtATPase complex was isolated by immunoprecipitation using a specific antiserum raised against oligomycin-sensitive mtATPase purified from a wild-type strain, and mitochondrially synthesised subunits of the enzyme complex were analysed by electrophoresis on SDS–polyacrylamide gels (fig.1). In the wild-type, two mitochondrial translation products were associated with the immunoprecipitate. These are the proteolipid subunit 9 (M_r 7600), which is also present as an oligomer (M_r 50 000), and subunit 6 (M_r 20 000) of the enzyme complex. This is in agreement with other studies in our laboratory and in [24].

Subunit 6 is absent from the immunoprecipitates of the mutants Mal and Mb12, and is very much decreased in intensity in strain Ma30. In one of the mutant strains, Mal, two new bands close together with app. mol. wt ~16 000 can be seen. In strains Mb12, a new band with mol. wt ~19 000 is present. In the third strain, Ma30, a new band, also with mol. wt ~16 000, can be seen. The presence of these new mitochondrial translation products in the immunoprecipitates indicates that they either cross-react with

Table 1
Mitochondrial enzyme activities of wild-type and *mit*⁻ strains

Strain	Specific activities (μmol. min^{-1} . mg^{-1})				
	NADH:cytochrome *c* reductase	cytochrome *c* oxidase	ATPase		ATP-P_i exchange
			no oligomycin	+ oligomycin	
J69-1B	0.41	0.42	1.53	0.21	0.156
Ma1	0.43	0.21	0.30	0.23	0.000
Mb12	0.32	0.06	0.18	0.13	0.001
Ma30	0.57	0.39	0.21	0.19	0.004

Published analytical procedures were used to determine the activities of NADH-cytochrome *c* reductase [19] and cytochrome *c* oxidase [20]. The ATPase activity was determined by a modification of the method in [21]: yeast mitochondria (20–40 μg protein) were suspended in 1 ml Tris–HCl buffer (50 mM, pH 8.0) containing ATP (1 mM), NADH (0.3 mM), $MgCl_2$ (3.3 mM), antimycin A (2 μg/ml), phosphoenol pyruvate (1 mM), lactate dehydrogenase (5 units/ml), and pyruvate kinase (2.5 units/ml), at 28°C. Oxidation of NADH was followed spectrophotometrically at 340 nm. Oligomycin, where present, was 30 μg/ml. The ATP–P_i exchange activity was measured as in [22], except that 0.25 M sucrose was added, and dithiothreitol was not included in the reaction mixture

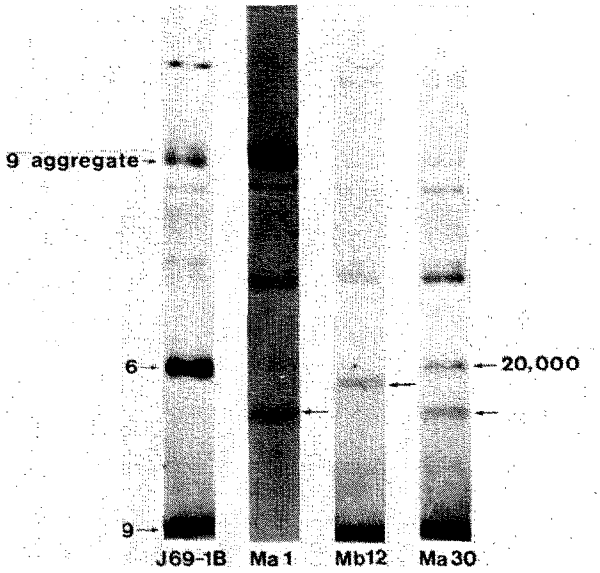

Fig.1. Mitochondrial translation products associated with mtATPase immunoprecipitates of the *mit*⁻ strains Mal, Mb12 and Ma30. The strains were labelled with $^{35}SO_4^{2-}$ in the presence of cycloheximide, and mitochondria were isolated. The mtATPase was extracted from the mitochondria with Triton X-100 (0.5%) and precipitated with rabbit antiserum raised against purified wild-type oligomycin-sensitive mtATPase. Samples of the immunoprecipitates were solubilised in SDS, and electrophoresed on a 12.5% polyacrylamide slab gel. The labelled bands were detected by scintillation autoradiography [23]. Mitochondrially synthesised ATPase subunits 6, 9 and the aggregate of subunit 9 are indicated on the left of the gel. Small arrows indicate new mitochondrial translation products associated with the mtATPase of the mutant strains. The apparent molecular weight is indicated on the right of the gel.

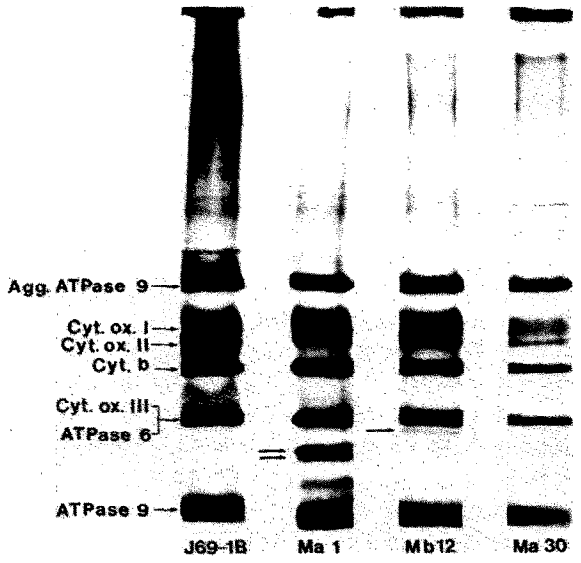

Fig.2. SDS–polyacrylamide gel electrophoresis of mitochondrial translation products in the *mit*⁻ strains Mal, Mb12 and Ma30. Cells were labelled, as in fig.1, and mitochondria were isolated and solubilised SDS. The samples were electrophoresed in a 10–20% polyacrylamide gradient gel. Mitochondrially-synthesised protein components of the mtATPase (subunits 6, 9 and aggregated subunit 9), cytochrome oxidase (cyt. ox. I, II and III) and the cytochrome *b* apoprotein (cyt. *b*) are indicated on the left of the gel. Small arrows indicate additional mitochondrial translation products found in the mutants.

the anti-ATPase antibodies, or are strongly associated with those ATPase subunits which are antigenic. When the mitochondrial translation products of the mutant strains were analysed in the whole mitochondria, the new mitochondrial translation products were also observable, albeit less clearly defined than in the immunoprecipitates in strains Mb12 and Ma30 (fig.2). The absence of subunit 6 is not immediately evident in the whole mitochondrial products, as subunit 6 has a similar molecular weight to that of the mitochondrially synthesised cytochrome oxidase subunit III, and cannot easily be separated from it on SDS–polyacrylamide gels.

The above observations suggest that the mutations in the *mit*⁻ strains affect the expression of a gene specifying subunit 6 of the mtATPase. The disappearance of subunit 6 and the appearance of new bands with higher mobilities in the immunoprecipitates of the mutant strains suggest that these strains carry termination mutations in the structural gene for subunit 6, resulting in the production of shorter polypeptide chains. Termination mutations have also been shown to be relatively common in other regions of the mitochondrial genome [9–11,25].

The inability of the mitochondria from the mutant strains to catalyse ATP–P_i exchange suggests that a functional subunit 6 is required for the coupling of oxidative phosphorylation, and may be directly involved in energy-transfer reactions. An energy-transfer factor (factor B) from beef heart mitochon-

dria has been isolated [26]. Factor B is a water soluble, colourless protein which contains active thiols [27], and which stimulates the energy-linked functions of submitochondrial particles treated with ammonia and EDTA. Several preparations with factor B-like properties, with app. mol. wt 11 000–47 000, have been reported (see [28,29]). However, it is not known whether there is any relationship between factor B and subunit 6.

Alternatively, the modification or loss of subunit 6 in the mutant strains may indirectly cause the uncoupling of oxidative phosphorylation, by preventing the correct assembly of the mtATPase. Yeast strains carrying mutations in the *oli2* region of the mitochondrial DNA now provide a means of studying the role of subunit 6 in the energy-linked functions and assembly of the mtATPase.

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