NUCLEAR GENE AFFECTING MITOChONDRIAL PROTEIN SYNTHESIS

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A nuclear mutant is described which carries a mutation influencing mitochondrial protein synthesis. The mutation causes a diminishing of the visible band of subunit I of cytochrome oxidase, but does not influence the presence of cytochrome oxidase. The latter appears in the low temperature spectrum as a peak at 602 nm and moreover its quantity is nearly the same as in the wild type.

The biogenesis of functional mitochondria, which are the area of respiratory activity of the cell, remains under the control of two genetic systems: mitochondrial and nuclear. Mitochondrial DNA represents a limited number of genes, and has been extensively studied [1, 2, 3]. However, data on the contribution of nuclear system are extremely scarce, especially with respect to the influence of nuclear genes on the expression of mitochondrial protein synthesis [4 - 11].

The present data deals with a mutant which in some way influences the efficiency of mitochondrial protein synthesis, although its effect is limited to the action on the enzymatic activity.

MATERIALS AND METHODS

Strains used. Yeast strains are listed in Table 1.

Media. The following media were used: YPG full medium (1% yeast extract Difco, 1% peptone Difco, 2% glucose), YP10 (the same as YPG but with 10% glucose), YPGAL (the same as YPG but with 2% galactose instead of glucose), N3 (the same as YPG but with 2% glycerol instead of glucose), NL5 (the same as YPG but with 4% DL-lactate instead of glucose), GO minimal medium (0.67% yeast nitrogen base w/o amino acids Difco, 2% glucose), sporulation medium (0.25% yeast extract, 0.98% potassium acetate).
### Table 1

**List of strains used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1-4A/8</td>
<td>his&lt;sup&gt;+&lt;/sup&gt;, a, rho&lt;sup&gt;+&lt;/sup&gt;, PET</td>
<td>Dr. A. Kruszewska</td>
</tr>
<tr>
<td>AB1-4C</td>
<td>ade&lt;sup&gt;+&lt;/sup&gt;, meth&lt;sup&gt;-&lt;/sup&gt;, x, rho&lt;sup&gt;+&lt;/sup&gt;, PET</td>
<td>Dr. A. Kruszewska</td>
</tr>
<tr>
<td>WY-91</td>
<td>arg&lt;sup&gt;-&lt;/sup&gt;, x, rho&lt;sup&gt;+&lt;/sup&gt;, PET</td>
<td>Dr. P. Slonimski</td>
</tr>
<tr>
<td>D225-5A/60</td>
<td>ade&lt;sup&gt;-&lt;/sup&gt;, liz&lt;sup&gt;-&lt;/sup&gt;, x, rho&lt;sup&gt;0&lt;/sup&gt;, PET</td>
<td>museum of Microbiological Institute</td>
</tr>
<tr>
<td>AB1-4A/8/55</td>
<td>his&lt;sup&gt;-&lt;/sup&gt;, a, rho&lt;sup&gt;0&lt;/sup&gt;, PET</td>
<td>Dr. P. Slonimski</td>
</tr>
<tr>
<td>A0150</td>
<td>his&lt;sup&gt;-&lt;/sup&gt;, a, rho&lt;sup&gt;+&lt;/sup&gt;, pet</td>
<td>mutant pet isolated from the strain AB1-4A/8</td>
</tr>
<tr>
<td>GZ107-9D</td>
<td>ade&lt;sup&gt;-&lt;/sup&gt;, meth&lt;sup&gt;-&lt;/sup&gt;, x, rho&lt;sup&gt;+&lt;/sup&gt;, pet</td>
<td>glic&lt;sup&gt;-&lt;/sup&gt; segregant from crose A0150 × AB1-4C</td>
</tr>
</tbody>
</table>

GO medium was supplemented with amino acids and/or adenine at the concentrations: adenine and methionine 40 μg/ml and histidine 20 μg/ml. When necessary the media were solidified with 2% Bacto agar.

**Methods.** Yeast strains were kept during the experiments in liquid YP10 medium at 4°C. Growth of cultures was conducted at 30°C. When necessary, liquid cultures were incubated and shaken. EMS mutagenesis was performed according to the modified method described by Fink [12]. Subsequent to the mutagenesis the cells were plated on YPGAL medium on which respiratory mutants form small colonies. They were picked up randomly and checked for the ability to grow on N3 medium (gly<sup>-</sup>).

Those cells which exhibited gly<sup>-</sup> phenotype were crossed with rho<sup>-</sup> strain and resulting diploids were replica plated on N3 medium. Clones which complemented rho<sup>-</sup> strain were isolated and classified as nuclear mutants.

The first classification of nuclear mutants was based on the analysis of the cytochrome spectra, which were recorded according to the method described by Claissé & Pajot [13]. Respiration of mutants was examined according to Estabrook [14] using Gilson oxygenograph. Yeast cells grown on solid galactose medium were used. After three days of incubation they were harvested, resuspended in saline and used for measurements of respiration.

Tetrad analysis and complementation test were done according to Fincham [15].

Activity of the respiratory enzymes was estimated according to the method described by Claissé [16]. The measurements of enzyme activities were done using isolated mitochondria. They were performed in 28°C and

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<sup>1</sup> Abbreviations used: ade<sup>-</sup>, arg<sup>-</sup>, his<sup>-</sup>, meth<sup>-</sup>, liz<sup>-</sup>, auxotrophs for adenine, arginine, histidine, methionine, lizyme, respectively; PET, nuclear gene responsible for respiratory activity; pet, its mutated allele; rho<sup>-</sup>, cytoplasmic determinant of respiratory activity; rho<sup>0</sup>, its mutated form; a/x, mating type.
the activity was expressed as moles of substrate which was oxidized within one minute by one milligram of protein.

Mitochondrial translation products were examined using the protocol described by Claisse et al. [17, 18]. Yeast cells were cultivated on galactose medium to which $^{35}\text{SO}_4$ was added; after labelling in the presence of cyclohexamide mitochondria were isolated and electrophoresis was performed on 15% polyacrylamide gel in the presence of SDS.

RESULTS

It is very well known that several nuclear genes are involved in the biogenesis of mitochondria. Some of them act directly like, for instance, genes for ribosomal proteins or enzymes of Krebs cycle or respiratory chain, others have an indirect effect causing alterations in the expression of mitochondrially coded proteins. In this paper we described an example of the second type of genes.

The nuclear mutant AO150 is affected in the synthesis of subunits I of cytochrome oxidase. This is evidenced by the examination of its mitochondrial translation products, the results of which are presented in Plate 1.

Plate 1. Mitochondrial protein synthesis of A, wild type AB1-4A/8; B, mutant pet AO150 and C, rho– Ab1-4A/8/55 strains. 1, cytochrome subunit oxidase 1; 2, cytochrome oxidase subunit II; 3, cytochrome b; 4, cytochrome oxidase subunit III.
In mutant AO150 we observed very strong bands of subunit II and III, but only a very negligible band of subunit I of cytochrome oxidase. For comparison, the data on the mitochondrial protein synthesis in the wild type and the rho<sup>+</sup> mutant are also included.

The nuclear mutation which apparently affects the expression of the mitochondrial gene coding for the subunit I of cytochrome oxidase was studied further both genetically and biochemically.

Figure 1 shows the cytochrome spectra of the mutant and the wild type. As shown in Fig. 1 the mutant AO150 possesses a complete cyto-

![Cytochrome spectra of wild type and mutant strains.](image)

chrome spectrum which does not differ from the cytochrome spectrum of the wild type. To elucidate the problem of the character of mutation an analysis of 20 tetrads was made. The 2:2 segregation of auxotrophic markers indicates that all spores in each tetrad result from a single meiotic event.

The segregation of ability to grow on glycerol, resulting in two spores growing and two non-growing, proves very strongly that the mutant AO150 is caused by single-gene nuclear mutation.

The ability to grow on different carbon sources was checked by spreading spots of mutant cells (approximately 10<sup>5</sup> cells) on appropriate media. Three carbon sources were examined: galactose, glycerol and lactate. According to the growth tests the mutant AO150 is completely unable to utilize glycerol and lactate, while on galactose it grows very poorly as compared with wild type.
The data of respiration of whole cells are presented in Table 2. Five carbon sources were examined and endogenous respiration was measured as well. The respiration of the mutant AO150 is not observed. The results strongly indicate that the mutation affects the functional organization of the respiratory chain.

Table 2

Respiration of mutants and wild type strains with different carbon sources

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon sources</th>
<th>glucose</th>
<th>ethanol</th>
<th>lactate</th>
<th>galactose</th>
<th>glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol O₂/h/mg protein</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>AB1-4A/8</td>
<td>59</td>
<td>88</td>
<td>118</td>
<td>88</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A0150</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

On the basis of cytochrome spectrum we calculated cytochrome content in the mutant AO150. The results are presented in Table 3. There is no significant difference in the cytochrome content between the mutant and the wild type. The only exception is cytochrome c, the content of which in the mutant is about 50% higher than in the wild type. The remaining cytochromes appear at almost equal concentration in both strains.

Table 3

Cytochrome content in mutant and wild type strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>c</th>
<th>c₁</th>
<th>b</th>
<th>a+a₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g of wet mass</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>AB1-4A/8</td>
<td>5.6</td>
<td>2.3</td>
<td>5.7</td>
<td>2.6</td>
</tr>
<tr>
<td>A0150</td>
<td>7.9</td>
<td>2.8</td>
<td>5.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

We tried to established the influence of the mutation on respiratory chain enzymes by measuring enzymatic activity. The results are summarized in Table 4. All activities are low, especially those concerning NADH - oxidase, NADH - cytochrome c reductase, succinate dehydrogenase and succinate - cytochrome c reductase. The activity of cytochrome oxidase, which probably is a target of mutation, amounted to around 20% of the wild type activity. The highest activity was recorded for NADH - dehydrogenase, giving in the mutant about 40% of the activity of the wild type.
Table 4

Measurements of respiratory enzymes activities of mutant and wild type strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>NADH oxidase</th>
<th>NADH dehydrogenase</th>
<th>NADH:cytochrome c reductase</th>
<th>Succinate dehydrogenase</th>
<th>Succinate cytochrome c reductase</th>
<th>Cytochrome oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of control</td>
<td>% of control</td>
<td>% of control</td>
<td>% of control</td>
<td>% of control</td>
<td>% of control</td>
</tr>
<tr>
<td>AB1-4A/8</td>
<td>2096</td>
<td>100</td>
<td>3396</td>
<td>100</td>
<td>1485</td>
<td>100</td>
</tr>
<tr>
<td>A0150</td>
<td>37</td>
<td>1.8</td>
<td>1336</td>
<td>39.4</td>
<td>50</td>
<td>3.4</td>
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<td></td>
<td></td>
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</table>

μmol/min/mg protein
DISCUSSION

In this paper we described a nuclear mutation influencing the yield of mitochondrially synthesized protein. In the mutant AO150 the synthesis of subunit I of cytochrome oxidase is inhibited. The inhibition is not complete and there still remains a weak band of this subunit. Apparently the mutation in some way affects the mitochondrial protein synthesis of subunit I. Its effect is unusual because, apart from the reduced subunit I of cytochrome oxidase, the cytochrome spectra of the mutant are complete. Moreover, when examining the cytochrome content we did not observe any marked quantitative difference between the mutant and the wild type strain. The only effect of the mutation observed is a reduction of enzymatic activities of respiratory chain enzymes. Another gene influencing mitochondrial protein activity was described by Dickemann et al. from Tzagoloff laboratory [9]. However, in this particular case the mutation affected the synthesis of apocytochrome b and is supposed to block maturation of pre-mRNA.

Michaelis et al. [10] described temperature sensitive mutants which at a non-permissive temperature of 36°C affected the synthesis of several mitochondrially coded subunits of cytochrome oxidase resulting in the lack of cytochrome oxidase peak at low temperature spectrum. Recently, another group of nuclear genes affecting the expression of mitochondrial genes in a positive way has been described. These genes belong to the class of nuclear suppressors and were found to be involved in pre-mRNA processing [19, 20, 21, 22]. Kruszewska & Slonimski [23, 24] have described another class of suppressors which preferentially act on intron mutations. The authors suggest that their suppressors are mutations in nuclear gene coding for a protein of a small subunit of mitochondrial ribosomes. Their action would cause the decreasing of fidelity of translation, leading to a misreading of some ochre codons. The case presented in the present paper is more complex, the mutation influences the appearance of the band of subunit I, and all other enzymatic activities connected with the respiratory chain. The low enzymatic activities could have appeared as a secondary effect, where the impairment of cytochrome oxidase influences in some way the rest of enzymatic activities.

The phenomenon can not be explained as a result of repression by galactose, because if this were the case, the synthesis of all mitochondrial subunits should be suppressed also. Our gene can be compared to the gene which has been described recently by Rödel & Fox [25]. These authors discovered the gene which is called CBS1 and which is involved in translation of the cob mRNA. This gene probably encoded a protein which in some way acts on untranslated 5' leaders of cob mRNA enabling its translation. The inhibition is not total because in strain bearing mutated allele CBS1 one can observe a very fine band of cytochrome b, the situation
which can be compared to our ones. Since Rödel & Fox [25] did not record the spectra of mutants we can not say that our gene acts in a similar manner as those described by those authors. Further experiments are in progress to elucidate this specific effect.

REFERENCES


