Assembly of the Mitochondrial Membrane System

CHARACTERIZATION OF NUCLEAR MUTANTS OF SACCHAROMYCES CEREVISIAE WITH DEFECTS IN MITOCHONDRIAL ATPASE AND RESPIRATORY ENZYMES*

(Received for publication, April 4, 1975)

ALEXANDER T'ZAGOLOFF, ANNA AKAI, AND RICHARD B. NEEDLEMAN

From the Public Health Research Institute of the City of New York, Inc., New York, New York 10016

In a previous paper we described a selection procedure for the isolation of mutants of Saccharomyces cerevisiae with specific lesions in enzymes of the respiratory chain and of the mitochondrial ATPase complex (1). Approximately 4000 strains showing lack of ability to grow on the fermentable substrate, glycerol, have been examined and of these a number of nuclear mutants have been found with specific defects in the ATPase, coenzyme Q\textsubscript{H}\textsubscript{2}-cytochrome c reductase, or cytochrome oxidase. In addition other mutants have been found which appear to be defective in coenzyme Q. Some of the biochemical and genetic properties of the mutants are reported here.

MATERIALS AND METHODS

The prototrophic strain, Saccharomyces cerevisiae D277-108 was mutagenized with either nitrosoguanidine or ethylmethanesulfonate (1). The screening procedure has been described elsewhere (1). ATPase was assayed at 37°C in the absence or presence of rutamycin with 10 mM ATP serving as the substrate (3).

Cytochrome Spectra—Oxidized versus reduced spectra of the cytochromes were obtained in a Cary 14 spectrophotometer equipped with a 0 to 0.2 absorption slide-wire. Since the submitochondrial particles were too turbid for spectrophotometric measurements, spectra were recorded on deoxycholate extracts of the particles. The particles were extracted at a final protein concentration of 7 to 8 mg/ml in the presence of 1% deoxycholate, 1 mM KCl, and 50 mM Tris-HCl, pH 8.0. These conditions quantitatively solubilize mitochondrial cytochromes of yeast. Samples of the extract were either oxidized with ferricyanide or reduced with sodium dithionite and the difference spectra were measured at room temperature.

Enzymatic Assays—NADH-cytochrome c reductase and cytochrome oxidase activities of the particles were measured at 23°C, as described previously (1). ATPase was assayed at 37°C in the absence or presence of rutamycin with 10 mM ATP serving as the substrate (3). The following assays were used to measure NADH-cytochrome Q reductase and coenzyme Q\textsubscript{H}\textsubscript{2}-cytochrome c reductase. NADH coenzyme Q reductase was measured in an Eppendorf fluorimeter (Hg-366 nm filter) by following the oxidation of NADH. The reaction mixture contained 60 μmol of Na\textsubscript{2}HPO\textsubscript{4} (pH 7.5), 30 μmol of Na\textsubscript{2}EDTA, 1.3 mg of bovine serum albumin, 0.033 μmol of NADH, 0.063 μmol of coenzyme Q\textsubscript{H}\textsubscript{2}, and 22 mg of bovine serum albumin and suspended in 60 ml of the same buffer. The culture was aerated at 32°C in a 2-liter flask by flushing the medium with air through a gas disperser. Aeration for 15 min at 24 hours, the cells were harvested, washed with 500 ml of a buffer containing 0.2 M mannitol, 20 mM Tris-acetate (pH 7.5), and 1 mM EDTA, and suspended in 80 ml of the same buffer. The suspension was disrupted in a Bronwill cell homogenizer and mitochondria isolated as described previously (3). Submitochondrial particles were prepared from the mitochondria by sonic irradiation with a 10Kc Raytheon sonic oscillator (3).

* This research was supported by Grants 1RO1-HE 13003 and GM 18966 from the National Institutes of Health, United States Public Health Service.
proportional to the concentration of submitochondrial particles up to 50 μg of protein.

**Analysis of Mitochondrial Products by Slab Gel Electrophoresis**—Cells were grown at 32° in 5 ml of 5% glucose medium containing 0.3% yeast extract and the salts of Ephrussi and Slonimski (2). The cells were harvested in early stationary phase and inoculated into 5 ml of fresh medium containing 2% glucose and 2 mg/ml of chloramphenicol. After 2.5 hours of aeration at 32° the cells were harvested, washed with 5 ml of water, and inoculated into 2.5 ml of fresh medium containing 2% glucose, salts (no yeast extract), 5 × 10^{-4} M cycloheximide, and 20 μCi of [35S]methionine. After incubation for 1.5 hours, the cells were harvested, washed with 5 ml of 0.25 M mannitol, 0.02 M Tris-acetate (pH 7.5), 1 mM EDTA, 2 mg/ml of chloramphenicol, and 5 × 10^{-4} M cycloheximide. The washed cells were suspended in 0.4 ml of 1 M potassium acetate (pH 4.75), 0.05 M Tris-HCl (pH 6.8), 2 mM EDTA, and 10% glycerol. After 5 to 7 days of incubation at 30°, the same cultures were scored after 5 to 7 days incubation at 30°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°. The same cultures were scored after 3.5 hours of aeration at 32°.


**Table I**

Properties of coenzyme QH<sub>2</sub>-cytochrome c reductase mutants

| Strain     | Specific Activity | Group | Rho-Complexation | QH<sub>2</sub>-Cyt. C | Cyt. Ox. | ATPase | Spectrum |
|------------|------------------|-------|------------------|-----------------------|---------|--------|----------|
|            | (μmol/min/mg)    |       |                  |                       |         |        |          |
| D273-10B  | <1               | N.D.  | 0.30             | 0.55                  | 5.4     | normal |
| N5-7      | 500/10<sup>9</sup> | 6     | III              | <0.01                 | 0.4     | 2.8    | no b     |
| N5-26     | none             | <1    | IV               | <0.01                 | 0.22    | 3.2    | no b     |
| N5-96     | none             | 1-2   | II               | <0.01                 | 0.3     | 3.1    | normal   |
| N6-60     | none             | 34    | II               | 0.01<sup>*</sup>      | 0.18    | 3.1    | normal   |
| N6-70     | none             | 1     | V                | <0.01                 | 0.24    | 3.5    | no b     |
| N6-192    | none             | 5     | N.D.             | <0.01                 | 0.16    | 3.6    | reduced b|
| N7-67     | none             | 3     | II               | <0.01                 | 0.1     | 3.7    | normal   |
| N8-9      | none             | 2     | II               | 0.05                  | 0.32    | 5.2    | reduced b|
| N8-110    | none             | 4     | II               | <0.01                 | 0.22    | 1.6    | reduced b|
| N8-129    | none             | 7     | I                | <0.01                 | 0.1     | 3.1    | reduced b|
| N8-130    | 100/10<sup>9</sup> | 31    | II               | <0.01                 | 0.32    | 5.1    | normal   |
| N9-38     | 400/10<sup>9</sup> | 4     | II               | 0.036                 | 0.59    | 5.2    | reduced b|
| E2-250    | none             | 3     | I                | <0.01                 | 0.1     | 3.5    | reduced b|
| E2-95     | 5/10<sup>9</sup>  | 5     | VI               | <0.01                 | 0.31    | 1.9    | no b     |
| E2-115    | high             | 2     | N.D.             | 0.02                  | 0.65    | 3.6    | normal   |
| E4-67     | none             | <1    | VII              | <0.01                 | 0.39    | 1.7    | no b     |
| E4-181    | 700/10<sup>9</sup> | 4     | VII              | <0.01<sup>*</sup>     | 0.24    | 1.3    | reduced b|
| E4-250    | 100/10<sup>9</sup> | 19    | IX               | <0.01                 |         |        |          |

*The QH<sub>2</sub>-cyt. c reductase activity in these strains was too low to be detected in the assay.

**Mutants of S. cerevisiae** that have mitochondrial defects (7, 8). Tetrad analysis of the mutants indicated a 2:2 segregation of the phenotype, confirming the nuclear nature of the mutations. The coenzyme QH<sub>2</sub>-cytochrome c reductase mutants fell into at least nine different groups based on genetic complementation.

The NADH-cytochrome c reductase deficient strains also included mutants in which this activity could be restored by the addition of coenzyme Q<sub>10</sub> to the assay. All the mutants had nearly identical NADH-coenzyme Q reductase. The mutants also showed the presence of variable levels of coenzyme QH<sub>2</sub>-cytochrome c reductase (Table II). The stimulation of NADH-cytochrome c reductase could be elicited with either coenzyme Q<sub>10</sub> or the natural analogue of S. cerevisiae, coenzyme Q<sub>10</sub>. The reconstituted activity was sensitive to antimycin A (Fig. 2). The cytochrome spectra of all the mutants in this group showed the presence of cytochromes c, b, a, and a<sub>3</sub>. A few strains were found to be capable of growth on glycerol when coenzyme Q<sub>10</sub> was added to the growth medium. Although we have not examined the biochemical lesions of this group of mutants further, it appears to consist of strains defective in coenzyme Q.

All the presumptive coenzyme Q mutants showed a 2:2 segregation in tetrad analysis and variable degrees of stability, both with respect to reversion to wild type and conversion to cytoplasmic petites (Table II). At least seven distinct complementation groups were found.

The mitochondrial products elaborated by the NADH-cytochrome c reductase mutants were examined by slab gel electrophoresis in the presence of sodium dodecyl sulfate. The results of these analyses indicated that most of the mutants were capable of forming all the normal products of mitochondrial protein synthesis. Two of the mutants (N5-26 and N6-70), however, had one of the major mitochondrial synthesized proteins missing (Fig. 3). Interestingly, both of these strains were completely deficient in cytochrome b.

**Cytochrome Oxidase Mutants**—Among the specific nuclear mutants, approximately 20 were found to have defective cytochrome oxidase. The properties of some of these strains are listed in Table III. The specific activities of cytochrome oxidase in the mutants chosen were 5% or less of the wild type. Based on their spectral properties, the mutants either had no detectable α band in the a and a<sub>3</sub> region of the spectrum or had highly reduced levels of these cytochromes. Some representative spectra of the cytochrome oxidase mutants are shown in Fig. 4.

The genetic tests for revertants and for cytoplasmic petite production again indicated a broad range of stabilities. A few mutants (N5-114, N8-105, E2-163, E4-238) degenerated to cytoplasmic petites at a high frequency. Most of the strains, however, were stable (Table III). Tetrad analysis of the mutants indicated a 2:2 segregation of the meiotic spore progeny and complementation tests yielded at least 10 different genetic groups.

It is unlikely that the deficiency in cytochrome oxidase is due
TABLE II
Properties of presumptive coenzyme Q mutants

| Strain  | Spontaneous Revertants | Percent Rho- Group | Complementation Group | NADH-cyt.c -coQ +coQ | Cyt. Ox. | ATPase | CoQH2-cyt. c |
|---------|------------------------|--------------------|-----------------------|-----------------------|----------|--------|-------------|
| D273-10B (wild type) | - | <1 | - | (0.56)§ (0.60)§ | 0.55 | 5.4 | 0.38 |
| N5-91  | 1/10^7 | 5 | I | 0.05 | 0.55 | 0.42 | 4.4 | 0.19 |
| N6-71  | 59/10^7 | 10 | I | 0.06 | 0.27 | 0.23 | 4.7 | 0.19 |
| N6-84  | none | 1 | N.D. | 0.16 | 0.51 | 0.4 | 4.1 | 0.40 |
| N9-57  | none | 21 | II | 0.04 | 0.46 | 0.33 | 3.5 | 0.17 |
| N10-140 | none | 17 | II | 0.04 | 0.14 | 0.17 | 2.4 | 0.14 |
| E2-237 | 27/10^7 | 28 | I | 0.02 | 0.42 | 0.28 | 2.3 | 0.13 |
| E2-247 | 1/10^7 | 11 | III | 0.04 | 0.27 | 0.39 | 3.8 | 0.09 |
| E2-249 | none | 23 | V | 0.03 | 0.45 | 0.12 | 1.6 | 0.15 |
| E3-24  | none | 9 | V | 0.05 | 0.43 | 0.21 | 2.2 | 0.20 |
| E3-71  | none | 15 | I | 0.02 | 0.23 | 0.17 | 1.1 | 0.06 |
| E4-140 | 29/10^7 | 1 | VI | 0.05 | 0.45 | 0.18 | 4.2 | 0.20 |
| E4-159 | none | 10 | VII | 0.07 | 0.6 | 0.19 | 4.2 | 0.14 |

§ Values taken from another experiment
FIG. 3. Polyacrylamide gel electrophoresis of radioactively labeled mitochondrial products from wild type and mutant strains of Saccharomyces cerevisiae. Cells were grown and labeled with [35S]methionine as described under "Materials and Methods." The isolated mitochondria were dissociated in the presence of 1% sodium dodecyl sulfate and 2% mercaptoethanol and 20 (odd numbered wells) and 50 μg (even numbered wells) of particle protein were separated on a 15% polyacrylamide gel according to the method of Studier (5). The gel was dried under vacuum and exposed to no-screen x-ray film for 6 days. D2′73, wild type; D273 (p"), cytoplasmic petite derived from D273 by ethidium bromide treatment (1); N9-168, nuclear ATPase mutant; N5-26, nuclear coenzyme QH-cytochrome c reductase mutant. The upper topmost radioactive band seen in wild type particles corresponds to one of the mitochondrially synthesized subunits of the ATPase.

ATPase activity of the comparable fractions from the parental wild type, D273-10B, and a cytoplasmic petite derived from D273-10B by ethidium bromide treatment. Under our conditions of cell breakage most of the F₁ synthesized by the cytoplasmic petite mutant is recovered in the post-ribosomal fraction.

The three ATPase mutants did not show the presence of spontaneous revertants in 10⁶ cells plated on glycerol medium. Conversion to cytoplasmic petals was less than 1% for N9-84 and E2-126 and 8% for N9-168.

N9-84 and E2-126 synthesized all the mitochondrial products seen in wild type. N9-168, however, was completely deficient in one of the proteins. The missing polypeptide (Band / in Fig. 3) corresponds to subunit nine of the rutamycin-sensitive ATPase complex which was previously shown to be a product of mitochondrially synthesized subunits of the ATPase.

DISCUSSION

Three enzymes of the mitochondrial inner membrane, the rutamycin-sensitive ATPase (10), cytochrome oxidase (11-13), and coenzyme QH₂-cytochrome c reductase (14), have been shown to consist of subunit polypeptides, some of which are synthesized in mitochondria and others on cytoplasmic ribosomes. An understanding of how the two protein synthesizing systems interact and how the polypeptide components are integrated into the functional enzyme complexes is one of the current goals in studies of mitochondrial biogenesis.

An approach to this problem is through the use of mutants in which defective subunit proteins lead to a blockage of the assembly process. The detection of partially synthesized enzyme intermediates should permit the sequence in which the subunits are integrated to be reconstructed. With this aim in mind, we have sought to isolate strains of S. cerevisiae in which the defects would be restricted to a single mitochondrial enzyme.

The mutants reported in this study appear to be specific for either ATPase, cytochrome oxidase, or coenzyme QH₂-cytochrome c reductase. The complementation test with a cytoplasmic petite strain and the observed 2:2 segregation of the meiotic spore progeny indicate that the mutations are nuclearly inherited and may be presumed that in each case a nuclear gene product is affected. It is not clear, however, that the mutated proteins are constituent polypeptides of the enzymes in question. For example, it cannot be excluded that some of the cytochrome oxidase mutants which show a total lack of cytochromes a and a₁ may be blocked in heme a biosynthesis.

The cytochrome oxidase mutants fall into at least 10 different genetic complementation groups. The mutants can also be distinguished by their spectral properties and patterns of mitochondrial products. For instance, a large number of the strains show a total absence of spectral cytochromes a and a₁. Some of the strains in this group may be similar to the nuclear mutants of cytochrome oxidase reported by Sherman and Slonimski (15), Šubik et al. (16) and Ehber et al. (17). A few mutants in this group have a mitochondrial product of cytochrome oxidase missing as has also been reported by Ehber et al. (17) for their mutants. Another group of cytochrome oxidase mutants have reduced but still detectable amounts of
TABLE III
Properties of cytochrome oxidase mutants

| Strain                  | Spontaneous | Percent Rho | Complementation Group | Cyt. Ox. (umoles/min/mg) | NADH-Cyt. c (umoles/min/mg) | ATPase | Spectrum |
|------------------------|-------------|-------------|-----------------------|--------------------------|-----------------------------|--------|----------|
| D273-10B (wild type)   | -           | <1          | -                     | 0.55                     | 0.35                         | 5.4    | normal   |
| N6-59                  | 1/10^7      | 3           | I                     | <0.01                    | 0.24                         | 1.7    | reduced a,a^- |
| N6-114                 | 45/10^7     | 66          | N.D.                  | <0.01                    | 0.33                         | 1.1    | reduced a,a^- |
| N7-13                  | 5/10^7      | 12          | N.D.                  | <0.01                    | 0.33                         | 2.9    | reduced a,a^- |
| N7-103                 | 67/10^7     | 8           | III                   | 0.013                    | 0.34                         | 1.6    | reduced a,a^- |
| N7-189                 | 2,000/10^7  | 4           | IV                    | <0.01*                   | 0.47                         | 4.1    | reduced a,a^- |
| N7-211                 | 300/10^7    | 10          | V                     | <0.01*                   | 0.35                         | 3.7    | reduced a,a^- |
| N8-102                 | 2/10^7      | 1.2         | V                     | <0.01                    | 0.11                         | 2.6    | reduced a,a^- |
| N8-105                 | none        | 50          | IV                    | <0.01*                   | 0.23                         | 1.7    | reduced a,a^- |
| N8-128                 | 2/10^7      | 4           | III                   | <0.01*                   | 0.09                         | 2.5    | reduced a,a^- |
| N9-8                   | 8/10^7      | N.D.        | IV                    | <0.01                     | 0.19                         | 3.0    | reduced a,a^- |
| N9-142                 | none        | 11          | II                    | <0.01*                   | 0.74                         | 5.2    | reduced a,a^- |
| E1-83                  | 19/10^7     | 3           | VI                    | <0.01*                   | 0.13                         | 1.9    | reduced a,a^- |
| E2-27                  | <1/10^7     | 6           | N.D.                  | <0.01*                   | 0.13                         | 2.9    | reduced a,a^- |
| E2-116                 | <1/10^7     | 30          | VII                   | <0.01*                   | 0.09                         | 2.45   | reduced a,a^- |
| E2-163                 | 1/10^7      | 24          | VIII                  | <0.01*                   | 0.13                         | 2.8    | reduced a,a^- |
| E2-215                 | 5/10^7      | 1           | II                    | <0.01*                   | 0.10                         | 4.3    | reduced a,a^- |
| E4-218                 | none        | 49          | IX                    | <0.01*                   | 0.07                         | 2.2    | reduced a,a^- |
| E4-230                 | none        | 20          | X                     | 0.04                     | 0.26                         | 4.2    | reduced a,a^- |

*The cytochrome oxidase activity in these strains was too low to be detected in the assay.

TABLE IV
Incorporation of [35S]methionine by cytochrome oxidase mutants in presence of cycloheximide

Cells were grown and labeled with [35S]methionine as described in the legend to Fig. 3.

| Strain                  | Specific activity (cpm/mg mitochondrial protein) |
|------------------------|-------------------------------------------------|
| D273-10B (wild type)   | 364,000                                         |
| N6-59                  | 908,000                                         |
| N6-78                  | 256,000                                         |
| N6-114                 | 267,000                                         |
| N7-13                  | 444,000                                         |
| N7-103                 | 371,000                                         |
| N7-189                 | 375,000                                         |
| N7-211                 | 370,000                                         |
| N8-102                 | 295,000                                         |
| N8-105                 | 161,000                                         |
| N8-128                 | 338,000                                         |
| N9-8                   | 794,000                                         |
| N9-142                 | 445,000                                         |

spectral cytochromes a and a3. These mutants show normal patterns of mitochondrial products.

The coenzyme QH2-cytochrome c reductase-specific mutants consist of at least nine complementation groups. These mutants also form different classes which are distinguished by their spectral properties. One group shows a total absence of cytochrome b. These may be similar to the nuclear mutants reported by Šubik et al. (18). A second group has reduced levels of the cytochrome and a third exhibits a normal cytochrome spectrum. Two of the mutants (N5-26 and N6-70) have one of the major mitochondrial products missing. Both strains are also devoid of cytochrome b. We do not know at present whether the missing protein is identical to the heme-carrying polypeptide which has been found to be a mitochondrial product in Neurospora (14).

Among the mutants examined, those showing a deficiency in mitochondrial ATPase were the least common. The three that were found were genetically distinct by complementation criteria. Two of the mutants were characterized by an absence of mitochondrial ATPase when this activity was assayed in the mitochondrial and post-ribosomal fractions. These two mutants showed normal patterns of mitochondrial products and exhibited both NADH-cytochrome c reductase and cytochrome oxidase activities, although the latter were considerably lower than in wild type. Since neither strain possesses enzymatically detectable ATPase, it is probable that the mutations affect the synthesis of functional F1-ATPase. Ebner and Schatz (19) have previously reported the isolation of a mutant which lacks both enzymatically and immunologically detectable F1. Their mutant also lacked respiratory activities and probably represents yet another class of ATPase mutants. The third ATPase
Enzymes known to be jointly made by the mitochondrial and cytoribosomal systems of protein synthesis, several interesting observations have emerged. The first is the rather large number of genetic complementation groups that are found within the class of cytochrome oxidase and coenzyme QH$_2$-cytochrome c reductase mutants. Since cytochrome oxidase is known to contain only four polypeptides that are synthesized on cytoplasmic ribosomes and coded by nuclear DNA (12), this finding implies the presence of a substantial number of additional nuclear gene products that are necessary for the biosynthesis of the enzyme. A similar conclusion may be drawn about coenzyme QH$_2$-cytochrome c reductase.

Ebner et al. (17) recently have described nuclear mutants of cytochrome oxidase in which some of the mitochondrial products of the enzyme fail to be made. In a subsequent study Ono et al. (22) concluded that a nuclear gene product in some of the cytochrome oxidase mutants in some way influenced the synthesis of one of the mitochondrial products. Evidence presented in this study suggests that the control of mitochondrial protein synthesis by nuclear genes may be a more general phenomenon that applies as well to the biosynthesis of coenzyme QH$_2$-cytochrome c reductase and ATPase.

**REFERENCES**

1. Teagoloff, A., Aoki, A., and Needleman, R. B. (1975) J. Bacteriol. 122, 826
2. Ephrussi, B., and Slonimski, P. P. (1950) Biochim. Biophys. Acta 346, 101
3. Teagoloff, A. (1971) J. Biol. Chem. 246, 3050
4. Needleman, R. B., and Teagoloff, A. (1973) Anal. Biochem. 64, 545
5. Studier, F. W. (1973) J. Mol. Biol. 79, 237
6. Mortimer, R. K., and Hawthorne, D. C. (1969) in The Yeasts (Rose, A. H., and Harrison, J. S., eds) p. 385, Academic Press, New York
7. Sherman, F. (1963) Genetics 48, 375
8. Kováč, L. (1974) Biochim. Biophys. Acta 345, 101
9. Sierra, M. F., and Teagoloff, A. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3155
10. Teagoloff, A., and Meagher, P. (1971) J. Biol. Chem. 246, 7328
11. Mason, T. L., and Schatz, G. (1973) J. Biol. Chem. 248, 1355
12. Sebald, W., Weiss, H., and Ziganke, B. (1973) Eur. J. Biochem. 41, 63
13. Sherman, F., and Slonimski, P. P. (1964) Biochim. Biophys. Acta 90, 1
14. Šubik, J., Kůzela, Š., Kolarov, J., Kováč, L., and Lachowicz, T. M. (1970) Biochim. Biophys. Acta 205, 513
15. Sherman, F., and Slonimski, P. P. (1964) Biochim. Biophys. Acta 90, 513
16. Šubik, J., Kůzela, Š., Kolarov, J., Kováč, L., and Lachowicz, T. M. (1970) Biochim. Biophys. Acta 205, 513
17. Sherman, F. (1963) Genetics 48, 375
18. Ephrussi, B., and Slonimski, P. P. (1950) Biochim. Biophys. Acta 346, 101
19. Sierra, M. F., and Teagoloff, A. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3155
20. Teagoloff, A., Aoki, A., and Needleman, R. B. (1975) J. Bacteriol. 122, 826
21. Ephrussi, B., and Slonimski, P. P. (1950) Biochim. Biophys. Acta 346, 101
22. Ono et al. (22) concluded that a nuclear gene product in some of the cytochrome oxidase mutants in some way influenced the synthesis of one of the mitochondrial products.

**TABLE V**

### Properties of nuclear ATPase mutants

Mitochondria were prepared as described under "Materials and Methods" except that 2 mM ATP and 1 mM EDTA were included in all the buffers. The cell suspensions were homogenized in the Bronwill cell homogenizer for 30 s and all subsequent steps were carried out at room temperature. The specific activities reported are for the mitochondria rather than submitochondrial particles.

| Strain          | Spontaneous revertants | Per cent $\rho^-$ | Complementation group |
|-----------------|------------------------|-------------------|-----------------------|
| D273-10B (wild type) | 1                      |                   |                       |
| D273-10B ($\rho^-$) | 0.35                  | 0.90              | 1.45 ($\Delta = 3.9$) |
| E2-126          | 0.30                   | 0.2               | 0.34 ($\Delta = 0.09$) |
| N9-84           | 0.37                   | 0.37              | 0.34 ($\Delta = 0.03$) |
| N9-168          | 0.32                   | 0.34              | 0.21 ($\Delta = 0.05$) |
| Post-ribosomal supernatant | 0.55                  | 0.73              | 0.13 ($\Delta = 0.49$) |
| D273-10B (wild type) | 0.52                  | 0.11              | 0.11 ($\Delta = 0.41$) |
| E2-126          | 0.65                   | 0.07              | 0.07 ($\Delta = 0.00$) |
| N9-84           | 0.03                   | 0.05              | 0.05 ($\Delta = 0.00$) |
| N9-168          | 1.95                   | 1.95              | 1.95 ($\Delta = 1.95$) |

*The numbers in parentheses refer to the ATPase activity inhibited by the antiserum to F$_c$.

### ATPase activity of mitochondria and post-ribosomal fractions of ATPase mutants

Mitochondria were prepared as described in the legend to Table IV. The post-ribosomal supernatant was centrifuged at 168,000 $\times$ g for 60 min to obtain the post-ribosomal supernatant fraction. All centrifugations were carried out at room temperature in the presence of 2 mM ATP and 1 mM EDTA. The post-ribosomal supernatants were dialyzed versus a solution containing 10 mM Tris-acetate (pH 7.5), 2 mM ATP, and 1 mM EDTA prior to assay in order to remove endogenous inorganic phosphate.

| Strain          | Spontaneous revertants | Per cent $\rho^-$ | Complementation group |
|-----------------|------------------------|-------------------|-----------------------|
| D273-10B (wild type) | 1                      |                   |                       |
| D273-10B ($\rho^-$) | 0.35                  | 0.90              | 1.45 ($\Delta = 3.9$) |
| E2-126          | 0.30                   | 0.2               | 0.34 ($\Delta = 0.09$) |
| N9-84           | 0.37                   | 0.37              | 0.34 ($\Delta = 0.03$) |
| N9-168          | 0.32                   | 0.34              | 0.21 ($\Delta = 0.05$) |

### Post-ribosomal supernatant

| Strain          | Spontaneous revertants | Per cent $\rho^-$ | Complementation group |
|-----------------|------------------------|-------------------|-----------------------|
| D273-10B (wild type) | 0.55                  | 0.73              | 0.13 ($\Delta = 0.49$) |
| D273-10B ($\rho^-$) | 0.52                  | 0.11              | 0.11 ($\Delta = 0.41$) |
| E2-126          | 0.65                   | 0.07              | 0.07 ($\Delta = 0.00$) |
| N9-84           | 0.03                   | 0.05              | 0.05 ($\Delta = 0.00$) |
| N9-168          | 1.95                   | 1.95              | 1.95 ($\Delta = 1.95$) |

*Values taken from another experiment.
17. Ebner, E., Mennucci, L., and Schatz, G. (1973) J. Biol. Chem. 248, 5379
18. Šubik, J., Kováč, L., and Kolarov, J. (1972) Biochim. Biophys. Acta 283, 146
19. Ebner, E., and Schatz, G. (1973) J. Biol. Chem. 248, 5379
20. Goffeau, A., Colson, A. M., Landry, Y., and Foury, F. (1972) Biochem. Biophys. Res. Commun. 48, 1448
21. Goffeau, A., Landry, Y., Foury, F., Briquet, M., and Colson, A.-M. (1973) J. Biol. Chem. 248, 7097
22. Ono, B., Fink, G., and Schatz, G. (1975) J. Biol. Chem. 250, 775
Assembly of the mitochondrial membrane system. Characterization of nuclear mutants of Saccharomyces cerevisiae with defects in mitochondrial ATPase and respiratory enzymes.
A Tzagoloff, A Akai and R B Needleman

J. Biol. Chem. 1975, 250:8228-8235.

Access the most updated version of this article at http://www.jbc.org/content/250/20/8228

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/20/8228.full.html#ref-list-1