BIOCHEMICAL PROPERTIES OF A NUCLEAR THERMOSENSITIVE MUTANT OF *Saccharomyces cerevisiae*

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SUMMARY: A nuclear thermosensitive mutant is blocked in the coenzyme Q biosynthesis. The mutant lacks cytochromes b, aa_3 and several dehydrogenases respiratory-chain-linked. The addition of CoQ_6 to the medium allows growth at non-permissive temperature.

The respiratory chain in yeast is controlled by a cytoplasmic factor (rho) as well as by several unlinked nuclear genes (P) (3, 12). Some p mutants lack cytochrome a + a_3 and often cytochrome b, as shown by low-temperature spectra in the visible region. Moreover, many enzymatic activities of the respiratory chain are affected by these mutants (13).

Mounolou *et al.* (8) have raised the question: by what mechanism does a single mutation lead to a multienzymatic deficiency? It is very difficult to answer this directly by use of the conventional p mutants. It is clear in fact that the pleiotropic effect shown by such p mutants makes it impossible to identify the gene product of each of them.

We have, therefore, taken into consideration the possibility that thermsensitive mutants that behave as P at 23°

and as p at 36° can provide more information about the product of P determinant. The rationale behind this is that p_{ts}, behaving as P when grown at 23°, synthetize a fully respiratory chain. The mitochondria isolated from such cells ought to have all the enzymatic activity when assayed at 23° but should lack only the activity (or activities) whose determinant is mutated to produce a thermsensitive gene product, when assayed at 36°. In the present paper, we report studies on the p thermosensitive mutant 1511 that lacks coenzyme Q when grown at 36°, probably because of a block in its biosynthesis. This mutation causes the complete absence of all the respiratory-chain-linked enzymatic activities.

MATERIALS AND METHODS

Coenzyme Q_6, succinic acid, D- and L-lactic acid and ascorbic acid were purchased from California Corporation for Biochemical Research. Cytochrome c, 2,6-Dichlorophenol-indophenol and phenazine methosulfate were purchased from Sigma Chemical Co. N:N:N':N-Tetramethyl-p-phenylene-diamine-dihydrochloride was purchased from BDH Lab. Chem. Div. (Poole, England).

Yeast strain: strain 1511 and other p_{ts} mutants were obtained by treatment with nitrosoguanidine of the strain K8/bc, ad_{2-1} try_{8-18} (11). The p standard mutants were kindly supplied by Drs. R.C. Mortimer and F. Sherman.

Culture media: the cells were grown in a medium containing 20 gm of Difco Yeast Extract and 0.6 gm glucose in 1000 ml distilled water (YEDL-gluc.medium). When glucose was re-

placed by a non-fermentable carbon-source, glycerol was added to the medium at the final concentration of 2% (YEDL-glyc.), Solid medium (YED) was obtained by adding 2% agar.

Growth conditions: 100 ml subculture was inoculated from a stock culture grown at 23° on YEDL-glyc medium and kept at 4°. The subculture was allowed to grow overnight on an alternating shaker at 23°. A fermentor containing 20 l. of YEDL-glyc medium was inoculated by the subculture and allowed to grow to the early stationary phase at 23°. The aeration was obtained by bubbling 1.5 l. of sterilized air per minute. The same procedure was adopted for growing cells at nonpermissive temperature (36°) in YEDL-gluc.

Mitochondrial preparation: the cells, harvested by centrifugation at 5,000 rpm at 4°, were washed twice and made up to 25 ml with a solution containing 0.25M sucrose, 0.05 M Tris-HCl pH 7.4 and 2.5 mM EDTA, and mixed with 25 ml of ice-cold glass beads (N12, Minnesota Mining Co.). The mixture was shaken at top speed for 90 seconds in a mechanical cell homogenizer. The unbroken cells and the debris were discarded by double centrifugation: 5 minutes at 550g followed by 5 minutes at 850g. The supernatant was then centrifuged at 20,000g for 15 minutes and the precipitate was resuspended in a Tris-EDTA-sucrose medium, as described before.

The enzymes were assayed according to Mahler et al.(7) and Mackler et al. (6). The coenzyme Q was extracted from the mitochondria according to Lester and Crane (5).

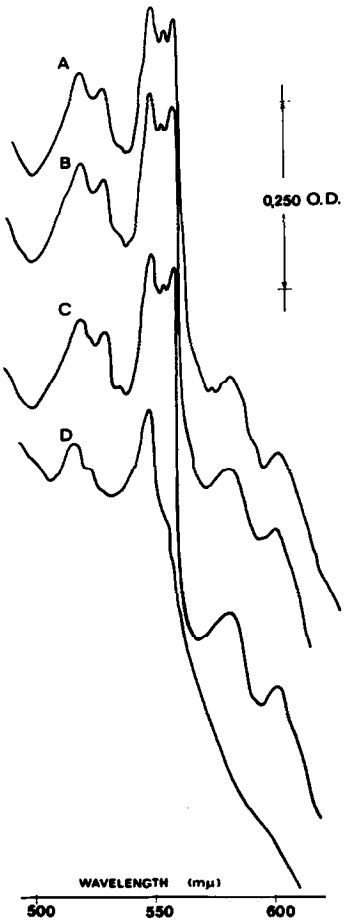

Fig. 1 - Low-temperature spectra. The spectra of cytochromes reduced by sodium dithionite were obtained at -190° with a Cary 14 Spectrophotometer specially equipped (11). 0.5 ml of a suspension of 1 x 10^{10} cell/ml was used for each spectra. A: wild-type S. cerevisiae grown at 23° ; B: wild-type at 36°, grown with galactose as carbon source; C: 1511 mutant grown at 23°, and D: some mutants grown at 36° with galactose.

RESULTS AND DISCUSSION

The 1511 mutant was obtained by treatment with nitrosoguanidine; it grows at 23° but not at 36° in presence of 2% glycerol as carbon source. The p_{ts} phenotype depends upon a mutation that occurred in a single chromosomal genetic deter-

minant as shown by its 2:2 segregation during meiosis from the heterozygous diploid (11). Figs. 1A and 1B show a typical low-temperature spectrum of the wild-type grown at 23° or 36° respectively in presence of non-repressive sugar concentration (0.6%) and, as expected, there is no difference in the reduced profile of the cytochromes at the two temperature, in agreement with the observation that the wild-type grows in presence of glycerol as carbon source both at 23° and 36°. Figs. 1C and 1D show the spectra of the p_{ts} mutant 1511 grown at 23° and 36°. The cytochromes b and a + a_3 are missing, as shown by the fact that the characteristic bands at 563 and 600 mμ are not present. The bands corresponding to reduced cytochrome c are present. These data clearly demonstrate that the mutation carried by strain 1511 shows a pleiotropic effect as in several p mutants described by Sherman and Slonimski (13).

The results relative to the enzymatic activities of the respiratory chain of the 1511 mutant are reported in Table 1. All the enzymes tested are present in the mitochondria isolated from the strain grown at 23°, when assayed at 23° or at 36°. On the other hand, in the yeast grown at 36° the isolated mitochondria show no detectable activity when assayed at 23° or 36° with all substrates used. No activity was present in the supernatant fraction of the strain grown at 36°, therefore excluding the possibility that at the nonpermissive temperature some mitochondrial proteins are not localized in the fraction precipitated by a 20,000g centrifugation.

TABLE 1

Mitochondrial enzymatic activities of 1511 mutant

	Experiments	Succinic dehydro-genase	Succinic oxidase	Cyto-chrome oxidase	L-lactic dehydro-genase	D-lactic dehydro-genase
I	Yeast grown at 23° Enzymes tested at 23°	2.78	1.41	1.83	2.0	1.3
II	Yeast grown at 23° Enzymes tested at 36°	2.64	1.29	1.85	1.95	1.3
III	Yeast grown at 36° Enzymes tested at 36°	0	0	0	0	0
IV	Yeast grown at 36° Enzymes tested at 23°	0	0	0	0	0
V	Yeast grown at 23° Mitochondria incu-bated at 36° for 30' before assay.	2.48	1.19	1.78	1.93	1.25

The activities are expressed as μmoles of substrate/min/mg protein. Correction has been made for the assays performed at different temperatures in order to compare directly the two sets of data.

The coenzyme Q is present only in yeast grown at the permissive temperature, as demonstrated by the absence in the differential spectrum of the ethanol-ether extract of the 1511 mutant grown at 36° of the peak corresponding to CoQ. The addition of the ethanol-ether extract from the mutant grown at 23° resulted in growth at nonpermissive temperature on YEDL-glyc medium (Fig. 2). The addition of commercial CoQ_6 resulted in growth of the mutant with the same division rate as the samples growing with the ethanol-ether extract.

The mutant grown at 36° on YEDL-glyc medium containing CoQ_6

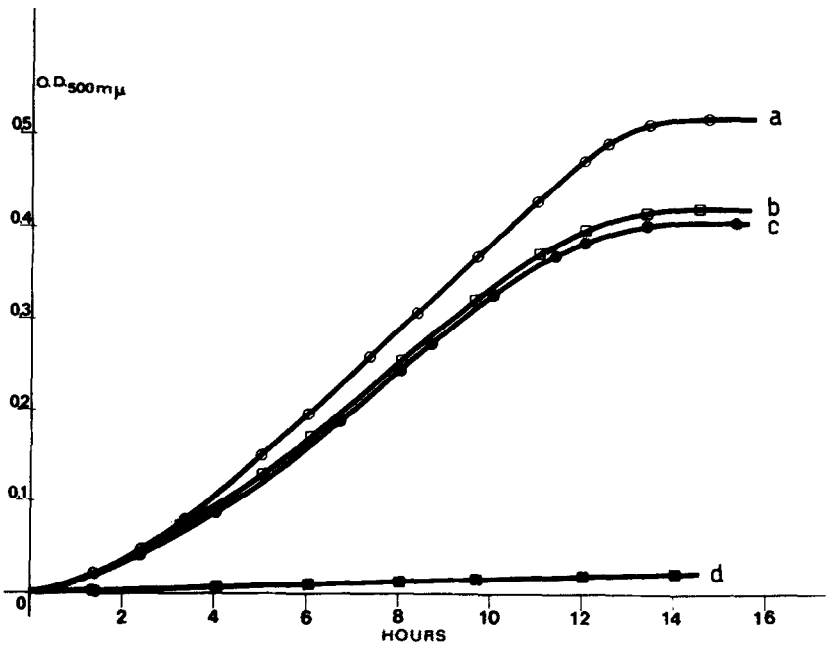

Fig. 2 - Growth of 1511 mutant at 36°. Samples of 10 ml each were grown on alternating shaker. A: growth in YEDL-gluc medium; after 14 hours the cell concentration was 4×10^7 cell/ml ; B: YEDL-glyc medium plus 20 mg/ml of ethanol-ether extract from the same mutant grown at 23°; C: 5 mg/ml of CoQ_6 were added to a YEDL-glyc medium; D: YEDL-glyc medium.

shows a spectrum superimposable on Fig. 1C; moreover all the enzymatic activities are present as in the mutant grown at permissive temperature.

The usefulness of mutants as a means of studying the fundamental mechanism of oxidative phosphorylation and electron transfer in the respiratory chain in yeast has proved to be a very important tool (1,2,4,10,14). The mutant described in this paper does not synthetize coenzyme Q_6 probably because its biosynthesis is blocked; the addition of either the commercial CoQ_6 or the coenzyme extracted from the same mutant grown at permissive temperature allows the strain to grow as P and the synthesis of all the components of the respiratory chain occurs.

It is interesting to note that the addition of coenzyme Q can catalyze the synthesis of the chain - as oxygen does during aerobiotic adaptation of anaerobiotic yeast. Worth noting also is the fact that the D-lactic ferricyanide activity is missing in the mutant. Therefore the Nygaard hypothesis (9) that this enzyme is a precursor of the other two lactic dehydrogenases (D-Lactic cyt. c reductase and the cytochrome b_2), both of them directly connected with the respiratory chain, finds further support in our data.

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