A TEMPERATURE SENSITIVE MITOCHONDRIAL MUTATION OF SACCHAROMYCES CEREVISIAE

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Received March 31, 1975

SUMMARY: A mitochondrially inherited temperature sensitive respiratory deficient mutant of yeast has been isolated. Detection of nuclear suppressor mutations indicates an interaction between the nuclear and mitochondrial genomes. A preliminary biochemical characterization is presented.

Mitochondrially synthesized components of the rutamycin sensitive ATPase and of the respiratory chain are essential for mitochondrial function; however, the cellular location of genes specifying these polypeptides is uncertain. While a few genes, e.g., those for mitochondrial rRNA and tRNA species (1), are known to be associated with mitochondrial DNA, the yeast mitochondrial genome with a molecular weight of 50×10^6 (1), could code for a number of additional products. Mitochondrially located genes coding for novel functions could be inferred by the analysis of strains carrying mitochondrial mutations resulting in temperature sensitive respiratory deficiency. A previously reported mutation of this class (2) is further described in this paper.

MATERIALS AND METHODS

Strain 2-38 (5-6) C (a/a leu1/leu1 ura1/ura1 + /ade2 trp5/ + /mal 1 + /suc 1 + /ser ADE 15/ + [OLI^{S}1]), obtained from E. Jones, was mutagenized with N-methyl- N^1-nitro-N-nitrosoguanidine (5mg/ml) for 5 min. at 23°C. The cells were washed free of the mutagen and plated at 23° on enriched medium (1% yeast extract plus 2% peptone) containing 3% glycerol and 1% ethanol (YPGE) and oligomycin (5μg/ml). Single colony isolates of antibiotic resistant clones were replica plated onto YPGE medium and incubated at permissive (23°) and non-permissive (36°) temperatures. Clones temperature sensitive on YPGE were mated with HK15 (lys1/lys1) obtained from G. Fink, and the resulting tetraploids were screened for somatic segregation of the temperature sensitivity.

YPGE = medium containing 1% yeast extract, 2% peptone, 3% glycerol, 1% ethanol and 2% agar.

Sporulation of tetraploid cells which exhibited somatic segregation gave rise to diploid clones. A temperature sensitive α diploid clone was finally sporulated to yield temperature sensitive haploid clones of which aa53-2d-a is representative.

Spontaneous revertants of the temperature sensitive respiratory deficient mutant were obtained by selecting clones of haploid derivatives of aa53 which grew after cells were incubated on YPGE plates at 36° for 5 days.

Cytoplasmic inheritance was determined by standard procedures of somatic segregation and tetrad analysis (3) in crosses with D6 (α arg met), a respiratory sufficient strain obtained from D. Wilkie. Respiratory deficient derivatives were obtained after growth in ethidium bromide (10μg/ml) at 23° for 18 hrs (4). The resulting respiratory deficient strains, aa53 2d-a EB and D6EB, lack detectable mitochondrial DNA as determined by analytical cesium chloride density gradient centrifugation.

Enzyme activities were assayed in lysates or crude mitochondrial fractions prepared from cells grown to stationary phase in broth containing 1% yeast extract, 2% peptone, and 1% galactose. Cells were washed and resuspended in buffer containing 0.25M sucrose, 10 mM N-2 hydroxyethylpiperazine-N^1-2 ethanesulfonic acid, 1 mM EDTA, pH 7.3 then shaken for 35 sec. in a Braun homogenizer in the presence of glass beads (0.45-0.50 mm). Activities were determined in the supernatant fraction present after 10 min. centrifugation at 1085 x g. Alternatively a crude mitochondrial pellet was obtained after an additional 10 min.centrifugation at 1085 x g followed by a 20 min.centrifugation at 12,100 x g. Standard spectrophotometric methods were used to assay NADH: cytochrome c reductase (EC 1.6.2.1) (5), succinate: cytochrome c reductase (5), cytochrome c oxidase (EC 1.9.3.1) (6) rutamycin sensitive ATPase (EC 3.6.1.3) (7) activities and for protein determination (8).

RESULTS

Growth curves of the temperature sensitive respiratory deficient haploid strain in enriched medium, containing either glucose or ethanol, at 23° and at 36° are presented in Fig. 1. Growth in ethanol is observed at 23° but not at 36°; however, glucose can be used as a carbon source at either temperature. Thus the strain carries a mutation which confers a temperature sensitive respiratory deficient phenotype. In addition, after 72 hours of growth at 36° on glucose medium, the strain retained its ability to grow on YPGE plates at room temperature.

The pattern of inheritance of the mutation was determined as follows: A heterogeneous population of mutant and wild type diploids was obtained from crosses of the temperature sensitive mutant with a respiratory competent tester strain (Table 1). Sporulation of these diploids resulted in tetrads in which 0:4 and 4:0 segregation of the mutant phenotype was observed (Table 1). In addition, sporulation of diploids having the

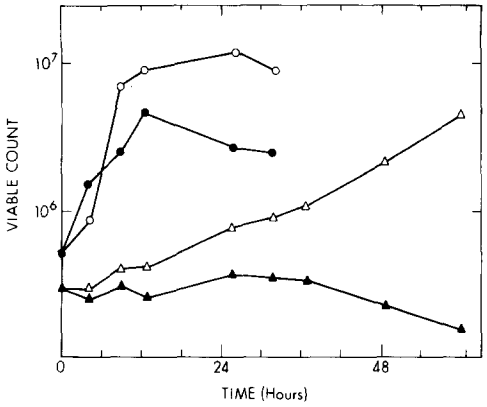

Figure 1. Growth of the mutant strain aa53-2d-a as a function of carbon source at two different temperatures.

Overnight cultures grown at room temperature in 1% yeast extract 2% peptone broth containing either 2% glucose or 2% ethanol as a carbon source were used to innoculate cultures grown in these media at either room temperature or 36°. At various times, aliquots of cells were removed, diluted and plated on enriched medium containing 2% glucose. Plates were incubated at room temperature and colonies were counted three days after plating. O, growth at room temperature, 2% glucose; •, growth at 36°, 2% glucose; Δ, growth at room temperature, 2% ethanol; ▲, growth at 36°, 2% ethanol.

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mutant phenotype resulted in 0:4 meiotic segregation while only wild type tetrads were derived from diploids having a respiratory sufficient phenotype. Both of the above mitotic and meiotic segregation patterns are consistent with cytoplasmic inheritance.

When the ρ^0 derivative aa53-2d-aEB was crossed with the tester strain, only wild type diploids and wild type tetrads were observed (Table 1). In contrast, when aa53-2d-a was crossed with D6EB, a respiratory deficient ρ^0 tester strain, only mutant diploids and mutant tetrads were observed (Table 1). Thus the cytoplasmic mutation appears to be associated with mitochondrial DNA.

The mutant strain was found to revert with a frequency of 10^{-8}. Two classes of

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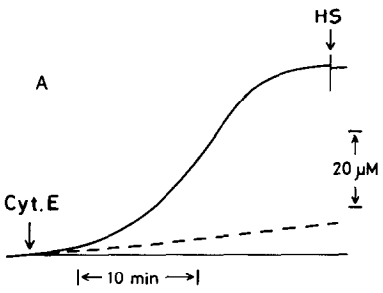
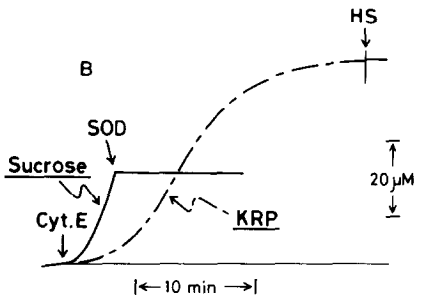
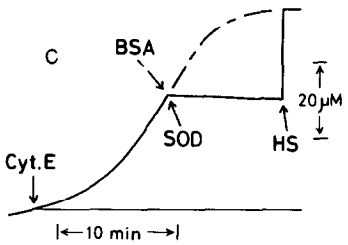
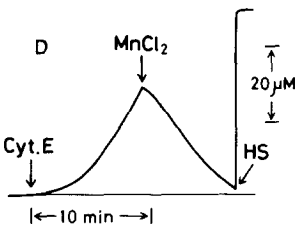
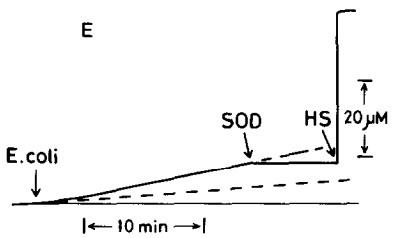
ρ^0 = respiratory deficient yeast strain lacking detectable mitochondrial DNA

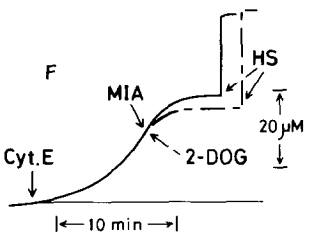

A
HS
20 μM
Cyt.E
|← 10 min →|

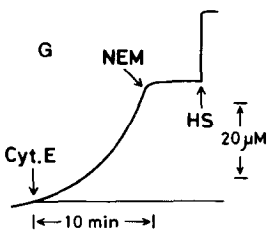

B
HS
SOD
Sucrose
20 μM
Cyt.E
KRP
|← 10 min →|


C
BSA
20 μM
SOD
HS
Cyt.E
|← 10 min →|


D
MnCl2
20 μM
Cyt.E
HS
|← 10 min →|


E
SOD
HS
20 μM
E.coli
|← 10 min →|


F
HS
MIA
20 μM
Cyt.E
2-DOG
|← 10 min →|


G
NEM
HS
20 μM
Cyt.E
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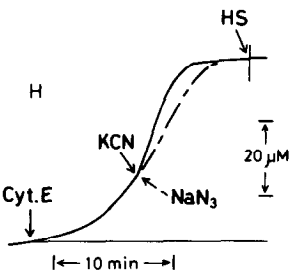
H
HS
KCN
20 μM
Cyt.E
NaN3
|← 10 min →|

spontaneous revertants have been isolated. Members of one class exhibit dominant Mendelian inheritance. This nuclear suppression of the temperature sensitive mitochondrial mutation is an interesting case of the interaction of nuclear and mitochondrial gene products. Members of the second class exhibit mitochondrial inheritance, probably due to reversion at the original mutant site.

Further analysis of the mutant may be summarized as follows: Mitochondrial protein synthesis and mitochondrial DNA synthesis were present at comparable levels in mutant and parent strains at both permissive and non-permissive temperatures. However, mitochondrial fractions derived from mutant cells grown at 36° were found to lack cytochrome c oxidase activity. In addition, these fractions exhibited a significant reduction in succinate:cytochrome c reductase and NADH:cytochrome c reductase levels (Table 2). In contrast, three to five-fold reductions in rutamycin sensitive ATPase activities were obtained from fractions isolated from either mutant or parent strain grown at 36°. Further details of studies of the mutation and revertants will appear elsewhere .

DISCUSSION

In studies of the function of the mitochondrial genome, the mutation described in this paper has the advantage of a stable, well-defined phenotype. In contrast, the common class of mitochondrial mutations, i.e., the petite mutation, exhibits loss or significant alteration of mitochondrial DNA accompanied by a lack of mitochondrial protein synthesis, absence of cytochromes $a+a_3$, b, c_1 and respiratory competence (1,9). Thus, it would appear that the alteration or loss of many loci could produce the petite phenotype. More limited alterations of mitochondrial DNA might be expected in the recently discovered mitochondrially inherited respiratory deficient strains which retain mitochondrial protein synthesis (10).

Another class of mitochondrially inherited mutation results in resistance to antibiotics which affect mitochondrial function. The use of such strains is a potentially promising

Table 2. Activities of cytochrome c oxidase, succinate: cytochrome c reductase, NADH: cytochrome c reductase in crude mitochondrial fractions obtained from mutant (ts) and parent strains (+) grown at two temperatures. Fractions were assayed at 24°C.

Strain	Growth temperature (°C)	Cytochrome c oxidase	Succinate: cytochrome c reductase	NADH: cytochrome c reductase
		(μM cytochrome c oxidized min^{-1} mg^{-1} protein)	(μM cytochrome c reduced min^{-1} mg^{-1} protein)	
aa53 (ts)	RT	0.354	0.152	0.610
aa53 (ts)	36	0.000	0.0024	0.368
2-38(5-6)C (+)	RT	0.412	0.180	0.810
2-38(5-6)C (+)	36	0.394	0.560	1.812

RT: room temperature, 23-25°C.

probe for the study of the function of mitochondrial genes, however, the nature of the relationship between resistance and a mitochondrial translation product remains to be demonstrated (9).

While a preliminary report of a mitochondrially inherited temperature sensitive mutation has been published by others (11), this paper presents a more detailed characterization of such a mutant. This temperature sensitive, respiratory deficient mutation should be useful in the formulation of a clearcut definition of a mitochondrial gene-protein relationship. Although it affects more than one mitochondrial enzyme, the mutation is revertable, and presumably represents a point mutation in a single gene. In addition, the study of the interaction of mitochondrial mutants and their suppressors should provide some interesting insights into the problem of organelle biogenesis and the interaction of nuclear and mitochondrially coded gene products. Mutations in other mitochondrial genes leading to temperature sensitive, respiratory deficiency will make it possible to construct a genetic and functional map of the mitochondrially located genes in yeast.

We thank William Perry and Jeffrey Chinsky for their excellent technical assistance, and Dr. Keng-Bon Lam for helpful discussion.

This research was supported by grants CA 12410 and GM 19100 from NIH. Elizabeth M. Storm was supported by an NIH Training Grant to the Department of Genetics, GM 00110.

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