A NUCLEAR MUTANT OF *S. CEREVISIAE* NON-TOLERATING THE CYTOPLASMIC PETITE MUTATION

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1. Introduction

Many nuclear mutants affecting specific mitochondrial functions in yeast have been identified. There are two such mutants in yeast *S. cerevisiae* of particular interest. These are the op_1 mutant [1,2] with affected adenine nucleotide translocase [3] and the *pet 936* mutant lacking mitochondrial adenosine triphosphatase [4]. The inability or drastic inhibition of cell growth in complex media after the superimposition of the cytoplasmic petite mutation over the nuclear one is the common but unexpected property of these mutants [5,4]. These findings with our previous suggestion [6] independently indicate that mitochondria in growing eukaryotic cells fulfills some vital function even in nonrespiring cells. Accordingly, the systematic use of similar but genetically different mutants of *S. cerevisiae* in the study of this mitochondrial function seems to be very promising.

In this report we describe properties of a new nuclear mutant of *S. cerevisiae* with repressed cytochrome *a* which does not tolerate the cytoplasmic petite mutation.

2. Experimental

The mutant used, designated *Saccharomyces cerevisiae* $Z1\times1$ (ad_1ly_2/ad_1tr_1), is an adenine-requiring diploid strain, homozygous for the affected nuclear gene, prepared from wild-type strain D 225-5A by ultraviolet light mutagenesis by Lachowicz et al. [7]. For comparison, diploid wild-type strain *S. cerevisiae* DT XII was employed.

Cells were grown at 30°C in a semi-synthetic medium [8] with 0.5–2% glucose (or galactose, glycerol, ethanol where indicated) as a carbon source, 1% peptone, 1% yeast extract and 0.004% adenine. A published method was used for growing cells in the absence of oxygen [9]. Growth was followed by counting cells in a haematocytometer chamber. Fermentation and respiration were determined by the conventional manometric technique or polarographically. Cytochrome spectra were measured in a SF-14 spectrophotometer.

3. Results

Mutant $Z1\times1$ was able to grow aerobically on fermentable (glucose, galactose) and nonfermentable (glycerol, ethanol) carbon sources both in the liquid and on the solid media. The growth yields of the mutant cells on these substrates (fig. 1) were substantially unaltered with respect to those of wild-type yeast [6,8]. On the other hand, the growth rate of $Z1\times1$ mutant was significantly lower than that of wild-type cells. The mean generation times of the mutant and wild-type cells growing on different substrates were 3.5 and 1.45 hr for glucose (or galactose), 7.2 and 2.6 hr for glycerol, 8.0 and 2.3 hr for ethanol, respectively.

The growth curve of mutant $Z1\times1$ growing on glu-

* Abbreviations: CCCP – carbonylcyanide *m*-chlorophenylhydrazone; DCCD – *N,N'*-dicyclohexylcarbodiimide; DNP – 2,4-dinitrophenol.

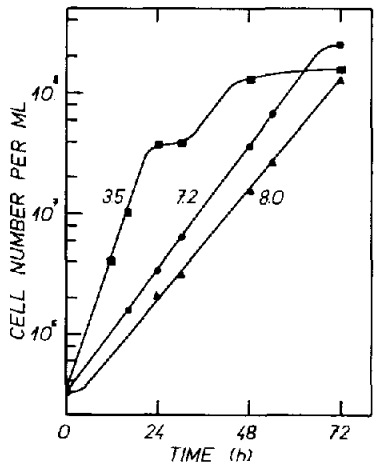

Fig. 1. The generation times of mutant *Z1×1*. *Z1×1* cells were grown aerobically in semi-synthetic medium with 2% glucose for 24 hr. Cells were then inoculated at a density 3 × 10^5 cells/ml into a semi-synthetic medium with 0.5% glucose ■; 2% glycerol ● or 2% ethanol ▲. The numbers at lines indicate the mean generation time.

cose as substrate displays a diauxic course (fig. 1). At higher glucose concentrations the second lag period was much longer. The mutant cells harvested at the beginning of the second lag period were supercatabolically repressed. Amounts of cytochromes *b* and *c* were reduced and cytochrome *a* was barely detectable both in the absolute and difference spectra (fig. 2). The content of cytochrome *a* was greatly reduced even after growth of mutant cells on glycerol or ethanol as carbon sources (fig. 2).

Some metabolic activities of the mutant *Z1×1* after growth under different conditions are summarized in table 1. The rate of glucose fermentation in the mutant cells was quite comparable to that of normal yeast. However, differences between the rates of glucose fermentation in nitrogen $\left(Q^{N_2}_{CO_2}\right)$ and that in air $\left(Q^{air}_{CO_2} \quad Q^{air}_{O_2}\right)$ of the mutant cells were substantially lower than that of wild-type yeast. The respiratory activity of mutant cells was dependent on

Table 1
Metabolic quotients of mutant *Z1×1* grown on fermentable and nonfermentable substrates

Strain and culture conditions	Substrate and inhibitor	$Q^{air}_{O_2}$	$Q^{air}_{CO_2}$	$Q^{N_2}_{CO_2}$
		(µl/h/mg dry wt)		
Wild-type yeast glucose grown for 24 hr	50 mM glucose	131	312	338
Z1×1 glucose grown for 24 hr	50 mM glucose	45.5	345	355
	+ 10 µM CCCP	50.0		
	+ 50 µM DNP	47.0	345	355
	+ 325 µM DCCD	34.3		
	+ 10 µM bongkrekic acid	13.0		
	+ 2 µg/ml antimycin A	0		
	+ 1 mM KCN	0		
Z1×1 glucose grown for 72 hr	50 mM glucose	51.1	260	246
	+ 50 µM DNP	58.0	260	
Z1×1 ethanol grown for 72 hr	50 mM glucose	168.0	327	218
	+ 50 µM DNP	200.0	327	
Z1×1 glycerol grown for 72 hr	50 mM glucose	37.6	263	280
	+ 50 µM DNP	37.6	263	

Cells were grown aerobically as indicated in fig. 1. Metabolic quotients of washed cells were determined manometrically at 30°C in 80 mM citrate–phosphate buffer, pH 4.3, containing yeast cells (1.9–2.4 mg dry wt), substrate and inhibitor as indicated in the table. The respiratory activity of 24 hr glucose-grown mutant cells was determined polarographically at 30°C in 50 mM potassium glutarate, 10 mM potassium phosphate, 100 mM KCl, final pH 4.3.

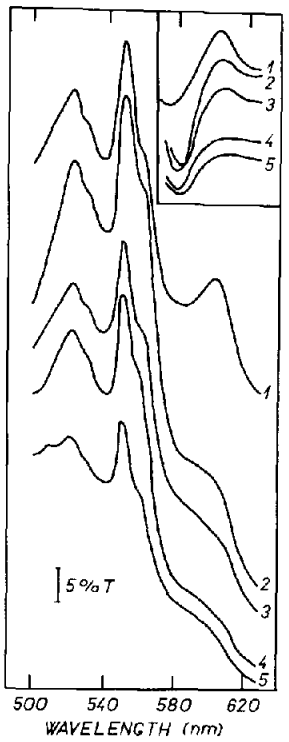

Fig. 2. Absorption spectra of mutant *Z1×1* grown on fermentable and nonfermentable substrates. Cells were grown aerobically as indicated in fig. 1. Insert represents difference spectra of cytochrome *a* (reduced by $Na_2S_2O_4$/oxidized by 50 mM H_2O_2). Cuvettes contained 2.5 ml of yeast suspension at room temperature: 1:24 hr glucose grown wild-type yeast (66 mg/ml); 2:72 hr ethanol grown mutant *Z1×1* (88 mg/ml); 3:72 hr glucose grown mutant *Z1×1* (78.3 mg/ml); 4:72 hr glycerol grown mutant *Z1×1* (98.8 mg/ml), 5:24 hr glucose grown mutant *Z1×1* (88.3 mg/ml).

the growth conditions, but, after growth on ethanol the rate of glucose oxidation was again comparable to that in wild-type yeast. In the mutant cells grown on glucose the oxidation of glucose was specifically inhibited by DCCD and bongkrekic acid. Complete inhibition of respiration was observed by antimycin A and cyanide. Contrary to wild-type yeast, the respiration of mutant cells was only slightly stimulated by uncoupling agents such as DNP and CCCP.

Table 2 summarizes the effect of agents interfering with functions and biogenesis of mitochondria on growth and multiplication ability of mutant

Table 2
Effect of drugs interfering with functions and biogenesis of mitochondria on growth and multiplication ability of mutant *Z1×1* cells

Inhibitor	Aerobic growth yields in the stationary phase (10^6 cells/ml)	Multiplication ability ('% of survival')
–	152.0	100
2 μg/ml Antimycin A	40.0	100
40 μg/ml Oligomycin	44.8	100
22 μM Bongkrekic acid	30.0	100
4 mg/ml Chloramphenicol	40.4	48
4 mg/ml Erythromycin	26.4	55
25 μg/ml Acriflavine	42.3	0
25 μM Ethidium bromide	18.4	0
2 μg/ml Antimycin A + 4.4 μM Bongkrekic acid	4.4	1

Cells were grown aerobically as indicated in fig. 1 in semi-synthetic media with 0.5% glucose as a carbon source and inhibitor. Growth yields of cells grown anaerobically in the absence of inhibitor was 3.2×10^7 cells/ml. Multiplication ability of *Z1×1* cells grown for 24 hr in the presence of inhibitor was tested by plating the cells after washing and proper dilution on solid semi-synthetic media with 2% glucose as substrate. '% of survival' indicates the percentage of colonies formed in relation to the total number of cells determined before dilution by counting in haemocytometer.

Z1×1 cells. All the inhibitors tested reduced the aerobic growth yields of cells growing on 0.5% glucose as a carbon source to the level of anaerobic one. However, contrary to wild-type yeast, the mutant cells have lost their multiplication ability after growth in the presence of acriflavine, ethidium bromide as well as antimycin A plus bongkrekic acid. The same was observed also after interaction of *Z1×1* cells with ethidium bromide under the non-growing conditions (24 hr, 50 mM phthalate buffer, 1% glucose, 25 μM ethidium bromide, pH 5.0). The multiplication ability of mutant cells was significantly reduced also after the growth of mutant *Z1×1* in the presence of chloramphenicol or erythromycin as well as after growth in the presence of bongkrekic acid (22 μM) and higher concentration of glucose (2%) ('% of survival' = 32). On the other hand, the loss of multiplication ability was not observed in the culture of the mutant cells growing under strictly anaerobic conditions (0.5–2% glucose) or growing in the presence (a) 2% glucose and 2 μg/ml antimycin A; (b) 2% glucose and 40 μg/ml oligomycin; (c) high glucose concentration (6%).

4. Discussion

Z1×1 is a single nuclear gene mutant of *S. cerevisiae* with repressed cytochrome *a*, non-tolerant of the cytoplasmic petite mutation. This mutant was able to grow with apparently normal growth yields both on fermentable and nonfermentable carbon sources. The oxidation of glucose in mutant cells was under control of phosphorylation reactions and its rate was limited probably by cytochrome *a*. The sufficient capacities of glycolysis or respiration of mutant *Z1×1*, however, do not explain the increased generation time of mutant cells growing on fermentable or nonfermentable substrates. It may be possible that growth of mutant cells under tested conditions is limited by some anabolic reaction. The unbalanced breakdown of the utilized carbon source may result in accumulation of catabolites with subsequent increased repression of mitochondrial enzymes synthesis.

The loss of multiplication ability in complex medium after the superimposition of the cytoplasmic petite mutation over the nuclear *Z1×1* mutation is the most striking feature of *Z1×1* cells. Properties of this mutant, however, are not identical with those of op_1 mutant [1,5] or *pet 936* mutant [4]. The genetic analysis [7] also revealed that the *Z1×1* mutation is nonallelic with both op_1 mutation and respiratory deficient mutations belonging to the following classes: p_1, p_2, p_3, p_4, p_5, p_6, p_7, p_{10} (see [10]). The growth of mutant *Z1×1* was not dependent either on respiration (growth in the presence of antimycin A) or on the presence of some respiratory chain components (growth under anaerobic conditions). On the other hand, the growth of this mutant was absolutely dependent on the proper functioning of the mitochondrial genetic and protein synthesizing systems. In these respects *Z1×1* is more similar to op_1 mutant than to mutant *pet 936*.

At present there is no adequate explanation for the unusual properties of *Z1×1* mutant. Some indications (increased generation time, diminished Pasteur effect, particular sensitivity of cytochrome *a* synthesis to catabolite repression, loss of multiplication ability under specific conditions) would be in favour of assumption that the *Z1×1* is a mutant with a slightly affected mitochondrial adenine nucleotide translocation system. The superimposition of the cytoplasmic petite mutation over the nuclear *Z1×1* one may then results in the profound modification of the properties of this system so that its operation from the point of view of the vital function of mitochondria [6] become insufficient. Studies to confirm or disprove this hypothesis are currently underway.

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