GLUCOSE SUPERREPRESSED AND DEREPRESSED RESPIRATORY MUTANTS IN A "PETITE-NEGATIVE" YEAST : SCHIZOSACCHAROMYCES POMBE 972h⁻

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SUMMARY

Two mutants modified in their sensitivity to glucose repression have been obtained from a "petite-negative" yeast species : Schizosaccharomyces pombe 972h⁻. Compared to glycerol grown cells, the respiration of cells grown in the presence of 10% glucose is decreased by a factor of 3 for the wild strain and by a factor of 24 for strain COB5 which is therefore designated as a superrepressed mutant. Respiration of a derepressed mutant COB6 is totally insensitive to glucose repression. The effects of glucose on the absorption peaks of respiratory pigments parallel the effects of glucose on respiration. Using a derepressing medium that limits cellular division, full restoration of respiration and respiratory pigments of the superrepressed strain was achieved.

INTRODUCTION

Glucose repression of the synthesis of catabolic enzymes has been known for many years in bacteria and yeasts (1, 2). Many yeast species grown on high concentrations of glucose show decreased respiration rates and low levels of mitochondrial enzymes and cytochromes. Whereas many detailed descriptions of enzymatic deficiencies resulting from glucose repression have been made, very little is known concerning the molecular mechanisms that regulate the synthesis of glucose-repressible enzymes in yeasts.

Although some yeast species or strains have been reported to be insensitive to glucose repression (3 to 6) no yeast mutants specifically affected in glucose repression of mitochondrial functions and development have been described previously. Such mutants should prove to be very useful for genetic and biochemical studies of the molecular mechanism of glucose repression.

In this report, we describe the properties of two mutants of a "petite-negative" yeast species, Schizosaccharomyces pombe 972h⁻, affected in their responses to glucose. Respiration of the mutant COB5 is subject to more severe repression than the wild strain and may be specifically derepressed in the absence of cellular division. On the other hand,

respiration of COB6 is not repressed by high glucose concentrations.

MATERIAL AND METHODS

Strains. S. pombe 972h$^-$ was used as parental strain. Isolation of mutants COB5 and COB6 on glucose medium containing 2 mM cobalt sulphate was described previously (7).

Growth conditions. Cells were grown at 30°C, either on a glucose medium containing : 5.8% (w/v) glucose, 2% (w/v) yeast extract (DIFCO) or on a glycerol medium containing : 3.6% (w/v) glycerol, 0.1% (w/v) glucose, 2% (w/v) yeast extract. Both media were brought to pH 4.5 with HCl. Cells were harvested in exponential phase of growth (about 60 x 10^6 cells/ml).

Respiratory derepression. Glucose repressed cells were transferred at a final dilution of 60 x 10^6 cells/ml into a derepressing medium containing: 3% (w/v) ethanol, 0.1% (w/v) glucose, 2% (w/v) yeast extract, pH 4.5. Derepression was carried out for 15 hours at 30°C with efficient aeration.

Respiration. Oxygen uptake of whole cells was measured at 30°C with a Clark electrode in a 3 ml closed chamber containing : 0.3% (w/v) glucose, 10 mM phthalate-NaOH, pH 4.5 and a total of about 10^8 cells. Respiration was expressed as $\mu l\ O_2 \times hr^{-1} \times (10^8\ cells)^{-1}$.

Low temperature spectra. Absolute spectra of a suspension of about 3.5 x 10^9 cells/ml reduced by dithionite were carried out at liquid nitrogen temperature with an Acta V Beckman spectrophotometer fitted with cells of 2 mm optical pathway.

RESULTS

Table 1 compares the respiratory rates of exponential phase cells grown either on 5.8% glucose or 3.6% glycerol. The three strains exhibit approximately the same high level of respiration when grown on glycerol. Compared to this latter rate, the respiration of cells grown on glucose is decreased by a factor of three in the wild strain and by a factor of eight in COB5. Moreover in glucose-repressed cells, the oxygen uptake is antimycin A sensitive in the wild strain but partly antimycin A insensitive in COB5. On the other hand, glucose-grown COB6 exibits high respiration rates, similar to those of the glycerol-grown cells. These effects on respiration are reflected in the cytochrome content qualitatively measured by low temperature spectra of cell suspensions. Figure 1A shows that the spectra of the three strains are similar in the glycerol-grown cells. Figure 1B shows that in S. pombe 972h$^-$ grown on 5.8% glucose, mitochondrial cytochrome absorption peaks are lowered but still detectable. In glucose grown COB5, the decrease

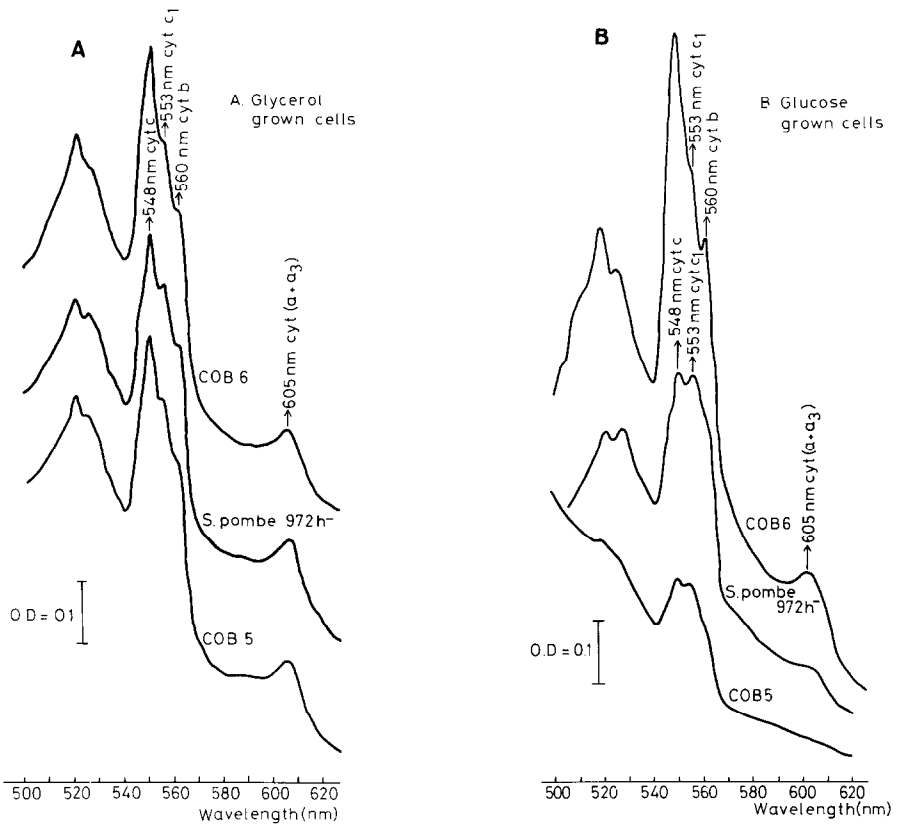

Figure 1. Absolute low temperature spectra of S. pombe 972h⁻, COB5 and COB6 grown on glycerol or glucose.
All cells were harvested during exponential growth.

of the mitochondrial cytochrome absorption peaks is accentuated. However the 553 nm peak which might be partly due to cytochrome c_1 and to the α_2 band of cytochrome b_1 is much less affected than the other cytochromes.

In glucose grown COB6, cytochrome $a+a_3$, b and c_1 absorption peaks are as pronounced as in the glycerol grown cells for the cytochrome c peak is even more pronounced.

Figure 2 shows that S. pombe 972h⁻, COB5 and COB6 vary not only in the extent of repression produced by a given concentration of glucose, but also in their responses to increasing glucose concentrations. The cells were grown for 5 or 6 generations on media containing initially 2%, 5.8% or 10% glucose. Both the cell respiratory rates and the residual glucose concentrations were measured. Increasing initial glucose concentrations from 2% to 10% do not change the respiration rates of wild type cells indicating that final concen-

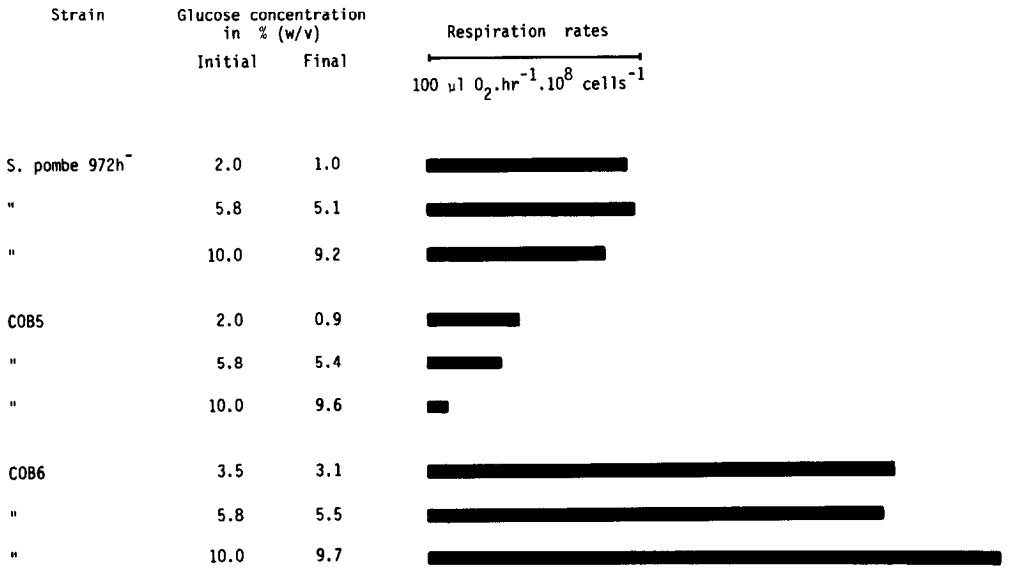

Figure 2. Effects of glucose concentrations on respiratory rates of S. pombe 972h⁻, COB5 and COB6. Cells were collected at 25 to 30 x 10^6 cells/ml for COB5, 65 x 10^6 cells/ml for S. pombe 972h⁻ and COB6. The glucose concentration of the culture medium was measured by the colorimetric, enzymatic method of Raabo and Terkildsen (8).

trations of glucose lower than 1% are already fully repressive. However, similar increases in glucose concentrations decrease markedly the respiration of COB5 and slightly increase respiration of COB6. Thus, for cells grown on 10% glucose, the differences between the three strains are most accentuated: the respiration of COB6 is 27 times higher than that of COB5.

The generation time of the wild strain is 2 hr for all glucose concentrations. Low respiration of COB5 results in slow growth; the generation time of COB5 increases from 2.5 hr in 2% glucose to 3.8 hr in 10% glucose. In COB6, the cell yield per mole of glucose is high due to high respiration/fermentation ratio; however growth is slow (generation time 3.5 hr) due to slow glucose utilization.

In order to use the COB5 mutant to study the biochemical bases of glucose repression it was of interest to determine conditions where the respiration of this strain can be specifically and fully derepressed in the absence of cellular division.

Derepression of glucose-repressed COB5 cells was achieved by aeration for 15 hours at 30°C in a medium containing : 3% ethanol, 2% yeast extract

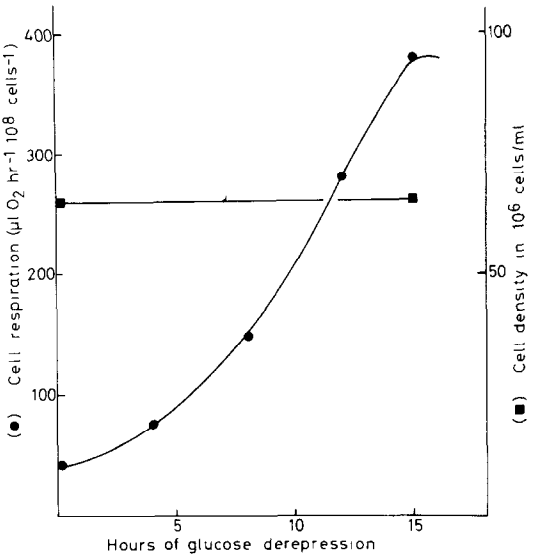

Figure 3. Time curve of respiratory derepression of COB5. Cells were collected at 60 x 10^6 cells/ml and transferred into the derepressing medium.

and 0.1% glucose. Since S. pombe oxidizes ethanol but is unable to utilize this substrate as carbon source for growth (9) no cellular division is obtained under these conditions (fig. 3). Derepression is inhibited by cycloheximide and chloramphenicol and is thus the result of de novo protein synthesis (data not shown).

In figure 4, low temperature spectra of derepressed cells show induction of the mitochondrial cytochromes. All cytochrome peaks increase with the exception of the 553 nm peak.

Table 2 shows that full respiratory derepression of COB5 is achieved only by combined addition of ethanol, yeast extract and low glucose concentrations. That the derepression requires decrease in the glucose concentration is shown by the total absence of respiratory derepression when 5.8% glucose is added to the derepressing medium.

DISCUSSION

Yeast may be divided into those species or strains which are not subject to glucose repression of respiration and those strains which are repressed by glucose (3 to 6). Reilly and Sherman have reported that cytochrome $a+a_3$ is extremely sensitive to glucose repression in several cytochrome c and b-deficient yeast mutants of Saccharomyces cerevisiae (10).

Table 1. Respiration rates of S. pombe 972h⁻, COB5 and COB6 grown in glucose and glycerol

Strains	Respiration[a] of cells grown on 5.8% glucose[b]	3.6% glycerol[c]	Glucose repression factor[d]
S. pombe 972h⁻	84	250	3.0
COB5	30	245	8.2
COB6	240	232	1.0

a. Expressed in $\mu l\ O_2 \times hr^{-1} \times 10^8\ cells^{-1}$ (Antimycin A sensitive)
b. Exponential phase cells harvested for S. pombe 972h⁻ at 100.10^6 cells/ml, for COB5 at 65.10^6 cells/ml, for COB6 at 90.10^6 cells/ml.
c. Exponential phase cells harvested at 65.10^6 cells/ml
d. Ratio of respiration of cells grown on glycerol to respiration of cells grown on glucose.

Table 2. Effects of ethanol, glucose and yeast extract on respiratory glucose derepression of COB5.

Conditions	Respiration(Antimycin A sensitive) in $\mu l\ O_2.hr^{-1}.10^8\ cells^{-1}$
Glycerol grown cells[a]	250
Repressed glucose grown cells[a]	32 ± 10[c]
Derepression in complete medium[b]	332 ± 70
Derepression medium minus glucose	152
Derepression medium minus yeast extract	230
Derepression medium minus ethanol[d]	276
Derepression medium plus 5.8% glucose	37

a. Exponential phase cells harvested at 65 x 10^6 cells/ml.
b. Derepression for 15 hr in 0.1% glucose, 3% ethanol, 2% yeast extract, pH 4.5.
c. Standard deviation from twelve experiments.
d. Average stimulation of derepression by 3% ethanol is 34% (three experiments).

However, no respiratory-deficient yeast mutants obtained from a single strain and showing specific modifications of mitochondrial response to glucose environment have been reported previously. Our results clearly show that such mutants can be obtained from S. pombe 972h⁻. Compared to the wild strain, respiration and respiratory pigments are much more repressed by glucose in COB5 than in COB6 which is totally unrepressed by concentrations

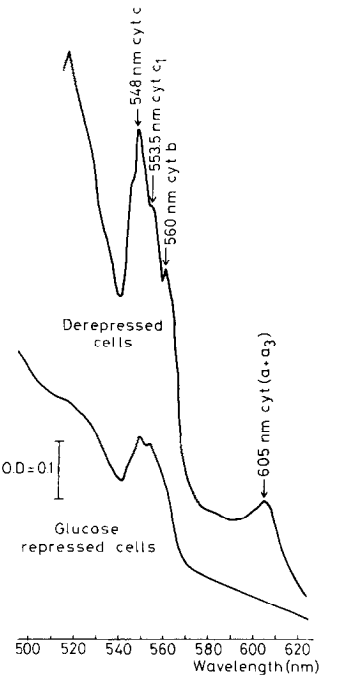

Figure 4. Absolute low temperature spectra of COB5 before and after derepression.
Cells were collected at 60 x 10^6 cells/ml and transferred into the derepressing medium for 15 hrs.

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of glucose up to 10%. Moreover, respiratory derepression of COB5 can be specifically obtained in the absence of cellular division.

De Deken reported a close correlation between the presence of glucose repression and the ability of a strain to produce cytoplasmic respiratory-deficient mutants by acriflavin treatment (4). This correlation holds for Saccharomyces fragilis (5). However our present data clearly show that this is not the case for S. pombe 972h⁻. It has previously been reported that S. pombe is a "petite-negative" yeast because no viable cytoplasmic respiratory-deficient mutants can be induced, even after extensive acriflavin or ethidium bromide treatments (7, 11). Nevertheless, respiration of S. pombe 972h⁻ is repressed by glucose and both a superrepressed mutant (COB5) and a derepressed mutant (COB6) have been isolated.

Further work is currently underway to determine whether the COB5 and COB6 mutations are affecting regulatory nuclear or mitochondrial genes controlling the synthesis of mitochondrial proteins or whether the mutations are located on structural genes coding for enzymes responsible for the rate

of glucose catabolism relative to cell growth. In any case, these mutants seem to offer convenient tools to investigate the role of mitochondrial DNA during glucose repression and derepression. They might be used, for instance, in conjunction with inhibitors of mitochondrial DNA replication and transcription such as ethidium bromide or acriflavine which are not mutagenic in S. pombe.

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