MITOCHONDRIAL DNA AND GLUCOSE REPRESSION IN YEAST

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SUMMARY

The amount of mitochondrial DNA, oxygen uptake, cytochrome content and number of mitochondria were examined in Saccharomyces carlsbergensis subjected to various degrees of glucose repression in chemostate cultures. The proportion of mitochondrial DNA varied from 11.5% of nuclear DNA in nonrepressed to 3.3% in repressed cells. Even at 0.2% glucose in the medium a decline in all parameters is observed. From calculations of the number of mitochondrial genomes per mitochondrion a constant number of 3 - 4 appears.

Our results contradict recent reports which claim the proportion of yeast mitochondrial DNA to be constant under all degrees of glucose repression.

INTRODUCTION

The biogenesis of mitochondria and their extent of genetic autonomy are problems very often approached with Saccharomyces as a eucaryotic type of cell. One fundamental question concerns variations in mitochondrial DNA (mitDNA) content relative to nuclear DNA (nDNA) and has recently become subject to dispute. In earlier studies diminished mitDNA was found as a consequence of glucose repression (1) (2) (3). However, Fukuhara finds a constant mitDNA:nDNA ratio under all growth conditions (4). Similar observations are reported by Williamson (3) and used by Hollenberg and Borst in conclusions concerning glucose inhibition of ethidium bromide induced formation of 'petite' mutants (5). The usual description of the conversion of mito-

chondria going from repressed to derepressed conditions shows repressed cells with few and large mitochondria with a relatively high density. Derepressed conditions lead to more and smaller mitochondria with lower density (6). It is difficult to maintain the idea of a constant mitDNA when considering these observations.

We have used a different experimental design by growing cultures of S. carlsbergensis in a chemostate with constant glucose concentration, keeping the cells in an exponential growth phase. The glucose repression was followed by determination of oxygen uptake, cytochromes, relative amount of mitDNA and number of mitochondria.

The number of DNA molecules per mitochondrion is calculated from the known genome size of mitDNA (7) and nDNA (8) and compared with the results of other methods, e.g. estimation of target numbers in UV-induced 'petite' mutations (9).

MATERIALS AND METHODS

A diploid strain of Saccharomyces carlsbergensis NCYC 74s was used throughout these studies. Cells were grown in a chemostate at 30°C in an aerated medium containing 1% Difco Yeast Extract, 1% Difco Bactopeptone, 3% sodium lactate in a 50 mM phosphate 'Sørensen' buffer at pH 6.25. Cells were harvested from media containing glucose between 0 and 20%. The flow rate was adjusted for the various glucose concentrations used. Glucose concentration in the outflowing medium was determined by the glucose oxidase method (Boehringer, Mannheim, GFR).

Total DNA was extracted by two different procedures (10) (11) and the DNA was examined in CsCl gradients in a Beckman model E analytical ultracentrifuge equipped with monochromator and photoelectric scanner. Double sector cells

were used with pure CsCl-solution in the reference sector. The two extraction procedures gave essentially identical results. Cytochrome spectra were taken as absolute spectra on a Shimadzu MPS-50 spectrophotometer with mechanical attenuation of the reference light path. Cell pastes in 3 mm cuvettes were reduced with dithionite and the relative contents of cytochrome c calculated according to Claisse et al. (12). Total contents of cytochromes were expressed as the area of the absorption spectra over the tangent drawn from 500 to 610 nm. The areas were measured with a planimeter.

Oxygen uptake was measured polarographically with ethanol as a substrate and without correction for cyanide insensitive respiration. The number of particles stained blue with Janus Green B were considered as mitochondria (average of counts on 30 cells). Cellular protein was determined by a biuret method after treatment of the cells for 5 min. at 100°C with 1 N sodium hydroxide.

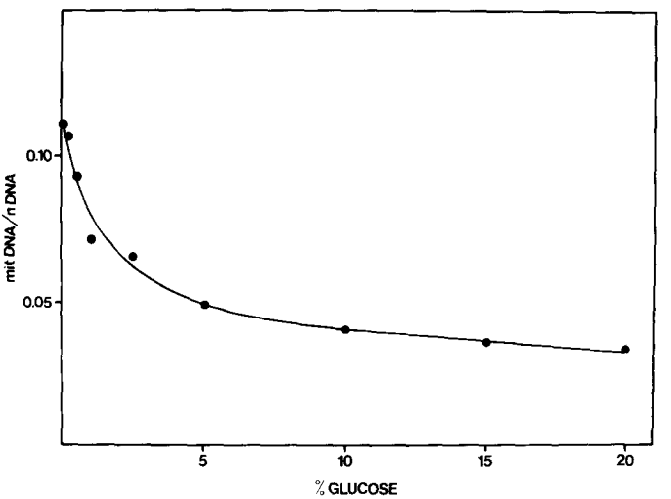

Fig. 1. Effect of increasing glucose concentration on the relative proportion of mitDNA to nDNA. The values represent means of results from two different extraction procedures.

RESULTS AND DISCUSSION

As shown in fig. 1 there is a decline in the relative proportion of mitDNA with increasing glucose concentration. From initial values of about 11.5% mitDNA there is a gradual decline to the lowest value of 3.3% mitDNA at 20% glucose, half of the decrease of mitDNA having occurred at 1% glucose.

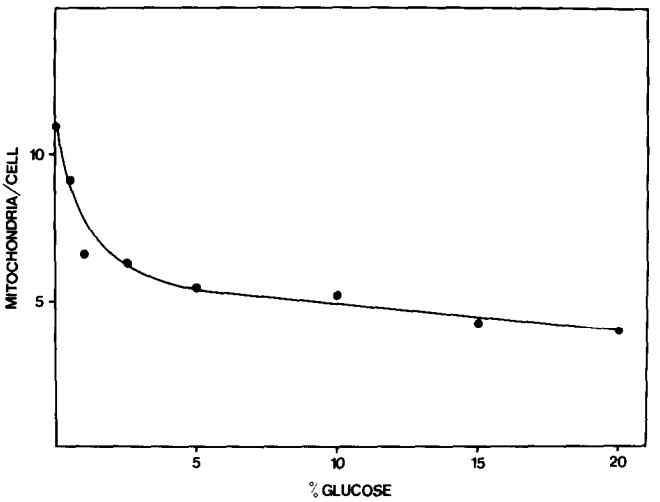

Fig. 2. Effect of increasing glucose concentration on the number of mitochondria per cell (determined by counting Janus Green B stained particles).

A maximum of 11.5% mitDNA is comparable to values obtained by others from nonrepressed yeast in logarithmic growth phase. In stationary phase the yeast cell may contain about 20% mitDNA (3).

The genome in yeast mitochondria has recently been shown to consist of circular DNA with a contour length of 25 μ (7). The nuclear genome is 9.4×10^9 daltons (8). The proportion of mitDNA in our diploid strain corresponds to about 43 mitochondrial genomes in nonrepressed cells, 12 mitochondrial genomes in the state of maximal repression.

The number of mitochondria per cell varied from 11 to 4 (fig. 2). It is seen that the ratio between the number of

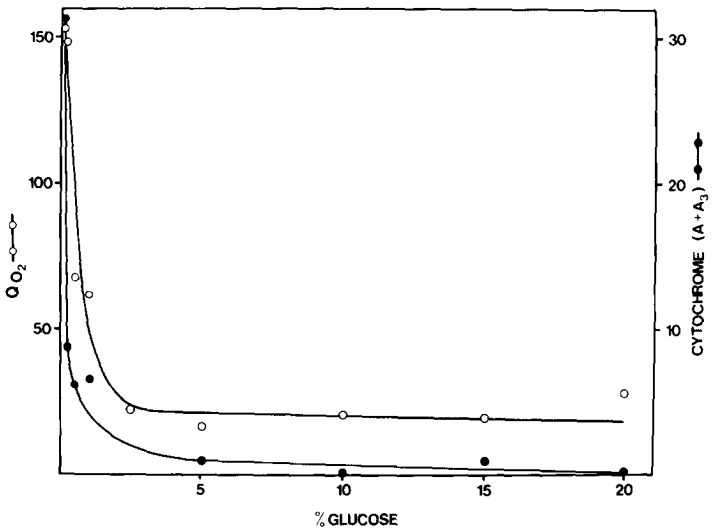

Fig. 3. Effect of increasing glucose concentration on oxygen uptake (Q_{O_2} = $\mu l O_2 \cdot h^{-1} \cdot mg\ protein^{-1}$) and cytochrome ($a + a_3$) content (arbitrary units).

mitochondrial genomes and the number of mitochondria is almost constant, corresponding to 3 - 4 genomes per mitochondrion.

Glucose caused a decrease in the number of targets in UV-induced 'petite' mutations as described by Allen and MacQuillan (9). The number of targets varied from 20 in nonrepressed to 3 in repressed cells. Equivalence was found between mitochondrial counts and number of targets (9).

The initially very steep decrease of Q_{O_2} shown in fig. 3 confirms the idea, that the intactness of the respiratory chain is very sensitive to glucose repression. Cytochromes ($a + a_3$) disappear totally (fig. 3) whereas cytochrome c is less sensitive to glucose repression and only diminishes to 40% of the nonrepressed value (fig. 4). When total cytochrome content is calculated a similar picture emerges (fig. 4). The cytochrome c content per mitochondrion may thus be constant.

Many conclusions in the context of mitochondriogenesis have been drawn from results of experiments with simultaneous glucose ('catabolite') repression and anaerobiosis. Glucose

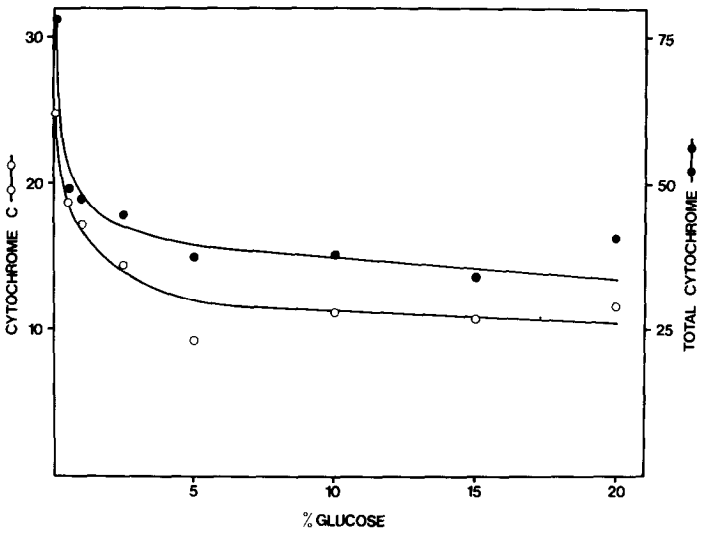

Fig. 4. Effect of increasing glucose concentration on cytochrome c and total cytochrome content (arbitrary units).

repression is very probably mediated via the adenosine 3',5'-cyclic monophosphate (cAMP) regulatory system described for bacteria (13) since it has been shown to counteract glucose repression of aerobic adaption in Saccharomyces as well (14). Anaerobiosis on the other hand is a condition which causes partial degradation of existing mitochondria, but restoration of functioning mitochondria is a process independent of de novo protein synthesis. It has been demonstrated by Luzikov et al. that neither chloramphenicol nor cycloheximide inhibited the restoration of mitochondria that were non-functioning after a period of anaerobiosis. On anaerobic incubation of nonrepressed cells Luzikov et al. found no changes in total cytochrome content (15).

The findings of Fukuhara of the same amount of mitDNA in cells from aerobic and anaerobic cultures (4) may therefore be due to the use of cells from stationary growth phase where glucose had been exhausted. In glucose repressed cells from exponential growth Fukuhara still finds the same percentage

of mitDNA. The reason for this seems unclear at the moment.

Our results are in agreement with other observations of mitDNA replicating independently of the replication of nDNA (2) Independent replication would allow regulation of mitDNA and cellular mitochondrial equipment according to metabolic conditions and enzymic demands.

It still remains to be established what the regulatory mechanism is that governs mitDNA replication. A likely candidate could be mitDNA polymerase which is known to have a nuclear gene (16). It might be regulated via the cAMP system since it is known that cAMP can relieve yeast cells from glucose repression (14).

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