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VARIATIONS IN MITOCHONDRIAL DNA CONCENTRATION AS A FUNCTION OF DIFFERENT GROWTH CONDITIONS IN YEAST

STEPHEN F. COTTRELL

Department of Biology, Brooklyn College of
The City University of New York
Brooklyn, New York 11210

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SUMMARY

The relative concentration of mitochondrial DNA in the yeast, Saccharomyces cerevisiae has been examined under a variety of different growth conditions by means of an isotope dilution procedure which is shown to yield accurate estimates of mitochondrial DNA content in small samples of this yeast. Under a derepression scheme in which only limited cell proliferation occurs, mitochondrial DNA exhibited nearly a doubling in relative amount. The concentration of mitochondrial DNA was also observed to fluctuate depending upon the strain, growth phase and carbon source included in the growth media. Our results indicate that the relative proportion of mitochondrial DNA does indeed vary according to a variety of different conditions that the cells are subjected to.

INTRODUCTION

While most populations of exponentially growing eukaryotic cells appear to exhibit relatively constant ratios of mitochondrial DNA (mtDNA) to total cellular DNA, the situation in the facultatively anerobic yeast, S. cerevisiae remains unclear. The question of possible fluctuations in mtDNA content in this yeast has been the subject of continuing dispute with numerous workers reporting alterations in the relative concentration of yeast mtDNA (1-5) while others report mtDNA concentrations to remain nearly constant under different environmental conditions (6,7).

Since most procedures used for determining mtDNA content may be subject to possible selective losses of either nuclear or mtDNA, we have reexamined mtDNA content under different culture conditions by employing a modification of the classical isotope dilution procedure as first adapted to yeast by

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Fukuhara (6) in conjunction with the separation of total cell DNA into nuclear and mtDNA fractions by poly-L-lysine (PLK) chromatography (2,8). This procedure appears ideally suited to this study in that it is shown to be quantitative and is independent of possible selective losses of either species of DNA.

MATERIALS AND METHODS

The haploid strains ATCC 42029 (9), A364A (10) and 20BB (4) of S. cerevisiae were used in these studies. Cells were grown in either the media of Mahler et al. (11) under their conditions of repression and derepression or in media containing 1% yeast extract, 2% bacto-peptone with carbon sources and concentrations as well as harvesting times as designated in the text. During the derepression scheme total cell protein, DNA, wet weight and cell turbidity were monitored according to previously reported procedures (12).

The proportion of total DNA that was of mitochondrial origin was determined by the following method. Labeled reference cells were prepared by inoculating Wickerham's medium (13) containing 20 uCi/ml of (2-^{3}H) adenine (Schwarz/Mann, 28 Ci/mmole) with the appropriate strain of yeast (corresponding to that strain being tested). Cells were grown to late stationary phase, harvested, resuspended in starvation medium (14) and stored at refrigeration temperature. An aliquot of labeled reference cells equal to approximately 1% of the unlabeled cells under examination was added and nuclear and mtDNAs co-isolated from a 1.5 g wet weight aliquot of the mix according to the PLK column chromotography methods described elsewhere (2, 8). Since some cross-contamination of the mtDNA by nuclear DNA was frequently observed, pooled mtDNA fractions were routinely rerun on fresh PLK columns. Similar purification of nuclear DNA generally was not required because of the extremely low levels of cross-contamination of this species of DNA by mtDNA. The specific radioactivity (cpm/unit of DNA absorption at 260 nm) in the pooled peak tubes of isolated nuclear and mtDNAs were determined with all values expressed here as the means of 3 independent determinations. Since the labeled reference yeast was added prior to nuclear and mtDNA isolation, our isotope dilution procedure is independent of possible selective losses of either species of DNA. The ratio of nuclear to mtDNA specific radio-activities for each sample were then multiplied by the actual percent mtDNA in the labeled reference cells as determined by analytical ultracentrifugation (12). The resulting values give the absolute percent mtDNA in the unlabeled cell samples under study. A more detailed description of this isotope dilution procedure is now in preparation.

To evaluate the quantitative nature of the isotope dilution procedure outlined here, purified fractions of both yeast nuclear and mtDNA were obtained by PLK chromatography and the 2 species of DNA mixed together to produce samples containing known relative concentrations of mtDNA. These samples were then processed according to the isotope dilution procedure reported here and the actual values for percent mtDNA in the artificial mixes were plotted against those values derived by isotope dilution analysis.

TABLE 1
CALCULATION OF PERCENT mtDNA BY ISOTOPE DILUTION ANALYSIS IN SAMPLES CONTAINING KNOWN CONCENTRATIONS OF mtDNA[a]

Percent mtDNA in mix	Calculated percent mtDNA
0	0
5	5.3
10	10.6
15	14.9
20	21.2

[a]DNA samples were prepared by mixing different proportions of purified nuclear and mtDNA previously isolated by PLK chromatography. Calculated values for percent mtDNA were determined as outlined in the text.

RESULTS AND DISCUSSION

An evaluation of the accuracy of the isotope dilution procedure used here for determining mtDNA concentrations is shown in Table 1. Close agreement between the actual values for percent mtDNA in mixtures of both nuclear and mtDNA and those values determined by the isotope dilution procedure is apparent. Based on this experiment and others (Cottrell, manuscript submitted), we conclude that this procedure can be employed to accurately measure the true relative concentrations of mtDNA in this yeast.

Strain A364A was shifted from conditions of repression (exponential growth in 5% glucose) to a derepression medium (0.25% glucose plus 3% ethanol) according to published procedures (11). Changes in total cell DNA, protein, wet weight and Klett optical density (Fig. 1) indicated only limited cell proliferation and agreed closely with parameters measured by others under the same conditions of derepression (11). During this derepression scheme mtDNA concentration was observed to increase rapidly during the first 3 hr and exhibited nearly a doubling during this time (Fig. 2). These values were reproducible in replicate experiments and differed considerably from that pattern of mitochondrial enzyme

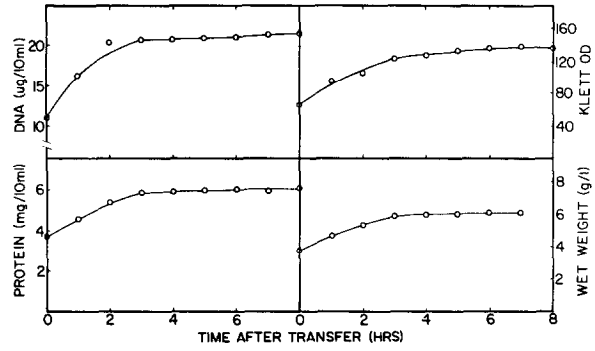

Fig. 1. Changes in various cell parameters during derepression. Repressed cells grown in 5% glucose were harvested in mid-log phase, washed and transferred to derepression medium containing 0.25% glucose plus 3% ethanol.

elaboration observed by others (11) in which enzymes known to be at least partly coded for by the mitochondrial genome were shown to exhibit nearly a steady rate of increase in activity from 1.5 hr to 7 hr after the shift to derepression medium. A causal relationship between mitochondrial DNA replication and the elaboration of these enzymes during this derepression scheme seems unlikely since Mahler and associates have considerable evidence that mitochondrial enzyme induction during derepression is independent of mitochondrial gene dosage.

In addition to examining mtDNA concentrations during derepression, we have also measured mtDNA content as a function of strain, growth phase and carbon source. The data reported in Table 2 indicate that the concentration

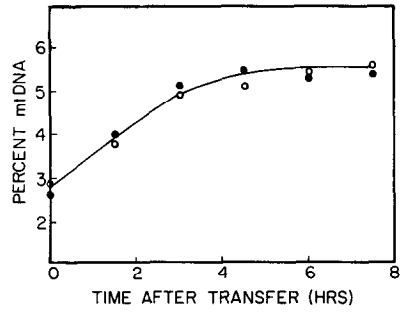

Fig. 2. Changes in percent mtDNA during derepression. Different symbols represent the results obtained in duplicate runs.

TABLE 2
EFFECTS OF CARBON SOURCE, GROWTH PHASE AND STRAIN ON PERCENT mtDNA AS DETERMINED BY ISOTOPE DILUTION ANALYSIS

	Carbon Source and Growth Phase			
	10% glucose		1.5% glycerol	
Strain	mid-log	late stationary	mid-log	late stationary
ATCC 42029	5.5 ± 0.4	9.5 ± 0.2	6.6 ± 0.1	7.8 ± 0.2
A 364A	3.2 ± 0.1	4.5 ± 0.1	5.2 ± 0.2	7.9 ± 0.1
20 BB	4.6 ± 0.4	5.5 ± 0.1	7.8 ± 0.4	11.3 ± 0.2

Values shown are means ± S.D. for 4 separate determinations

of mtDNA does vary in different haploid strains and in the same strain under different culture conditions. It is also apparent that the magnitude of change in mtDNA content in response to altered conditions also differs according to the strain of yeast employed. Such strain-dependent variations may be responsible for at least some of the discrepancies reported in the literature.

Numerous observations suggest that the synthesis of nuclear and mtDNA is not a tightly coupled process in this yeast (12, 15-17). Our data support this view and since the data reported here were obtained by an isotope dilution method that is independent of possible selective loss of either species of DNA, these data confirm the observations of others which have shown mtDNA content to vary according to strain (18) as well as culture conditions (1-5).

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