NUCLEAR GENE DOSAGE EFFECTS ON MITOCHONDRIAL MASS AND DNA

GARY W. GRIMES, HENRY R. MAHLER, and PHILIP S. PERLMAN

From the Departments of Zoology and Chemistry, Indiana University, Bloomington, Indiana 47401. Dr. Grimes's present address is the Department of Biology, Hofstra University, Hempstead, New York 11550. Dr. Perlman's present address is the Department of Genetics, Ohio State University, Columbus, Ohio 43210.

ABSTRACT

In order to assess the effect of nuclear gene dosage on the regulation of mitochondria we have studied serial sections of a set of isogenic haploid and diploid cells of *Saccharomyces cerevisiae*, growing exponentially in the absence of catabolite repression, and determined the amount of mitochondrial DNA per cell.

Mitochondria accounted for 14% of the cytoplasmic and 12% of the total cellular volume in all cells examined regardless of their ploidy or their apparent stage in the cell cycle. The mean number of mitochondria per cell was 22 in the diploid and 10 in the haploids. The volume distribution appeared unimodal and identical in haploids and diploids. The mitochondrial DNA accounted for 12.6 ± 1.2% and 13.5 ± 1.3% of the total cellular DNA in the diploid and haploid populations, respectively. These values correspond to 3.6×10^{-15} g, 2.2×10^{9} daltons, or 44 genomes (50×10^{6} daltons each) per haploid and twice that per diploid cell. On this basis, the average mitochondrion in these cells contains four mitochondrial genomes in both the haploid and the diploid.

INTRODUCTION

A great deal of effort has been devoted recently to studies of the mitochondrial genome of *Saccharomyces cerevisiae* in order to understand its possible functions and limitations in the specification of the organelle (reviews in Borst and Kroon, 1969; Rabinowitz and Swift, 1970; Borst, 1972; Linnane et al., 1972; Borst and Flavell, 1972; Sager, 1972). One of the most fundamental parameters required is $\bar{n}$, the average number of mitochondrial genomes per mitochondrion, and the effect upon this parameter of a variety of genetic and physiological determinants such as nuclear ploidy and cell physiology. In principle, $\bar{n}$ can be determined from: (*a*) the total DNA per cell; (*b*) the fraction of this constituted by mitochondrial (mt) DNA; (*c*) the size of the mitochondrial genome, or particle weight of mtDNA, (*d*) the number of mitochondria per cell. Of these (*c*) is now known with considerable accuracy and reliability (Borst, 1972; Borst and Flavell, 1972; Blamire et al., 1972). Determination of (*a*) and (*b*) presents no inherent difficulty (Bhargava and Halvorson, 1971; Goldring et al., 1970; Williamson et al., 1971; Nagley and Linnane, 1972) but needs to be performed in each instance because of inherent strain-dependent variations (Fukuhara,

1969; Williamson, 1970; Christiansen et al., 1971; Nagley and Linnane, 1972). Concerning (*d*) a different situation prevails. It is frequently impossible to count the mitochondrial profiles seen in thin sections with sufficient accuracy, and in any event, because of well-known stereological considerations, this parameter does not always provide the investigator with an objective estimate of the number of organelles per cell.

In this communication we report on such measurements that have provided us with the number, size, and volume (and hence mass) of these and other organelles found in isogenic haploid and diploid cells of *S. cerevisiae* growing exponentially on lactate in the complete absence of catabolite repression. We have also determined the number of molecules of mtDNA in the same cells and thereby obtained an estimate of the number of mitochondrial genomes per unit mitochondrion, all within a *constant* genetic background. The results show that both the fraction of cellular volume and DNA represented by the mitochondrial component are independent of gene dosage.

MATERIALS AND METHODS

Strains and Culture Conditions

The strains used were provided by Professor H. Halvorson (Brandeis University), who had obtained them from Professor S. Fogel (University of California, Berkeley). The consisted of two isogenic heterothallic, haploid prototrophic strains X2180 1A (mating type allele a) and X2180 1B (mating type allele α) as well as HOH-2, a diploid strain originating from their conjugation. They were maintained on agar slants and grown with vigorous aeration (gyratory shaker) on a semisynthetic medium consisting of (per liter): 2 g yeast extract (Difco Laboratories, Detroit, Mich.), 1 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, and 0.5 g NaCl with either 5% glucose (repressed) or 3% lactate (derepressed) as carbon source. Samples (500 ml) were harvested in midexponential phase, at a turbidity (A_{600}) equal to 0.50. The relevant growth data are described in greater detail in Table I.

Electron Microscopy

Cells of defined physiological states were washed two to four times in twice distilled water, suspended in 2% aqueous $KMnO_4$ (wt/vol) at room temperature, and placed at 4°C for 12–16 h. Cells were then washed three to four times in twice distilled water, embedded in 0.5%, dehydrated in an alcohol series plus propylene oxide, and penetrated with Epon 812 resin mix. Penetrated agar blocks were then spread onto a polystyrene Petri dish in a thin layer of resin and polymerized for 2 days at 60°C.

After polymerization, embedded clusters of cells were cut out of the Epon layer and glued onto a blank block for sectioning. Blocks were hand trimmed with nonparallel faces so that circular ribbons were obtained upon sectioning. Sections were cut with a Porter-Blum MT-2 ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.) using a DuPont diamond knife (E. I. DuPont de Nemours & Co., Wilmington, Del.), and picked up on single hole grids (1 mm inside diameter) by patting. As many as 150 sections were collected on individual grids. Cells were photographed at original magnifications of 3,500–7,500 on a Philips EM-300 at 60 kV.

Analysis of Photographs

Each set of serial sections was first spread out and each mitochondrion was numbered and followed from section to section until the full dimensions had been obtained. Accurate measurements were thus obtained of both number and shape of *all* mitochondria in each cell.

The *relative* volume fractions of organelles can be calculated directly from complete measurements of the *areas* occupied by these organelles in all sections, assuming average equal section thickness throughout the series. On each photograph, area determinations were made of mitochondria, nucleus, vacuole, and total cell (excluding cell wall) by either counting the area (in square millimeters) on acetate overlay graph paper or by the "best fit" to varied shapes and sizes of circles and ellipses of varying axial ratios, all with premeasured areas, on acetate overlays. In general, mitochondrial area was estimated to the nearest 5 mm^2, the nucleus and vacuole to the nearest 25 mm^2, and the cell to the nearest 100 mm^2. If folds in the sections precluded direct measurements of the parameters, the values were estimated by averaging the measurements obtained on sections immediately before and after the folded section. After scoring the entire cell, relative volume fractions of the various cell constituents could be easily obtained by division of *total* areas after summation.

The accurate absolute volume determination depends upon knowledge of areas, final magnification of photographs, and section thickness; accurate estimation of the latter is essential for accurate determinations of absolute volume. Section thickness was therefore determined by two means. First, the interference color of floating sections gives an estimate of section thickness (e.g., silver = 60±90 nm). Secondly, dimensions of whole embedded cells were determined with oil immersion light microscopy. After photographing, the number of sections required to pass through a complete cell in a given orientation divided into the corresponding cell dimensions yields an average and close approximation to exact section thickness.

Once the mean section thickness is determined, absolute organellar volumes are obtained by equating measured square millimeters to square micrometers of absolute area; i.e., by dividing the area equivalent of 1 μm^2 into the measured area. Multiplying the average section

thickness yields an estimate of absolute volume: e.g., at × 10,000, an area equivalent to 1 μm^2 equals 100 mm^2. For sections 0.1 μm^2 thick a volume of 1 μm^3 is represented by 1,000 mm^2 of measured area (i.e.,

$$\frac{1{,}000\ mm^2}{100\ mm^2/1\ \mu m^2} \times 0.1\ \mu m = 1\ \mu m^3).$$

Characterization of DNA

Whole cell DNA was determined as described by Bhargava and Halvorson (1971). DNA was extracted from cells previously converted to spheroplasts (Perlman and Mahler, 1971); with the cells (approximately 1 g wet weight per sample) used in these studies recovery was routinely >75%. Any calculations of the amount of purified DNA extracted are based on the actual recovery in each experiment. The method used is a modification of standard procedures (Marmur, 1961; Smith and Halvorson, 1967). It uses washed spheroplasts as starting materials and lyses them in 6 ml of 0.15 M sodium chloride, 0.015 M trisodium citrate, pH 7.0 (SSC/10) by the addition of sodium lauryl sarcosinate (Sarkosyl, Geigy Pharmaceuticals, Div. Ciba-Geigy Corp., Ardsley, N. Y.) to a final concentration of 2% and heating the mixture at 60°C for 10 min. Pronase (Calbiochem, San Diego, Calif., 100 μg/ml, previously self-digested at 37°C for 30 min at 1 mg/ml) was then added and incubated at 33°–34°C overnight. 2.2 ml of 5 M $NaClO_4$ was then added to give a final concentration of 1 M, followed by an equal volume of chloroform (24 parts) to isoamyl alcohol (1 part), shaken gently for 15 min, and the phases separated by centrifugation. The remainder of the procedure followed standard procedures. The usual yield for derepressed cells was of the order of 300 μg of purified DNA as determined by either the diphenylamine reaction (Burton, 1968) or by absorbance at 260 nm, corresponding to 70–80% of the amount determined in the starting cell suspension.

Analysis by analytical ultracentrifugation used 2-ml samples in SSC with a final DNA concentration of 10 μg/ml. Solid CsCl (2.571 g) was added and the refractive index adjusted at 25°C to 1.3995 ± 0.0002. Centrifugation was performed at 25°C for ~24 h at 44,770 rpm in a model E Beckman ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) equipped with multiplexer and an ultraviolet scanning and recording system. For estimation of the relative areas under the peaks corresponding to nuclear (n) DNA and mtDNA, the scans were transferred to acetate sheets, enlarged six times onto data paper, cut out, and weighed on an analytical balance.

RESULTS

Cellular Parameters

GROWTH CHARACTERISTICS: The data in Table I are based on at least two growth curves determined for each strain. The generation time, and its reciprocal, the specific growth rate, appears to be the same. Cells growing on lactate (or glycerol) exhibit a growth rate less than on 5% glucose, which in turn is lower than that obtainable on 1% glucose (data not shown). They are also considerably less dense as indicated by their mg/*A*.

MORPHOMETRIC PARAMETERS: A total of seven cells of the diploid and six of the X2180 1A haploid, all growing exponentially on lactate, were subjected to serial section and morphometric evaluation. The mitochondria in all cells examined appeared to be irregular, convoluted cylinders and tubules. The results of the measurements are summarized in Table II and Figs. 1 and 2. One of the cells from the haploid culture was clearly diploid in size and other characteristics and has

TABLE I

Values of Growth Parameters for the Three Isogenic Strains Used

	C source	5% glucose				3% lactate			
Strains	Parameter	μ_A	μ_D	mg/*A*	Cells/*A*	μ_A	μ_D	mg/*A*	Cells/*A*
HOH		0.70	0.79	1.05	4.5×10^7	0.33	0.31	0.90	3.4×10^7
X2180 1Aa		0.80	0.69	1.1	6.1×10^7	0.28	0.26	0.78	5.4×10^7
X2180 1Bα		0.72	0.80	1.1	5.2×10^7	0.27	0.31	0.84	5.6×10^7

μ_A = specific growth rate in reciprocal hours (reciprocal of generation time) based on turbidity (A_{600}).

μ_D = same as μ_A but based on dry weight.

mg/*A* = dry weight per unit of turbidity (A_{600}) per milliliter determined at A_{600} = 0.5 (mean of three determinations).

Cells/*A* = cell count by hematocytometer per unit of turbidity, determined at A_{600} = 0.5 (mean of five determinations); for cells grown on lactate the fraction of cells constituted by single cells (devoid of buds) was 0.12 for HOH-2, 0.20 for X2180 1A, and 0.15 for X2180 1B; the fraction of cells with large buds corresponding in size to the mother cell was 0.083 for HOH-2, 0.098 for X2180 1A, and 0.08 for X2180 1B.

TABLE II

Morphometric Data for Representative Cells

Cell no.	Volume V (μm^3)					Mitochondria				
	Nucleus	Vacuole	Mitochondria	Cytoplasm*	Total cell	Volume percent of cytoplasm	Volume percent of cell	Number (N_m) per cell	V_m/N_m	Stage
A. Diploids										
1	4.001	2.492	3.051	20.98	27.47	14.5	11.0	19	0.16	Early S
2	2.505	0.565	2.955	21.80	24.87	13.6	11.9	23	0.13	
3 (total)	3.553	1.130	3.562	23.69	28.37	13.6	12.5	29	0.12	Late S
Main	*3.553*	*0.991*	*2.109*	*15.09*	*19.63*	*14.0*	*10.7*	*17*	*0.12*	
Bud	—	*0.139*	*1.453*	*8.60*	*8.74*	*14.9*	*16.6*	*12*	*0.12*	
4	1.953	1.68	3.062	21.85	25.48	14.0	12.0	24	0.13	G_1
5	1.974	0.821	2.731	18.72	21.52	14.6	12.7	15	0.18	G_1
6 (total)	3.118	2.051	3.226	23.24	28.41	13.9	11.3	23	0.14	Mid S
Main	*3.118*	*2.051*	*2.952*	*19.50*	*24.67*	*13.9*	*12.0*	*20*	*0.15*	
Bud	—	—	*0.274*	*3.74*	*4.01*	*7.3*	*6.8*	*3*	*0.091*	
Mean	2.90	1.46	3.10	21.7	26.0	14.2	11.9	22	0.14	
Y‡ (total)	3.963	3.006	5.215	35.72	42.69	14.6	12.2	35	0.15	Mid S
Main	*3.555*	*3.006*	*3.396*	*20.97*	*27.53*	*16.2*	*12.3*	*20*	0.17	
Bud	*0.408*		*1.810*	*14.75*	*15.16*	*12.3*	*12.1*	*15*	*0.12*	
B. Haploids										
1	1.292	0.804	1.867	11.596	13.7	16.1	13.6	17	0.11	Early S
3	1.312	0.626	1.721	11.822	13.8	14.6	12.5	8	0.22	Early S
4	1.446	1.067	2.004	14.336	16.9	14.0	11.8	10	0.20	Early S
5§	1.099	0.931	1.618	12.069	14.1	13.4	11.4	8	0.20	M
6	1.147	0.879	1.364	9.469	11.5	14.4	11.8	7	0.19	G_1
7‖	1.81	0.533	1.26	10.14	12.5	12.4	10.1	10	0.13	Late S
Mean	1.35	0.807	1.64	11.6	13.8	14.2	11.9	10	0.16	

* Cytoplasm = cell − (nucleus + vacuole).

‡ Cell Y (originally cell no. 2 of that set) was a diploid found among the haploid population; such cells arise by conversion of the mating type gene $\alpha \rightarrow a$ followed by conjugation and propagation by mitosis (see Mortimer and Hawthorne, 1969).

§ Large bud containing part of the nucleus.

‖ Mother cell, bud was not measured.

therefore been included as cell Y. The presumptive stage in the cell cycle is based on the nuclear volume as well as the presence and size of buds.

The data lead to several conclusions. (*a*) The mean cell volume (always taken as mother cell plus bud) of the diploid is twice that of the haploid. (*b*) Although the values encompass a much greater range because of their dependence on the cell cycle this inference appears equally valid for nuclear volumes. As a corollary the mean cellular volume fraction occupied by the nuclei is directly dependent on ploidy. (*c*) The volume fraction, either of the cellular or the cytoplasmic volume, occupied by the mitochondria of any cell is *constant* and *independent of nuclear ploidy*. (*d*) As a corollary it also appears to be independent of the cell cycle. (*e*) This constancy does not reflect a constancy of the number of mitochondria per cell, although the

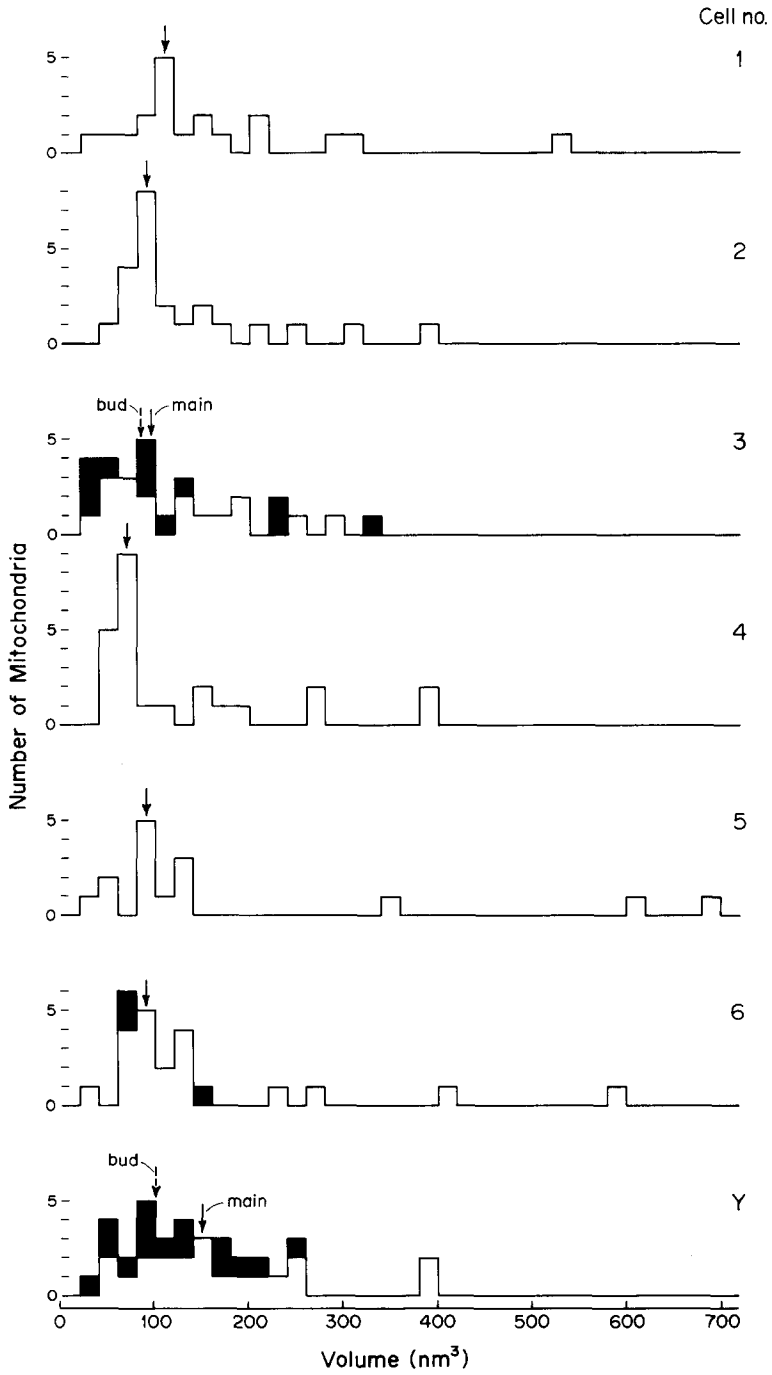

FIGURE 1 Mitochondrial volume distribution in diploid cells (strain HOH-2). Shaded and unshaded areas correspond to mitochondria in buds and mother cells, respectively. The arrow indicates the median size of mitochondria in that cell or bud. Additional parameters of these cells are presented in Table II.

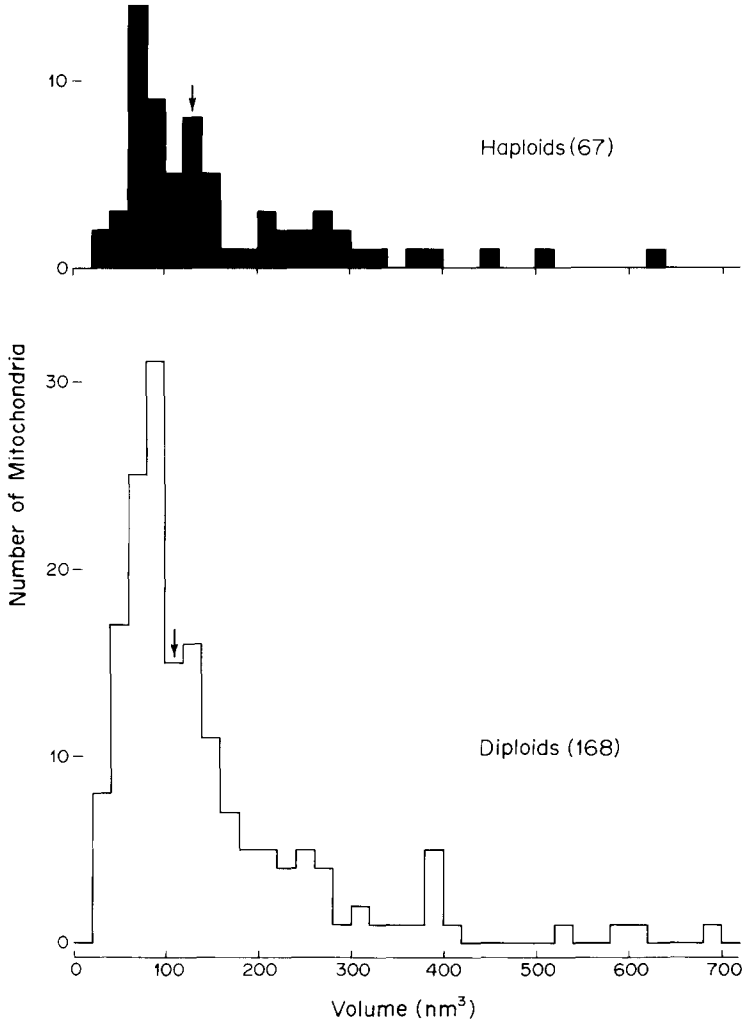

FIGURE 2 Comparison of mitochondrial volume distribution in haploid (X2180 1A) and diploid (HOH-2) cells. Since the sizes of mitochondria in different cells from the same ploidy are similar the pooled data for all haploid and diploid cells are presented. The arrow indicates the median size of mitochondria in each cell sample. 67 mitochondria were scored in haploid and 168 in diploid cells.

mean value of this parameter in the population of diploid cells does appear to be twice that in the haploids. Hence the mean number of mitochondria per unit cytoplasmic volume is also constant and independent of ploidy; it equals 1,020 and 850 per 100 μm^3 of cytoplasm for strains HOH-2 and X2180 1A, respectively.

The volume distributions of the individual mitochondria in several of the diploid cells are shown in Fig. 1. Although they exhibit a great deal of variation between cells not readily referable to their size or history, their median values, indicated by arrows, appear to coincide. A comparison of the cumulative distribution of all mitochondrial volumes in diploids to that in the haploids is presented in Fig. 2. The inference appears warranted that the two distributions coincide. Therefore the volume (and hence mass) of *individual* mitochondria is also independent of nuclear ploidy and gene dosage.

In addition the volume distribution for the mitochondria of three diploid cells with buds (Fig.

TABLE III
Composite Data for Nuclear and Mitochondrial DNA

	DNA per cell ($g \times 10^{14}$)		Weight percent in		Mitochondrial		
	Total	Purified	nDNA	mtDNA	DNA/cell ($g \times 10^{15}$)	Genomes/cell* ($\bar{n}$)	$\bar{n}/p$‡
Diploid§ (2)	5.40	4.3 ± 0.3	88.4 ± 1.2	12.6 ± 1.2	6.80	83	35
Haploids§ (4)	2.86	1.7 ± 0.2	86.5 ± 1.3	13.5 ± 1.3	3.82	47	38
Diploid‖	5.0	2.9		12			
Haploids ‖		1.7		13			

Number of determinations in parentheses; values represent means ± standard deviations for the experimentally determined parameter.

§ Derepressed cells, number of independent determinations in parentheses.

‖ Repressed cells (5% glucose); presented for purposes of comparison only, full details will be presented in the second paper of this series.

* Assuming a mitochondrial genome with a particle weight of 5.0×10^7 daltons (8.25×10^{-17} g) (see Borst, 1972; Borst and Flavell, 1972).

‡ Mitochondrial genomes per nuclear genome with the particle weight of the latter assumed to equal 1.25×10^{10} daltons (2.7×10^{-14} g).

1, cell 3, 6, and Y) are also presented to permit a comparison of the bud and the mother cell. On the basis of this limited sample it appears that mitochondria in buds are indistinguishable from those in the mother cell; bud cytoplasm (Table II) contains a relatively normal amount of mitochondrial mass even when the bud is quite small (for instance diploid cell 6).

Amount of DNA and Number of Genomes

The amount of DNA per cell was measured both on whole cells and after its partial purification and the relative proportions of nDNA and mtDNA were determined. The analytical tracings of the density distributions of the DNA from haploid and diploids were superimposable. The relevant data are summarized in Table III, including the values for total cellular DNA, the yield of the latter recovered after purification, as well as the weight fraction of mtDNA as determined by analytical ultracentrifugation. The fraction of mtDNA appears constant, and therefore the absolute amount of mtDNA and the number of mitochondrial genomes in the diploid are twice those found in the isogenic haploid cells. As will be shown in the next publication of this series for these strains under the particular conditions used here, i.e., with all cells harvested in midexponential phase, the ratio mtDNA to nDNA also appears to be relatively insensitive to variations in the extent of catabolite repression.

DISCUSSION

Possible Source of Errors

ELECTRON MICROSCOPY AND MORPHOMETRY: The results obtained demonstrate the power of serial sectioning for the analysis of basic biological questions. However, the technique is subject to certain limitations. For example, the analysis assumes preservation of cellular details in an unaltered state. Comparisons of a number of strains, in addition to the three used here, grown under a variety of physiological states and fixed by several different procedures described in the literature (permanganate and glutaraldehyde) all indicate that $KMnO_4$ fixation preserves cellular and organellar size and shape. These parameters are, however, strain dependent and when differences do exist they are retained during the treatment.

Other factors could also affect the accuracy of the data. Folds in the sections, variations in section thickness, and inaccurate estimates of measured areas could easily bias the data, both for volumetric estimates and mitochondrial numbers. Comparison of volume determinations of whole cells with the light microscope (assuming a prolate spheroid shape) with those from the sections shows

a close agreement between the results of the two procedures.

DNA DETERMINATIONS: The amounts of DNA per cell determined in this investigation agree with other values reported recently (Schweizer and Halvorson, 1969; Bhargava and Halvorson, 1971). These are all somewhat higher than the ones usually quoted as authoritative in this field (Ogur and Rosen, 1950; Ogur et al., 1952). The proportion of the total DNA that can be assigned to the nucleus is also greater than indicated by independent estimates of the haploid nuclear genome size determined from renaturation kinetics (Bicknell and Douglas, 1970; Christiansen et al., 1971), which leads to a value of 1.0×10^{10} daltons (equivalent to 1.65×10^{-14} g) for this parameter. However, as pointed out by Bicknell and Douglas (1970) this apparent discrepancy is probably due to the presence of newly replicated DNA in rapidly dividing cells, many with attached buds.

However, the greatest source of uncertainty is the possibility of preferential loss of mtDNA in the course of isolation and extraction. This possibility is rendered unlikely, based on three lines of evidence: (*a*) The recoveries of total DNA were high, and reproducibly amounted to approximately 70% of the total. (*b*) The heavy satellite (γDNA; with a bouyant density in CsCl equal to 1.705 g/ml) (Cramer et al., 1972) was present in all preparations as a discrete and reproducible component. Extensive degradation of DNA of relatively low molecular weight, such as mtDNA, would have been expected to lead to an analogous deterioration of γDNA as well. (*c*) The values obtained are within the range established for a large number of strains by other investigators (Fukuhara, 1969; Williamson, 1970; Bleeg et al., 1972; Nagley and Linnane, 1972; Finkelstein et al., 1972).

Size and Mass of Mitochondria

Although volumes of individual mitochondria vary widely in all cells, their actual distribution appears very similar. This is readily apparent for diploids (Fig. 1). Although the total number of mitochondria per haploid cell is too small for such a detailed analysis, it is evident from their cumulative distribution shown in Fig. 2, that they are subsumed in the volume distribution of the diploid. It is also evident that there is no significant difference apparent in this distribution between mother cells and buds, nor between cells varying widely in the size of their nuclei and buds, and hence their presumed stage in the cell cycle. We conclude therefore that:

(*a*) Apparently mitochondrial division is not tightly coupled to the cell cycle. This confirms conclusions concerning the same organism but using a quite different technique (Sena, 1971; Williamson and Moustacchi, 1971).

(*b*) The apportioning of mitochondria between bud and mother cell probably involves a representative sample of the total cytoplasm. It therefore is not likely to proceed by the sequestration of a single mitochondrion, the function of which is to act as progenitor of all the mitochondria produced in the new cell.

(*c*) In any one genetic constitution and physiological state the percentage of cellular mass in mitochondria is completely independent of nuclear gene dosage, cell size, and stage in the cell cycle. It is therefore one of the most stable of all cellular parameters.

Recently Hoffman and Avers (1973) have reported, also using analysis of serial sections, that cells of a diploid strain, Iso-N, contain but a single highly branched mitochondrion even when cells were grown in the absence of catabolite repression and were actively dividing. They therefore proposed that this morphological feature might be a general property of mitochondria in many if not all eukaryotes. The data presented in this and our other studies should suffice to disprove the generality of this hypothesis; we have shown, using four different yeast strains, that cells growing exponentially on nonrepressing carbon sources contain rather large numbers of discrete mitochondria. Buds of these cells contain mitochondria that resemble but are rarely extensions of those in the mother cell. We have also examined cells of one strain released from catabolite repression by growth into early stationary phase on 1% glucose and failed to observe branching structures in this case also. We do find, however, that the actual number of mitochondria is highly strain dependent and we have observed branching structures and relatively small numbers of mitochondria, as few as one per cell, in fully repressed cells of certain strains. Iso-N, therefore, may represent a particularly extreme form of such strain dependence.

The Number of Mitochondrial Genomes Per Cell and Per Mitochondrion

The ratio of mtDNA to nDNA or its fraction of total cellular DNA in these isogenic strains is in-

dependent of nuclear gene dosage. This confirms for strains of identical genetic constitution and physiological state a hypothesis derived earlier from studies on several cell lines under less carefully controlled conditions (Williamson, 1970). This constancy of mitochondrial DNA/total DNA together with the constancy of mitochondrial mass (volume)/cellular mass suggests that the amount of mtDNA per unit mitochondrial volume, or the number of mitochondrial genomes per unit of mitochondrial mass, is also constant. The inference appears also permissible that the number of genomes per mitochondrion exhibits a similar constancy. So far this parameter, however, is really only applicable to the totality of the cells in a population and describes the properties of an "average mitochondrion," an entity that may be devoid of any physical meaning. The mean number of mitochondrial genomes per cell (Table III) equals 47 ± 5 in a haploid and 83 ± 8 in a diploid cell and corresponds to about 36 per haploid chromosome set and four per individual mitochondrion.

The data presented do not permit a choice whether the observed constancy can be explained in terms of a constant amount of DNA per organelle (regardless of size) or per unit mitochondrial mass. The latter alternative, originally suggested by Bahr (1971) for rat liver mitochondria, may signify that the number of mitochondrial genomes per cell is more than sufficient to apportion at least one such genome to all mitochondria including the smallest. On the other hand, if what is constant is the number of mitochondrial genomes per mitochondrion independent of its size, this number may be related to or required for their function as an extranuclear genophore (see e.g. Slonimski et al., 1968; Mahler et al., 1971).

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