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Separation and Characterization of a Satellite DNA from a Yeast Cytoplasmic "Petite" Mutant

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DNA prepared from the particular cytoplasmic "petite" mutant of *Saccharomyces cerevisiae* investigated in this work shows, when centrifuged in a caesium chloride density-gradient, two bands: a main band having the same density ($\rho = 1{\cdot}700$ g/cm^3) as nuclear DNA from wild-type cells, and also showing a heavier shoulder; and a satellite band which has a much lower density ($\rho = 1{\cdot}672$ g/cm^3) than mitochondrial DNA from wild-type cells ($\rho = 1{\cdot}685$ g/cm^3).

The light satellite DNA has been isolated using hydroxyapatite column chromatography, a method which is also effective for isolating mitochondrial DNA from the wild-type cell DNA. Enzymic digestion of the light satellite DNA to nucleosides, and analysis of these on polyacrylamide gel columns have shown that it is formed by A and T in equimolar amounts, to the extent of 96%; G and C form only about 4% of the digest. When examined by electron microscopy, the light satellite DNA shows both linear and circular molecules, in contrast with nuclear DNA which shows only linear molecules.

Chromatography of total DNA from both wild-type cells and the cytoplasmic petite mutant has also revealed the presence of a small nucleic acid component which is extremely rich in guanine; its origin is still unknown.

1. Introduction

The presence of DNA in the mitochondria of *Saccharomyces cerevisiae* has been established by Schatz, Halsbrunner & Tuppy (1964). When DNA preparations from wild-type cells are examined by caesium chloride density-gradient centrifugation, they show a main band of density 1·700 g/cm^3, corresponding to nuclear DNA, a satellite band of density 1·685 g/cm^3 (Carnevali & Tecce, 1965), identified with mitochondrial DNA (Tewari, Jayaraman & Mahler, 1965; Corneo, Moore, Sanadi, Grossman & Marmur, 1966), and a component of density 1·704 g/cm^3, which is incompletely resolved from nuclear DNA, and probably has a non-mitochondrial origin (Corneo *et al.*, 1966; Moustacchi & Williamson, 1966).

In cytoplasmic petite mutants no changes were observed in the major band ($\rho = 1{\cdot}700$ g/cm^3), nor in its shoulder ($\rho = 1{\cdot}704$ g/cm^3) (Corneo *et al.*, 1966). In contrast, mitochondrial DNA exhibits remarkable differences in buoyant density, being, for example, lighter in a neutral petite ($\rho = 1{\cdot}683$ g/cm^3), and heavier in a suppressive petite strain ($\rho = 1{\cdot}695$ g/cm^3) (Mounolou, Jakob & Slonimski, 1966,1967). In a particular mutant, Carnevali, Piperno & Tecce (1966) observed a satellite component having an extremely low density ($\rho = 1{\cdot}670$ g/cm^3).

We report here an investigation on the DNA from the cytoplasmic mutant studied

by Carnevali *et al.* (1966) and its corresponding wild type. Two newly developed techniques were used and proved to be extremely valuable in this work: chromatography of nucleic acids on hydroxyapatite columns (Bernardi, 1965,1968), and analysis of nucleosides on polyacrylamide columns (Carrara & Bernardi, 1968*a*).

2. Materials and Methods

(a) *Yeast cells*

Cytoplasmic mutant DM_1 (ρ^-) was obtained from the diploid strain DM of *S. cerevisiae* by acriflavine treatment. Both strains were kindly provided by Professor Giorgio Morpurgo, of the Istituto Superiore di Sanità, Rome.

Yeast cells were grown in aerobiosis at 28°C in a medium containing: 3·3 g $NaNO_3$, 1·0 g $MgSO_4 \cdot 7\ H_2O$, 0·5 g KCl, 18 mg $FeSO_4 \cdot 7\ H_2O$, 1·0 g KH_2PO_4, 6·0 g yeast extract, 40 g glucose, per litre of distilled water. Cells were harvested by centrifugation and washed twice in 0·2 M-NaCl–0·2 M-EDTA (pH 8·0).

(b) *DNA preparation*

This was done according to a modification of the method of Marmur (1961). All operations were carried out at 0 to 4°C, except where otherwise stated. Washed yeast cells were added to twice their weight of Celite 503 (Johns-Manville, New York, N.Y.; washed with 0·2 M-NaCl–0·2 M-EDTA, pH 8·0) and crushed in a mechanical mortar cooled to −10°C.

The paste obtained from 100 to 130 g of cells was extracted with 300 ml. of 0·15 M-NaCl–0·1 M-EDTA (pH 8·0). The extract was centrifuged at 2000 *g* for 20 min: 5 M-$NaClO_4$ was added to the supernatant fraction (about 170 ml.) in order to reach a final concentration of 1 M-$NaClO_4$. One volume of chloroform–isoamyl alcohol (24: 1, v/v) was added; the mixture was shaken at room temperature for 30 min and the 2 phases were then separated by centrifugation at 2000 *g* for 30 min. The aqueous phase was precipitated with 2 vol. of 95% ethanol. The flocculent precipitate was collected by centrifugation and dissolved in 30 ml. SSC†. This solution was treated again with chloroform–isoamyl alcohol and centrifuged for 2 hr at 14,000 *g*. The clear aqueous supernatant fraction was precipitated with 2 vol. of 95% ethanol. Fibres were collected with a glass rod, dissolved in 10 ml. SSC, and digested at 37°C for 4 hr with 1·5 mg of crystalline pancreatic RNase (Worthington, Freehold, N. J.) dissolved in 3 ml. of 0·15 M-NaCl and heated at 80°C for 10 min. The solution was then treated again with chloroform–isoamyl alcohol, centrifuged for 3 hr at 14,000 *g*, and precipitated with 95% ethanol. Fibres were dissolved in 9 ml. 0·1 SSC; this was added to 1 ml. of 3 M-sodium acetate–0·001 M-EDTA (pH 7·0). DNA was precipitated with 5·4 ml. of isopropyl alcohol. Fibres were washed with 70, 80 and 95% ethanol and dissolved in SSC.

(c) *Chromatography of nucleic acids on hydroxyapatite columns*

This was performed according to Bernardi (1965,1968). The experimental conditions used in the present work are given in detail in the legends of the Figures.

(d) *Caesium chloride density-gradient centrifugation*

Analytical density-gradient centrifugation (Meselson, Stahl & Vinograd, 1957) was carried out in a Spinco model E ultracentrifuge equipped with ultraviolet optics. CsCl (Merck, Darmstadt, Germany; Suprapure) was added to 0·6 ml. DNA solutions to obtain a density at 25°C of 1·690 g/cm^3. Samples were centrifuged at 44,750 rev./min for 21 hr. Several ultraviolet absorption photographs were taken in the last hours of the run using a Kodak film (Kodélio épais; Kodak, Metz, France). These were analysed with a Joyce–Loebl D 31 microdensitometer. The densities of the DNA samples were determined as described by Schildkraut, Marmur & Doty (1962) using phage 2C DNA (ρ = 1·742 g/cm^3; Szybalski, 1968) and *Streptomyces fradiae* DNA (ρ = 1·730 g/cm^3) as density markers.

† Abbreviations used: SSC, standard saline citrate, is 0·15 M-NaCl–0·015 M-sodium citrate (pH 7·2); HA, hydroxyapatite; NaP, sodium phosphate buffer (pH 6·8).

(e) *Sedimentation velocity analysis*

This was done in a Spinco model E instrument, using the ultraviolet optics. Nucleic acid concentrations used ranged from 6 to 15 μg/ml. in about 0·3 M-NaP. Median S values were calculated.

(f) *Enzymic digestion of DNA samples to nucleosides*

DNA samples were dialysed against 0·05 M-ammonium acetate (pH 5·5), and digested with spleen acid DNase and exonuclease preparations obtained according to Bernardi, Bernardi & Chersi (1966) and Bernardi & Bernardi (1968), respectively. Digestions were carried out at room temperature; they were stopped by emulsifying the incubation mixture with 1/20 vol. of chloroform–isoamyl alcohol (5:1 v/v), when the absorbancy increase reached the plateau value of about 70% (Carrara & Bernardi, 1968*b*). Emulsions were broken by centrifugation; the aqueous phase was removed with a pipette and digested with spleen acid phosphomonoesterase II (G. Bernardi, A. Chersi & A. Bernardi, manuscript in preparation). Digests were then chromatographed on DEAE cellulose (acetate form) columns, in order to determine the amount of nucleotides still present. Generally, 1 ml. solutions having an A_{260} value of 4 to 7 were loaded on 0·8 cm × 10 cm DEAE columns; nucleosides were washed through with water in a vol. of 5 to 10 ml.; nucleotides were eluted with 1 M-ammonium acetate. Nucleosides were then dried at about 30°C in a rotary evaporator.

(g) *Nucleoside analysis on polyacrylamide gel columns*

This was done as described by Carrara & Bernardi (1968*a*), using BioGel P2 (Bio-Rad, Richmond, Calif.) columns.

(h) *Electron microscopy*

Samples were prepared according to Kleinschmidt & Zahn, (1959). 0·3 ml. of the DNA–cytochrome *c* mixture in 2 M-ammonium acetate was poured along a glass rod (Trurnit, 1960) on the hypophase formed by 0·3 M-ammonium acetate containing 0·5% formaldehyde or by 0·2 M-ammonium acetate. The available surface (about 400 cm^2) was 4 to 5 times larger than that covered by the protein–DNA film. The film was compressed by a motor-driven bar until a pressure of 1 dyne/cm was reached. Platinum shadowing was in two directions at right angles. Pictures were taken using a Siemens Elmiskop on areas chosen at random on the grids, using a magnification of 9500 (as measured with a grating). Length measurements were done at least in duplicate, on pictures having a final magnification of 28,500 or 40,000. No differences were found whether formaldehyde was used or not.

3. Results

(a) *Fractionation of yeast DNA on hydroxyapatite*

When DNA preparations obtained from wild-type yeast cells and the cytoplasmic petite mutant were fractionated on HA columns, they showed essentially the same chromatographic patterns. These were characterized by the presence of the following fractions.

(1) A fraction formed by material not retained by the columns equilibrated with 0·1 M-NaP; when DNA preparations were loaded on columns equilibrated with 0·001 M-NaP, most of this fraction was again not retained, the rest being eluted at NaP molarities below 0·05 M; this elution behaviour is that found for nucleotides and oligonucleotides, respectively (Bernardi, 1964,1965,1968).

(2) A fraction eluting at a NaP molarity close to 0·20 M, having a nucleic acid spectrum and giving a negative reaction with diphenylamine; this fraction is identified with RNA, since it can be digested with pancreatic RNase, although at a lower rate than sRNA from yeast, a finding which explains its presence after the RNase digestion done during preparation of DNA.

The percentage of the two fractions described so far varied somewhat from one DNA preparation to another; these fractions were not investigated further.

(3) A large fraction eluting at about 0·25 M-NaP (fraction *a*)

(4) A small fraction eluting at 0·27 to 0·30 M-NaP (fraction *b*)

(5) A small fraction eluting at about 0·37 M-NaP (fraction *c*)

Fractions *a*, *b* and *c* were investigated in detail in the present work and identified, respectively, as nuclear DNA, mitochondrial or satellite DNA, and as a small nucleic acid of unknown origin. In contrast with fraction *b*, which was present in a constant

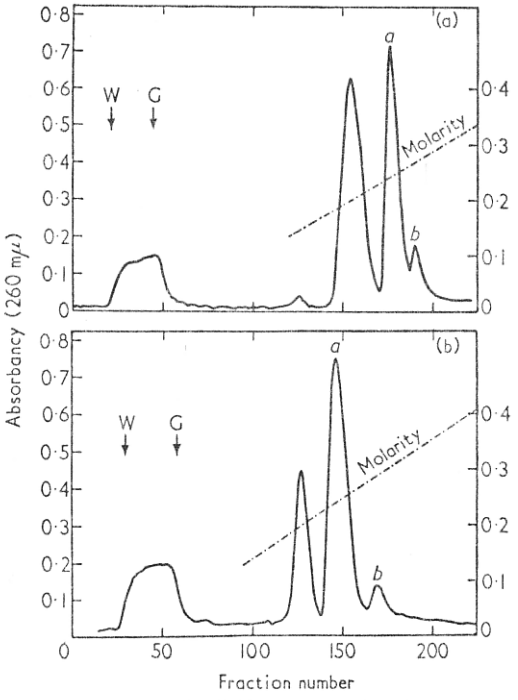

FIG. 1. (a) Chromatography of a DNA preparation from wild-type yeast cells.

100 ml. of DNA solution in 0·1 M-NaP, $A_{260} = 0{\cdot}820$, were loaded on a 2 cm × 40 cm HA column; the column was then washed with 100 ml. of 0·1 M-NaP; elution was carried out with a linear gradient (450 ml. + 450 ml.) of NaP (0·1 to 0·5 M). Loading was started at fraction 0, washing at fraction marked by arrow W, gradient at fraction marked by arrow G. 3·8-ml. fractions were collected. Flow rate was about 55 ml./hr. A_{260} recovery was 98%.

(b) Chromatography of a DNA preparation from cytoplasmic petite mutant.

100 ml. of DNA solution in 0·1 M-NaP, $A_{260} = 0{\cdot}800$, were loaded on a 2 cm × 34 cm HA column. 3·5-ml. fractions were collected. Flow rate was about 50 ml./hr. A_{260} recovery was 100%. All other indications as in (a).

ratio to fraction *a*, fraction *c* varied according to the preparation procedure used and was practically absent from DNA preparations which had been submitted to a greater number of precipitations with ethanol and isopropanol than that described in the previous section. Figure 1(a) and (b) shows chromatograms obtained with DNA preparations of this type.

(b) *Caesium chloride density-gradient centrifugations of fractions* a *and* b

(i) *Petite mutant*

Figure 2(a) shows the CsCl density-gradient centrifugation of an unfractionated cytoplasmic petite mutant DNA. Two components are present, a major one

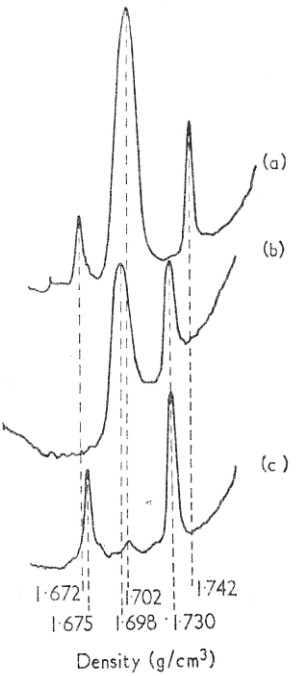

FIG. 2. Microdensitometer tracings of DNA preparations from cytoplasmic petite mutant yeast cells centrifuged at 44,770 rev./min for 21 hr in a CsCl density gradient. DNA's from phage 2C (1·742 g/cm³) and *Streptomyces fradiae* (1·730 g/cm³) were used as density markers.
(a) Unfractionated DNA; (b) fraction *a* (nuclear DNA); (c) fraction *b* (satellite DNA).

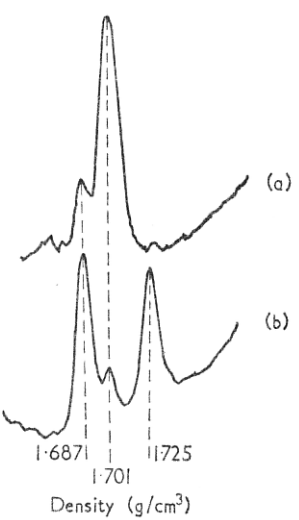

FIG. 3. Microdensitometer tracings of DNA preparations from wild-type yeast cells centrifuged at 44,770 rev./min for 21 hr in a CsCl density-gradient. *E. coli* denatured DNA (1·725 g/cm³) was used as density marker.
(a) Unfractionated DNA; (b) fraction *b* (mitochondrial DNA).

($\rho = 1{\cdot}702$ g/cm^3) corresponding to nuclear DNA, and a minor one ($\rho = 1{\cdot}672$ g/cm^3), as already reported by Carnevali *et al.* (1966). Fraction *a* can be identified as nuclear DNA on the basis of its buoyant density (Fig. 2(b)), whereas fraction *b* contains the satellite component.

(ii) *Wild type*

Figure 3(a) shows a CsCl density-gradient centrifugation of an unfractionated wild-type DNA preparation, showing a major component ($\rho = 1{\cdot}701$ g/cm^3), corresponding to nuclear DNA, and a minor component ($\rho = 1{\cdot}687$ g/cm^3), corresponding to mitochondrial DNA; this finding is in agreement with observations by previous investigators. Figure 3(b) shows that fraction *b* contains the satellite DNA ($\rho = 1{\cdot}687$ g/cm^3) of mitochondrial origin; a small amount of contaminating nuclear DNA ($\rho = 1{\cdot}701$ g/cm^3) can also be seen. The results in Figure 3(a) and (b) show that fraction *a* is formed by nuclear DNA.

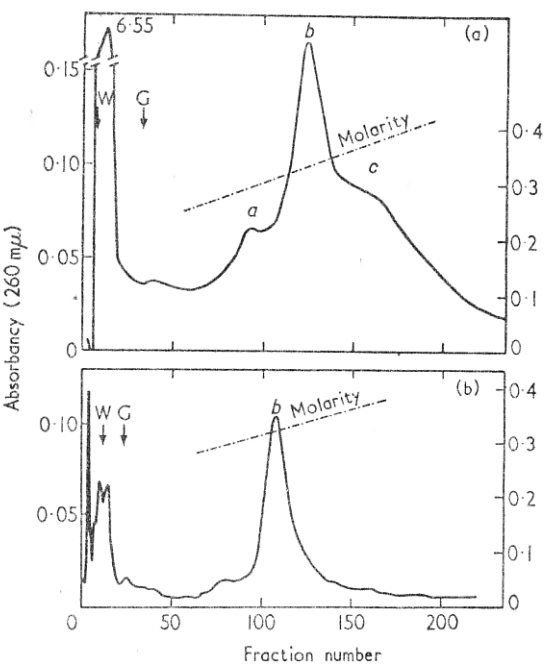

FIG. 4. (a) Chromatography of a DNA preparation from cytoplasmic petite mutant. 108 ml. of DNA solution in 0·25 M-NaP, $A_{260} = 5{\cdot}99$, were loaded on a 2 cm × 40 cm HA column. The column was then washed with 470 ml. of 0·25 M-NaP; elution was carried out with a linear molarity gradient of NaP (0·25 to 0·50 M); the total volume of eluent was 900 ml. Loading was started at fraction 0, washing at fraction 8 (arrow W), gradient at fraction 33 (arrow G). 18·3-ml. fractions were collected from fractions 1 to 32; 4·0-ml. fractions were collected from fractions 33 to 235. Flow rate was 75 ml./hr. A_{260} recovery was 95%.

(b) Rechromatography of fractions 106 to 140 from the previous experiment. Pooled fractions were adjusted by addition of water to 0·25 M-NaP and loaded on a 2 cm × 40 cm HA column. This column was then washed with 250 ml. of 0·25 M-buffer; elution was carried out with a linear molarity gradient of NaP (0·25 to 0·50 M); the total volume of eluent was 900 ml. Loading was started at fraction 0, washing at fraction 11 (arrow W), gradient at fraction 23 (arrow G). 19·5-ml. fractions were collected from fractions 1 to 24; 3·6-ml. fractions were collected from fractions 25 to 220. Flow rate was 55 ml./hr.

(c) *Chromatographic purification of fraction* b

(i) *Petite mutant*

An improved chromatographic separation (Fig. 4(a)) of fraction *b* was obtained by changing the experimental conditions (see legend to Fig. 4). Almost the whole of the material preceding fraction *b* was washed through the column equilibrated with 0·25 M-NaP. The molarity gradient subsequently applied to the column eluted first a small peak belonging to fraction *a* (nuclear DNA) and then resolved fraction *b*, eluted as a rather sharp peak at 0·33 M, from a broad shoulder formed by fraction *c*. The re-chromatography of fraction *b* is shown in Figure 4(b).

(ii) *Wild type*

A chromatographic separation of fraction *b* similar to that just reported for the petite mutant was obtained; the separation of fraction *b* from fraction *c* was also observed. These fractions have been studied in less detail, so far, than the corresponding ones from the petite mutant.

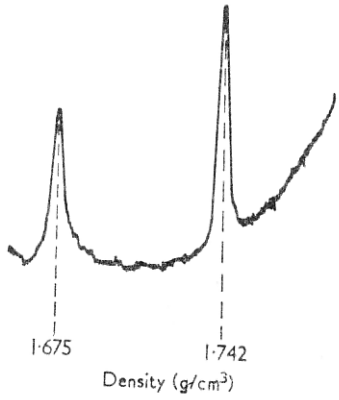

FIG. 5. Microdensitometer tracing of fraction *b* (pooled tubes 106 to 140 from the chromatogram of Fig. 4(a)) from the cytoplasmic petite mutant centrifuged at 44,770 rev./min for 21 hr in a CsCl density-gradient. DNA from phage 2C (1·742 g/cm³) was used as a density marker.

(d) *Characterization of fraction* b *from the petite mutant*

(i) *Caesium chloride density-gradient centrifugation*

Figure 5 shows the CsCl density-gradient centrifugation of pooled fractions 106 to 140 from the HA chromatogram shown in Figure 4(a). A band of $\rho = 1{\cdot}675$ g/cm³ was the only component present.

(ii) *Nucleoside analysis*

Fractions 98 to 119 from the chromatogram shown in Figure 4(b) were pooled and digested to nucleosides. The analysis of the nucleosides is shown in Figure 6(a). Table 1 shows in detail the analytical results obtained for the fraction *b* component of petite mutant DNA. A and T form 96·3% of the digest and are present in equimolar amounts, within experimental error; G and C are only present to the extent of 3·8%. It is noteworthy that the yield in nucleosides obtained from fraction *b* as from the DEAE cellulose column was only 84%, instead of being higher than 90 to 95%, as usual with enzymic digests obtained from nuclear yeast DNA, or from DNA preparations from other sources. This result will be commented upon in the discussion.

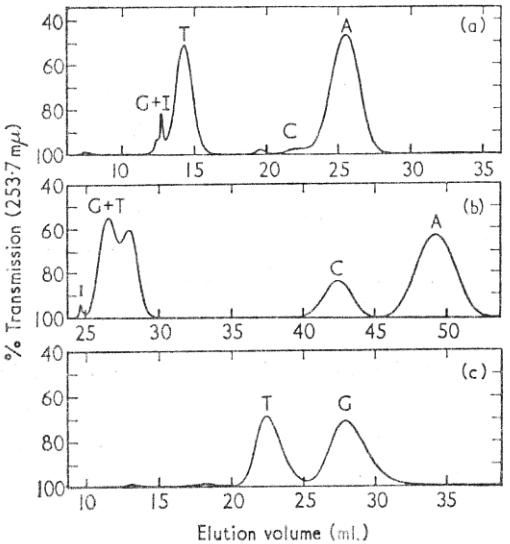

FIG. 6. Chromatography on Bio-Gel columns of the deoxyribonucleosides obtained by enzymic digestion from chromatographic fractions of cytoplasmic mutant DNA, as recorded by a Uvicord (LKB, Stockholm, Sweden) using a cell with an optical path equal to 0·3 cm.

(a) Fraction *b* (pooled fractions 98 to 119 of the chromatogram shown in Fig. 4(b)). 4·42 A_{271} units in 0·2 ml. of 2×10^{-3} M-ammonium carbonate (pH 10·2) were chromatographed on a 0·7 cm × 70 cm column equilibrated with the same buffer. The two main peaks are formed by thymidine and deoxyadenosine, respectively; the two minor components preceding T are formed by deoxyguanosine and deoxyinosine (derived by deamination of A), respectively; of the two minor components preceding A, the first one was not identified, and the second one is cytidine.

(b) Fraction *a* (nuclear DNA). 5·97 A_{271} units in 0·2 ml. of 2×10^{-3} M-ammonium carbonate (pH 10·2) were chromatographed on a 0·9 cm × 80 cm column equilibrated with the same buffer.

(c) Rechromatography on a Bio-Gel P-2 column of the G + T fraction from the previous experiment. The chromatographic fraction was dried *in vacuo*, dissolved in 0·2 ml. of 2×10^{-4}M-sodium phosphate (pH 7·2), and chromatographed on a 0·6 cm × 50 cm column equilibrated with the same buffer. The amount loaded was 2·76 A_{271} units.

The analysis of nuclear DNA (fraction *a*) from the petite mutant was also carried out (Fig. 6(b) and (c)); the results obtained are shown in Table 2. Data of Tewari, Vötsch, Mahler & Mackler (1966) are presented for comparison.

(iii) *Electron microscopy*

Plate I shows a typical electron micrograph of fraction *b* (from pooled fractions 98 to 119 from the chromatogram shown in Figure 4(b)). Two different kinds of molecules could be observed: (a) linear molecules; (b) circular molecules. Aggregates of different sizes were also present.

For the sake of comparison, a preparation of nuclear DNA from the petite mutant and biosynthetic dAT:dAT and dA:dT were also examined; in all cases only linear molecules having the thickness and the appearance of duplex DNA molecules were observed. The lengths of the molecules from fractions *a* and *b* were measured and the histograms obtained are shown in Figure 7(a) and (b), respectively. Very interestingly, in all cases studied, namely linear and circular molecules from fraction *b* and linear molecules from nuclear DNA, the most frequent class was formed by molecules having an average length of 0·5 μ (corresponding to a molecular weight

PLATE I. Electron microscopy of DNA preparations from the cytoplasmic petite mutant: component *b* from pooled fractions 98 to 119 (see Fig. 4(b)). ×28,500.

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TABLE 1

Nucleoside analysis of fraction b *from the cytoplasmic petite mutant*†

λ (mμ)	250	255	258	260	267	280	$\frac{250}{260}$	$\frac{280}{260}$	Molar fraction
T	1·38			2·05	2·23	1·48	0·67 (0·65‡)	0·72 (0·72‡)	48·8
A	2·59		3·18	3·14		0·830	0·83 (0·83)	0·26 (0·24)	47·5
G§	0·125	0·110		0·095		0·075	1·32 (1·02)	0·79 (0·70)	1·9
C§	0·065			0·095		0·120	0·68 (0·43)	1·26 (2·16)	1·9

† Values reported are absorptions, at pH 2·0, at the specified wavelengths, multiplied by the volumes of the nucleoside fractions (10 ml. for A and T; 5 ml. for G, C and I).

‡ Theoretical values given by Calbiochem.

§ Deoxyguanosine and deoxycytidine were completely separated by rechromatography of G + I + T and C + A on a column at pH 7·2. The disagreement between some of the absorption ratios and the theoretical values is due to experimental error unavoidable at the extremely low concentrations investigated.

TABLE 2

Nucleoside analysis of yeast nuclear DNA

	Molar fraction	
	(a)	(b)
T	30·9	34·7
A	31·4	31·2
G	19·2	15·6
C	18·6	18·5

(a) Present work; nuclear DNA from the cytoplasmic petite mutant.
(b) Data of Tewari *et al.* (1966) for the nuclear DNA of wild-type yeast cells.

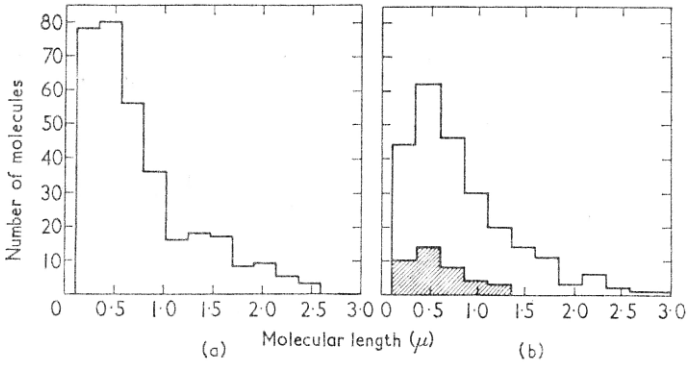

FIG. 7. Contour length distribution of DNA preparations from the cytoplasmic petite mutant. (a) Nuclear DNA; (b) component *b* from pooled fraction 98–119 (see Fig. 4 (b)). Hatched area, circular molecules.

of 1×10^6 daltons), and the large majority of molecules had lengths shorter than 1·0 μ (2×10^6 daltons). Length measurements done on several electron micrographs of fraction *b* showed that circular molecules formed close to 10% by weight of the non-aggregated material; the same results were obtained when making these measurements on pooled fraction *b* and the peak tube of the fraction. Aggregates were estimated to form a sizeable amount of total material.

(iv) *Sedimentation*

Analytical ultracentrifugation of fraction *b* showed it to have $S_{20,w} = 11{\cdot}5$ s; nuclear DNA from the petite mutant had $S_{20,w} = 10{\cdot}9$ s.

(e) *Characterization of fraction* c

Some of the properties of fraction *c* from both wild-type cells and petite mutant cells were investigated. The following results were obtained.

(1) The ultraviolet spectrum showed a maximum at 255 mμ, a minimum at 230 mμ and a 280:260 ratio equal to 0·53 (0·05 M-ammonium acetate, pH 5·6, was used as the solvent).

(2) The sedimentation coefficient was determined in 0·05 M-ammonium acetate (pH 5·6) and found to be $S_{20,w} = 2{\cdot}9$ s, for fraction *c* from the petite mutant cells.

(3) Fraction *c* did not band in a density gradient formed by a CsCl solution of density equal to 1·700 g/cm^3, and could not be seen by electron microscopy. These findings are scarcely surprising in view of the low sedimentation coefficient of fraction *c*.

(4) Fraction *c* was resistant to pancreatic DNase, acid spleen DNase, pancreatic RNase, acid spleen RNase (Bernardi & Bernardi, 1966), and spleen exonuclease, as judged by the absence of hyperchromic shift under experimental conditions where DNA and sRNA samples, respectively, were completely digested in a very short time. The sedimentation coefficient did not change after digestion with acid DNase, acid RNase or exonuclease from spleen. This resistance of fraction *c* to nucleolytic enzymes can be used to purify it from contaminating fraction *b*.

(5) Fraction *c* showed a hyperchromic shift of 17% after heating for 20 minutes at 100°C in 0·05 M-ammonium acetate (pH 5·3). After thermal treatment, the material was still resistant to acid DNase and acid RNase, but could be degraded to nucleotides with spleen exonuclease.

(6) Nucleoside analysis of heated and exonuclease-digested fraction *c* from the cytoplasmic petite mutant showed it to have the following composition: U, 11·3; A, 14·6; G, 66·2; C, 8·0%. This analysis was obtained on only one preparation of fraction *c*, and should be considered as preliminary.

4. Discussion

(a) *Fractionation of yeast nucleic acids on hydroxyapatite*

The results obtained by fractionating yeast DNA preparations on HA columns are interesting in several respects. The separation of small fragments (mono- and oligo-nucleotides, probably of RNA origin) from high-molecular weight nucleic acids and of RNA from nuclear DNA are as expected from previous work (Bernardi, 1964,1965, 1968). The elution molarities observed in the present work are higher than those reported previously (0·20 and 0·25 M, instead of 0·15 and 0·22 M, for RNA and DNA, respectively). This is due to the use of sodium buffers instead of potassium buffers as the eluents; the latter being more dissociated than the former, they have a higher

eluting power at comparable molarities (Bernardi, 1968). A surprising finding is that two fractions, called here *b* and *c*, were eluted at higher molarities than nuclear DNA.

Fraction *b* has been shown to be formed by mitochondrial DNA in the case of DNA from wild-type cells and by the satellite DNA (also, probably, of mitochondrial origin) in the case of the DNA from the cytoplasmic mutant. HA columns provide therefore the first chromatographic method to separate mitochondrial DNA from nuclear DNA. The separation on these columns of nuclear and mitochondrial DNA suggests that the latter may differ in their secondary structures. In fact, it appears from work carried out in Strasbourg (Bernardi, 1962,1965,1968; Chevallier & Bernardi, 1968; Bernardi & Kawasaki, 1968) that in the chromatography of polynucleotides on HA, their secondary structure has a fundamental role in determining their elution molarity from the columns, and that the elution molarity of rigid macromolecules (like native double-stranded DNA) increases with the number of chemical groups (phosphate groups) interacting with HA per surface unit of the macromolecule in contact with HA. Another interesting point concerning fraction *b* is the finding that no chromatographic separation takes place between circular and linear molecules, as shown by electron microscopy. This result, far from surprising in view of the size of the DNA molecules, is in keeping with the recent report that, in the case of polyoma DNA, untwisted circular and open molecules do not differ in their chromatographic behaviour on HA columns (Bourgaux-Ramoisy, Van Tieghem & Bourgaux, 1967).

The high elution molarity of fraction *c* suggests that this small, guanine-rich, nucleic acid of unknown origin has very high phosphate concentration on its surface interacting with HA and a structure different from that of double-stranded nucleic acids. It may be recalled here that, of all polynucleotides investigated so far, only triple-stranded poly A–2 poly U is eluted at a higher molarity than fraction *c* (Bernardi, 1965,1968).

(b) *Characterization of the* b *component from the cytoplasmic petite mutant*

Component *b* from the cytoplasmic petite mutant is the satellite component already described by Carnevali *et al.* (1966), since it shows a buoyant density equal to 1·675 g/cm^3 when centrifuged in a CsCl density-gradient. This density is slightly lower than that reported for dAT:dAT ($\rho = 1{\cdot}678$ to 1·679 g/cm^3) by Schildkraut *et al.* (1962), Erikson & Szybalski (1964), and Wells & Blair (1967), but much higher than that of dA:dT, which has a density $\rho = 1{\cdot}647$ g/cm^3 (Szybalski, 1967; Wells & Blair, 1967).

The buoyant density of component *b* is explained by the finding that 96% of its bases are A and T, in equimolar amounts. The fact that component *b* has a buoyant density slightly lower than that of dAT:dAT, in spite of its small but significant GC content, may be due to the presence of stretches of lighter dA:dT in it. As far as the origin of G and C in component *b* is concerned, the only possibility is that they belong indeed to the satellite DNA. In fact, G and C cannot derive from a contamination from nuclear DNA, since 9 to 10% of nuclear DNA in fraction *b* would have been detected by CsCl density-gradient centrifugation (see, for example, Fig. 2(c)). G and C cannot derive from fraction *c* which may contaminate fraction *b*, since this is resistant to nucleolytic enzymes and remains on DEAE cellulose columns (causing the observed low recovery in nucleosides); furthermore, the G/C ratio of fraction *c* is higher than 8, whereas G and C are present in equimolar amounts in fraction *b*.

Electron microscopy of component *b* showed it to be composed of linear and circular molecules as well as aggregates. The aggregates are artefacts, since they do not appear in sedimentation velocity experiments; furthermore, they are formed by material which has chromatographic properties identical with those of linear and circular molecules. Their formation, however, seems to be associated with the particular composition and/or structure of fraction *b* since they do not appear in either nuclear, dAT:dAT or dA:dT materials. As far as linear and circular molecules are concerned, it is very interesting to note that both electron microscopy (Fig. 7) and sedimentation velocity have shown that these molecules, as well as those of nuclear DNA, have a low molecular weight, of the order of 1×10^6 daltons. The agreement between the molecular weights as estimated from the sedimentation coefficient and electron microscopy suggests that aggregates are not formed by a special class of molecules.

The low molecular weights of both nuclear and satellite DNA indicate that they have undergone enzymic degradation during the preparation procedure. The circular and the linear DNA molecules of fraction *b* may well have the same origin, deriving from a polydisperse, circular DNA population (such as that described by Avers, 1967), some of its smaller members having survived the degradation.

In conclusion, the main findings of this work are the following:

(a) The separation of mitochondrial and satellite DNA from nuclear DNA on HA columns, a finding suggesting the possible existence of structural differences between mitochondrial and nuclear DNA.

(b) The demonstration in a cytoplasmic petite mutant of an A,T-rich satellite DNA; the mitochondrial origin of this satellite DNA has not yet been shown by actual isolation from mitochondria, but is likely.

(c) The isolation of a small G-rich nucleic acid, of unknown origin; further results on this material will be published elsewhere in the near future.

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