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The Mitochondrial Genomes of *Ustilago cynodontis* and *Acanthamoeba castellanii*

Elena MERY-DRUGEON, Edwin J. CROUSE, Jürgen M. SCHMITT, Hans-Jürgen BOHNERT, and Giorgio BERNARDI

Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, and Botanisches Institut der Universität Düsseldorf

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Mitochondrial DNA from *Ustilago cynodontis* has been investigated in several of its properties. Its dG + dC content is equal to 33.5%; its buoyant density (1.698 g/cm^3) is higher, by 5 mg/cm^3, and its melting temperature (82.5 °C) is lower than expected for a bacterial DNA having the same base composition; the first derivative of its melting curve indicates a large compositional heterogeneity, its molarity of elution from hydroxyapatite is high, 0.28 M phosphate, and allows its partial separation from nuclear DNA. Degradation by micrococcal nuclease indicates that about 25% of the DNA is formed by stretches having no more than 15% dG + dC. Finally, the unit size of mitochondrial genome is about 50×10^6. In most of its properties, the mitochondrial genome of *U. cynodontis* presents strong analogies with that of *Saccharomyces cerevisiae*.

A parallel investigation on mitochondrial DNA from *Acanthamoeba castellanii* which has as genome unit size of only 27×10^6, has shown that this shares with the former the dG + dC content (32.9%), the melting temperature (82.5 °C), a large compositional heterogeneity and a very similar pattern of micrococcal nuclease degradation; its buoyant density (1.692 g/cm^3) and its molarity of elution from hydroxyapatite (0.25 M phosphate) are, however, normal, probably because of a different short-sequence pattern and the fact that its dA + dT-rich stretches are shorter, on the average.

Investigations carried out over the past years have shown that the mitochondrial genome of *Saccharomyces cerevisiae* has a eukaryotic type of organization, in that its coding sequences are interspersed with non-coding sequences; these consist of long dA, dT spacers (dG + dC < 5%) [1] containing short dG, dC clusters (dG + dC > 80%); (see [1, 2] for two short reviews). An important question of obvious evolutionary interest is how general is such an organization for mitochondrial genomes having a large genome unit size, such as those of most protists and plants. It is evident that in order to provide an answer to such a question, one requires organelle genomes in which non-coding sequences have molecular properties which can be easily distinguished from those of coding sequences, as is the case for the mitochondrial genome of *S. cerevisiae*.

The first mitochondrial genome studied for this purpose was that of *Euglena gracilis*. Having a quite low dG + dC content (25%), not very much higher than that of *S. cerevisiae* (18%), and a high buffer concentration for elution from hydroxyapatite [3], like the latter, this genome was a good candidate for containing dA, dT spacers. dA + dT-rich stretches were indeed found in the mitochondrial genome of *E. gracilis*, though less abundant in dA, dT and in amount than those of *S. cerevisiae* [4]; a small amount of sequences very rich in dG + dC were also found in the mitochondrial genome of *E. gracilis* [4].

Another case which has been studied in detail is that of *Torulopsis glabrata* [5]. This yeast has a mitochondrial genome which has a unit size of 12.5×10^6, only one fourth of that of *S. cerevisiae*, but shares with the mitochondrial genome of *S. cerevisiae* a number of properties, buoyant density, base composition, buffer concentration for elution from hydroxyapatite and multimodal melting. It is therefore very likely that the organization of the mitochondrial genome of *T. glabrata* is similar to that of *S. cerevisiae*. Mitochondrial DNAs from other yeasts, *Brettanomyces anomalus* and *Kloeckera africana*, have been characterized to a lesser extent, yet they share with the mitochondrial DNA of *S. cerevisiae* at least a very low buoyant density and a high molarity of elution from hydroxyapatite [5]. The available evidence, therefore is that at least three and possibly five dA + dT-rich mitochondrial genomes contain long stretches extremely high in dA, dT.

In the present work, we have applied the methodology developed to study the compositional heterogeneity of the mitochondrial genome of *S. cerevisiae* to two other mitochondrial genomes, richer in dG + dC than those previously studied, (≈ 33%), those of *Ustilago cynodontis*, a fungus belonging to the class of Basidiomycetes (in contrast to *S. cerevisiae*, which is an Ascomycetes) and of *Acanthamoeba castellanii* a protozoan. While the latter DNA had already been investigated in some of its properties, namely buoyant density, genome size and complexity [6–10], the former one had never been studied before.

In the case of *U. cynodontis* DNA we have studied two points in addition to its sequence organization. The first one was the size of the mitochondrial genome units; the second was whether there are any detectable differences between the mitochondrial genomes of the mycelial and the yeast-like forms of this fungus. The study of this latter point was prompted by the following considerations. *U. cynodontis*, a plant pathogen, is a dimorphic fungus, able to grow both as a mycelium or as a yeast; growth as a yeast is induced by chloramphenicol and ethidium bromide [11, 12], two specific inhibitors of mitochondrial functions. Chloramphenicol also promotes the transition from mycelial to yeast-like forms in other dimorphic fungi belonging to the classes of Phycomycetes and Deuteromycetes [13, 14]. In contrast to these fungi, however, *U. cynodontis* undergoes, upon chloramphenicol and ethidium bromide action, a transition which is long-lasting and even permanent, and

which is accompanied by a change from prototrophy to auxotrophy, with lysine and arginine requirements [11, 12]. In view of the specific mitochondrial action of these products and of the apparent mitotic segregation of mycelial forms from yeast-like forms, it has been suggested that mitochondrial functions are involved in these modifications [11].

MATERIALS AND METHODS

Cell Cultures and DNA Preparation of U. cynodontis

Both the mycelial form of *Ustilago cynodontis*, strain 4001 (ATCC 28990), and the yeast-like cells derived from them by treatment with ethidium bromide were used.

Cells were grown in 4% glucose, 1% yeast extract (Difco Labs, Detroit, MI) at 28 °C, using a New Brunswick (New Brunswick, NJ, USA) rotary shaker operated at 350 rev./min. Exponential phase cells were inoculated into 10-l or 15-l volumes of the same medium and grown in a New Brunswick FS-614 fermentor, operated at 500 rev./min, with an air flow rate of 11 min^{-1} (l culture)$^{-1}$, using 100 µl polypropylene glycol/l (P2000; Touzart et Matignon, Paris, France) as an antifoam agent.

Cells were harvested during the exponential phase of growth by centrifugation at 6000 rev./min in a Sharpless centrifuge type TI. They were washed twice with sterile water and collected by centrifugation at $16000 \times g$ for 10 min in a RC2-B Sorvall centrifuge (Sorvall, Norwalk, CT, USA). Cultures were checked for absence of contamination and phenotypic reversion by plating on solid medium containing 4% glucose, 1% yeast extract.

A cell suspension (0.2–0.4 g/ml) in 1.2 mM sorbitol, 2 mM EDTA, 10 mM Tris/HCl, pH 5.6, was incubated at 30 °C for 2.5–4 h with a mixture of 20–40 mg helicase/g cell (Industrie Biologique Française, Gennevilliers, France) and 80–85 mg cellulase/g cells (British Drug Houses, Pool, England). The protoplasts so prepared were then washed twice in the same medium to eliminate the enzymes and lysed by osmotic shock.

Nucleic acids were prepared according to Prunell et al. [15]; mitochondrial DNA was prepared by chromatography on hydroxyapatite [16] and further purified by two preparative centrifugations in a CsCl density gradient [17].

Cells Cultures and DNA Preparation of A. castellanii

Acanthamoeba castellanii (Neff strain) was grown in aerated 8-l fermenting flasks and harvested when still in the logarithmic growth phase. Cells were broken by means of a Dounce homogenizer and mitochondria obtained by differential centrifugation. Mitochondrial DNA was extracted as will be described (Bohnert and von Gabain, unpublished). After purification through two cycles of CsCl density gradient centrifugation (at 40000 rev./min for 24 h using a Beckman vertical rotor), DNA was dialysed and concentrated under reduced pressure at room temperature. The yield was about 2 mg mitochondrial DNA/100 g fresh-weight cells. About 80% of the mitochondrial DNA molecules were found to be open circular molecules (average contour length = 12.7 µm).

Absorbance/Temperature Profiles

DNA solutions ($A_{270} = 0.5 - 1.0$) in standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7 [18]) were used. Absorbance/temperature profiles were determined with a Gilford 250 spectrophotometer. The standard deviation measurements of 50 consecutive readings at zero absorbance varied between 1×10^{-4} and 2×10^{-4}. The temperature of the cuvette (330-µl volume) was controlled by a Gilford 2527 thermoprogrammer. To minimize temperature gradients in the cuvette, the cuvette chamber was kept at a temperature lower, by about 5 °C, than the cuvette, by a Haake FT circulating bath with a Haake PC II programmer. Data (time, absorbance with five-figure readings and temperature with four-figure readings) were collected automatically using a Dialog interface and a Facit tape puncher, as well as a chart recorder to monitor the course of the experiment directly. About 20 measurements were made per sample and per degree with a linear heating rate of 0.1 °C/min and a dwell time of 0.3 s. Experimental set-up and data handling largely followed the recommendations of Ansevin et al. [19]. Data reduction was done using program MELT, written in Fortran IV: absorbance measurements were smoothed over 9, 13 or 19 points (see figure legends), using the procedure of Savitzky and Golay [20] with the convolution coefficients of Ziessow [21]. Temperature measurements were smoothed linearly over a corresponding number of points. In some cases, the program NOISE was used to average several profiles. The smoothed values were differentiated according to $Y = (dA/A_{25})/dT$, (where A is the absorbance, T the temperature, A_{25} the absorbance at 25 °C) and plotted off-line with a Calcomp or Tektronix 4662 plotter, using standard library software. Numerical calculations were performed on the TR 445 computer of the computing center of the Düsseldorf University. The Fortran IV programs used are available, upon request, from J.M.S.

Other Methods

Sedimentation coefficients and buoyant densities in CsCl were determined as described [22, 23]. Micrococcal nuclease degradation was performed according to Prunell and Bernardi [24]. Base composition was determined, after enzymatic degradation to nucleosides [25] by chromatography on columns of Aminex A6 (Bio-Rad, Richmond, CA, USA) [26]. Restriction enzyme degradation, gel electrophoresis of restriction fragments, microdensitometry of the negative pictures of the gels were done as described [15]. Alternatively, restriction fragments were separated on 0.4–2.2% horizontal agarose (Marine Colloids, Rockland, MA, USA) or 3–6% vertical polyacrylamide gels.

RESULTS

Ustilago cynodontis

Chromatography of Mitochondrial DNA on Hydroxyapatide. Fig. 1 shows a chromatogram on hydroxyapatite of a nucleic acid extract obtained after protoplast lysis, deproteinization and alcohol precipitation from ethidium-bromide-induced yeast-like cells of *U. cynodontis*. The DNA fractions shown in the figure form a main peak eluting at about 0.25 M phosphate and a shoulder eluting at about 0.28 M phosphate. Analytical ultracentrifugation in CsCl showed that the main peak is essentially formed by DNA having a buoyant density of 1.713 g/cm^3, whereas the shoulder is mainly formed by DNA having a buoyant density of 1.697 g/cm^3. The latter DNA corresponds to mitochondrial DNA, as indicated by its properties (see below) and by the fact that mitochondrial DNA from a closely related species, *U. maydis*, has the same buoyant density [27]. The DNA forming the bulk of the main peak has been

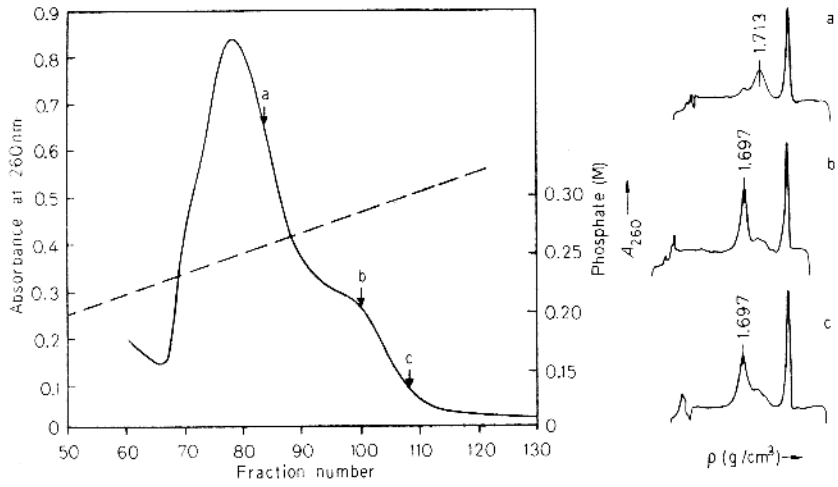

Fig. 1. *Chormatography of mitochondrial DNA from* U. cynodontis *on hydroxyapatite.* A nucleic acid extract (92 ml in 0.15 M sodium phosphate, pH 6.8) obtained from 192 g (wet weight) of yeast-like cells of *U. cynodontis* was adsorbed on 40 ml of (packed) hydroxyapatite equilibrated with 500 ml of 0.15 M sodium phosphate. The suspension was loaded on a column (1.7 cm × 50 cm) of hydroxyapatite equilibrated with 0.15 M sodium phosphate; the column was washed with 650 ml of the same buffer and then a linear 0.15 – 0.40 M gradient was applied; flow rate was 24 ml/h. 9.6-ml fractions were collected using an LKB Ultrorac fraction collector (Stockholm, Sweden); transmission at 253.7 nm was monitored using an LKB Uvicord recorder. Arrows indicate fractions used for analytical CsCl density gradient centrifugations (on right). Fractions 84 – 130 were pooled and used in a preparative CsCl density gradient centrifugation

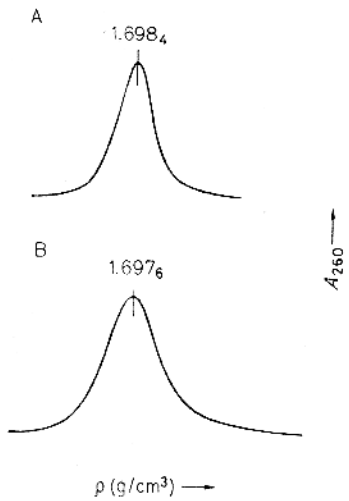

Fig. 2. *Analytical CsCl density gradient centrifugation of mitochondrial DNA from (A) mycelial and (B) yeast-like cells of* U. cynodontis. The wider band of DNA from the yeast-like form is due to its lower molecular weight: sedimentation coefficients were 15.7 S for the DNA of mycelial forms and 13.5 S for the DNA from yeast-like forms; these values correspond to molecular weights of 3.8×10^6 and 2.6×10^6, respectively

Table 1. *Physical properties of mitochondrial DNA from* U. cynodontis, A. castellanii, *and other protists*

ϱ is the buoyant density, t_m the melting temperature and [Phosphate] the concentration of sodium phosphate at the peak of mitochondrial DNA as eluted from hydroxyapatite

DNA source	ϱ	t_m	[Phosphate]
	g/cm³	°C	M
U. cynodontis			
Mycelial cells	1.698_4	82.5	0.28
Yeast-like cells	1.697_6	82.5	0.28
A. castellanii	1.692_2	82.5	0.25
S. cerevisiae [30]	1.683	74.7	0.31
E. gracilis [4]	1.690	77	0.29
T. glabrata [5]	1.684		0.31
B. anomalus [5]	1.682		0.31
K. africana [5]	1.683		0.31

identified with nuclear DNA. Chromatographic results essentially identical to those just described were obtained for DNA preparations from mycelial cells.

Properties of Mitochondrial DNA. The hydroxyapatite fractions containing mitochondrial DNA were pooled, purified by two preparative density gradient centrifugations in CsCl and used for determining a number of properties. The analytical buoyant density profiles in CsCl of mitochondrial DNA preparations from yeast-like and mycelial cells are shown in Fig. 2. In both cases, nuclear DNA contamination appears to be lower than 5% (compare the results of Fig. 2 with the profiles of Fig. 1). The absorbance/temperature differential profile is shown in Fig. 3 for mitochondrial DNA from mycelial cells; identical results were obtained for the DNA from yeast-like cells. In both cases, the profiles were characterized by a rather wide thermal transition and by an evident skewness on the low-melting side. In addition, small amounts of both low-melting and high-melting material were seen. Table 1 presents the values of buoyant density in CsCl, melting temperature t_m and molarity of elution from hydroxyapatite. Nucleoside analysis is shown in Table 2. dG + dC contents as obtained by chemical analysis and as calculated from ϱ [28] and t_m [29] for mitochondrial DNAs derived from mycelial and yeast-like cells are shown in Table 3.

Degradation by Micrococcal Nuclease. Fig. 4 shows the dG + dC contents of the excluded and retarded fractions obtained by running micrococcal nuclease digests of *U. cynodontis* mitochondrial DNA on Sephadex columns [14]. About 25% of the material could be obtained as small oligonucleotides (Fig. 4B) having a dG + dC level close to 15%; about 20% of the large fragments (Fig. 4A) had dG + dC contents in excess of 55%.

Restriction Enzyme Analysis. Three restriction enzymes *Hae*III, *Hpa*II and *Hind*II + III were used to degrade the mitochondrial DNAs from mycelial and yeast-like cells of *U.*

Table 2. *Nucleoside composition of mitochondrial DNAs from* U. cynodontis *and* A. castellanii

DNA source	dA	dC	dG	dT
	%			
U. cynodontis				
Mycelial cells	33.9	17.0	16.5	32.6
Yeast-like cells	32.8	16.9	16.5	33.9
A. castellanii	33.3	16.6	16.3	33.9

Table 3. *dG + dC contents of mitochondrial DNA's from* U. cynodontis, A. castellanii *and other protists*

DNA source	Estimated by		
	analysis	ϱ	t_m
	%		
U. cynodontis			
Mycelial cells	33.5	39.2	31.15
Yeast-like cells	33.4	38.2	31.15
A. castellanii	32.9	33.0	31.6
S. cerevisiae [30]	17.4	23.5	17.6
E. gracilis [4]	24.8	33	18.8
T. glabrata [5]	19	24	

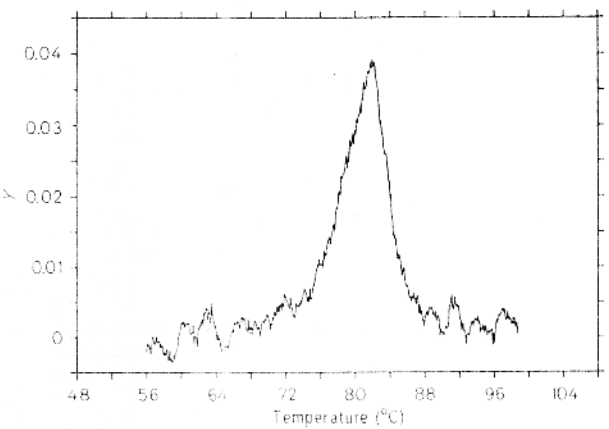

Fig. 3. *Differential melting profile of mitochondrial DNA from mycelial cells* of U. cynodontis. Absorbance data were smoothed over 19 points

cynodontis. Identical restriction patterns were obtained for both DNAs (Fig. 5) ruling out that the transition between the two forms is due to large changes in mitochondrial DNA; obviously, point mutations, small sequence alterations, or changes in other cytoplasmic genetic elements cannot be ruled out by the methods used. Estimates of the genome sizes were obtained by adding up the molecular weights of all fragments; the fact that some bands contained two fragments was taken into account. Values close to 50×10^6 were obtained from all three enzymatic digests (Table 4).

Acanthamoeba castellanii

Chromatography of Mitochondrial DNA on Hydroxyapatite. In this case chromatography was not used to

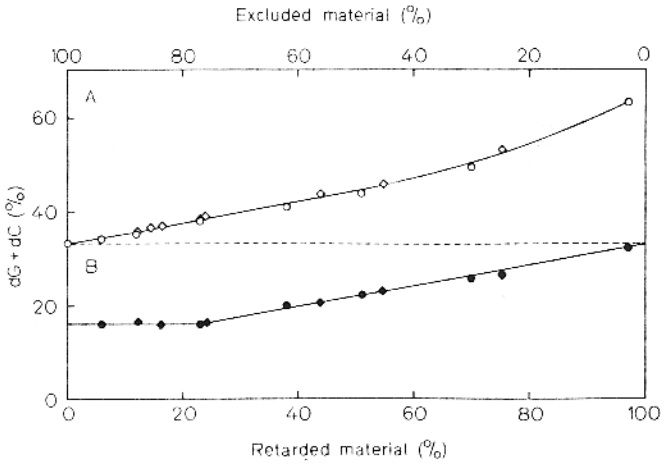

Fig. 4. *Degradation of mitochondrial DNA of* U. cynodontis *and* A. castellanii *by micrococcal nuclease.* Mitochondrial DNAs from mycelial cells of *U. cynodontis* and from *A. castellanii* were degraded at 6° C by micrococcal nuclease. The dG + dC levels of the fractions which were (A) excluded (open symbols) and (B) retarded (close symbols) by Sephadex G-25 columns are plotted against the relative amounts of these fractions. Squares refer to *U. cynodontis* DNA, circles to *A. castellanii* DNA. The retarded material corresponds to mono and oligonucleotides released by the enzyme, the excluded material to the large fragments formed by the enzyme. The enzyme has a strong preference for dA + dT-rich segments, which are first degraded to small fragments

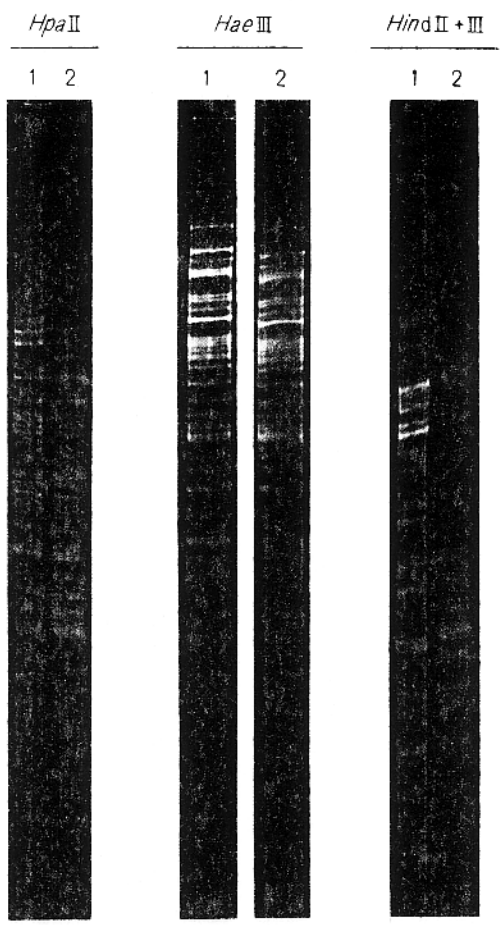

Fig. 5. *Electrophoretic patterns of restriction enzyme digests of mitochondrial DNA from* U. cynodontis. The enzymes used are indicated. 1 and 2 refer to mycelial cells and yeast-like cells, respectively. 2% polyacrylamide/0.5% agarose [10] gels were used

Table 4. *Unit sizes of the mitochondrial genomes of* U. cynodontis *and* A. castellanii
For *U. cynodontis*, analysis was done on 2%, 3% and 6% gels, except in the case of the *Hae*III digest, where only the 2% gel was used; the smallest fragments were, therefore, not taken into account in this latter estimate

Restriction enzyme	Digest of *U. cynodontis*			Digest of *A. castellanii*		
	no. of bands	no. of fragments	genome unit size	no. of bands	no. of fragments	genome unit size
*Hpa*II	48	54	50×10^6	40	40	27×10^6
*Hind*II	42	53	49×10^6	29	29	27×10^6
*Hind*III				26	26	27×10^6
*Hae*III	38	44	46×10^6	31	31	27×10^6

Table 5. *Unit size of the mitochondrial genome of* A. castellanii

Fragment number	$10^{-6} \times M_r$ of fragment obtained by restriction enzyme									
	*Hind*III	*Eco*RI	*Sal*I	*Bam*HI	*Pst*I	*Eco*RI + *Sal*I	*Eco*RI + *Bam*HI	*Bam*HI + *Sal*I	*Sal*I + *Pst*I	*Eco*RI + *Pst*I
1	5.9	10.3	20.4	11.8	19.4	10.3	8.6	10.7	12.4	5.7
2	2.5	7.5	3.6	10.6	7.3	3.5	4.6	8.2	7.3	4.0
3	2.38	3.5	3.2	4.6	0.58	3.45	3.5	3.5	3.6	4.0
4	2.35	2.25		0.20		3.2	2.25	3.1	3.2	3.5
5	1.75	2.05				2.05	2.05	1.5	0.58	3.4
6	1.54	1.05				1.75	1.96	(0.1)[a]	–[b]	2.25
7	1.43	0.54				1.05	1.82	(0.1)	(0.2)	2.05
8	1.20					0.78	1.05			1.05
9	1.13					0.54	0.76			0.58
10	1.09					0.49	0.54			0.54
11	0.89						0.20			
12	0.84									
13	0.74									
14	0.70									
15	0.60									
16	0.59									
17	0.50									
18	0.35									
19	0.34									
20	0.20									
Genome unit size	27.02	27.19	27.20	27.20	27.28	27.11	27.13	27.20	27.08	27.07

[a] Fragment *Bam*H1-4 is cleaved in the double digestion, the two products were not resolved.
[b] Not resolved.

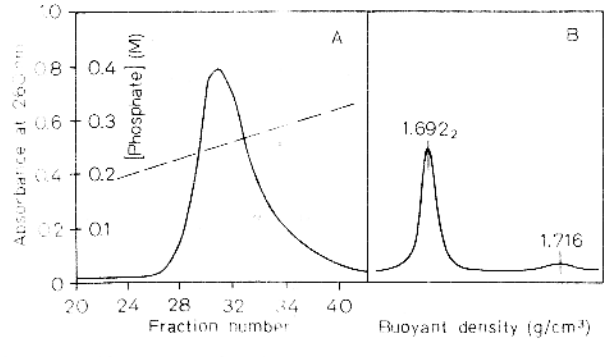

Fig. 6. *(A) Chromatography of mitochondrial DNA from* A. castellanii *on a hydroxyapatite column. (B) Analytical CsCl density gradient centrifugation of* A. castellanii *mitochondrial DNA*

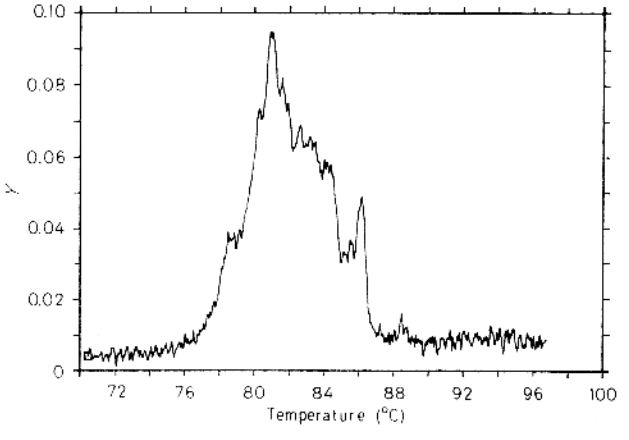

Fig. 7. *Differential melting profile of mitochondrial DNA from* A. castellanii. Absorbance data were smoothed over 19 points

purify mitochondrial DNA, but only to determine its molarity of elution; this was found to be equal to 0.25 M sodium phosphate (Fig. 6).

Properties of Mitochondrial DNA. The DNA obtained from hydroxyapatite was analyzed by density gradient centrifugation (Fig. 6) and shown to exhibit a single, symmetrical peak. The contamination of nuclear DNA ($\varrho = 1.716\,g/cm^3$) was extremely low. The absorbance/temperature profile (Fig. 7) was very complex, with some DNA melting ahead of the main peak and a large amount of DNA melting at several higher temperatures. Tables 2 and 3 present the physical and chemical properties of mitochondrial DNA from *A. castellanii*.

Degradation by Micrococcal Nuclease. The results are shown in Fig. 4. They are similar to those exhibited by the mitochondrial DNA from *U. cynodontis*.

Restriction Enzyme Analysis. The results obtained with several enzymes are presented in Table 4 and 5. They all lead to an estimate of the genome unit size equal to 27×10^6.

DISCUSSION

The chromatographic behaviour of *Ustilago cynodontis* mitochondrial DNA on hydroxyapatite is characterized by a very high buffer concentration for elution, 0.28 M phosphate, which allows this DNA to be partially separated from nuclear DNA, which is eluted at 0.25 M. This behaviour is very similar to those already described for the mitochondrial DNAs of *Saccharomyces cerevisiae* [30], *Euglena gracilis* [4], *Torulopsis glabrata*, *Brettanomyces anomalus* and *Kloeckera africana* [5]. In contrast, the chromatographic behavior of *Acanthamoeba castellanii* mitochondrial DNA was 'normal', the molarity of elution being 0.25 M sodium phosphate (Table 1).

The analytical dG+dC content of mitochondrial DNA from *U. cynodontis*, 33.4 %, was lower than expected on the basis of its buoyant density, but higher than expected from its t_m. In fact, if this DNA was to follow the (dG+dC)/ϱ relationship established for bacterial DNAs [28] one would calculate a content of 38 %; likewise, if it was to follow the (dG+dC)/t_m relationship of bacterial DNAs [29], one would calculate a content of 31 %. These discrepancies cannot be attributed to trivial reasons, like glucosylation or methylation for reasons already given [4, 30]. The trends shown, higher buoyant density and lower t_m than expected, are the same as these already found for the mitochondrial DNAs from *S. cerevisiae*, *E. gracilis* and *T. glabrata* (Table 1). Again, the *A. castellanii* mitochondrial DNA differed from its *U. cynodontis* counterpart in that its analytical dG+dC content was that expected on the basis of its buoyant density; yet it was slightly higher than expected from its t_m (Table 1).

The differential melting profile of mitochondrial DNA from *U. cynodontis* was not strikingly multimodal as in the cases of *S. cerevisiae* or *E. gracilis*, yet provided evidence for a remarkable compositional heterogeneity, in that it was characterized by a skewness on the low-melting side and also by the presence of small amounts of both very low and very high-melting material. In agreement with the melting profile, degradation by micrococcal nuclease revealed the presence of about 25 % od sequences as low as 15 % in dG+dC, as well as that of high-dG+dC material. Both of these features were found previously in the other two mitochondrial DNAs, except that the amounts and the dG+dC levels of dA+dT-rich stretches were much more striking in them. The *A. castellanii* mitochondrial DNA exhibited a melting profile characterized by the presence of several components, and its pattern of degradation by micrococcal nuclease was identical to that of *U. cynodontis* mitochondrial DNA.

It appears therefore that the two DNAs investigated, though having the same base composition, differ in that the DNA from *U. cynodontis* exhibits properties (high molarity of elution from hydroxyapatite, high buoyant density) already found in other mitochondrial DNAs containing dA + dT-rich stretches (Table 1), whereas the DNA from *A. castellanii* does not. In order to understand the reasons for such a difference, one should recall that the frequency of short oligonucleotides affect the buoyant density of DNA [31–33], and that DNAs containing stretches of non-alternating dA, dT show a high molarity of elution from hydroxyapatite [16]. This suggests that the DNA from *A. castellanii* does not have in its dA + dT-rich stretches a particular abundance of the sequences causing an increase in buoyant density and in elution buffer concentration. Concerning this latter point, it should also be mentioned that the dA + dT-rich stretches of *A. castellanii* DNA are shorter, on the average, than those of *U. cynodontis* DNA since their percentage is the same but the genome size of the former is about half that of the latter. This situation may also influence the elution molarity from hydroxyapatite.

The main conclusion of this work is that mitochondrial DNAs from *U. cynodontis* and *A. castellanii* do contain long stretches very rich in dA · dT base pairs, like those from *S. cerevisiae*, *E. gracilis*, *T. glabrata* and, very probably, *B. anomalus* and *K. africana*. Since very recent sequence determinations have shown the existence of dA,dT spacers in the minicircles of kinetosplast DNA from *Trypanosoma brucei* [34] and in the mitochondrial DNA of *Aspergillus nidulans* [35], it appears that such a feature is very widespread and concerns the mitochondrial genome of species which are phylogenetically very distant from each other. A subsequent paper will show that the presence of dA,dT spacers is also found in chloroplast genomes.

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F. Mery-Drugeon and G. Bernardi, Laboratoire de Génétique Moléculaire,
Institut de Recherche en Biologie Moléculaire du Centre National de la Recherche Scientifique,
Université de Paris VII, Tour 43, 2 Place Jussieu, F-75221 Paris-Cedex-05, France

E. J. Crouse, Institut de Biologie Moléculaire et Cellulaire du Centre National de la Recherche Scientifique,
15 Rue René-Descartes, Esplanade, F-67084 Strasbourg-Cedex, France

J. M. Schmitt, Lehrstuhl Botanik I der Justus-Maximilians-Universität Würzburg,
Mittlerer Dallenbergweg 64, D-8700 Würzburg, Federal Republic of Germany

H.-J. Bohnert, Botanisches Institut der Universität Düsseldorf,
Universitätsstraße 1, D-4000 Düsseldorf, Federal Republic of Germany