ESTIMATION OF THE MOLECULAR LENGTH OF PETITE NEGATIVE YEAST MITOCHONDRIAL DNA

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1. Introduction

It was reported that the size of mitochondrial DNA of protists ranges from 12.8 μm in *Paramecium* to 25 μm in *Saccharomyces cerevisiae* and *S. carlsbergensis* [1–3] whereas animal mitochondrial DNA consists of a closed circular molecule with a contour length of about 5 μm [4]. Recently a gross size difference in mitochondrial DNA between *S. cerevisiae* and some petite negative yeasts was reported by Sanders et al. [5] and O'Connor et al [6]. They found quite a small quantity of circular DNA molecules with a homogeneous contour length two to four fold shorter than that of petite positive strain in electron micrographs of mitochondrial DNAs of petite negative yeasts. In this report, the size of mitochondrial DNA of another typical petite negative yeast *Torulopsis colliculosa* was estimated unambiguously by the restriction endonuclease analysis.

2. Materials and methods

2.1. *Preparation of yeast mitochondrial DNA*

T. colliculosa (petite negative strain) was kindly provided by Dr S. Nagai of Nara Women's University. It was grown at 28°C with constant shaking in the medium of Ohnishi et al. [7], and harvested at a mid-log phase. Yeast spheroplasts were prepared with glusulase (β-giucuronidase) as described by Duell et al. [8], and lysed using a mechanical homogenizer. Liberated mitochondria were treated for 20 min at 0°C with 50 μg per ml DNase-1 [9] in 20% sucrose–10 mM $MgCl_2$–1 mM EDTA in 5 mM potassium phosphate buffer (pH 6.8) and washed three times with 0.25 M sucrose–0.1 M EDTA (pH 8.0), and lysed in 15 mM NaCl–1.5 mM sodium citrate containing 1% SLS (sodium lauryl sulfate) by heating at 60°C for 10 min. Pronase was added to a final concentration of 100 μg per ml. The mixture was incubated for 2 h at 37°C and then frozen overnight. After thawing, the mixture was treated with an equal volume of phenol equilibrated with 1% SLS-20 mM Tris-buffer (pH 9.0). The separated aqueous fraction was treated with pancreatic RNase for 30 min at 37°C to digest any contaminating RNA. After removing RNase by the phenol treatment, the solution was dialyzed against 0.1 mM EDTA–5 mM potassium phosphate buffer (pH 6.8) and then passed through a Sepharose 2B column equilibrated with the same buffer used for dialysis.

2.2. *Restriction endonucleases*

Restriction endonucleases Hind II, Hin H-I, Hap II, and Hga I were kindly provided Dr M. Takanami of Kyoto Univeristy.

2.3. *Polyacrylamide gel electrophoresis of DNA fragments*

About 100 units of restriction endonucleases were added to a reaction mixture (0.3 ml) containing 7 mM Tris (pH 7.6), 7 mM $MgCl_2$, 7 mM mercaptoethanol and 0.2 A_{260} unit of DNA. After incubation for 4 h at 37°C, the reaction was terminated by adding EDTA to 10 mM. The hydrolysates were layered on 5% gel columns formed in 0.036 M Tris–0.032 M KH_2PO_4–0.1 mM EDTA (pH 7.8) and electrophoresed for 16 h at 2 mM per tube. The gels

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were immersed for 1 h in a solution of 2 g of ethidium bromide per liter in 0.2 M acetic acid and 0.2 M sodium acetate. Excess stain was removed by rinsing the gel with 20% methanol solution overnight.

3. Results and discussion

T. colliculosa mitochondrial DNA was prepared as described in the text. This preparation showed heterogeneous length distribution ranging from 16 S to 30 S which was estimated by sucrose density gradient centrifugation. Whether mitochondrial DNA is cleaved by several restriction endonucleases was investigated by polyacrylamide gel electrophoresis (fig.1). Whereas the DNA preparations treated with restriction endonucleases Hin H-I and Hga I as well as the untreated appeared one broad band at the top of the electrophoregram, the one treated with restriction endonuclease Hap II appeared nine discrete bands although weak background absorbance was also observed. The electrophoretic profile of the DNA digested with endonuclease Hap II indicates that the specific fragments, both termini of which were introduced by the restriction endonuclease, are excised predominantly over unspecific fragments when the slightly and unspecifically degraded DNA preparation is used. The small quantity of the unspecific fragments heterogeneous in length may form the background absorbance. Consequently, the molecular length of the intact mitochondrial DNA can be estimated by totaling the length of each DNA fragment produced by the restriction endonuclease. It was reported that a plot of the logarithm of the DNA length versus the logarithm of its mobility on electrophoresis gave a straight line [10]. The lengths of the mitochondrial DNA fragments were calculated using this correlation. The standard curve was obtained with the fd RF DNA fragments produced by the restriction endonucleases, the lengths of which were calculated by the electron micrography [10]. The results are shown in fig.2 and table 1. It was con-

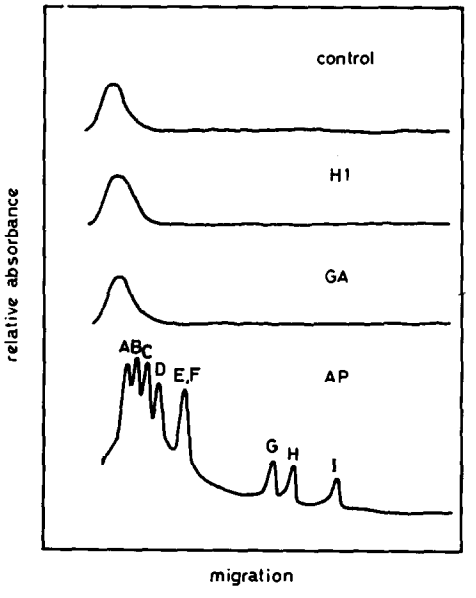

Fig.1. Polyacrylamide gel electrophoresis of *T. colliculosa* mitochondrial DNA digested by restriction endonucleases. Yeast mitochondrial DNA was digested by restriction endonucleases Hin H-I, Hga I, and Hap II as described in the text. The hydrolysates were layered on 5% gel columns and electrophoresed. Gels were stained with ethidium bromide and the color was traced by a densitometer.

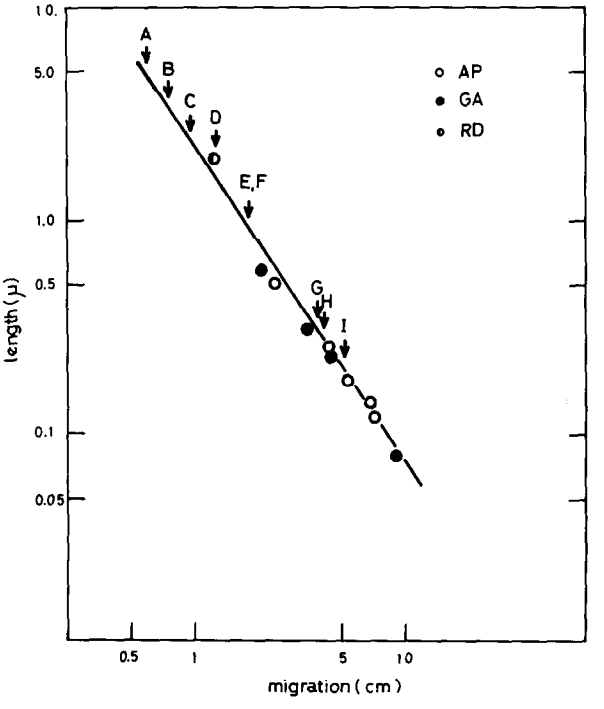

Fig.2. Estimation of molecular length of *T. colliculosa* mitochondrial DNA. The standard curve was obtained with the fd RF DNA fragments produced by the restriction endonucleases Hap II (○), Hga I (●) Hind II (◐). Each arrow shows the migration distance of the mitochondrial DNA fragment produced by the restriction endonuclease Hap II.

Table 1
Molecular length of *T. colliculosa* mitochondrial DNA

Fragment	Length (μm)
A	4.7
B	3.4
C	2.4
D	1.6
E	0.92
F	0.92
G	0.31
H	0.27
I	0.20
Total	14.7 μm

cluded that the length of intact *T. colliculosa* mitochondrial DNA is 15 μm.

The results presented in this paper confirms that the sizes of mitochondrial DNA of petite negative yeasts are grossly smaller than that of *S. cerevisiae*. Restriction endonuclease analysis employed in this report is applicable to establish a physical map of mitochondrial DNA of petite negative yeast. It will be contributive to elucidate the significance of size difference of mitochondrial DNA between petite-negative and -positive yeast strains. In the course of such investigation information on petite formation or even on evolutionary process of mitochondrial DNA may be obtained.

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