THE GENETIC COMPLEXITY OF *SCHIZOSACCHAROMYCES POMBE* MITOCHONDRIAL DNA

Henk F. TABAK and Peter J. WEIJERS

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Eerste Constantijn Huygensstraat 20, Amsterdam, The Netherlands

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1. Introduction

There is a strong conservation in size and genome complexity in mitochondrial DNAs (mtDNAs) of related organisms. Yeasts are an exception to this rule since the mtDNA differs in size and complexity from 50×10^6 daltons in *Saccharomyces cerevisiae* and *S. carlsbergensis* [1] to 22×10^6 daltons in *Kluyveromyces lactis* [2]. There are even indications that in *Schizosaccharomyces pombe* [3] and *Torulopsis glabrata* [4] the mtDNA may be as small as 12.5×10^6 daltons. This would result in a 4-fold difference in mtDNA size within a group of related organisms without apparent reason why this should be so. Evidence is accumulating that the gene products of *Saccharomyces* mtDNA account for not more than 10×10^6 daltons [5] and it is not clear if the 40×10^6 daltons left code for RNA or protein or have a so-called spacer function [6]. A yeast mtDNA of lower genetic complexity may be a simpler tool to study the function of yeast mtDNA and may shed light on the possible functions of spacer or extra DNA, present in *Saccharomyces* mtDNA.

We have measured the molecular weights of restriction endonuclease fragments of *S. pombe* mtDNA. The total molecular weight of the fragments adds up to a genetic complexity of 17×10^6 daltons, somewhat higher than the original value reported by O'Connor et al. [3].

We consider it likely that the DNA characterized by O'Connor et al. is not the mtDNA of *S. pombe*.

2. Methods and materials

S. pombe 972 h^- was obtained from Dr A. Goffeau, Laboratoire d'Enzymologie, Université Louvain, Belgium. Cells were grown overnight at 30°C in 2% yeast extract, 5.8% glucose and 0.9% NaCl (pH 4.5) and harvested at a cell density of 10^8 cells/ml. Spheroplasts and mitochondria were essentially prepared according to procedures described by Goffeau et al. [7]. For treatment with pancreatic deoxyribonuclease mitochondria were resuspended in 0.6 M sorbitol, 10 mM imidazole (pH 6.6), 1 mM EDTA, 0.1% bovine serum albumin and 5 mM $MgCl_2$ and incubated in 100 μg/ml deoxyribonuclease for 10 min at 0°C.

The mitochondrial suspension derived from 30 g of cells was diluted with 20 ml 50 mM Tris–HCl (pH 8), 0.1 M EDTA and lysed by addition of sodium dodecylsulphate to a final concentration of 2%. The lysate was deproteinized by extraction with phenol (containing 14 ml cresol and 0.1 g hydroxyquinoline per 100 g phenol) and chloroform-isoamylalcohol. The aqueous phase was made 2% in Na-acetate and nucleic acids were precipitated by the addition of 2.5 volumes of alcohol. The preparation was stored overnight at −20°C. The precipitate was recovered by centrifugation at −20°C in the Sorvall Hb4 rotor for 15 min at 10 000 rev/min. The pellet was dissolved in 1 ml 0.1 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate (pH 7.0)) and treated with pancreatic ribonuclease (50 μg/ml) at 30°C for 30 min, followed

by pronase treatment (100 μg/ml) in the presence of 0.2% sodium dodecylsulphate for 60 min at 30°C. The mixture was deproteinized by extraction with phenol equilibrated with 50 mM Tris–HCl (pH 8), 1 mM EDTA. The aqueous phase was chromatographed over a Sephadex G-100 column in 0.1 × SSC and the fractions containing high-molecular weight DNA were pooled.

Analytical CsCl equilibrium density gradient centrifugation was performed as described by Borst et al. [8].

Electron microscopy was carried out according to the Kleinschmidt technique [9] with phage PM2 DNA as internal standard. The contour length of phage PM2 DNA was taken to be 3.02 μm [10].

DNA·RNA filter hybridization was performed as described by Tabak et al. [11]. Filters were incubated in 0.5 ml containing 3 × SSC, Qβ RNA (10 μg/ml), 0.2% sodium dodecylsulphate and ^{125}I-labelled *S. carlsbergensis* mtRNA for 40 h at 60°C. Filters were rinsed with 3 × SSC and washed twice with 3 × SSC at 60°C.

Approximately 1 μg mtDNA was incubated for 1 h at 30°C with enough restriction endonuclease to ensure a limit of digestion in 100 μl solutions in the incubation media described for BamI [12], HaeIII [13], HindII and HindIII [13]. After addition of glycerol the DNA fragments were analysed by disc or slab gel electrophoresis in 0.5% agarose as described by Sanders et al. [14].

3. Results

3.1. *Identification of mtDNA*

Mitochondria were isolated by differential centrifugation from a spheroplast lysate. DNA was extracted from this mitochondrial fraction with and without prior treatment of the mitochondria with 100 μg/ml of pancreatic deoxyribonuclease. Both DNA fractions were analysed by equilibrium centrifugation in CsCl in an analytical ultracentrifuge (fig.1). Only the DNA with a density of 1.689 g/cm^3 is protected against degradation and confirms the density reported by Bostock [15] for mtDNA of *S. pombe*. It differs from the result of O'Connor et al. [3] who reported the absence of a density difference between nuclear and mtDNA of *S. pombe*.

Studies on DNA sequence homology between mtDNAs of different yeast strains have shown that the genes coding for mitochondrial ribosomal RNA are conserved in evolution [16]. This feature allows

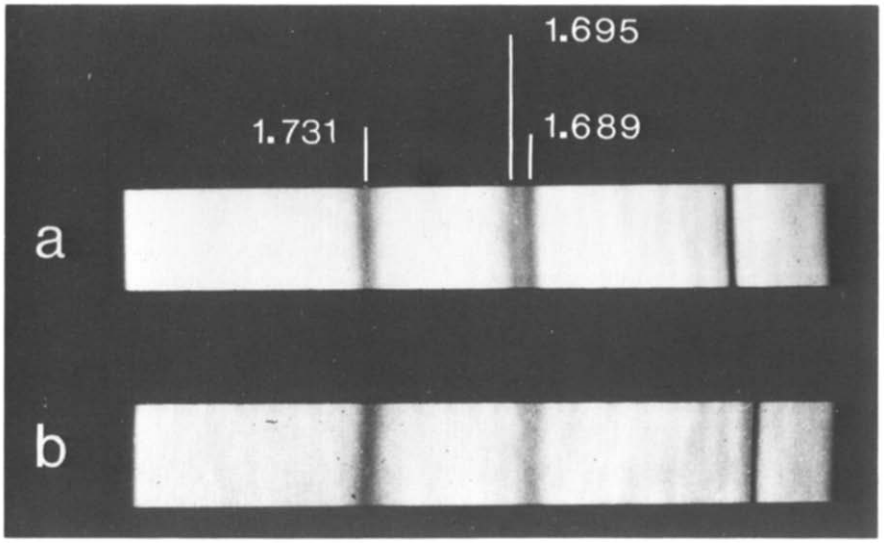

Fig.1. Analytical CsCl equilibrium centrifugation of *S. pombe* mtDNA with *Micrococcus luteus* DNA as reference. A: DNA isolated from mitochondria not treated with deoxyribonuclease. B: DNA isolated from mitochondria treated for 10 min at 0°C with 100 μg/ml of pancreatic deoxyribonuclease before extraction of nucleic acids.

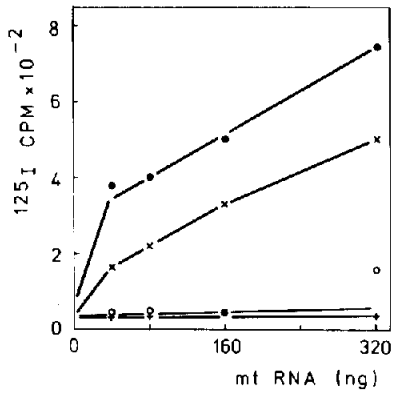

Fig.2. Hybridization of *S. pombe* mtDNA with mtRNA of *S. carlsbergensis*. Filters were loaded with 1 μg of DNA hybridized with in vitro ^{125}I-labelled *S. carlsbergensis* mtRNA as described in Methods. •, mtDNA of *S. pombe;* ×, mtDNA of *S. carlsbergensis;* ○, nuclear DNA of *S. cerevisiae* (petite mutant RD1A); +, DNA of *Escherichia coli*.

Table 1

Fragment number	Molecular weight of restriction endonuclease fragments (base pairs)			
	HaeIII	BamI	HindII	HindIII
1	12 300	17 300	5800	10 425
2	4700	7100	4366	3310
3	2300	2325	3400	3008
4	2000		2783	2585
5	1550		2116	2012
6	1100		1807	1822
7	960		1783	1600
8	900		1557	
9	860		1073	
Total no. of base pairs	26 670	26 725	24 685	24 762

us to identify the mitochondrial nature of the *S. pombe* DNA by its positive hybridization with the well-characterized mtRNA from *S. carlsbergensis*. Figure 2 shows that the *S. carlsbergensis* mtRNA hybridizes even more efficiently to the same amount of *S. pombe* DNA than to the homologous *S. carlsbergensis* mtDNA, which can be explained by the lower complexity of the *S. pombe* mtDNA (see below).

3.2. *Measurement genetic complexity*

The restriction endonucleases BamI, HaeIII, HindII and HindIII or HsuI cleave the mtDNA of *S. pombe* in a number of specific fragments, small enough to be measured by agarose gel electrophoresis against a set of DNA fragments of known size. Restriction endonuclease fragments of *S. pombe* mtDNA were analysed by electrophoresis on slab gels together with the HindII+III fragments of *S. carlsbergensis* mtDNA as standard [14]. In fig.3 the HaeIII DNA fragments are shown together with the HindII+III fragments of *S. carlsbergensis* mtDNA as example. The molecular weights of the *S. pombe* mtDNA fragments obtained with four restriction endonucleases are summarized in table 1. In all cases the total molecular weight of all the fragments of a particular restriction endonuclease digestion is 17×10^6 ($\pm 1 \times 10^6$) daltons. We conclude that the genome complexity of the bulk of the mtDNA of *S. pombe* is approximately 17×10^6 daltons.

4. Discussion

We took interest in the study of the mtDNA of *S. pombe* as a result of the report of O'Connor et al.

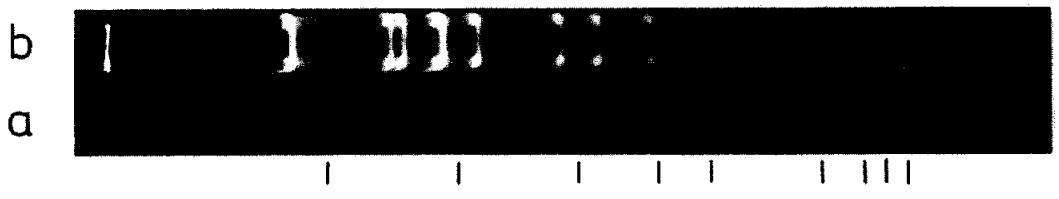

Fig.3. Slab gel electrophoresis on 0.5% agarose of restriction endonuclease fragments of *S. pombe* mtDNA digested with HaeIII and *S. carlsbergensis* mtDNA digested with HindII+III. A: *S. pombe* mtDNA digested with HaeIII. B: *S. carlsbergensis* mtDNA digested with HindII+III.

[3], who isolated intact 6-μm circles from a mitochondrial fraction of *S. pombe* by ethidium CsCl equilibrium centrifugation and suggested that *S. pombe* has the smallest mitochondrial genome found as yet in yeast. We tried to confirm their results by a more detailed analysis.

Mitochondria were carefully isolated from a spheroplast lysate and the DNA isolated from this fraction fulfilled two criteria for a bona fide mtDNA: the DNA with the lower density in CsCl was protected against degradation when mitochondria were treated with pancreatic deoxyribonuclease and the DNA hybridized efficiently with mtRNA of *S. carlsbergensis*. The genetic complexity of the DNA was measured with four restriction endonucleases – BamI, HaeIII, HindII and HindIII. The molecular weights of the fragments were determined by agarose gel electrophoresis with the HindII+III fragments of *S. carlsbergensis* mtDNA as standard. The sum of the molecular weights of the fragments adds up to at least 17×10^6 ($\pm 1 \times 10^6$) daltons. We cannot exclude that the genetic complexity is slightly higher because of loss of small fragments not resolved in the 0.5% agarose gels.

This complexity of 17×10^6 daltons is significantly higher than the complexity of 12.5×10^6 daltons found for the 6-μm circles by O'Connor et al. [3]. Also the density of the DNA in CsCl that we have studied is lower than that of nuclear DNA, as was found by Bostock [15], while the 6-μm circles have the same density in CsCl as nuclear DNA. After extensive screening of mtDNA preparations by electron microscopy we have been able to find only 12 open circular DNA molecules, all having a contour length of 5.98 μm (± 0.62 μm). Since O'Connor et al. do not state which percentage of the total DNA is recovered as intact circles in the ethidium CsCl gradient, we favour the idea that the circles that O'Connor et al. have enriched represent a minor mtDNA species or a DNA contaminating the mitochondrial fraction. This could be analogous to the 2-μm DNA circles of nuclear density that occur in *S. cerevisiae* and can be isolated as contaminants from the mitochondrial fraction.

Our data show that the bulk of the mtDNA of *S. pombe* has a genetic complexity of at least 17×10^6 daltons. This shows that within the yeasts there is nearly a 3-fold difference in genetic complexity of mtDNAs, which remains a remarkable exception to the general rule of size conservation of mtDNA in evolution.

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