

## CHAPTER II.3

# AN ANALYSIS OF THE MITOCHONDRIAL GENOME OF YEAST WITH RESTRICTION ENZYMES

G. BERNARDI, A. PRUNELL and H. KOPECKA

*Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire du C.R.N.S. et de l'Université Paris VII, 2 Place Jussieu, 75005 Paris*

Several years ago we did some physical and chemical investigations on the mitochondrial DNA from both wild-type yeast (*Saccharomyces cerevisiae*) cells and from cytoplasmic "petite" mutants (1-3). On the basis of the results obtained, we thought that the mitochondrial DNA from wild-type yeast cells was characterized by an interspersion of A+T-rich and G+C-rich stretches, and that the "petite" mutation was essentially the result of deletions originating from internal crossing-overs in the A+T-rich stretches, which were visualized as internally repetitive. Subsequent work on the fragments obtained by enzymatic degradation of wild-type mitochondrial DNA showed that this was very heterogeneous in base composition (4-6) and formed, in roughly equal amounts, by "spacers" having a G+C level lower than 5% and by "genes" having an average G+C level of about 32% (7). According to the model mentioned above, the basis for the high frequency of spontaneous "petite" mutation in yeast is the high level of sequence homology in the AT-rich stretches; in addition, deletions not leading to the petite phenotype could be expected to occur. Both predictions could be put to a direct experimental test by studying the fragments released from wild-type and "petite" mitochondrial DNAs by restriction enzymes.

We would like to report here the results obtained by degrading the mitochondrial DNAs from three *S. cerevisiae* and one *S. carlsbergensis* wild-type strains and three petite *S. cerevisiae* mutants. The *S. cerevisiae* wild-type strains used were strains A, B and DM, already used in previous work (1-3); the *S. carlsbergensis* strain was strain C (NCY 74 S) kindly provided by Dr. C. Christiansen. The *S. cerevisiae* petite mutants were strains  $a_1^*$  (D 243-2B-R<sub>1</sub>(A<sub>1</sub>)), provided by Dr. J.C. Mounolou, b and DM<sub>1</sub> already described (1-3); they were derived from wild-type strains A, B and DM, respectively, and had G+C contents equal to 17%, 12% and 4%, respectively. Although four different restriction enzymes were used in our work, namely Eco R<sub>1</sub>, Hind II + III, Hpa II and Hae III, we will restrict this presentation to the results obtained with Hpa II (all abbreviations of restriction enzymes are according to (8)), except where otherwise stated.



Endo R	Hind	HpaII				Hind	EcoRI
	II+III	_____				II+III	
DNA	SV40	A	B	DM	C	SV40	$\lambda$

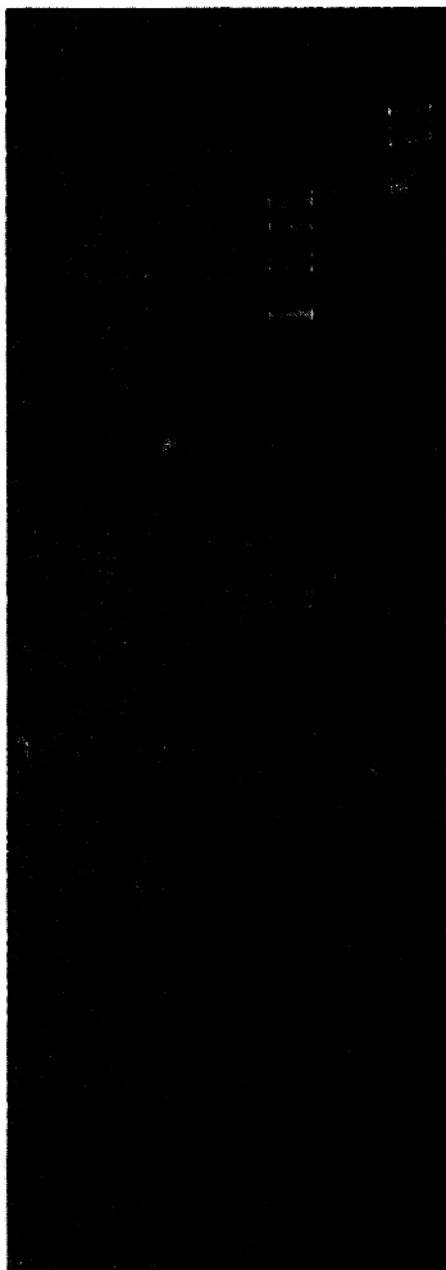


Fig. 2. Band pattern obtained with mitochondrial DNAs as degraded by Hpa II.  $\lambda$  and SV40 DNA digests were used as molecular weight standards. A 2.5% polyacrylamide-0.5% agarose gel was used.

Resolution of the Hpa II fragments was obtained by gel electrophoresis on  $0.4 \times 16 \times 40$  cm slabs of 2 to 6% polyacrylamide containing 0.5% agarose. Microdensitometry of the negative pictures of the gels stained with ethidium bromide permitted very precise estimations of the electrophoretic mobility of the fragments. Molecular weights of the fragments were determined by comparing their  $R_f$  values with those of DNA markers of known molecular weight, mostly restriction enzyme fragments from lambda phage and SV40 DNAs.

The results obtained with the mitochondrial DNAs from the petite strains can be summarized as follows: a) the fragment pattern of the  $a_1^+$  strain DNA was very similar to that of the parent wild-type strain A, but a number of bands were missing or reinforced in the mutant DNA which also showed a few novel bands (fig. 1a, b); b) the fragment pattern of the b strain DNA was characterized by a very small number of bands, compared to the parent wild-type strain B, one of which was amplified a very large number of times (fig. 1c); c) no bands were observed for the DM<sub>1</sub> strain DNA.

The results obtained with the mitochondrial DNAs from the wild-type strains can be outlined as follows: a) a very large number of bands (about 100) could be resolved in all cases; the molecular weights of the fragments ranged from about  $4 \cdot 10^6$  to about  $4 \cdot 10^4$ ; b) differences were found in the band patterns shown by the DNAs from the different *S. cerevisiae* strains; such differences were much larger between the *S. carlsbergensis* and the *S. cerevisiae* strains (fig. 2); c) a number of bands showed twice or three times the expected amount of DNA; in several cases this could be shown to arise from a lack of resolution; in other cases duplications could be responsible for this phenomenon; d) the genome size could be evaluated by summing up the molecular weights of all fragments; values ranging from 47 (C; DM) to 49 (B), to 54 million daltons (A) were found for the DNAs originating from the different strains used; these values are in general agreement with electron microscopic estimates of the physical size of yeast mitochondrial DNA (9).

Since the enzyme used specifically splits the sequence CCGG (10) and since the frequency of such sequence is negligible in the spacers ( $G+C < 5\%$ ) and remarkably lower in the ribosomal genes ( $G+C = 23\%$ ; (11)) compared to the other genes, three main sorts of fragments can be expected: a) fragments formed by the G+C-rich genes; these fragments ought to be the smallest ones, since they have the highest frequency of CCGG; b) fragments of intermediate size, formed by enzyme-resistant spacers flanked by G+C-rich genes; c) fragments derived from the ribosomal genes; these might well be the largest fragments, because the number of sites for the enzyme is very low compared to that of the G+C-rich genes. These expectations are borne out by the composition of the fragments as resolved by Sepharose 2B columns (fig. 3).

In conclusion, we have obtained for the first time direct evidence for the deletions and amplifications characterizing the mitochondrial DNAs from

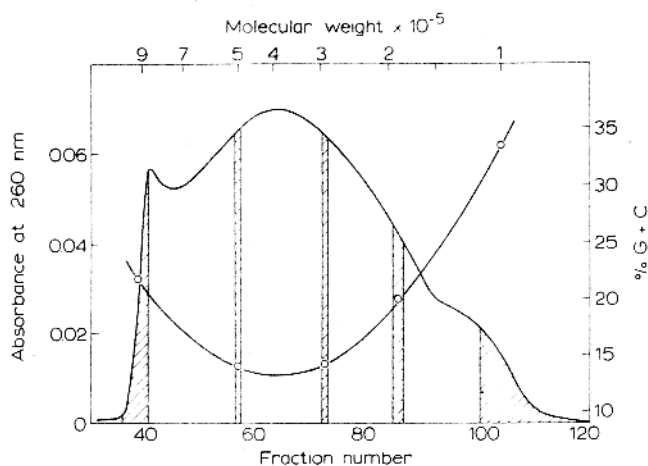


Fig. 3. Sepharose 2B chromatogram of strain B mitochondrial DNA as degraded by Hpa II. Hatched areas indicate the fractions pooled for the base composition.

the petite mutants; novel bands, arising as a result of elimination or insertion of sites recognized by the enzymes, were also found; the results with DM<sub>1</sub> DNA can be explained by the enzyme specificity.

Remarkable differences in nucleotide sequences of the mitochondrial DNAs from different *S. cerevisiae* strains and from the *S. carlsbergensis* strain are reported here for the first time. Fragment patterns are too different from each other to have been originated to more than a minor extent by point mutations at the cleavage sites. We suggest that the differences in nucleotide sequences of DNAs from different wild-type strains have the same molecular basis (deletions, amplifications) as the petite mutation. The significant differences in genome size of the different mitochondrial DNAs support this conclusion.

## References

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Dr. SCHATZ:

What is the effect of glucose repression on the cleavage patterns?

Dr. BERNARDI:

This point has not been tested yet.

Dr. SCHATZ:

Was the digestion by restriction enzymes complete?

Dr. BERNARDI:

Yes. This was tested by studying cleavage patterns at different enzyme concentrations.

Dr. SCHATZ:

Can recombinant molecules be shown by this method?

Dr. BERNARDI:

I think so. We plan to check this point.