THE CYTOPLASMIC "PETITE" MUTATION IN SACCHAROMYCES CEREVISIAE

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INTRODUCTION

Several years ago it was shown, (1,2), that the mitochondrial DNAs from two genetically unrelated, acriflavine-induced, cytoplasmic "petite" mutants of Saccharomyces cerevisiae had a grossly altered base composition, (GC = 4 %), compared to the DNAs from the parent wild-type cells, (GC = 18 %). These findings unequivocally established that massive alterations in the nucleotide sequences of the mitochondrial genome may accompany the "petite" mutation, and be responsible for it.

Subsequent investigations, (3), showed that the mitochondrial DNAs from three different, spontaneous, suppressive "petite" mutants : 1) had GC levels lower, to different extents, (13.0-16.8 %), than the DNAs from the parent wild-type strains ; 2) had melting profiles which contained the main, low-melting component of DNAs from wild-type cells, but had lost, again to different extents, high-melting components ; 3) renatured very rapidly. These results demonstrated that cytoplasmic suppressive "petite" mutants had defective mitochondrial genomes in which large segments of the parental wild-type genomes had been deleted. Four years ago, direct evidence was provided for both the deletions (4), and the accompanying sequence amplifications (4,5).

On the basis of our early results (3), we proposed that the deletions in the mitochondrial genomes of "petite" mutants arise by an excision mechanism of the Campbell type , involving site-specific, illegitimate recombination events in the AT-rich spacers of mitochondrial DNA. These spacers, (3,6), form 50 % of the mitochondrial genome, have a GC content lower than 5 %, contain short repetitive nucleotide sequences, and are likely to be endowed with some sequence homology over stretches long enough as to allow recombination,(7-10).

The subsequent discovery (11), of other, shorter, mitochondrial DNA segments also endowed with some extent of sequence homology, the (CCGG, GGCC) clusters, raised the possibility that the site-specific, illegitimate recombination events, supposed to underlie the excision mechanism originating the defective genomes of "petite" mutants, could take place not only in the AT-rich spacers, but also in the latter sequence elements. In either case, an interesting aspect of the model outlined above is that it attributes the instability of the mitochondrial genome of yeast, (the spontaneous "petite" mutation has a rate of 1-5 % per generation), to the existence in each genome unit of a number of nucleotide sequences endowed with enough homology as to allow site-specific, illegitimate recombination events to take place.

In the present work, we have investigated the molecular mechanism underlying the "petite" mutation by studying in some detail the organization of the mitochondrial genome of several "petite" mutants.

RESULTS

The experimental work essentially consisted in studying the restriction enzyme degradation of three "petite" genomes of low complexity and their hybridization on restriction fragments from the corresponding parental genomes; in addition, we studied the restriction patterns of one heterogeneous population of "petite" genomes and of

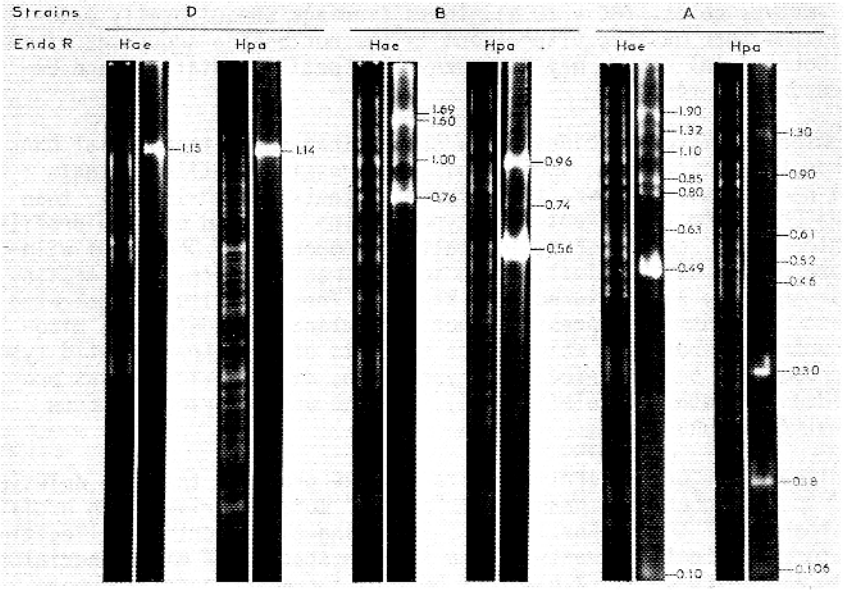

Fig. 1. Hybridization patterns of mitochondrial DNAs from strain d, b and a on Hpa II and Hae III restriction fragments of mitochondrial DNAs from parental wild-type strains D, B and A.

some of its sub-clones.

Strain d. The mitochondrial DNA from this acriflavine-induced "petite" had a GC content of only 4 % (1). No degradation could be obtained with any of 9 restriction enzymes tested : Eco RI, Hind II + III, Hpa II, Hae III, Hha I, Taq I, Mbo I, Hinf I, Alu I; (Hpa II and Hae III will be indicated as Hpa and Hae hencefrom). The DNA from strain d hybridized to 1 Hpa band of $1.14.10^6$ and 1 Hae band of $1.15.10^6$ of the DNA from the parent strain D (fig. 1).

Strain b. Only 2 of the 9 restriction enzymes tested (see above), Hpa and Alu I, were able to split this mitochondrial DNA (GC=13.0 %; 3). Both enzymes released a single fragment, having a molecular weight of $5.6.10^5$ (fig. 2). Partial digests were characterized by a series of fragments having molecular weights which were exact multiples, (within 1 %, namely within 8 base pairs), of the basic unit of $5.6.10^5$, (4). A double hydrolysate, Hpa + Alu I, produced a fragment 3.10^4 smaller in molecular weight than the basic unit of $5.6.10^5$. The results (fig. 2) clearly show that the genome of

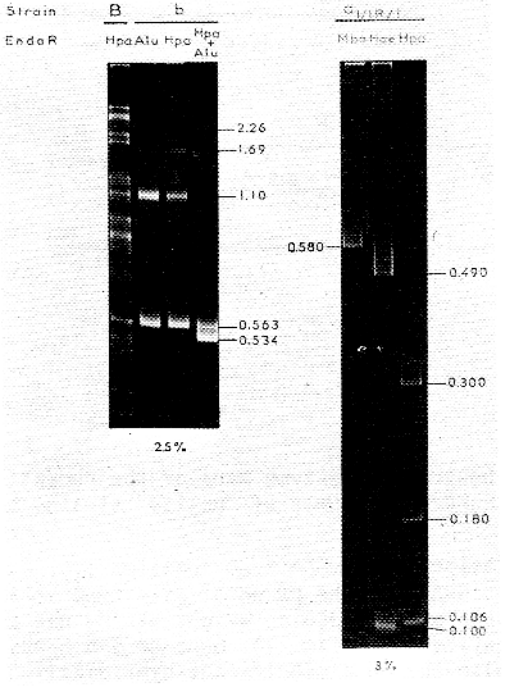

Fig. 2. Restriction patterns of mitochondrial DNAs from "petite" strains b and $a_{1/1R/1}$. Restriction endonucleases and polyacrylamide concentrations are indicated as well as the molecular weights (in millions) of the bands.

"petite" strain b is made up of a tandem repetition of the $5.6.10^5$ basic unit ; the very simple restriction map of such unit is shown in fig. 3. The DNA from "petite" strain b mainly hybridized to 1 Hpa band, MW = $5.6.10^5$, and 1 Hae band, MW = $7.6.10^5$, of the DNA from the parental strain B. A weaker hybridization on 2 (or 3) other Hpa and 3 other Hae bands was also founds, as well as a very weak hybridization on other bands (fig. 1).

Strain $a_{1/1R/1}$. Only 3 out of 19 restriction enzymes tested, (those mentioned above plus Bgl I, Bgl II, Sma I, Bam HI, Hpa I, Kpn I, Pst I, Sal I, Xba I, Xho I), were able to degrade this mitochondrial DNA. This was split by Mbo I into a single fragment of $5.80.10^5$, by Hae into 2 fragments of $4.9.10^5$ and $0.98.10^5$, and by Hpa into 3 fragments of $3.0.10^5$, $1.80.10^5$ (fig. 2). A study of partial and double digests led to the restriction map of the basic unit shown in fig. 3.

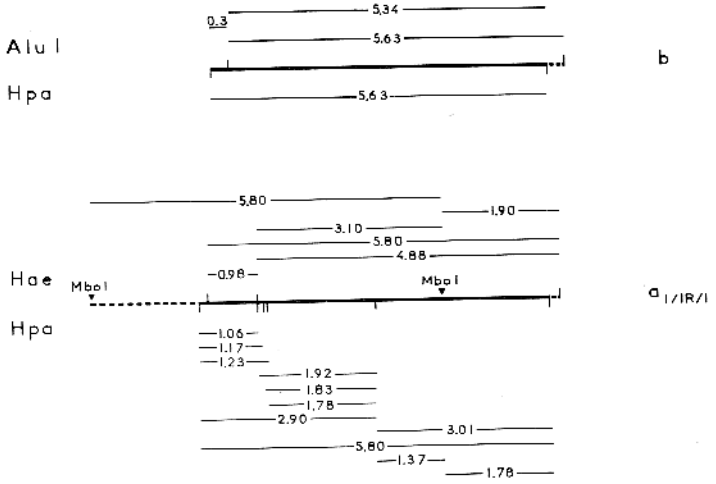

Fig. 3. Restriction enzyme maps of the repeating units of the mitochondrial genomes of "petite" strains b and $a_{1/1R/1}$.

The main hybridization of the DNA from "petite" $a_{1/1R/1}$ onto Hae and Hpa digests of the DNA from the parental wild-type strain A was on fragments having the same molecular weight as the Hae and Hpa fragments from the "petite" DNA (fig. 1). Another 5-6 bands in both Hae and Hpa digests also showed a significant hybridization.

Strain a_1^* and its sub-clones. The Hae and Hpa patterns of the mitochondrial DNA from strain a_1^* (fig. 4) was characterized by the following features : 1) roughly, one third of the bands from strain A were missing ; this corresponds to the deletion of about 27 millions of wild-type DNA. 2) Almost all bands had mobilities identical to those of the DNA from the parent strain A ; in fact, only

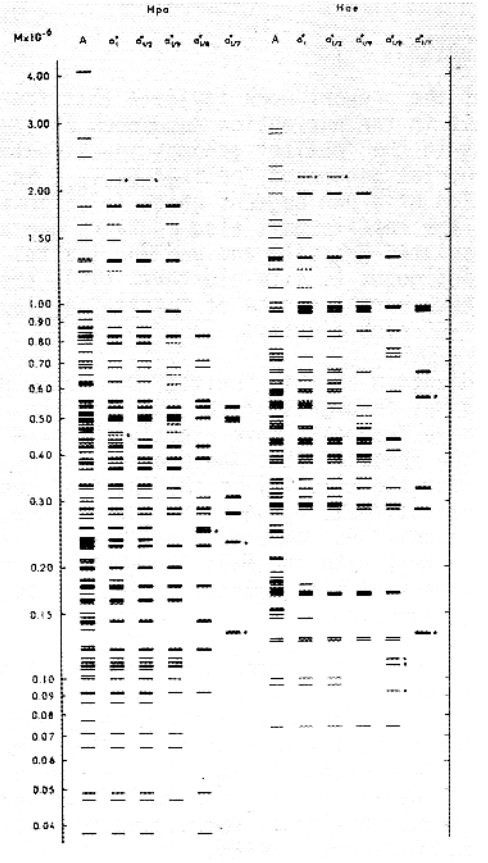

Fig. 4. Scheme of Hae III and Hpa II band patterns of mitochondrial DNAs from strains A, a_1^* and its sub-clones. Different line thicknesses indicate different band intensities ; broken lines indicate faint bands. Asteriks indicate bands not present in the DNA from the parental wild-type strain.

1 Hpa band, out of 53, and 1 Hae band, out of 42, had different mobilities, 3) some bands had higher intensities, some had weaker intensities than the corresponding bands from strain A.

The restriction patterns of four subclones of strain a_1^*, (fig. 4), as well as their different suppressiveness, confirm the heterogeneity of the mitochondrial genome in strain a_1^* indicated by its restriction pattern. All or almost all the bands present in a given sub-clone represented a sub-set of the bands of a_1^* ; very few bands

not present in A nor a_1^*, were also present.

DISCUSSION

The findings of the present work indicate that four different situations exist in the nucleotide sequences at the ends of the repeating units in the "petite" genomes investigated here.
i. The mitochondrial genome of "petite" $a_{1/1R/1}$ is formed by the tandem repetition of a DNA segment which is delimited by two Hae-Hpa clusters ; the repeats unit also contains one additional Hae-Hpa cluster, one isolated Hpa site and one Mbo I site. If the GC-rich clusters are contiguous to site clusters, (11), then the repeat unit of the mitochondrial genome of "petite" $a_{1/1R/1}$ should also contain several GC-rich clusters.

The main hybridization of this "petite" DNA takes place on 2 Hae fragments and 3 Hpa fragments of the DNA from the parental wild-type cells ; these fragments have exactly the same size as those obtained from the "petite" DNA. The Hae and Hpa fragments of the latter account for the totality of the "petite" genome, (except for joining oligonucleotides), as shown by the absence of other bands on the gels under conditions where the smallest double-stranded fragment, (stoichiometric with the main fragments), could be detected, and also by the partial hydrolysates, which can be accounted for in terms of known fragments.

These findings strongly indicate that excision of the repeating unit of $a_{1/1R/1}$ from the parental wild-type genome did take place at two Hae-Hpa site clusters. The most likely primary mechanism in this case can be considered to be a site-specific, illegitimate recombination at two Hae-Hpa site clusters located on the same or on two different genome units. In the first case, the nucleotide sequences involved in the process should basically correspond to inverted repeats ;in the second case, to direct repeats.

The Hae-Hpa site clusters, (11, 12), are present in the wild-type genomes in a large number, about 60 ; are scattered all over the genome ; and contain a number of symmetrical sequences, (the Hae and Hpa sites GGCC and CCGG), which might themselves be arranged symmetrically in the clusters. We suggested, (11), that these sequences, as well as the contiguous GC-rich clusters, might have sufficient sequence homology as to permit site-specific, illegitimate recombination. The spurious hybridization of the DNA from $a_{1/1R/1}$ with fragments of wild-type DNA which do not correspond to the excised segment is indicative, in our opinion, of the existence of such partial homology.

If one recalls that almost all Hae sites in the DNAs from wild-type cells are present in Hae-Hpa clusters (11), the presence of only very few "new" Hae bands namely of Hae fragments having different sizes compared to the corresponding parental ones in the DNAs from heterogeneous populations of spontaneous "petites" (fig. 4) very

strongly suggests that the situation found for $a_{1/1R/1}$ is the most current one.

ii. The case of the genome from "petite" mutant b differs from the previous one in that the repeat unit has the same length as a Hpa fragment of the parental, wild-type genome, but is considerably shorter, (by 2.10^5), than the corresponding Hae fragment. In this case, the sequences used in the recombinational process leading to the excision may be two GC-rich clusters, which are apparently contiguous to Hpa sites (or site clusters), as indicated by Prunell and Bernardi (11).

iii. Another situation is represented by "petite" genomes which are either formed by tandem repeats of "new" bands or by tandem repeats of "old" and "new" bands. In this case, the sites involved in the primary recombination event are different from Hae or Hpa sites. In this case we favor the idea that the sites different from the Hae-Hpa cluster are located on spacer sequences because of the results of Fonty et al. (13). This case is different from the previous one since none of the two ends of the repeat unit of b DNA originates from a spacer region (unpublished results).

iv. The last case is that of "petite" mutant d. The hypothetical repeat unit carries in this case either the totality or a fraction of the seryl-t-RNA gene (14) ; no Hae or Hpa sites are present ; over 90 % of the hypothetical repeat unit is made up of spacer sequences, as judged from results obtained with micrococcal nuclease digests. It is evident that in this case, if the original event was one of those postulated above, considerable sequence rearrangements have occurred ; alternatively, the original event was different from those just described. In any case, it is very significant, in our opinion, that this "petite", in contrast with the other ones already considered, is the result of mutagenization with acriflavine. It is well known that the tremendous increase in "petite" formation upon mutagenization, (from a few percent to 100 % per generation), is accompanied by a fragmentation of the wild-type DNA, (15). Investigations carried out in other laboratories concerning "petite" mutants induced by ethidium bromide confirm the idea that such "petites" fall into more complex excision patterns compared to those found here for the genome of $a_{1/1R/1}$.

REFERENCES

1. Bernardi G., Carnevali F., Nicolaieff A., Piperno G. and Tecce G. (1968) J. Mol. Biol., 37. 493-505

2. Mehrotra B.D. and Mahler H.R. (1968) Arch. Biochem. Biophys., 128. 685-703

3. Bernardi G., Faurès M., Piperno G. and Slonimski P.P. (1970) J. Mol. Biol., 48. 23-42

4. Bernardi G., Prunell A. and Kopecka H. (1975)
in Molecular Biology of Nucleocytoplasmic Relationships
(Puiseux-Dao S., ed.), pp. 85-90, Elsevier, Amsterdam.

5. Locker J., Rabinowitz M. and Getz G.S. (1974)
Proc. Nat. Acad. Sci. USA., 71. 1366-1370

6. Bernardi G. and Timasheff S.N. (1970)
J. Mol. Biol., 48. 43-52

7. Bernardi G., Piperno G. and Fonty G. (1972)
J. Mol. Biol., 65. 173-190

8. Piperno G., Fonty G. and Bernardi G. (1972)
J. Mol. Biol., 65. 191-205

9. Ehrlich S.D., Thiery J.P. and Bernardi G. (1972)
J. Mol. Biol., 65. 207-212

10. Prunell A. and Bernardi G. (1974)
J. Mol. Biol., 86. 825-841

11. Prunell A. and Bernardi G. (1977)
J. Mol. Biol., 110. 53-74

12. Prunell A., Kopecka H., Strauss F. and Bernardi G. (1977)
J. Mol. Biol., 110. 17-52

13. Fonty G., Goursot R., Wilkie D. and Bernardi G. (1978)
J. Mol. Biol., 119. 213-235

14. Carnevali F., Falcone C., Frontali L., Leoni L., Macino G.
and Palleschi C. (1973)
Biochem. Biophys. Res. Comm., 51. 651-658

15. Goldring E.S., Grossmann L.I., Krupnick D., Cryer D.R. and
Marmur J. (1970) J. Mol. Biol., 52. 323-335.