PUTATIVE ORIGINS OF REPLICATION IN THE MITOCHONDRIAL GENOME OF YEAST

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1. Introduction

The mitochondrial genomes of cytoplasmic spontaneous 'petite' mutants of *Saccharomyces cerevisiae* arise by a mechanism in which a segment of a mitochondrial genome unit of a wild-type cell is excised and tandemly amplified (reviewed [1]; fig.1). Excised segments may originate from different regions of the wild-type genome unit, may have different sizes, and are highly conserved in sequence [2]. It has been suggested that, in the case of spontaneous 'petite' mutants, such segments contain an origin of replication, implying that the wild-type genome unit contains several origins of replication [3]. In agreement with this suggestion, our recent work [4] has shown that 3 'petite' mutants (which we call supersuppressive) have mitochondrial genomes characterized by the following features:

(i) They are selectively transmitted to the progeny in crosses with wild-type cells;
(ii) They are made up of very short repeat units (400–900 basepairs);
(iii) They share a sequence of ~ 80 nucleotides, in spite of the fact that they derive from two different regions of the parental wild-type genome.

These results have been interpreted as indicating that the common sequence corresponds to an origin of replication; if this is so, the multiple copies of the origin of replication in supersuppressive genomes explain why these can compete out those of wild-type cells and become the only ones present in the progeny.

Here we have shown that the common 80 nucleotide sequence exhibited by the 3 supersuppressive 'petites' in [4] also exists in the genome of

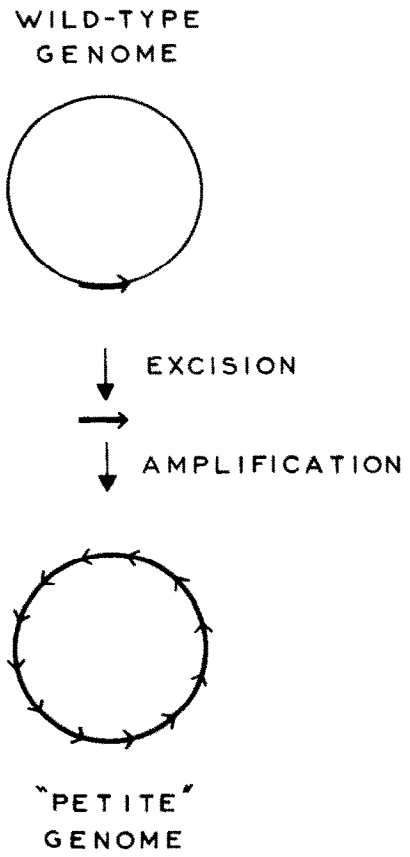

Fig.1. Scheme of the process leading to the formation of spontaneous 'petite' genomes. A segment of the mitochondrial genome unit from wild-type yeast cell is excised and tandemly amplified to yield the mitochondrial genome unit of a 'petite' mutant. The excised segment from the wild-type genome becomes the repeat unit of the 'petite' genome. This may in turn undergo further deletions leading to secondary 'petite' genomes having simpler repeat units.

another 'petite' $a^*_{1/7/8}$, originating from yet another region of the parental wild-type genome.

2. Materials and methods

The restriction maps of the repeat units of the mitochondrial genomes under consideration are given in fig.2; both these 'petite' genomes and the genomes of their parental wild-type strains (A,B) have been extensively investigated [2,5]. The regions of wild-type strain genomes from which the petite genomes of fig.2 originated are indicated in fig.3. The methods used have been detailed in [2,5].

3. Results and discussion

The common 80 nucleotide sequence present in the mitochondrial genomes of petites $a_{1/1R/1}$, $a_{1/1R/Z1}$ and b was found [4] to be centered around a very characteristic GC cluster made up of 3 partially

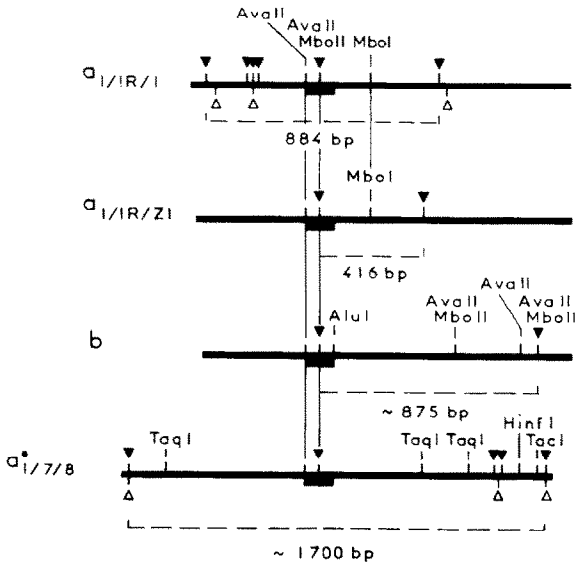

Fig.2. Restriction enzyme map of the repeating units of the mitochondrial genomes of the spontaneous petite mutants considered in the present work. The molecular weights of the repeat units are indicated along with the positions of *Hae* III (△), *Hpa* II (▼) and other restriction sites. The vertical lines indicate corresponding restriction sites in different repeat units.

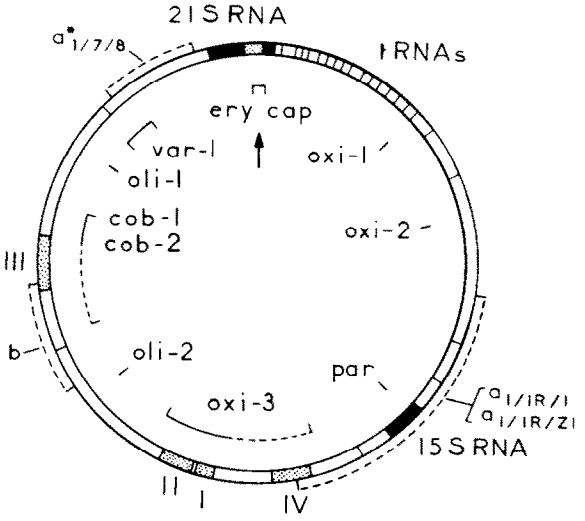

Fig.3. Regions of excision of the 'petite' genomes considered here are indicated on a general map of the mitochondrial genome of *Saccharomyces cerevisiae* (the map is adapted from [11]).

overlapping sequences which are split by *Hpa* II, *Mbo* II and *Ava* II; it should be noted that the recognition site of *Mbo* II is a pentanucleotide located 8 basepairs away from the splitting site (fig.4). Our first step was, therefore, to look for such a GC cluster in the mitochondrial genome of $a^*_{1/7/8}$. Such a cluster having been identified around the *Hpa* II site located in the center of the map shown in fig.2, the two flanking sequences were determined using the technique in [6].

The results so obtained (fig.4) indicate that the repeat unit of $a^*_{1/7/8}$ also contain the 80 nucleotide sequence previously found in the other 3 petite genomes. Several features of the sequence are worth mentioning:

(i) On the left border, patchy homology with the sequence of $a_{1/1R/1}$ taken as an arbitrary reference is found between positions 16–31;

(ii) The long central region (positions 32–109) is characterized by perfect homology, except for 4 basepair changes which are clustered in the hexanucleotide (62–67) separating the large AT palindrome (39–61) from the GC cluster on its right; interestingly, the only basepair change found in $a_{1/1R/Z1}$ is identical with one of these changes;

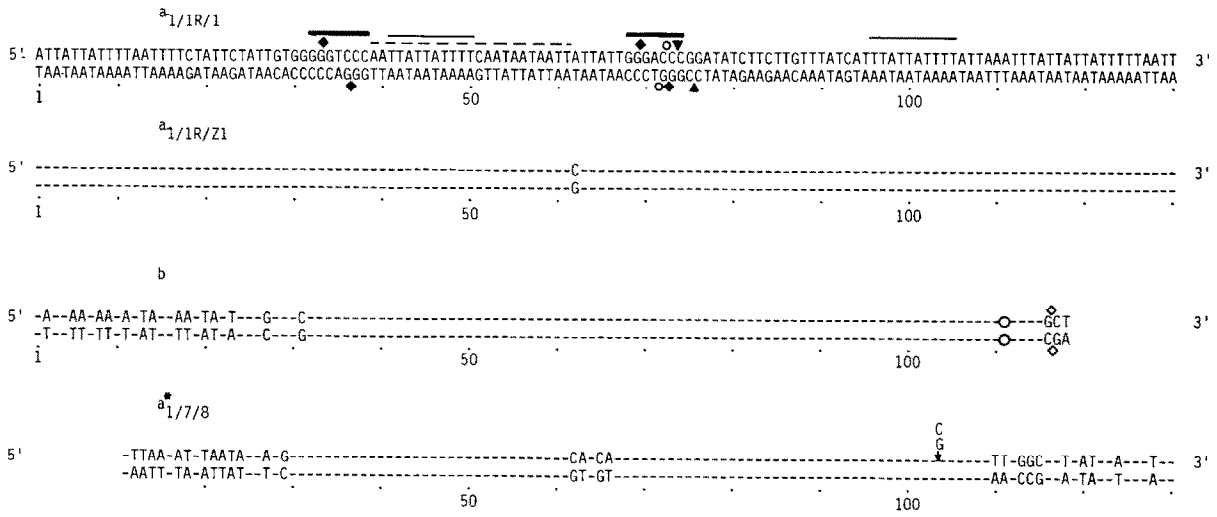

Fig.4. Primary structure of the mitochondrial genome of $a_{1/1R/1}$ between nucleotides 340 and 470 [12], of the corresponding segment of $a_{1/1R/Z1}$ [13], and of the region around the *Hpa* II, *Mbo* II and *Ava* II clusters of the repeat units of b [4] and $a_{1/7/8}$ (this work). Dashes indicate nucleotide identical to those found in $a_{1/1R/1}$ (taken as a reference). Symbols represent: *Hpa* II sites (▼); *Mbo* II sites (○); *Ava* II (♦); *Alu* I (◇). One basepair deletion in b is indicated by a double empty circle and one basepair addition in $a^*_{1/7/8}$ is indicated by an arrow. The 23 basepair palindrome is indicated by a broken line, the 7 basepair inverted repeats by heavy lines, the 10 basepair direct repeat by thin lines.

(iii) On the right border, a C:G basepair insertion is found after position 103 and homology becomes patchy again between positions 110 and (at least) 130.

The finding of the 80 nucleotide sequence in the mitochondrial genome of $a^*_{1/7/8}$ strongly suggests that this sequence is also present in the genomes of $a^*_{1/7/12/1}$ and $a_{3/1}$ (see [2]) since these encompass that of $a^*_{1/7/8}$. If this is the case, then all 6 spontaneous petite genomes studied by us [2] contain the sequence under consideration, in spite of their origin from 3 distinct regions of the parental wild-type genome. Interestingly, one of these petites (b) originated in fact from a different parental wild-type strain (strain B) compared to all the others which originated from strain A; its DNA showed, however, the same hybridization pattern on restriction fragments from the mitochondrial DNAs of both B and A.

No direct evidence is available yet to prove that the 80 nucleotide sequence corresponds to an origin of replication of mitochondrial DNA. The indirect evidence is, however, very strong and rests on the following facts:

(i) The regions of origin of the 4 'petite' genomes of fig.2, although in need of a better definition, appear to coincide with the regions from which spontaneous 'petites' arise with the highest frequency [7]; obviously, the simplest explanation for these preferential regions of excision is that they contain origins of replication;

(ii) Three of the 4 'petites' are supersuppressive, as already mentioned ($a^*_{1/7/8}$ has a suppressivity of 88% [2] and is being tested for supersuppressivity); again the simplest explanation for this phenomenon is that multiple replication origins are present in these 'petite' genomes.

If the 80 nucleotide sequence is indeed an origin of replication, as is highly probable, each mitochondrial genome unit of wild-type cells contains at least 3 such origins.

It is of interest to note that a 'petite', RD1A, is known [8] which has a mitochondrial genome mostly formed by short repeat units of 68 nucleotides, having nothing in common with the 80 nucleotide sequences discussed here. This 'petite' is neutral [9]; its cross with wild-type cells yields a purely wild-type progeny and is the result of a drastic ethidium bromide treat-

ment, a procedure known to cause degradation of mitochondrial DNA which may be followed by sequence rearrangements [10]. We suggest that this 'petite' genome does not carry an origin of replication per repeat unit, but carries instead an origin of replication per genome unit, probably as the result of a translocation, and that for this reason its genome is competed out by the wild-type genome in crosses.

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