EXCISION AND REPLICATION OF MITOCHONDRIAL GENOMES FROM SPONTANEOUS PETITE MUTANTS OF YEAST

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ABSTRACT

The sequences involved in the excision of mitochondrial genomes of spontaneous petite mutants of yeast from the genomes of wild-type cells have been found to correspond to (CCGG, GGCC) clusters and to sequences in the AT spacers. In addition, results have been obtained on nucleotide sequences which are likely to correspond to the origin of replication of the mitochondrial genome.

It is now well established that the mitochondrial genome of spontaneous cytoplasmic petite mutants of Saccharomyces cerevisiae originate from those of parental wild-type cells by a mechanism involving a) the excision of a segment of the latter genomes, and b) its subsequent tandem amplification, as shown in fig. 1; the repeat units of the petite genomes so formed may in turn undergo further deletions leading to secondary petite genomes having shorter repeat units (see Bernardi, 1979, for a brief review). Ten years ago, the excision mechanism was considered to be due to illegitimate, site-specific recombination events in the long AT-spacers (forming 50 % of the mitochondrial genome) which were supposed to contain sequence repetitions. The subsequent discovery in the mitochondrial genome of yeast of many short segments extremely rich in GC, the GC clusters, (several of which were likely to be homologous in sequence), raised the possibility that these sequences, later shown to be embedded in the AT spacers, were also involved in the recombination phenomena underlying the excision process. In any case, the basic idea of the model was that the instability of the mitochondrial genome of yeast, (the spontaneous petite mutation has a rate of 1-5 % per generation in most strains), was due to the existence in each mitochondrial genome unit of a number of nucleotide sequences having enough homology to allow illegitimate site-specific recombination to take place.

We present here recent results concerning the nucleotide sequences involved in the excision of the mitochondrial genomes of spontaneous petites. In addition we present data on nucleotide sequences which are likely to correspond to the origin of replication of the mitochondrial genome.

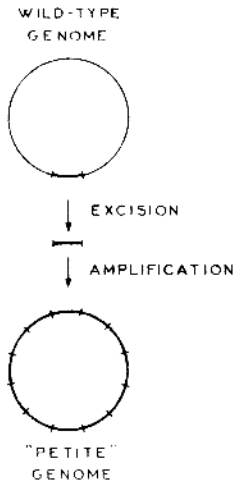

Fig. 1. Scheme of the process leading to the formation of spontaneous petite genomes. A segment of the mitochondrial genome unit from wild-type yeast cells is excised and amplified to yield the mitochondrial genome unit of a petite mutant. The excised segment from the wild-type genome becomes the repeat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

Restriction mapping and hybridization of mitochondrial genomes from spontaneous petites on restriction fragments from parental wild-type genomes (Faugeron-Fonty et al.,1979) have shown that the petite genomes belong in two classes. In the first case, the repeat units appear to be delimited by (CCGG, GGCC) clusters (one of the two classes of GC clusters ; see Prunell and Bernardi, 1977); in the second case, by other sequences. Petite genomes of the first class are exemplified by $a^{*}_{1/7/8}$ and $a_{1/1R/1}$ (fig. 2); these have been shown to hybridize on parental wild-type Hae III or Hpa II fragments which have the same size as the petite Hae III or Hpa II fragments (fig. 3), thus demonstrating the identity of petite genome fragments with fragments from the parental wild-type genomes. Petite genomes of the second class are exemplified by $a_{1/1R/Z1}$ and by b (fig. 2). The first of these genomes does not hybridize on a Hpa II parental fragment corresponding to the petite Hpa II fragment, but it

does so on the two largest Hpa II fragments of $a^{*}_{1/1R/1}$ (fig. 3). Incidentally, this hybridization is due to GC clusters, since it is not altered by the removal of AT spacers from this genome (fig. 3); GC clusters also appear to be responsible for the "spurious" hybridizations shown by all these petite genomes. Petite b also belongs in this second class, in spite of the fact that it hybridizes on Hpa II fragment(s) from parental wild-type genome apparently having the same size as the petite Hpa II fragment (results not shown; this conclusion is based on sequence data to be published elsewhere).

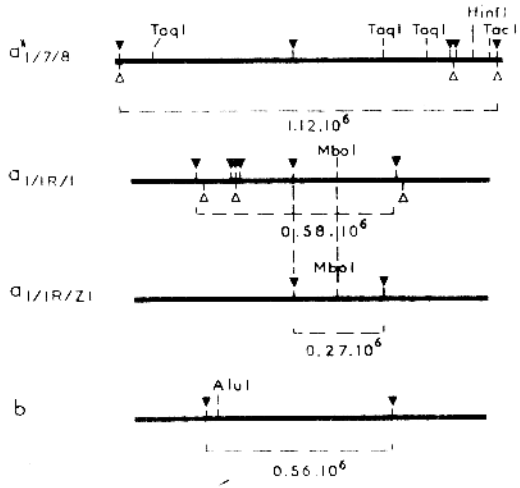

Fig. 2. Restriction enzyme maps of the repeating units of the mitochondrial genomes of four spontaneous petite mutants $a^{*}_{1/7/8}$, $a_{1/1R/1}$, $a_{1/1R/Z1}$, and b. The molecular weights of the repeat units are indicated, along with the positions of Hae III (Δ), Hpa II (▼) and other restriction sites.

Evidence exists for the sequence homology of several (CCGG, GGCC) clusters (Cosson and Tzagoloff, 1979; Macino and Tzagoloff, 1979 ; Gaillard et al.,1979), so that petite genomes of the first class are extremely likely to fit with the excision model discussed above. In contrast, no indications are available concerning the sequences involved in the excision of petite genomes of the second class. We decided therefore to investigate these genomes. The obvious choice was to sequence the repeat unit of $a_{1/1R/1}$, since several lines of evidence suggested that this contained the repeat unit of $a_{1/1R/Z1}$ and its flanking sequences : a) both petite genomes were originally present in the same spontaneous heterogenous petite $a_{1/1R}$, from which they could be isolated by subcloning; b) they originated from the same region (map positions 27 to 46 of Sanders et al., 1977) of the parental wild-type genome; c) they shared a

Hpa II - Mbo I fragment (fig. 2) and they showed overlapping hybridization patterns.

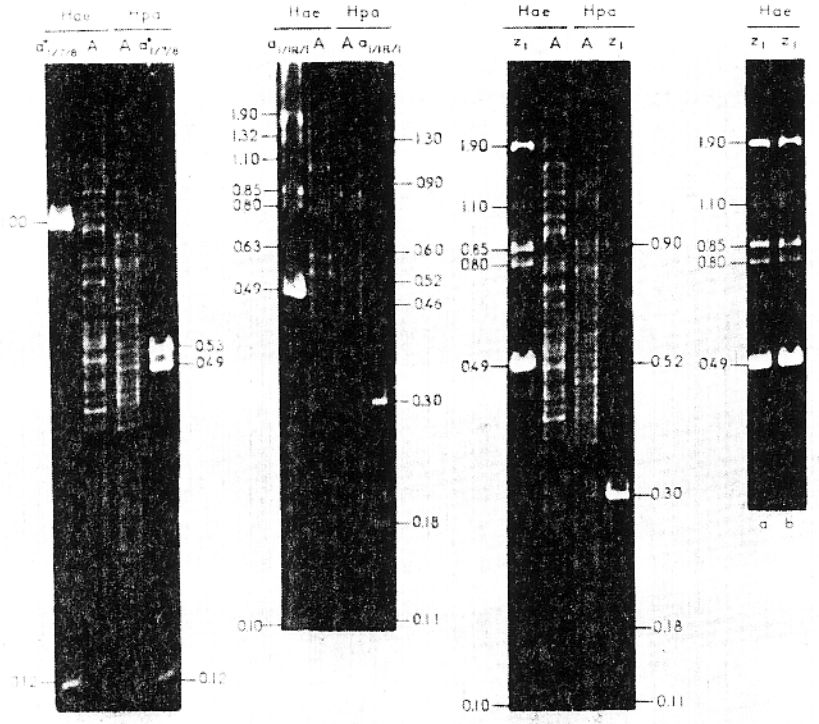

Fig. 3. Hybridization patterns of mitochondrial DNAs from petite mutants $a^{*}_{1/Z/8}$, $a_{1/1R/1}$, and $a_{1/1R/Z1}$ on Hae III and Hpa II fragments of the mitochondrial DNA from the wild-type strain A. Restriction enzymes, strains, and molecular weights ($x10^{-6}$) of the restriction fragments are indicated. Z1 stands for $a_{1/1R/Z1}$. a and b correspond to the hybridization of $a_{1/1R/Z1}$ DNA digested with micrococcal nuclease up to 33 % and 66 % degradation, respectively, to Hpa II fragments of DNA from strain A.

The 884 nucleotide sequence of the repeat unit of $a_{1/1R/1}$ (fig. 4) was indeed found (Gaillard et al., 1979) to contain a 416 nucleotide segment which is identical to that (previously sequenced by Gaillard and Bernardi, 1979) of $a_{1/1R/Z1}$, except for two base pair changes. The most important feature of the sequence of $a_{1/1R/1}$ is that the initial nonanucleotide of the repeat unit of $a_{1/1R/Z1}$, AATAATATA, is repeated just after the end of the repeat unit; if allowance is made for one A:T to T:A change, these direct repeats are in fact 13 nucleotides long. This situation is reminiscent of those found on both sides of insertion sequence IS1 and of transposon Tn9 in E.coli

(Carlos et al.,1978; Grindley, 1978; Johnsrud et al., 1978) and raises the possibility that segments like $a_{1/1R/Z1}$ can be transposed onto other mitochondrial genome units and so contribute to the changes in length observed in genome units belonging to different strains (Prunell et al., 1977).

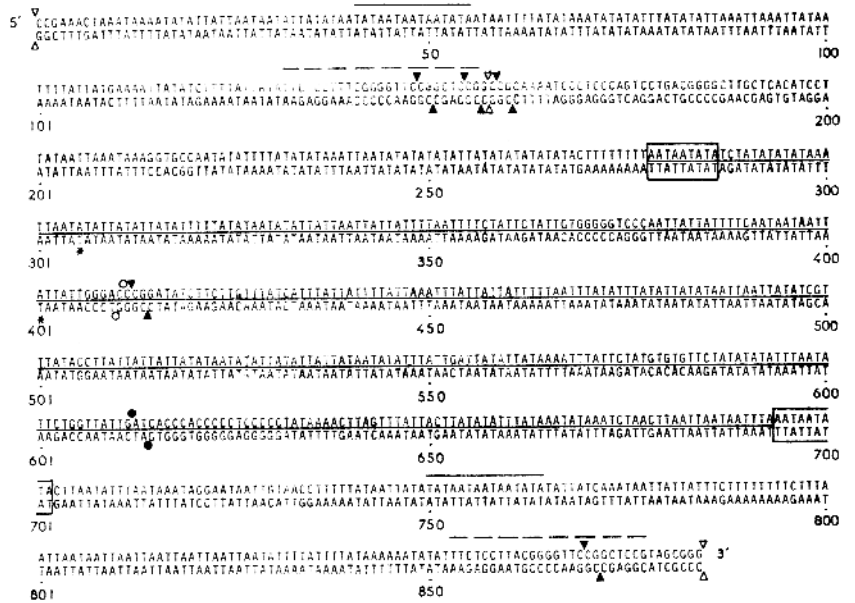

Fig. 4. Nucleotide sequence of the repeat unit of the mitochondrial genome of spontaneous petite mutant $a_{1/1R/1}$. Cutting sites of Hae III (Δ), Hpa II (▲), Mbo I (●), Mbo II (○) are indicated. The sequence between positions 278 and 693 is identical to that of $a_{1/1R/Z1}$ (Gaillard and Bernardi,1979) except for the asterisked base pairs which are replaced by G:C, and C:G, respectively, in the latter. Boxed sequences correspond to the nonanucleotides flanking the sequence of $a_{1/1R/Z1}$. Lines and broken lines indicate repeated sequences.

An inspection of the sequence of $a_{1/1R/1}$ fails to reveal the presence of any gene, a conclusion confirmed by the present knowledge of codons used for the synthesis of mitochondrially encoded proteins (Macino and Tzagoloff,1979) and of tRNA gene sequences (Martin et al., 1979). In all likelihood, the only function left in this petite genome (as well as in $a_{1/1R/Z1}$) is replication. Since the excision process leading to the formation of spontaneous petite genomes is extremely conservative as far as the excised sequence is

concerned (in contrast to that induced by ethidium, where sequence rearrangements of different kinds are frequent; Lewin et al., 1978), each repeat unit of $a_{1/1R/1}$, should contain an origin of replication.

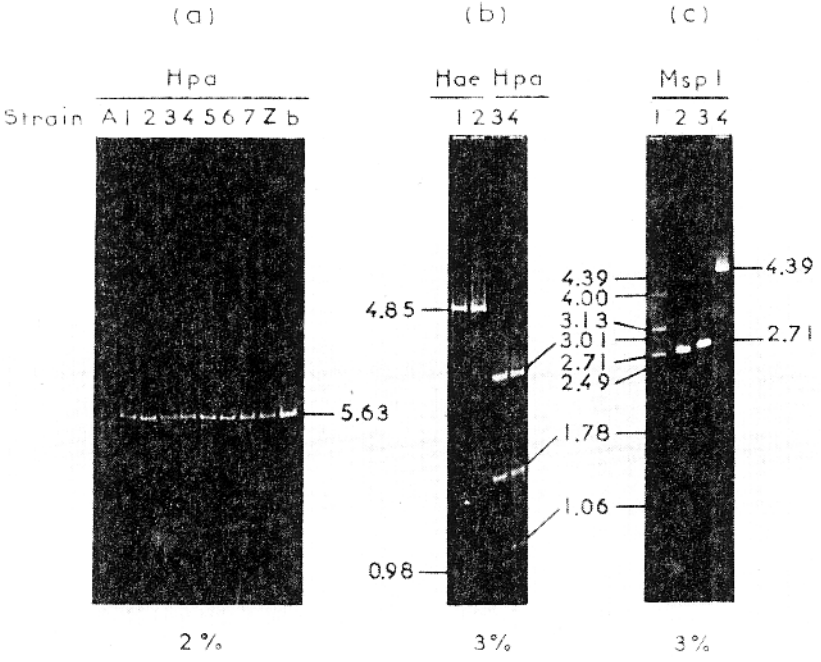

Fig. 5. (a) Hpa II restriction pattern of mitochondrial DNAs from parental wild-type strain A and petite mutant b and from clones obtained from 7 buds (1-7) and the residual zygote (Z) from a Axb cross. (b) Hae III and Hpa II restriction patterns of mitochondrial DNAs from parental petite $a_{1/1R/1}$(1,3) and from a random diploid obtained from a $Bxa_{1/1R/1}$ cross (2,4). (c) Msp I restriction pattern of mitochondrial DNAs from parental petite $a_{1/1R}$(1), from a clone derived from a residual zygote (2), and from 2 random diploids derived from a $Bxa_{1/1R}$(3,4) cross. The sequence split by Msp I, CCGG, is the same as that split by Hpa II. In all cases, the polyacrylamide gel concentration is indicated at the bottom of the patterns (the gels containing in addition 0.5 % agarose) and the molecular weights ($x10^{-5}$) of the fragments are indicated. Faint bands with no indication of molecular weight correspond to nuclear DNA.

A good candidate for this role is the 83 nucleotide sequence centered around position 412. In its left half this sequence contains a palindrome 23 nucleotide long (378-400) and a small symmetrical

sequence TTATT (402-406), which are flanked by two inverted repeats formed by G:C base pairs only; in its right half, the sequence contains a decanucleotide formed by A:T base pairs only, which is a direct repeat of another sequence present in the left half. The 83 nucleotide sequence not only is characterized by these remarkable features which are reminiscent of those found in the origin of replication of the mitochondrial genome from HeLa cells, but is also shared by $a_{1/1R/1}$, $a_{1/1R/Z1}$ and, more significantly, by another genome, that of petite b, which originated from a different region (map positions 64 to 76) of the parental wild-type genome.

If an origin of replication is present in each repeat unit of these petite genomes, which are all representing only 0.5-1 % of the wild-type genome a high replication rate may be expected. This appears to be indeed the case, since the three petites under consideration are "supersuppressive" (Goursot et al.,1979), namely petites whose mitochondrial genomes compete out those of wild-type cells in crosses and become the only ones found in the progeny (fig. 5).

In conclusion, these results provide evidence for the correctness of the deletion model proposed ten years ago (Bernardi, 1979); they strongly suggest that the origin of replication corresponds to the 83-nucleotide sequence shared by the three suppressive petites under consideration. Two additional implications of these data are : a) that the wild-type genome has more than one origin of replication, as previously suggested (Prunell and Bernardi, 1977); b) that petite mutants induced by massive ethidium treatment, like RD1A, are neutral (Moustacchi,1972), in spite of the fact that they contain mitochondrial DNA, because they do not contain an origin of replication per repeat unit (which is only 68 nucleotide long; Van Kreijl and Bos, 1977); instead, they may have only one origin of replication per genome unit, probably as a result of a translocation; this may explain why genomes of this kind are competed out in crosses by wild-type genomes.

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