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Short Communication

Supersuppressive "Petite" Mutants of Yeast

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Summary. A class of suppressive "petite" mutants of *S. cerevisiae*, called here *supersuppressive*, is characterized by a) the fact that their unmodified mitochondrial genomes are the only ones found in the progeny of crosses with wild-type cells; b) very short repeat units (400–900 base pairs) in their mitochondrial genomes. The repeat units of the three supersuppressive "petites" investigated here share a common 83 nucleotide sequence, which seems to correspond to an initiation site of DNA replication; the multiple copies of this site in the mitochondrial genomes of supersuppressive "petites" might explain why these genomes can compete out those of wild-type cells.

Key Words: MitDNA restriction analysis – Nucleotide sequences – Possible initiation site of DNA replication.

Introduction

When cytoplasmic "petite" mutants of *Saccharomyces cerevisiae* are crossed with wild-type cells, the progeny may either consist entirely of wild-type cells or both of wild-type cells and "petite" mutants; the "petites" entering the cross are called neutral in the first case and suppressive in the second one (Ephrussi et al., 1955); the degree of suppressivity (roughly equal to the percentage of "petites" in the progeny) varies according to the "petite" mutant and also, to some extent, to the wild-type strain used in the cross.

As far as neutral "petites" are concerned, it is well established that some of these correspond to a special class of "petites", the ρ° "petites" which lack mitochondrial DNA (Goldring et al., 1970; Nagley and Linnane, 1970); other ones, however, contain it (Moustacchi, 1972). The case of suppressive "petites" is still completely obscure. It has been suggested that suppressivity is due to a faster replication rate of the mitochondrial genomes of suppressive "petite" mutants compared to those of wild-type cells (Carnevali et al., 1969; Rank, 1970a, b); alternatively, suppressivity has been associated with the recombination of the defective mitochondrial genome of the suppressive "petite" with that of wild-type cells (Coen et al., 1970; Perlman and Birky, 1974).

We present here two main results concerning suppressive "petites": a) the existence of a class of suppressive "petite" mutants, whose unmodified mitochondrial genomes are the only ones found in the progeny of crosses with wild-type cells; we propose to call supersuppressive these "petites" (this finding was first reported at the Symposium on the Biochemistry and Genetics of Yeast, Sao Paulo, Brazil, in 1977); b) the presence in the repeat units of the mitochondrial genomes of the three supersuppressive "petites" so far analyzed of a common 83 nucleotide sequence which might correspond to an initiation site for DNA replication; the amplification of the repeat unit in these genomes might then account for their ability to compete out the mitochondrial genomes of wild-type cells.

Results and Discussion

In the present work we have studied the mitochondrial genomes of diploid clones issued from three crosses of wild-type cells x "petite" mutants: Axb; $Bxa_{1/1R/1}$ and $Bxa_{1/1R}$. All the parental strains have been extensively investigated in our laboratory (Prunell et al., 1977; Fonty et al., 1978; Faugeron-Fonty et al., 1979). These comprise two wild-type strains A and B, and three highly

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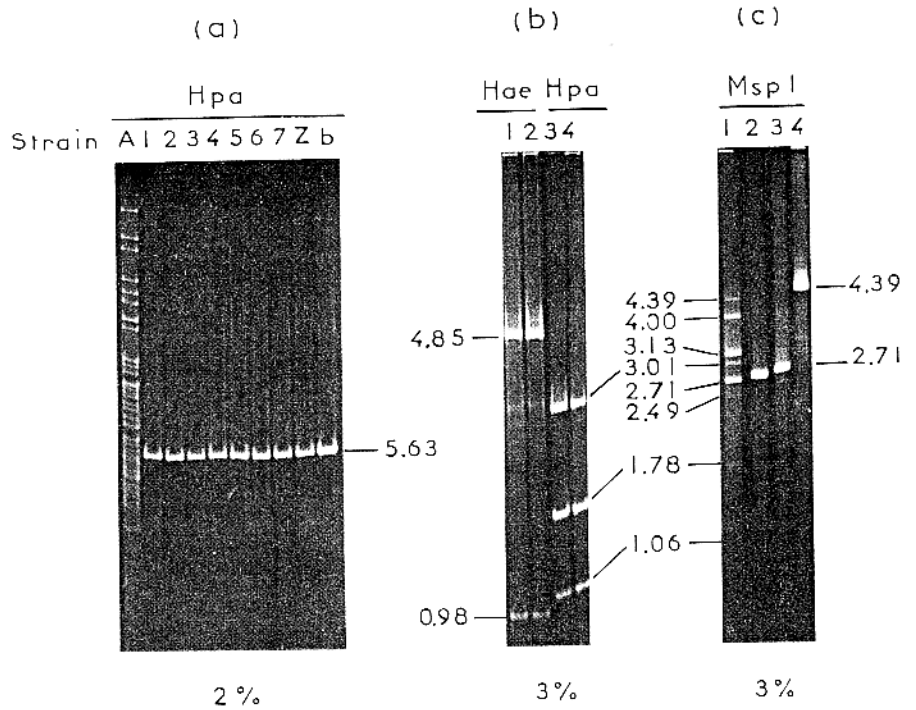

Fig. 1. (a) Hpa II restriction pattern of mitochondrial DNAs from parental wild-type strain A and "petite" mutant b and from clones obtained from 7 buds (1–7) and the residual zygote (*Z*) from a Axb cross. (b) Hae III and Hpa II restriction patterns of mitochondrial DNAs from parental "petite" $a_{1/1R/1}$ (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 ($\times 10^{-5}$) of the fragments are indicated. Faint bands with no indication of molecular weight correspond to nuclear DNA

suppressive spontaneous "petite" mutants (suppressivity $\geqslant$ 95%): b, derived from B; $a_{1/1R/1}$ and $a_{1/1R}$, derived from A ($a_{1/1R/1}$ is a sub-clone of $a_{1/1R}$).

In the case of the Axb cross, the mitochondrial genomes of 10 diploid clones were studied; 2 of the clones were random diploids derived from one cross, 7 originated from subsequent buds produced by one zygote, and 1 from the corresponding residual zygote, namely the zygote left after removal of the buds. In all cases, the Hpa II or Alu I restriction patterns of the mitochondrial genomes were those of the parental "petite" b (Fig. 1a). Similarly, in the $Bxa_{1/1R/1}$ cross the mitochondrial genomes from 3 random diploids and 1 residual zygote showed the Hpa II or Hae III restriction patterns of the mitochondrial genome of the parental "petite" $a_{1/1R/1}$ (Fig. 1b). In the third case, the "petite" used in the cross, $a_{1/1R/1}$, was a heterogeneous one; its cells contained 5 or 6 genomes, 4 of which have already been isolated by sub-cloning. In this case, we studied 2 random diploids from one cross and 1 residual zygote from another cross. The clone from the residual zygote, $a_{1/1R/Z1}$, and one of the random diploids contained the same mitochondrial genome, characterized by a repeat unit of $2.71 \cdot 10^5$ daltons. The other random diploid contained another of the genomes of $a_{1/1R}$; this had a repeat unit of $4.39 \cdot 10^5$ daltons. It should be mentioned here that the repeat units of the mitochondrial genomes of $a_{1/1R/1Z}$ and $a_{1/1R/1}$ have been completely sequenced (Gaillard and Bernardi, 1979; Gaillard et al., 1979) and that the former corresponds to a segment of the latter, with only 2 base pair changes.

These results demonstrate that suppressive "petite" mutants exist whose unmodified mitochondrial genomes are the only ones found in the progeny of crosses with wild-type cells. Such genomes appear to share the property of having very short repeat units corresponding to 0.5–1% of the wild-type genome. The exclusive presence of parental "petite" genomes in the progeny is, in all likelihood, due to a selective advantage in DNA replication of these genomes compared to wild-type genomes. Previous suggestions along this line, however, either did not include any molecular model (Rank, 1970a, b) or involved a wrong model, such as the premature detachment of DNA polymerase and the rapid replication of the incomplete genomes so formed (Carnevali et al., 1969). In our opinion, the most reasonable selective advantage could be the presence of an initiation site for DNA replication in each repeat unit (and therefore of a large number of initiation sites per genome unit) of the spontaneous "petite" genomes. These are known to be characterized by the absence of sequence rearrangements and to be faithful copies of the wild-type genome segments originally excised (Faugeron-Fonty et al., 1979), in contrast with the situation often found in ethidium-bromide induced "petites" (Lewin et al., 1978).

This hypothesis was submitted to the following experimental test. First of all, we looked for a region in the sequenced repeat units of $a_{1/1R/1}$ and $a_{1/1R/Z1}$ which might be involved in initiation of DNA replication. Both genomes contain no gene and are made up essentially of AT stretches; an outstanding feature they share is a

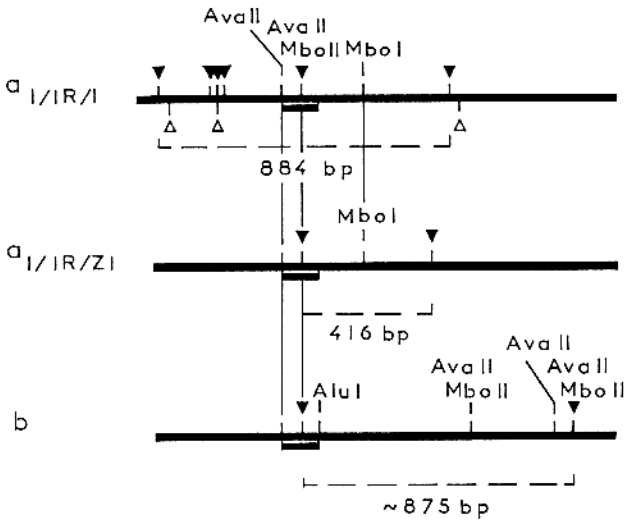

Fig. 2. Restriction enzyme maps of the repeating units of the mitochondrial genomes of "petite" mutants $a_{1/1R/1}$, $a_{1/1R/Z1}$ and b. The length of the repeat units, in base pairs, and the localizations of Hae III (△), Hpa II (▲), Ava II (♦), Alu I (◇) and other restriction sites are indicated. The 83 nucleotide sequences shown in Fig. 3 correspond to the regions around the isolated Hpa II sites (heavy bars)

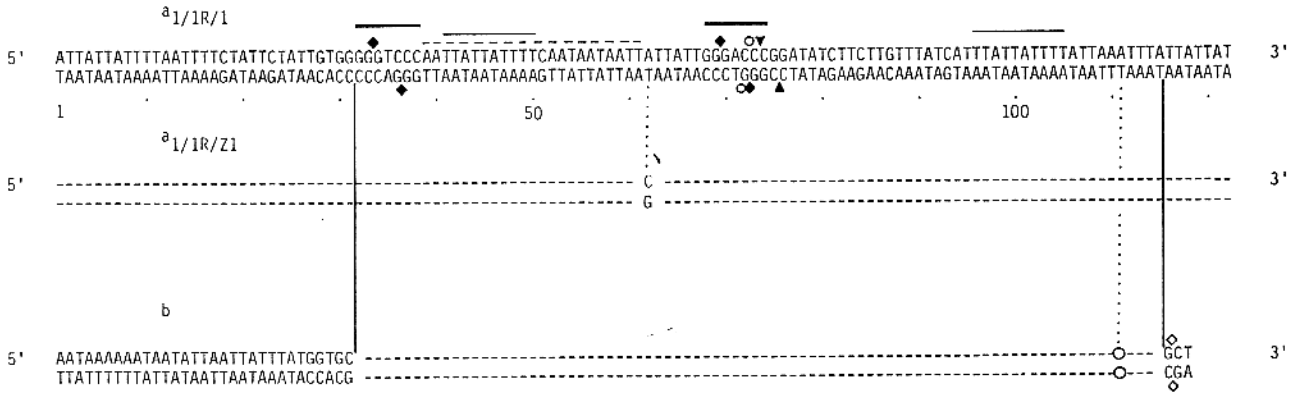

Fig. 3. Primary structure of the mitochondrial genome of $a_{1/1R/1}$ between nucleotides 340 and 461 (Gaillard et al., 1979), of the corresponding segment of $a_{1/1R/Z1}$ (Gaillard and Bernardi, 1979), and of the region around the Hpa II site of the repeat unit of b. Horizontal broken lines indicate the region of sequence identity of $a_{1/1R/Z1}$ and b with $a_{1/1R/1}$; in this region $a_{1/1R/Z1}$ presents a single base pair change, b a single base pair missing as indicated. To the left of this region b exhibits partial homology over a few base pairs. Restriction sites are indicated as in Fig. 2. The 23 base pair palindrome is indicated by the broken line, the 7 base pair inverted repeats by heavy lines, the 10 base pair direct repeats by thin lines

palindrome of 23A:T base pairs (with one G:C) flanked by two inverted repeats of 7 G:C base pairs. In view of the features of sequence involved in the initiation of DNA replication (see Crews et al., 1979, for example), this region of $a_{1/1R/1}$ and $a_{1/1R/Z1}$ was considered to be the best candidate. We then looked for an identical or similar sequence in the repeat unit of the mitochondrial genome of "petite" b. This "petite" was of particular interest for two reasons: a) its repeat unit had arisen from a different region of wild-type genome and from a different strain compared to $a_{1/1R/1}$ and $a_{1/1R/Z1}$; whereas the latter originated from the 15 S RNA gene region of strain A, the former arose from the cob-oli 2 region of strain B; b) the mitochondrial genome of b showed essentially identical hybridizations on Hae III and Hpa II restriction fragments from the genomes of strains A, B, D (another wild-type strain) and also of *S. carlsbergensis* (Faugeron-Fonty et al., 1979). The primary structure around the Hpa II site of the mitochondrial genome of b (see Fig. 2 for restriction maps of the three "petite" genomes) was found to be identical (except for an A:T base pair, which was absent in b) over 83 nucleotides with regions of $a_{1/1R/1}$ and $a_{1/1R/Z1}$ (Fig. 3). This 83 nucleotide sequence contained in its left half the remarkable features mentioned above, whereas its right half was almost exclusively made up of A:T base pairs with one 10 nucleotide direct repeat of a sequence present in the left half. These findings very strongly support the model proposed above to account for the supersuppressivity phenomenon; they suggest that more than one initiation site for DNA replication exists on the wild-type genome. Also, they hint that the massively mutagenized "petite" studied by Moustacchi (1972) is neutral, since it contains only a single

initiation site for DNA replication per genome unit as a result of a translocation.

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