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*; *Bxa1/IR1* and *Bxa1/IR* and *Bxa1/IR*. 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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 suppressive spontaneous "petite" mutants (suppressivity > 95%); b, derived from B; a1/I1R/1 and a1/I1R, derived from A (a1/I1R/1 is a sub-clone of a1/I1R).

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 Bxa1/I1R/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" a1/I1R/1 (Fig. 1b). In the third case, the "petite" used in the cross, a1/I1R/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, a1/I1R/2, and one of the random diploids contained the same mitochondrial genome, characterized by a repeat unit of 2.71 \times 10^5 daltons. The other random diploid contained another of the genomes of a1/I1R; this had a repeat unit of 4.39 \times 10^5 daltons. It should be mentioned here that the repeat units of the mitochondrial genomes of a1/I2/2 and a1/I2/1 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 (Fauceron-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 a1/I1R/1 and a1/I2/2 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 a1/R1, a1/R/Z1 and b. The length of the repeat units, in base pairs, and the localizations of Hae III (†), Hpa II (Δ), 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 a1/R1 between nucleotides 340 and 461 (Gaillard et al., 1979), of the corresponding segment of a1/R/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 a1/R/Z1 and b with a1/R1; in this region a1/R/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 23 A: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 a1/R1 and a1/R/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 a1/R1 and a1/R/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 a1/R1 and a1/R/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 Mous-tacchi (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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References


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