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Short Communication

The Nucleotide Sequence of the Mitochondrial Genome of a Spontaneous "Petite" Mutant of Yeast

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Summary. The nucleotide sequence of the repeat unit of the mitochondrial genome of a spontaneous petite mutant of *S. cerevisiae* is reported. The sequence provides direct information on the AT-spacers and GC-clusters of the mitochondrial genome of yeast.

Introduction

The mitochondrial genome of *Saccharomyces cerevisiae* wild-type cells has a GC content of 18% (Bernardi et al., 1970), the lowest reported so far for a functional genome, and contains, in addition to mitochondrial genes, two particular sequence elements: a) the AT spacers (GC < 5%) form about 50% of the genome, and are made up of short, alternating AT:AT and non-alternating A:T sequences (Bernardi et al., 1970; Prunell et al., 1974); b) The GC clusters, (GC = 45–60%), account for 10% or more of the genome. Two sorts of GC clusters have been distinguished (Prunell et al., 1977; Prunell and Bernardi, 1977): 1) The (CCGG, GGCC) clusters, characterized by a local concentration of Hpa II and Hae III restriction sites, are present in 60–70 copies per mitochondrial genome unit; 2) The GC-rich clusters do not contain those restriction sites but appear to be largely contiguous to the CCGG sequences, whether isolated or clustered with GGCC sequences. We have suggested that the AT spacers are to some extent internally repetitive and palindromic in sequence, and that the (CCGG, GGCC) clusters, and possibly the GC-rich clusters, are to some extent symmetrical and homologous in sequence (Prunell and Bernardi, 1974; Prunell and Bernardi, 1977).

The physiological role of AT spacers and GC clusters is not yet established. "Allelic" AT spacers appear to vary in length in different *Saccharomyces* strains and in the progeny of crosses originating from such different strains, probably because of unequal crossing-over events (Prunell et al., 1977; Fonty et al., 1978). On the other hand, the GC clusters might play regulatory roles and correspond to sequences involved in the initiation of replication, in RNA processing, and/or to promotor and operator sequences (Prunell and Bernardi, 1977). In any case, it has been shown that the GC clusters, and possibly the AT spacers, act as preferential sites for the excision (see Fig. 1) of the defective genomes of spontaneous "petite" mutants (Fonty et al., 1979). Under these circumstances, direct information on the nucleotide sequences of AT spacers and GC clusters is of great interest. With this purpose in mind, we have sequenced the repeat unit of the mitochondrial genome of a spontaneous "petite" mutant, $a_{1\ 1R\ Z1}$, already investigated in our laboratory (Fonty et al., 1979). This "petite" genome

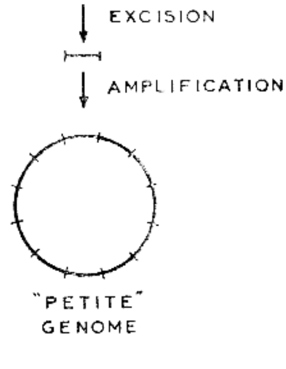

Fig. 1. Scheme of the process leading to the formation of spontaneous "petite" genomes. A segment of the mitochondrial genome from wild-type yeast cells is excised and amplified to yield a "petite" mitochondrial genome unit

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is made up of perfect tandem repetitions of a DNA segment which arose from a sector corresponding to map positions 27 to 46 on the wild-type genome of strain KL14-4A (Fonty et al., 1979), (see Sanders et al., 1977).

Results and Discussion

Fig. 2 shows the complete sequence of the repeat unit of $a_{1/1R/Z1}$. The sequence is 416 nucleotides long and contains 60 GC base pairs (GC = 14.4%). 30 of these are clustered in: a) a central row, (A), of three penta-C repeats, (the first one of which contains an A), each of which is preceded by an A or a T; this cluster contains part of the Mbo I site; b) two symmetrical heptanucleotides, (B, C), GGGTCCC, GGGACCC, externally flanked by two GG doublets; one of these sequences includes the Hpa II site and the sequence cut by Mbo II. The first cluster is 78%, the other two 89% in GC.

The 380 nucleotides outside the GC clusters contain 30 GC base pairs (GC = 7.6%), and are formed by short alternating AT:AT and non-alternating A:T sequences with sparse GC base pairs, never present in sequences longer than 2 nucleotides. The long AT stretches include a number of repeated, symmetrical, and palindromic sequences, some of which are indicated in Fig. 2. The 47 base pairs forming the right end of the repeat unit, as presented in the figure, are remarkable in that they are formed by a large palindrome, 23 nucleotides long, and a small symmetrical sequence, TTATT, flanked by the two symmetrical GC clusters.

These findings are interesting for two different series of reasons. First, they confirm several previous results and suggestions: that AT spacers are made up of short alternating and non-alternating AT sequences (Ehrlich et al., 1972) and contain repeated sequences and palindromes (Prunell et al., 1977); that GC-rich clusters are largely contiguous to Hpa II sites (Prunell and Bernardi, 1977); and that the buoyant density of yeast mitochondrial DNA is higher than expected from the ρ vs. GC relationship established for bacterial DNAs (Bernardi et al., 1970). If the latter (Schildkraut et al., 1962) were used, the buoyant density of the $a_{1/1R/Z1}$ DNA, ($\rho = 1.683$ g/cm^3, Fonty et al., 1979), would indicate a GC content of 23%, a value 9% higher than the analytical one. No information was obtained on the (CCGG, GGCC) clusters since none of these was present in $a_{1/1R,Z1}$. Cosson and Tzagoloff (1979) have just shown, however, that these sequences fit our predictions (Prunell and Bernardi, 1977), in being palindromic, in being present in more than one copy per genome, and in being contiguous to GC-rich clusters.

On the other hand, these results open the way to the study of replication and excision of mitochon-

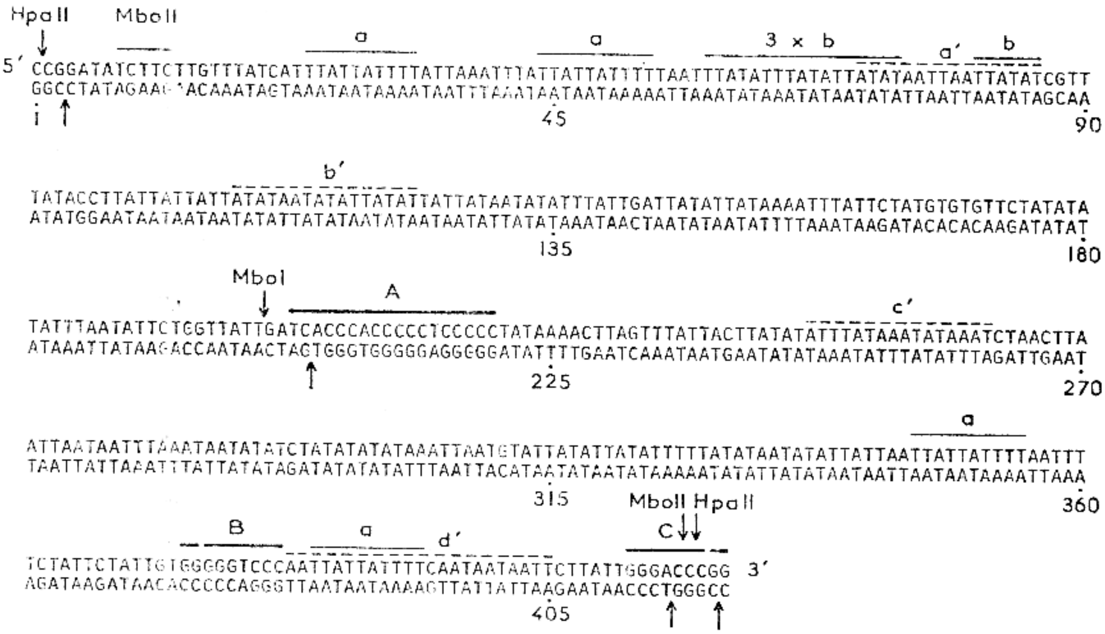

Fig. 2. Nucleotide sequence of the repeat unit of the mitochondrial genome of spontaneous "petite" mutant $a_{1/1R/Z1}$. This DNA was degraded by either Hpa II, Mbo I, or Mbo II, dephosphorylated with *E. coli* alkaline phosphatase, rephosphorylated with ^{32}P-γ labelled ATP using polynucleotide kinase, and degraded again with Mbo I, Hpa II and MboI, respectively. Restriction fragments were then separated by gel electrophoresis and sequenced according to Maxam and Gilbert. A, B and C indicate the GC-rich clusters; a and b, a repeated decanucleotide and a repeated hexanucleotide, respectively; a′, b′, c′ and d′, palindromic sequences in the AT stretches. The restriction sites of HpaII, MboI and MboII are indicated by arrows; the recognition site of the latter enzyme is also indicated

drial DNA in yeast. a) The genome of $a_{1/1R/Z1}$ does not contain any gene or gene segment, but is capable of replication. The excision process leading to the formation of spontaneous "petite" genomes is highly conservative as far as the excised sequence is concerned (Fonty et al., 1979), in contrast to that leading to ethidium induced "petite", where sequence rearrangements are frequent (Lewin et al., 1978). It is likely, therefore, that the repeat unit of $a_{1/1R/Z1}$ contains a site for the initiation of DNA replication. b) Sequence work on the genome of another "petite", $a_{1/1R/1}$, whose repeat unit contains that of $a_{1/1R/Z1}$ (Fonty et al., 1979), should shed some light on the nucleotide sequences involved in the excision of $a_{1/1R/Z1}$, since it should provide information on the nucleotides flanking the repeat unit of $a_{1/1R/Z1}$.

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