

THE PETITE MUTATION : EXCISION SEQUENCES, REPLICATION ORIGINS AND SUPPRESSIVITY.

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Introduction

Last year we reported (1) restriction mapping and DNA: DNA hybridization experiments on the mitochondrial genomes of spontaneous petite mutants and of the corresponding parental wild-type cells of Saccharomyces cerevisiae. The main conclusions arrived at were : a) that the petite genomes are made of tandem repetitions of the DNA segment originally excised from the wild-type genome; b) that the excision mechanism is extremely conservative, in that the excised segment does not show any sign of the rearrangements found in ethidium-induced petites (2); this conclusion implies that every repeat unit of the petite genome contains a replication origin and, since petite genomes can arise from many regions of the wild-type genome, that the latter contains several origins of replication; c) that excision most frequently takes place in GC clusters; alternatively, it occurs elsewhere, most probably in AT spacers. These conclusions seem to apply to the vast majority, but not to the totality of spontaneous petite mutants. Fig. 1 shows the restriction map of the mitochondrial genomes of some of the spontaneous petites used in our work and the localization of some excision sites.

Direct repeats are used in the excision of spontaneous petite genomes

Because of the sequence conservation in the mitochondrial genomes of spontaneous petites, excision sequences can be determined by comparing the primary structure around the excision sites, or that of the entire repeat unit, of given petite genomes and of other petite genomes encompassing them. Using this approach, a comparison of the sequences around the excision sites of $a^{-1/7/8}$ and of $a^{-3/1}$ indicated (fig. 2) that the excision of the repeat unit of petite $a^{-1/7/8}$ took place at two direct repeats, 23 nucleotides long, located in two GC clusters (3). On the other hand, a comparison of the repeat units of petites $a^{-1/1R/21}$ (4) and $a^{-1/1R/14}$ (5) with that of $a^{-1/1R/1}$ (6) showed (fig. 3) that the excision sequences are two direct repeats, 13 nucleotides long (with

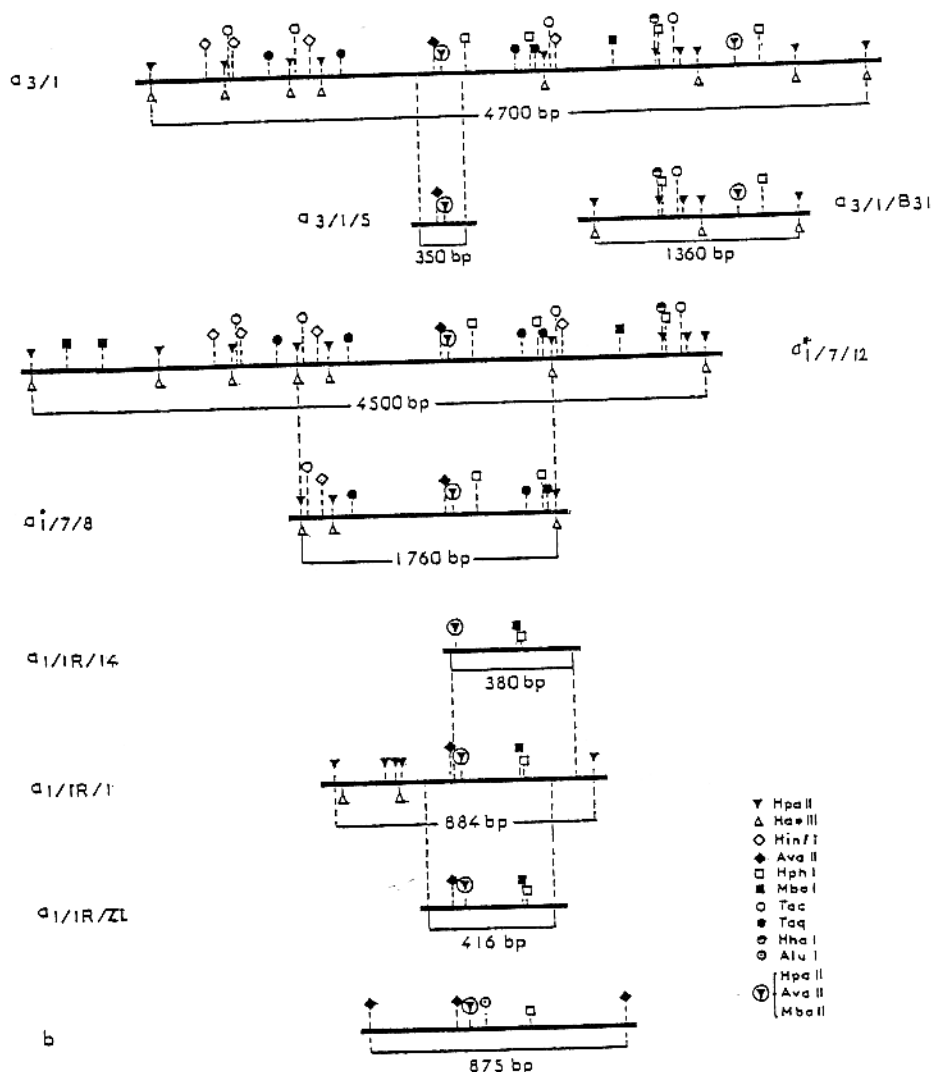


Fig. 1. Restriction enzyme maps of the repeating units of mitochondrial genomes of spontaneous petite mutants. The molecular weights, in base pairs, of the repeat units are indicated. Only some Mbo II and Ava II sites are indicated. The previous maps of a-3/1, a⁻1/7/12 and a⁻1/7/8 (1) were revised in the light of recent work. Sequenced excision sites are indicated by vertical broken lines. All sequences are aligned on Hpa II, Ava II, Mbo II site clusters.

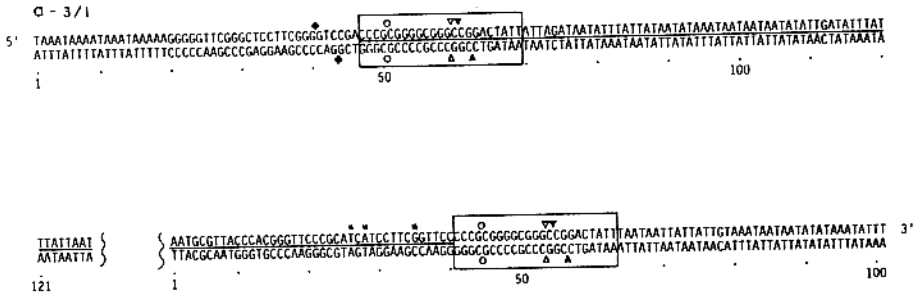


Fig. 2. Primary structure around the putative excision sequences used in the formation of the repeat unit of a⁻1/7/8. The a⁻1/7/8 sequence is identical with that of a-3/1 in the region indicated by the continuous line between base pairs, except for two base pair changes (A:T and G:C replace T:A and A:T) and one deletion (G:C) at the positions indicated by asterisks.

one mismatch in the case of a-1/1R/Z1), located in the AT spacers. The longer sequence of the excision repeat and its higher GC content appear to account for the higher frequency of excisions at GC clusters as opposed to AT spacers. Obviously, more excision sequences need to be studied in order to generalize this conclusion, as well as to check whether inverted repeats are also involved in the excision process.

These results fully confirm a hypothesis according to which repeated sequences in the mitochondrial genome of yeast are responsible for the excision of petite genomes by a site-specific illegitimate recombination process (7). The very high frequency of long repeats in the wild-type genome (8) accounts, again as predicted, for the very high rate of the spontaneous petite mutation.

Genomes without genes

An analysis of the sequence of a-1/1R/1 (fig. 3) fails to reveal the presence of any gene or gene segment in its repeat unit. Very recent results (to be presented at this Meeting) indicate that the genome of a-1/1R/1 is transcribed. Genomes like a-1/1R/1 are, therefore, able to replicate and to be transcribed, and yet they do not have any apparent usefulness to the cell. In this respect, and in their capacity to spread to other cells *via* crosses (see below), these genomes are the best examples of what has been called, rightly or wrongly, selfish DNA (9,10).

Origins of replication

A search for an origin of replication on the repeat unit of the genome of a-1/1R/1 (fig. 3) has focused our attention on the region comprised between positions 370 and 630. This is characterized by (4-6) two short GC clusters, A and B, flanking a 23-nucleotide AT palindrome, and one long GC cluster, C; the first two clusters are mainly formed by two symmetrical heptanucleotides, GGGTCCC and GGGACCC, and the third one by three penta-C repeats (the first one of which contains an A), each one of which is preceded by an A or a T. It should be noted that cluster A contains an *Ava* II site, cluster B contains a *Hpa* II, an *Ava* II and an *Mbo* II cutting site (the recognition site of the latter enzyme is a pentanucleotide located 8 base pairs away from the cutting site); cluster C contains an *Mbo* I, a *Mnl* I and a *Hph* I recognition site (the cutting site of the latter enzyme is located 7 nucleotide away from the recognition site). It is of interest to remark that the region comprised between clusters A and B can be folded in a way (fig. 4) similar to that reported for a region of the replication origin of HeLa cell mitochondrial DNA (11).

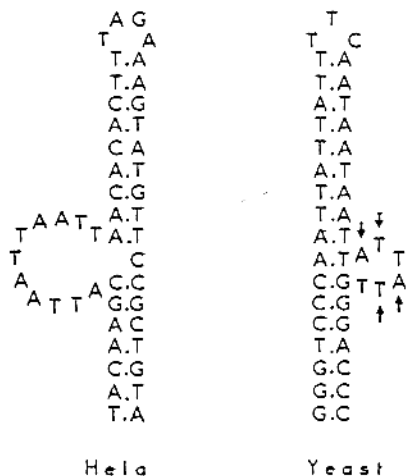


Fig. 4. Hypothetical secondary structure of the left end of the replication origins of mitochondrial DNA; the region corresponds to the sequence comprised between GC clusters A and B (see fig. 3); arrows indicate base changes found in different petite mutants. The palindromic sequence found in the replication origin of HeLa cell mitochondrial DNA (11) is shown for the sake of comparison.

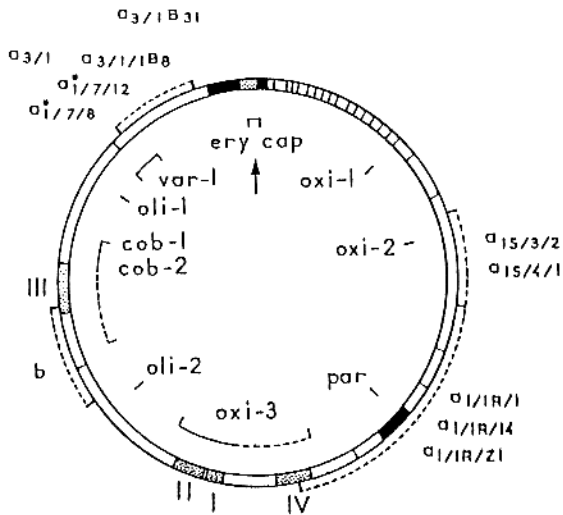


Fig. 5. Regions of excision of the spontaneous petite genomes studied here are indicated on a genome map of the mitochondrial genome of *Saccharomyces cerevisiae* (adapted from ref. 18).

Considering that the region just described might correspond to an origin of replication, we decided to look for it in several petite genomes having arisen from three different regions of the parental wild-type genomes (fig. 5) : the var-1-ery, the cob-oli 2, and the 15 S RNA regions. We first looked for the presence of the most characteristic cluster B in the repeat units of these petite genomes. Having found it, we sequenced both sides of the cluster, to discover that a stretch of about 80 nucleotides centered on the cluster was present in all cases, with only a few base pair changes (19,12 and present work; fig. 6), which were localized in the loop of fig. 4, or a short deletion, in the case of a-1/1R/14 (see below). On the left of the 80-base pair stretch, sequence homology became patchy after the Ava II site of cluster A; on the right, sequence work on the repeat unit of a-3/1 and a-1/7/12 showed that homology extended until cluster C with some base pair changes in the long AT stretch between B and C; in the repeat unit of a-3/1/5, the whole right end of this region was absent (see also below). The whole replication origin is therefore at least 260 nucleotide long. This region has also been partially sequenced or at least mapped in the repeat units of other petite genomes. Our present knowledge is summarized in Table 1.

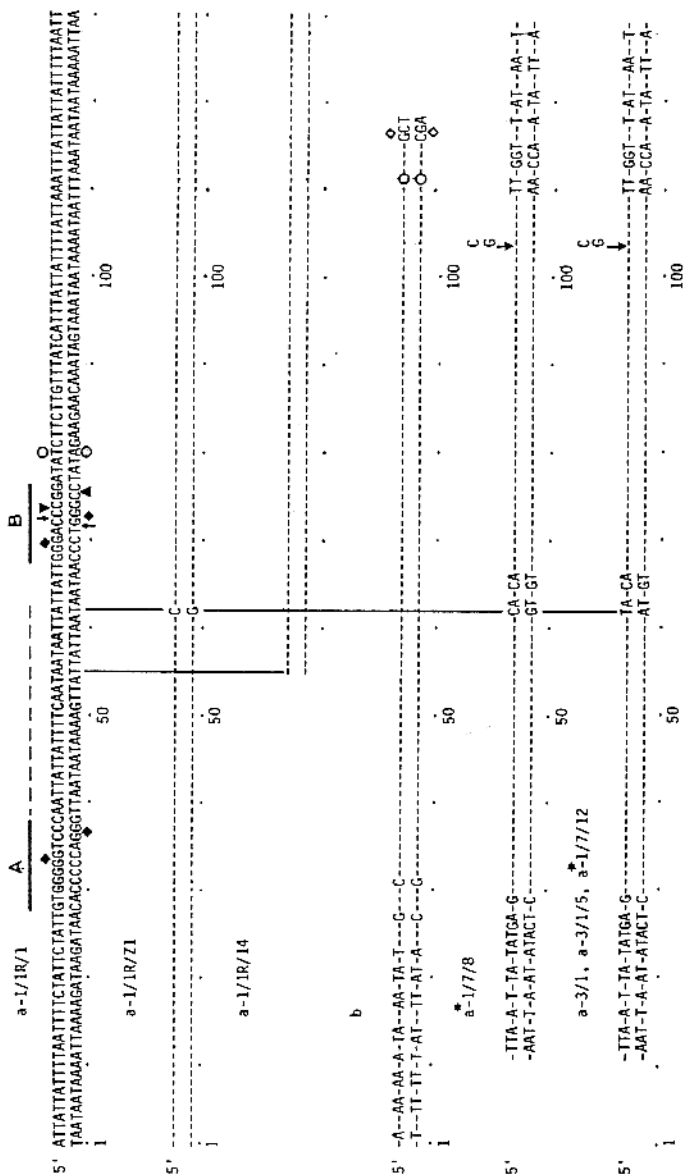


Fig. 6. Primary sequence of the left end of the replication origin of the mitochondrial genomes from several spontaneous petite mutants (see also fig.3). The sequence found in the DNA of a-1/1R/1 is taken as a reference; dashes indicate nucleotides identical to those of a-1/1R/1. Base pair changes are indicated; a double empty circle indicates a base-pair deletion, an arrow indicates an insertion. Restriction sites and other symbols as in fig.3.

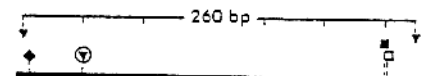










Recent experiments have provided additional information on the origins of replication. The repeat units of two spontaneous petite genomes, a-15/3/2 and a-15/4/1 were shown to have arisen from the *oxi-2* region and to contain at least the Hpa II, Ava II, Mbo II cluster and the Hph I site at the expected position. It is of interest that hybridization of these petite genomes took place on the same or on corresponding restriction fragments originated from the genome of four different wild-type strains; this result is reminiscent of similar ones found in the hybridization of the genome of petite b (1) and stresses the conservation of the localization of the origins of replication in wild-type genomes. A second series of results obtained with nick-translated DNA from a-1/1R/Z1 (the repeat unit of this mitochondrial genome is only slightly larger than the origin of replication) showed that hybridization of this DNA took place on the repeat unit of petite b, and on the Hae III and Hpa II fragments from the DNAs of a-3/1, a-3/1/B31, a^x-1/7/8, a-15/3/2, a-15/4/1 which contained the Ava II, Hpa II, Mbo II site cluster. A very interesting finding was that in the case of a-3/1, two Hae III fragments bound the probe; one of these two fragments is present on the repeat unit of a^x-1/7/8, the other one on that of a-3/1/B31. This indicates that a-3/1 contains in fact not one but two origins of replication, as characterized by hybridization and restriction map. The results available so far show, therefore, that at least five origins of replication are present on the mitochondrial genome of wild-type cells.

Suppressivity

Crosses of the petite mutants of fig. 1 with wild-type cells have proved extremely useful in two respects : a) in providing a functional evidence that the sequences discussed above are indeed the origins of replication of the mitochondrial genome ; b) in helping to understand the phenomenon of suppressivity.

Three years ago we observed, and reported at the Symposium on the Biochemistry and Genetics of Yeast held in Sao Paulo, Brazil, that crosses of petites a-1/1R/1 and b with wild-type cells produced diploids harboring the mitochondrial genome of the petite used in the cross. Both petites are very highly suppressive (95 % suppressivity) and have genomes characterized by repeat units corresponding to only 1 % of the wild-type genome. We proposed to call supersuppressive these petites and suggested that the reason for the petite genome being the only one found unaltered in the progeny was that these petite genomes contained multiple copies of the origin of replication and could,

TABLE 1.

| Petite Strain | Repeat Unit (bp) | Origin of replication ^(c) | Hybrid. ^(b) Supp., % | P:M ratio ^(a) |
|------------------------|------------------|---|---------------------------------|--------------------------|
| a-1/1R/1 | 884 |  | 95 | 4 : 0 |
| a-1/1R/Z1 | 416 |  | + | |
| a-1/1R/14 | 380 |  | 80 | |
| a-3/1 | 4700 |  | ++ | 60-70 28 : 4 |
| a-3/1/B31 | 1360 |  | + | |
| a-3/1/5 | 350 |  | <5 | 5 : 0 |
| a ^x -1/7/8 | 1760 |  | + | 85 14 : 0 |
| a ^Δ -1/7/12 | 4500 |  | 60-80 | 36 : 0 |
| a-15/3/2 | 4300 |  | + | 50 12 : 0 |
| a-15/4/1 | 4800 |  | + | <10 |
| b | 875 |  | + | 95 10 : 0 |

(a) This is the ratio of genomes of petite diploids having the repeat unit of the parental petite to those having modified repeat units; the latter may either be recombinant genomes or minority genomes (excised from the parental petite) transmitted to the progeny.

(b) Hybridization of nick-translated DNA from a-1/1R/Z1 on Hae III (or Alu I in the case of petite b) fragments.

(c) Thick lines indicate sequenced stretches. In the case of a-3/1/5 the right end of the repeat unit is located within the broken line. Restriction site symbols as in Fig. 1. The Ava II and Mbo I sites on a-3/1/B31, a-15/3/2 and a-15/4/1 have not been mapped.

therefore, compete out the genomes of wild-type cells (12), a view similar to that originally hypothesized by Rank (13).

We have now extended these investigations to other petite mutants characterized by moderate or low degrees of suppressivity, to understand which relationships, if any, exist between supersuppressive and suppressive petites on one hand, and between suppressivity and the putative origins of replication discussed above, on the other. The results are summarized in Table 1 and lead to two main conclusions. The first one is that even when petites of moderate or low suppressivity are used in crosses with wild-type cells, their mitochondrial DNA is transmitted unaltered in its restriction map to the petite diploids issued from the cross. The number of petite genomes showing evidence of recombination is very small and certainly does not account for suppressivity, ruling out explanations based on this notion (14, 15). The second conclusion is that there is a correlation between conservation or alteration of the origin of replication and suppressivity, and probably also between length of the repeat unit and suppressivity. The first point is illustrated by the case of a-3/1/5 where the loss of the whole right end of the origin of replication is associated with an almost complete loss of suppressivity; another case, though less striking, is that of a-1/1R/14 where the loss of the leftmost 25 base pairs of the origin of replication is accompanied by a decrease in suppressivity. The second point seems to be supported by the suppressivities of a^x-1/7/8 and a^x-1/7/12, two genomes with origins of replication probably identical; in this case, the genome with a longer repeat unit is less suppressive than that with a shorter repeat unit. The case of a-3/1 is more complex because of the presence of two close origins of replication.

Two final points should be made. The first one is that, if the sequence of the origin of replication is the most important factor in determining the degree of suppressivity as suggested above, and the length of the repeat unit is another possible factor, other features may also play a role, like the amount of mitochondrial DNA in the petite used in the cross. The second one is that petites exist, essentially among ethidium-induced ones, which do not contain an origin of replication in every repeat unit. This is the case of neutral petite RD1/A (16,17). In this case we postulate that the repeat units forming the majority of each genome carry with them an origin of replication picked up from an excision event different from that concerning the majority repeat units. Current investigations should clarify this point.

REFERENCES

1. Faugeron-Fonty G., Culard F., Baldacci G., Goursot R., Prunell A. and Bernardi G. (1979) *J. Mol. Biol.*, 134. 493-537.
2. Lewin A., Morimoto R., Rabinowitz M., and Fukuhara H. (1978) *Mol. Gen. Genet.*, 163. 257-275.
3. Baldacci G., de Zamaroczy M., and Bernardi G. (1980) *FEBS Letters*, 114. 234-236.
4. Gaillard C., and Bernardi G. (1979) *Mol. Gen. Genet.*, 174. 335-337.
5. Bernardi G., Baldacci G., Culard F., Faugeron-Fonty G., Gaillard C., Goursot R., Strauss F., and de Zamaroczy M. (1980) in *Mobilization and Reassembly of Genetic Information* (Scott W.A., Werner, R., and Joseph D.R., eds.), Academic Press, New York, (in press).
6. Gaillard C., Strauss F., and Bernardi G. (1980) *Nature*, 283. 218-220.
7. Bernardi G. (1979) *Trends Biochem. Sci.*, 4. 197-201.
8. Bernardi G., and Bernardi G. (1980) *FEBS Letters* (in press).
9. Doolittle W.F. and Sapienza C. (1980) *Nature*, 284. 601-603.
10. Orgel L.E., and Crick F.H.C. (1980) *Nature*, 284. 604-607.
11. Crews S., Ogala D., Posakony J., Nishiguchi J., and Attardi G. (1979) *Nature*, 277. 192-198.
12. Goursot R., de Zamaroczy M., Baldacci G., and Bernardi G. (1980) *Current Genet.*, 1. 173-176.
13. Rank G.H. (1970) *Can J. Genet. Cytol.*, 12. 129-136.
14. Perlman P.S., and Birky C.W., Jr. (1974) *Proc. Natl. Acad. Sci. USA.*, 71. 4612-4616.
15. Slonimski P.P., and Lazowska J. (1977) in *Mitochondria 1977* (Bandlow W. *et al.*, eds.), pp. 39-52, de Gruyter, Berlin, 1977.
16. Van Kreijl C.F. and Bos J.L. (1977) *Nucleic Acids Res.*, 4. 2369-2388.
17. Moustacchi E. (1972) *Biochim. Biophys. Acta*, 277. 59-60.
18. Borst P. and Grivell L.A. (1978) *Cell*, 15. 705-723.
19. de Zamaroczy M., Baldacci G., Bernardi G. (1979) *FEBS Letters*, 108. 429-432