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The mitochondrial genomes of spontaneous *ori*^{r} petite mutants of yeast have rearranged repeat units organized as inverted tandem dimers

(*Saccharomyces cerevisiae*; excision sequences; recombination; genome rearrangements)

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SUMMARY

We have investigated the structure and organization of the mitochondrial genomes of two related *ori*^{r} (*ori*-rearranged) spontaneous petite mutants of *Saccharomyces cerevisiae*. In these mutant genomes every repeat unit contains an inverted terminal duplication harboring a second (inverted) *ori* sequence, and tandem pairs of repeat units alternate with tandem pairs in inverted orientation. We have shown that *ori*^{r} genomes are organized as the genomes with inverted repeat units of ethidium bromide (EtBr)-induced petites, and we have clarified the mechanism by which such mutant mitochondrial genomes arise.

INTRODUCTION

Investigations carried out in our laboratory (Bernardi et al., 1970; 1976; 1978; 1980; 1982; Faugeron-Fonty et al., 1979; Marotta et al., 1982; de Zamaroczy et al., 1983) have clarified the molecular basis of the cytoplasmic spontaneous "petite" mutation in the yeast *S. cerevisiae*. This is a case of extreme genomic instability, in which segments of the mitochondrial genome units of wild-type cells are excised and tandemly amplified to form the defective genome units of the petite mutants. Excision takes place by an internal recombination process between direct nucleotide repeats present in the abundant noncoding sequences of the wild-type genome units. The high number of potential excision sequences in such sequences accounts for the extremely high rate (about 1% per generation in most laboratory strains) of the spontaneous petite mutation (see Bernardi, 1979; 1982, for two brief reviews).

The excised segments of wild-type genomes,

Abbreviations: bp, base pairs; EtBr, ethidium bromide; *ori*, origin of replication; *ori*$^+$, *ori*$^-$, *ori*^{o}, *ori*^{r}, *ori*^{s}, see INTRODUCTION.

which become the repeat units of the mitochondrial genome units of spontaneous petites, can derive from different regions of the former and contain, therefore, different sequences where DNA replication starts. In most cases (*ori*$^+$ petites), the origin of DNA replication is one of the seven canonical *ori* sequences of the wild-type genome (de Zamaroczy et al., 1981; Bernardi, 1982). Otherwise, it may be a partially deleted *ori* sequence in *ori*$^-$ petites (de Zamaroczy et al., 1981), or a surrogate origin of replication (*ori*^{s} sequence) in *ori*^{0} petites (Goursot et al., 1982); in the latter case, the repeat units contain no intact or partial canonical *ori* sequence. In general, the relative efficiency of replication decreases in the order *ori*$^+$ to *ori*$^-$ to *ori*^{0}. It should be noted that the evidence for *ori* sequences being origins of DNA replication has been strong but indirect so far; direct biochemical evidence has, however, been obtained in our laboratory (G. Baldacci and G. Bernardi, paper in preparation).

In the present work, we have studied the rearranged structure and the complex organization of the mitochondrial genomes from yet another class of spontaneous petite mutants, the *ori*^{r} (*ori* rearranged) petites and we have clarified the mechanism by which these genomes arise. Although *ori*^{r} petites are rare among spontaneous petites, their mitochondrial genomes are of special interest because, as shown by these investigations, they are organized as the genomes with inverted repeat units of EtBr-induced petites. This is one of the two major classes of mitochondrial genomes found in EtBr-induced petites, the other consisting of genomes with tandemly arranged repeat units (Bos et al., 1980). Whereas the latter genomes are identical to those of *ori*$^+$ spontaneous petites, the former have a more complex organization. The present detailed study of *ori*^{r} genomes provides, therefore, a new insight into the structure, organization and mechanism of formation of this class of mitochondrial genomes. Furthermore, investigations on subcloning and crosses of *ori*^{r} petites have shed light on the correlation between *ori* sequences and replication of the mitochondrial genome of yeast (see the accompanying paper by Mangin et al., 1983).

MATERIALS AND METHODS

(a) Yeast strains

The parental wild-type *S. cerevisiae* strain was strain D-243-2B-R1 (called here, as in our previous papers, strain A; see Faugeron-Fonty et al. (1979), for the properties of this strain). Petite strains were spontaneous cytoplasmic respiratory-deficient petite mutants, derived from wild-type strain A (see RESULTS, sections **a**, **c**). Growth media and culture conditions were as described by Faugeron-Fonty et al. (1979).

(b) Mitochondrial DNA

Mitochondrial DNA was purified by centrifugation in CsCl density gradients, using a method modified from Lang et al. (1977). Restriction enzyme degradations, gel electrophoresis, nick translation of DNA probes, transfer of DNA to nitrocellulose films, and filter hybridization were performed essentially as described by Faugeron-Fonty et al. (1979). The primary structure of DNA was determined according to Maxam and Gilbert (1977).

RESULTS

(a) The mitochondrial genomes of *ori*^{r} spontaneous petite mutants

The mitochondrial genomes studied here were those of four spontaneous petite mutants. The *ori*$^+$ petite a-15/3/2 and *ori*^{r} petite a-15/4/1 were derived by subcloning from a-15/3 and a-15/4, two petites endowed with heterogeneous mitochondrial genomes derived in turn from the same parental spontaneous petite a-15 (Faugeron-Fonty et al., 1979). The other two petites, *ori*^{r} a-15/4/1/23 and *ori*$^+$ a-15/4/1/1, were subclones of a-15/4/1 (Mangin et al., 1983) and will be denoted henceforth as a—23 and a—1, respectively.

Petites a-15/3/2 and a-15/4/1 had mitochondrial genomes formed by repeat units about equal in length, which were derived from the same re-

gion of the wild-type genome; the former had, however, a suppressivity of about 50%, whereas the latter had a suppressivity of about 5% (see Mangin et al., 1983). As a first approximation, suppressivity can be operationally defined as the percentage of petites found in the progeny of a cross with a wild-type strain; we know that suppressivity is basically determined by the replication efficiency of the petite genome relative to that of the wild-type genome (Bernardi et al., 1980; de Zamaroczy et al., 1981; Goursot et al., 1982; Mangin et al., 1983). Restriction mapping of the a-15/3/2 genome showed (Fig. 1) that this was formed by tandem repeat units 4200 bp long containing an *ori* sequence (Fig. 1), which was localized and oriented on the wild-type genome map, *ori*5 (de Zamaroczy et al., 1981). Its left end was located at about 160 bp after the end of the *oxi*2 gene (Thalenfeld and Tzagoloff, 1980). Its right end was at about 1850 bp and 3250 bp, respectively, from a landmark *Hin*cII site and from the rightmost *Hha*I site of the genome segment of the parental wild-type strain A (Fig. 1). Incidentally, these *Hin*cII and *Hha*I sites correspond to the

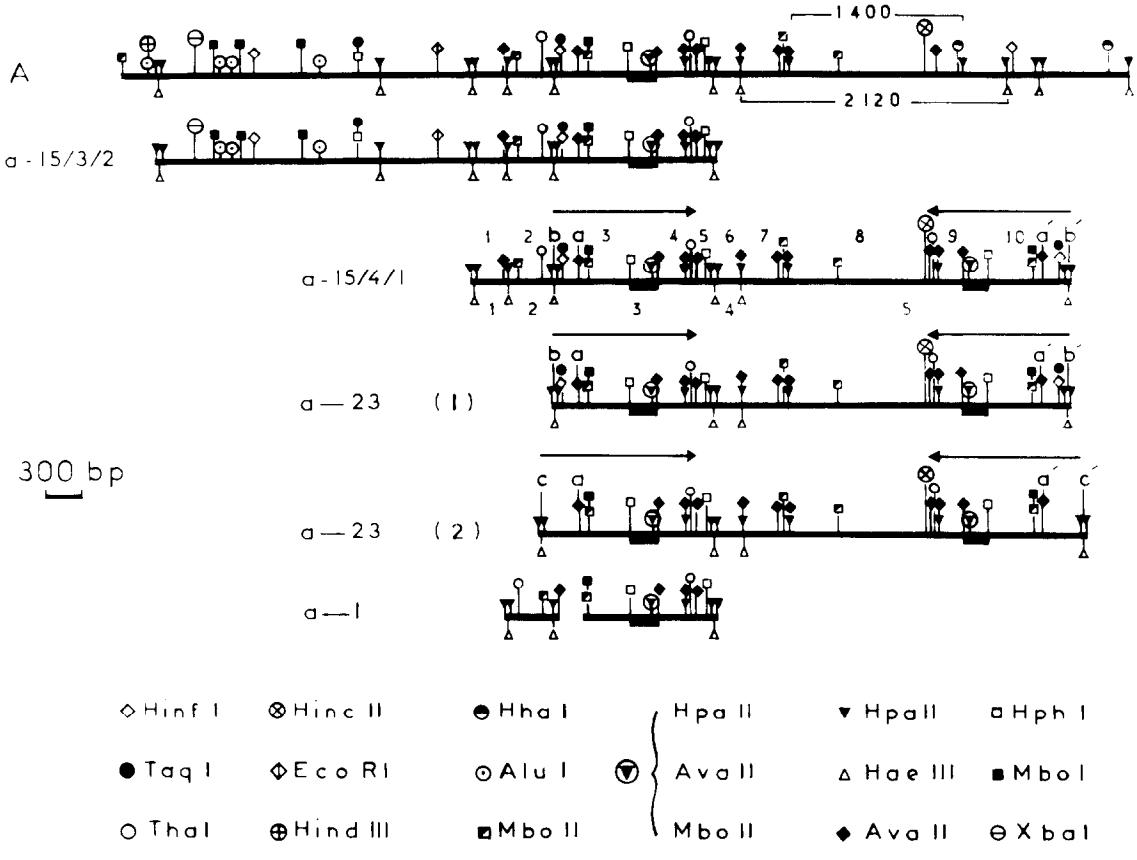

Fig. 1. Restriction maps of the repeat units of four petite mitochondrial genomes derived from the *ori*5 region of the genome of wild-type strain A. The restriction map of this region (the left-to-right direction corresponds to the clockwise orientation of the circular genome map; see Bernardi, 1982) is derived from our results and also from data of Thalenfeld and Tzagoloff (1980) for its left end and of Martin et al. (1982) for its right end. The *Hin*cII and the rightmost *Hha*I sites correspond to the methionine and proline tRNA genes, respectively (Newman et al., 1980). The lengths (in bp) of the *Hpa*II (1400 bp) and *Hae*III (2120 bp) fragments referred to in RESULTS, section **a**, are given. Not all restriction sites investigated have been mapped. Arrows indicate inverted segments in the repeat units of petite genomes. The *Hpa*II (upper designations) and *Hae*III (lower designations) fragments are numbered in the case of a-15/4/1. This fragment numbering corresponds to that of Fig. 2. Both types of repeat units of a—23 are presented (see Fig. 7 and RESULTS, section **c**); some sites on both a-15/4/1 and a—23 repeat units are indicated by letters; these sites are referred to in RESULTS, section **c**. The deleted segment of the repeat unit of a—1 is represented by a gap (see Fig. 8). The *ori*5 sequences are underlined (double-thickness lines).

genes of methionine and proline tRNAs, respectively (Newman et al., 1980). In spite of the well-established restriction fragment length polymorphism of the mitochondrial genomes harbored by different yeast strains (Bernardi et al., 1975; Prunell et al., 1977), the map distances given above and deduced from work on other strains apply to the case of strain A since restriction maps of the

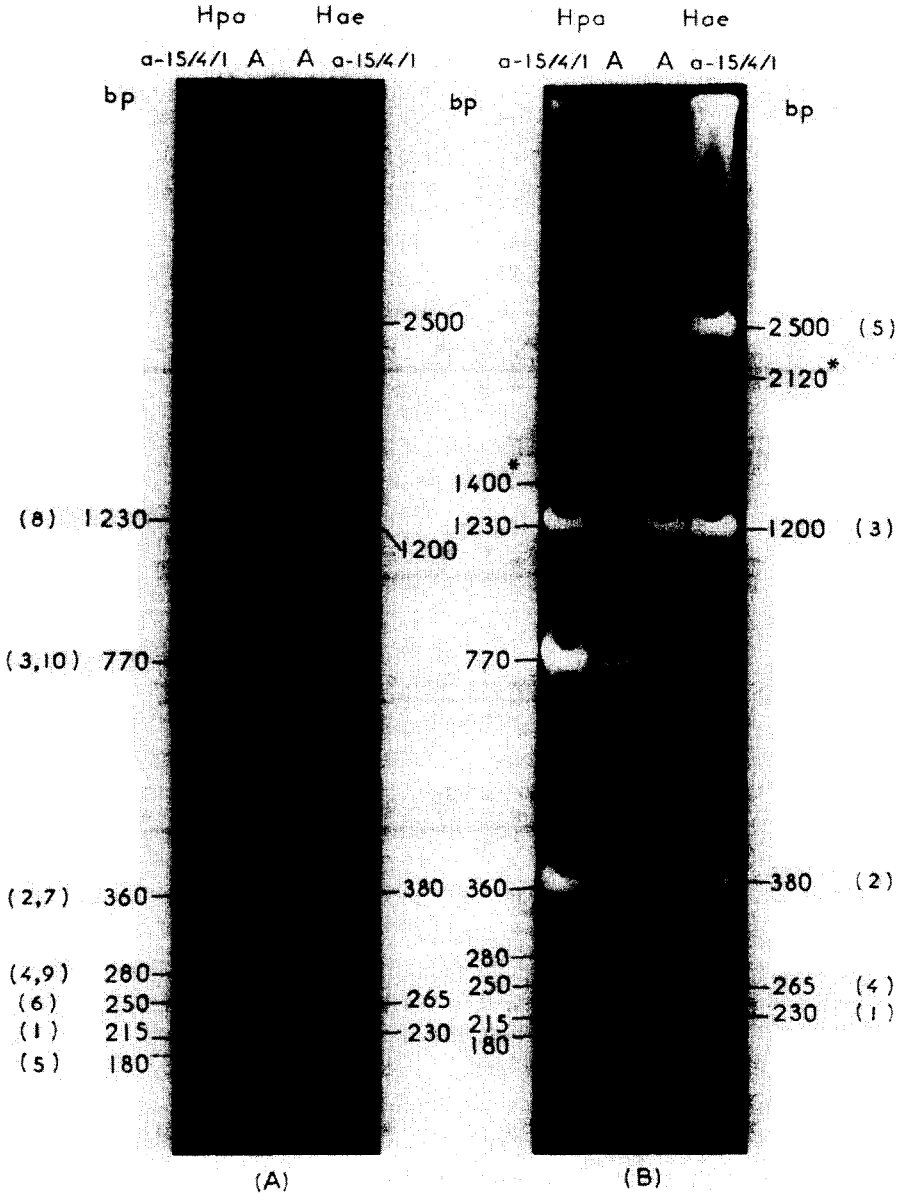

Fig. 2. (A) Electrophoretic pattern on 2% agarose gel of the *Hae*III and *Hpa*II restriction fragments of mitochondrial DNAs from petite mutant a-15/4/1 and its parental wild-type genome A. Two petite DNA *Hpa*II bands (770 and 280 bp) correspond to two pairs of identical fragments (3,10 and 4,9 of Fig. 1), another one (360 bp) to two comigrating fragments (2 and 7 of Fig. 1). Two petite DNA fragments (1230-bp *Hpa*II and 2500-bp *Hae*III) do not correspond to parental fragments, since they encompass the terminal inverted duplication of a-15/4/1 absent from the parental wild-type genome (see Fig. 1). (B) Hybridization of ^{32}P-labelled mitochondrial DNA from a-15/4/1 to nitrocellulose transfers from the gel shown in panel (A). Hybridization bands on petite fragments coincide with those seen in (A). Hybridization to wild-type DNA fragments takes place on fragments having the same size as petite fragments, with the exception of a 1400-bp *Hpa*II fragment and a 2120-bp *Hae*III fragment (asterisks; these fragments, indicated in Fig. 1, have their right ends in a region not present in the petite repeat unit). Faintly hybridizing fragments are not visible.

region under consideration are extremely similar. To sum up, petite a-15/3/2 is a "normal" spontaneous *ori*$^+$ petite, with repeat units arranged in a tandem fashion, each containing one *ori*5 sequence.

In contrast, the organization of the genome of a-15/4/1 was complex, as shown by detailed investigations involving restriction mapping with a large number of enzymes and hybridization experiments with appropriate probes. The results obtained can be summarized as follows. The *Hpa*II pattern of the repeat unit of a-15/4/1 showed 7 bands (Fig. 2A). Of these, 5 corresponded to 5 fragments (numbered 1–5 in Fig. 1 and 1810 bp in overall length), identical with those forming the right half of a-15/3/2. One *Hpa*II band (360 bp) could be shown to correspond to two comigrating fragments (2 and 7 of Fig. 1); 2 other *Hpa*II bands (770 and 280 bp) corresponded to 2 doublets of identical fragments (3,10 and 4,9, respectively; Fig. 1). Since fragments 3 and 4 flank the *Hpa*II site of GC cluster B (de Zamaroczy et al., 1981) of *ori*5 the repeat unit of a-15/4/1 must contain a duplication harboring a second *ori*5 sequence. The total number of *Hpa*II fragments in the repeat unit of a-15/4/1 is, therefore, 10 and corresponds to an overall length of 4450 bp. As shown in Fig. 2A, the *Hpa*II fragments of a-15/4/1 comigrated with those of the parental wild-type genome A, with the exception of a 1230-bp fragment (fragment 8 of Fig. 1) which joins the left side (*Hpa*II fragments 1–7) of the repeat unit with its right-end side terminal duplication (comprising *Hpa*II fragments 9 and 10). This duplication (i) followed the *Hin*cII site; (ii) contained, as mentioned above, a second *ori*5 sequence; and (iii) had an inverted orientation relative to the first one, as shown in Fig. 1. Likewise, of the 5 *Hae*III fragments of a-15/4/1 (Fig. 2A), 3 (1–3 of Fig. 1) were identical to those forming the right half of a-15/3/2; 4 (1–4 of Fig. 1) were identical to those of the parental wild-type genome, and 1 (2500-bp fragment 5 of Fig. 1) was different from any of the wild-type fragments; this fragment encompassed the terminal inverted duplication of a-15/4/1, which obviously does not exist on the parental wild-type genome.

Hybridization experiments using labeled mitochondrial DNAs from a-15/4/1 (Fig. 2B) and

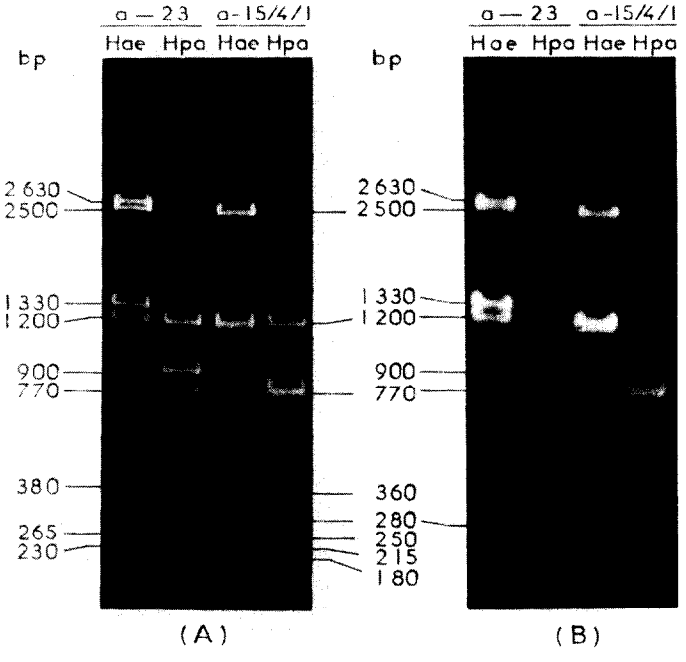

Fig. 3. (A) Electrophoretic pattern on 2% agarose gel of the *Hae*III and *Hpa*II restriction fragments of mitochondrial DNAs from petite a-15/4/1 and its subclone a—23. (B) Hybridization pattern of ^{32}P-labelled mitochondrial DNA from petite a-1/1R/Z1, used as an *ori* probe, to restriction fragments of (A). The hybridization band corresponding to the 280-bp *Hpa*II fragment of a-15/4/1 is very faint.

from a-1/1R/Z1 (Fig. 3; the latter contains barely more than *ori*1; Gaillard and Bernardi, 1979) showed the following. The a-15/4/1 DNA probe, hybridized as expected to all *Hpa*II and *Hae*III fragments of a-15/4/1 and to the corresponding fragments of the wild-type genome (faintly hybridizing fragments are not visible in Fig. 2B); in the latter case, however, the probe hybridized to a 1400-bp *Hpa*II fragment and a 2120-bp *Hae*III fragment instead of the 1230-bp and 2500-bp fragments of petite DNA; these parental fragments (whose lengths in bp are specified in Fig. 1) have their right ends in a region not represented in the petite repeat unit. The a-1/1R/Z1 probe indicated that each one of the two largest *Hae*III fragments (3 and 5) contained an *ori* sequence and that the same was true for each one of the two pairs of identical *Hpa*II fragments (3,10, 4,9; Fig. 3).

(b) The mechanism of the *ori*^{r} rearrangement

The mechanism of formation of the repeat units of a-15/4/1 can be reconstructed as follows (Fig. 4): (i) first, a segment is excised by an internal recombination process between two direct repeats located to the right of the *Eco*RI and *Hin*cII sites, respectively, of the wild-type genome (Fig. 4, a–b); the latter sequence may coincide with sequence L (see below); (ii) this segment is tandemly amplified (Fig. 4b); (iii) one of the repeat units of the primary petite genome undergoes an inversion (as in a—1; see below) through a crossing-over mechanism acting on two inverted repeats, L and R, located to the right of the *Hin*cII and of the *Tha*I sites, respectively (Fig. 4, c–d). The sequence of the L repeat, starting at 69 bp to the right of *Hin*cII, was found in data of D. Miller and N. Martin (personal commun.) and that of the R repeat, starting at the rightmost bp of the *Tha*I site, in data of M. de Zamaroczy (personal commun.). Both sequences are presented in Fig. 5 and belong in the *ori*^{s} family (Goursot et al., 1982); (iv) an excision takes place (Fig. 4d) between two *Hpa*II-*Hae*III site clusters, 1 and 3′, which are two *ori*^{s} sequences (Goursot et al., 1982), originally located on two subsequent repeat units. Incidentally, sequence 3′ is a sequence also used in the excision of the repeat units of a—1 (sequence A2

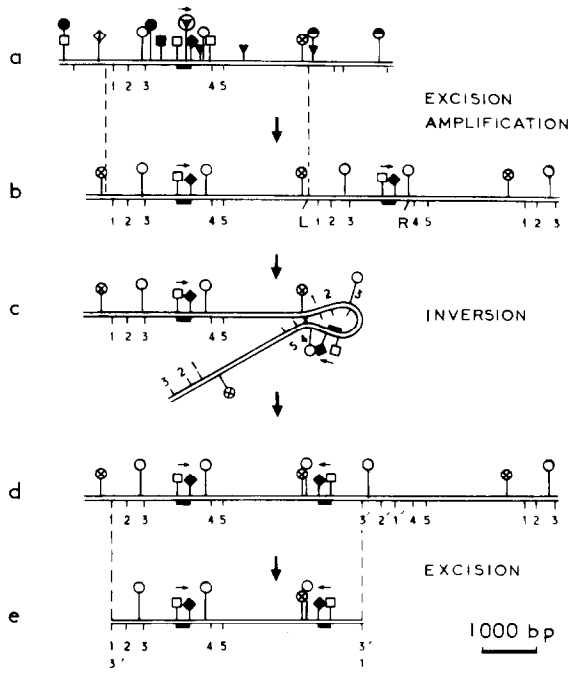

Fig. 4. Possible mechanism for the formation of the repeat unit of the mitochondrial genome of a-15/4/1. (a) Segment of the mitochondrial genome of strain A in the *ori*5 (underlined) region. Numbers and short vertical lines indicate *Hae*III-*Hpa*II site clusters; other restriction sites are as in Fig. 1. (b–e) Steps leading to the formation of the a-15/4/1 repeat unit. L and R are the inverted sequences used in the inversion process. 1′, 2′, 3′ correspond to the inverted 1, 2, 3 sequences, respectively. Short horizontal arrows indicate the orientation of *ori*5 sequences. Vertical broken lines delimit the genome segment which is excised to become the repeat unit of the resulting petite genome. Not all restriction sites are indicated in b–c. See RESULTS, section **b**, for further details.

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L    5' TATCTCCTTCGGGGTTCGGTCCCCCTC 3'
R       GAGGGGG-CGGACCCCCGAAGGAGATA

B1      ACTCCTTTGGGGT
B2      ACCCCAAAGGAGT

A1      TCCTTTCGGGG-TTCC-GGCTCCCGTAGCCGGGCCCCGGAACTTT
A2      ATAGTTCCGGGGCCCGGCCACGGGTGCCCGGAACCCCCGAAGGGA
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Fig. 5. Sequences of inverted repeats L and R (see Fig. 4), B1, B2 and A1,A2 (see Fig. 8). Restriction sites are as in Fig. 1. Vertical thick lines indicate mismatches, horizontal dashes single-base deletions. Mismatches between L and R are likely to be due to strain differences. The L and R sequences were determined by D. Miller and N. Martin (L) and by M. de Zamaroczy (R) (unpublished results).

of Fig. 5) and of a-15/3/2. It should be noted that excisions encompassing two subsequent repeat units are known in other petite genomes (de Zamaroczy et al., 1983); (v) this excision leads to the formation of the repeat unit of a-15/4/1 (Fig. 4e), which is, therefore, a secondary petite.

The amplification of the repeat units of a-15/4/1 does not occur in the usual tandem fashion, as shown by the following results. Degradation with *Hin*cII produced three bands (Fig. 6). The stoicheiometry of bands A : B : C was 1 : 2 : 1, as determined by microdensitometry of negatives of EtBr-stained gels. Band B corresponded to two fragments, whose size (4450 bp) was identical and equal to that of the repeat unit, whereas the sum of the sizes of bands A and C (6100 and 2800 bp) corresponded to two repeat units. As shown in Fig. 7, B fragments derive from cuts at *Hin*cII sites located on tandem repeat units, whereas A and C fragments derive from cuts at *Hin*cII sites located on contiguous inverted repeats. The results of Fig. 6 can be explained if the repeat units of a-15/4/1 are arranged in tandem pairs, which, in turn, are arranged in an inverted fashion (Fig. 7), an organization leading to an inverted orientation of all subsequent *ori* sequences. An alternative explanation of the stoicheiometry of *Hin*cII bands involving a very particular combination (possibly in different genome units) of simple inverted repeats with tandem repeats is not compatible with the fact that petite genome units are polydisperse in size, and is not supported by partial *Hin*cII digests (not shown) which only reveal the bands expected on the basis of the first model.

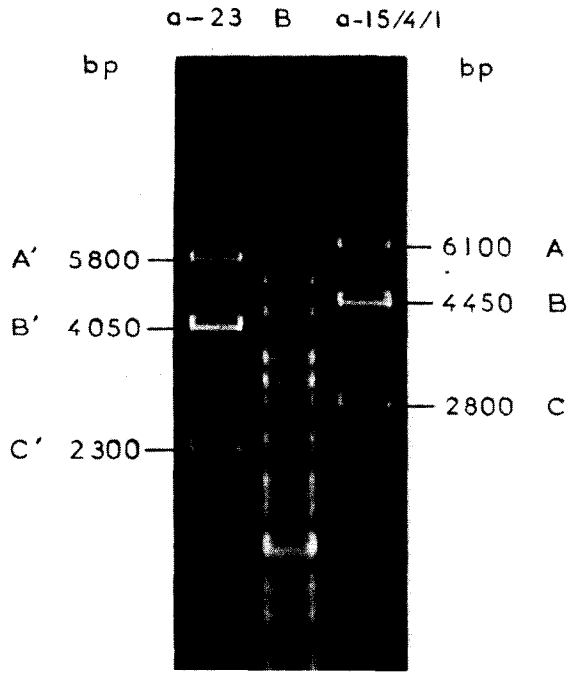

Fig. 6. Electrophoretic pattern on 0.8% agarose gel of *Hin*cII digests of mitochondrial DNAs from petite mutant a-15/4/1 and its subclone a—23. *Hae*III fragments of the DNA of wild-type strain B were used as molecular weight markers in this particular experiment, but other fragments were used in other experiments. Bands B and B′ correspond to pairs of fragments having the size of the repeat units and the average size of repeat units 1 and 2 of a—23, respectively. The sums A + C and A′ + C′ correspond to two repeat units, of the two classes in the case of a—23.

(c) The mechanism of further genome rearrangements

In the case of the mitochondrial genome of a—23, a petite derived from a-15/4/1 by subcloning, the repeat units were significantly different from those of the parental genome and belonged to two classes (Figs. 1, 3, 6 and 7). Class 1 (3950 bp long) lacked the two small *Hae*III and *Hpa*II fragments (1 and 2) forming the left end of the repeat unit of a-15/4/1 (Figs. 1 and 3). In class 2 (4210 bp long), the distance between the *Ava*II sites a,a′ and the terminal *Hae*III-*Hpa*II site clusters c,c′ was larger by 130 bp than that between a,a′ and the nearest *Hae*III-*Hpa*II site clusters b,b′ on the repeat unit of a-15/4/1 (Figs. 1 and 7); furthermore, the *Hin*fI-*Taq*I site cluster at 25 bp from b,b′ was missing, and the repeat unit was shorter on its left end than that of a-15/4/1. The deletions undergone by repeat units 1 and 2 of a—23 relative to those of the parental petite a-15/4/1 can be accounted for by two excisions taking place on a circular tandem dimer of a-15/4/1 (represented in linearized form in Fig. 7), whereas no excision on a circular inverted dimer of a-15/4/1 can lead to the increased length of a′c′ relative to a′b′.

On the basis of the *Hin*cII digestion patterns (Fig. 6), the overall arrangement of repeat units in the a—23 genome consisted, as proposed for a-15/4/1, of two tandem repeats followed by two tandem repeats in opposite orientation. Interest-

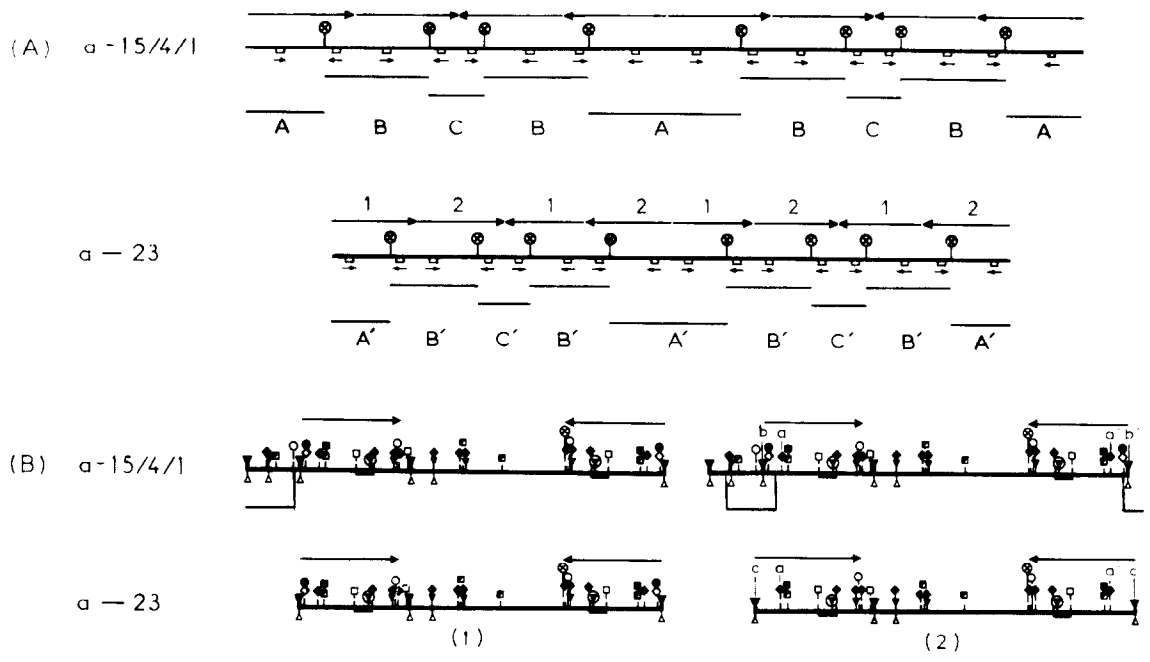

Fig. 7. (A) Mitochondrial genome organization of petite mutants a-15/4/1 and a—23. Orientation of repeat units is given by the upper arrows; *ori*5 sequences are indicated by boxes, their orientation by small arrows; *Hin*cII fragments (A, B, C and A', B', C'; see Fig. 6) are denoted by horizontal bars. *Hin*cII sites are as in Fig. 1. (B) Restriction maps of the repeat units of a-15/4/1 and a—23 employ the symbols of Fig. 1. Small-case letters indicate some particular restriction sites (see RESULTS, section c). (1) and (2) represent the two different repeat units present in the genome of a—23; arrows indicate inverted repeats. The deletions supposed to lead from the repeat units of a-15/4/1 to repeat units 1 and 2 of a—23 are indicated below the a-15/4/1 restriction maps (see RESULTS, section c).

ingly, the size of B' fragments corresponded to the distance of two *Hin*cII sites located on repeat units arranged in a tandem fashion and belonging to class 1 and class 2, respectively, showing that class 1 and class 2 repeat units are arranged in the order 1-2-1-2 (Fig. 7) and not in the order 1-2-2-1. This was confirmed by the sizes of other restriction fragments encompassing repeat units 1 and 2 (such as those produced by *Mbo*I, *Ava*II, *Hin*fI, *Taq*I and *Hin*cII). As in the case of a-15/4/1, partial *Hin*cII digests were only compatible with the repeat unit arrangement shown in Fig. 7.

Another subclone of a-15/4/1, petite a—1 (Fig. 1), was an *ori*$^+$ secondary petite exhibiting a genome formed by tandem repeat units which presented, however, a deletion and an inversion relative to the wild-type genome. Mapping and sequence analysis of the repeat unit of a—1 of the corresponding region of the parental wild-type strain A made possible a precise reconstruction of the process leading to these repeat units (Fig. 8): (i) the repeat unit of a primary petite (a-15/4/1 or a similar one) exhibits two pairs of inverted repeats, A1,A2 and B1,B2 (Fig. 8a); sequences A1 and A2 belong in the *ori*^{8} sequences, sequences B1 and B2 in the *ori*^{8} family (Goursot et al., 1982); these sequences are specified in Fig. 5; sequence A2 corresponds to the right excision sequence of a-15/3/2 (de Zamaroczy et al., 1983); sequences A1, B1 and B2 correspond to nucleotides 465-508, 87-99 and 641-653, respectively, of the repeat unit of petite a—10/3 (Goursot et al., 1982); (ii) an inversion takes place between B1 and B2, and changes A1,A2 into two direct repeats (Fig. 8 b, c); (iii) an excision takes place by internal recombination between A1,A2 (Fig. 8d), leading to the formation of the repeat unit of a—1 (Fig. 8 e, f).

To sum up, the excision process generating the repeat unit of a—1 is the classical one (de Zamaroczy et al., 1983), and the special features of the a—1 repeat units are simply due to the fact

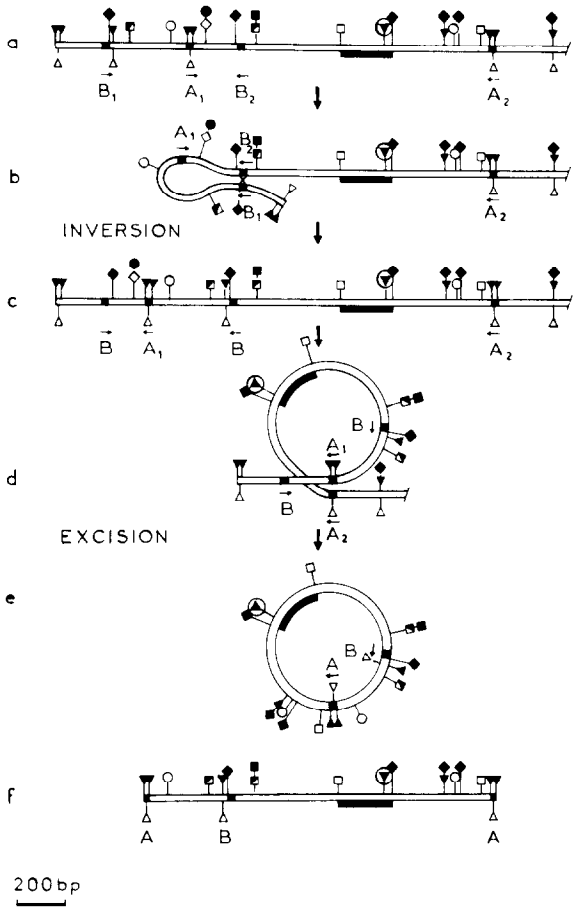

Fig. 8. Possible steps leading to the formation of the repeat unit of the mitochondrial genome of petite mutant a—1. (a) Left end of the repeat unit of a-15/4/1. Restriction sites are as in Fig. 1. Horizontal arrows indicate the relative orientations of the two pairs of inverted repeats A1,A2 and B1,B2. (b–e) Steps leading to the formation of the a—1 repeat unit. A and B are the hybrid sequences generated by the recombination processes which involved A1,A2, and B1,B2, respectively. Not all restriction sites are indicated in (b, d). (f) Linearized form of the repeat unit of a—1. The inverted and the deleted segments of a —1 correspond to segments B1-A1, and A1-B2 from the repeat unit of the primary petite shown in (a). See RESULTS, section c, for further details.

that excision occurs on a petite genome which is already rearranged, in that a DNA segment has been inverted. Since excision involves, on one side, a sequence located on the inverted segment, the final result is that the repeat unit of a—1 presents a deletion beside an inverted segment.

DISCUSSION

The present investigations provide novel information on the structure, the mechanism of formation and the organization of repeat units from the mitochondrial genome of spontaneous petite mutants of yeast which we call *ori*^{r}. These petites are extremely rare (a-15/4/1 and its subclone a—23 were the only *ori*^{r} petites found among several hundred spontaneous petites studied in our laboratory) for reasons which are now understood and explained in the following paper (Mangin et al., 1983). The repeat units of *ori*^{r} petites are characterized by a terminal inverted duplication harboring a second (inverted) *ori* sequence. The *ori*^{r} petites can only arise as secondary petites since the mechanism leading to the formation of the rearranged repeat units involves inversions occurring by internal recombination at inverted nucleotide repeats (a point first demonstrated here for the mitochondrial genome of yeast) which are located on subsequent repeat units of a primary petite genome. We have shown that the repeat units of *ori*^{r} genomes are arranged as tandem dimers in inverted orientation relative to their nearest neighbors. The amplification mechanism leading to such arrangement cannot be a simple rolling circle mechanism acting on a circular monomer, as in the case of the tandemly arranged repeat units of *ori*$^+$ petites, but must be a more complex process. Some important points of the latter have been clarified by this work. First of all, the excisions leading to the formation of the two different repeat units of a—23 from those of a-15/4/1 appear to have taken place on tandem dimers of the latter; this indicates that tandem dimers and not monomers or inverted dimers are the initial templates for DNA amplification. Such tandem dimers can only be amplified into inverted tandem dimers. We will not speculate here on the mechanism underlying this process, but we suggest that it is linked with the presence of terminal inverted duplications containing a second inverted *ori* sequence. This is indicated by the following observations: (i) the removal of the inverted duplication systematically leads to the formation of tandemly amplified *ori*$^+$ genomes (Mangin et al., 1983); and (ii) the presence of a terminal, non-duplicated inversion not containing an *ori* se-

quence, as found in the genome of a—1, is not conducive to the repeat unit arrangement of *ori*^{r} genomes, but to the usual tandem one of *ori*$^+$ genomes.

The results on *ori*^{r} genomes just described lead to some new insights on the EtBr-induced petite mutation. Indeed, the restriction patterns obtained with enzymes like *Hin*cII, which cut asymmetrically in the nonduplicated part of the repeat units of a-15/4/1 and a—23, are identical to those reported by Heyting et al. (1979) and Bos et al. (1980) for the mitochondrial genomes with inverted repeat units of induced petites. These patterns were erroneously interpreted by Bos et al. (1980) as indicating "random mixed" direct and inverted repeats in those genomes. The 1:2:1 stoicheiometry of the *Hin*cII bands of Fig. 6, or of the equivalent bands from the genomes studied by Bos et al. (1980), indicates the regular organization of repeat units shown in Fig. 7, and we suggest that such is also the arrangement of repeat units in the induced petite genomes of Bos et al. (1980). A difference between the latter and the *ori*^{r} genomes is that most of the former do not seem, from the limited mapping data available, to contain any canonical *ori* sequence. We suggest that the role played by such sequences in the amplification of *ori*^{r} genomes is played in those EtBr-induced genomes by the ubiquitous surrogate origins of replication, the *ori*^{s} sequences (Goursot et al., 1982).

The above points lead to some more general considerations about the mitochondrial genome of induced petite mutants. We already know (de Zamaroczy et al., 1983) that direct nucleotide repeats, almost always located in the AT spacers and GC clusters of intergenic sequences, are used in the excision of both spontaneous genomes and of induced petite genomes with tandemly arranged repeat units. In other words, no difference can be seen between this class of induced petite genomes and spontaneous petite genomes. Now we see that, although very rare, rearranged genomes of spontaneous petites exist which are identical in the structure and organization of repeat units to the genomes with inverted repeats of EtBr-induced petites. Then the main difference between spontaneous and induced petites consists of the different frequency of such rearranged genomes in these two classes of mutants. Investigations presented in the following paper (Mangin et al., 1983) account for this difference.

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