The EMBO Journal Vol.1 No.6 pp.705–711, 1982

Surrogate origins of replication in the mitochondrial genomes of *ori*° petite mutants of yeast

Regina Goursot, Marguerite Mangin[1], and Giorgio Bernardi*

Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, 2, Place Jussieu, 75005 Paris, France

Communicated by G.Bernardi
Received on 27 May 1982

We have investigated the mitochondrial genome of eight *ori*° spontaneous petite mutants of *Saccharomyces cerevisiae*. The tandem repeat units of these genomes do not contain any of the seven canonical *ori* sequences of the wild-type genome. Instead, they contain one, or more, *ori*^{s} sequences. These 44-nucleotide long surrogate origins of replication are a subset of GC clusters characterized by a potential secondary fold with two sequences ATAG and GGAG, inserted in AT spacers, two AT base pairs just following them, a GC stem (broken in the middle, and, in most cases also near the base, by non-paired nucleotides), and a terminal loop. This structure is reminiscent of that of GC clusters A and B from canonical *ori* sequences and supports the view (Bernardi, 1982a) that the GC clusters of the mitochondrial genome arose, by an expansion process, from the canonical *ori* sequences. Like the latter, *ori*^{s} sequences are present in both orientations, are located in intergenic regions, and can be used as excision sequences when tandemly oriented. Again as in the case of canonical *ori* sequences, the density of *ori*^{s} sequences on the repeat units of petite genomes are correlated with the replication efficiency of the latter, as assessed by the outcome of crosses with wild-type or petite tester strains.

Key words: mitochondrial DNA/petite mutation/yeast

Introduction

The molecular genetics of the spontaneous cytoplasmic "petite colonie" mutation of *Saccharomyces cerevisiae* is now largely understood (see Bernardi 1979, 1982b for two brief reviews). The first event is the excision of a segment of a mitochondrial genome unit from a wild-type cell; excision takes place between two direct sequence repeats located in the non-coding regions of the genome (de Zamaroczy *et al.*, in preparation). This is followed by a tandem amplification process (in which the excised segment becomes the repeat unit of a defective genome unit), and by the replication of the genome unit within the wild-type cell. The last step is the segregation of defective genome units into the buds formed by the wild-type cell; further segregation of these genome units in the progeny leads to the formation of petite mutants, whose mitochondrial genome is formed by identical defective units. We have shown that the excised segment usually carries one (or more) of the seven canonical origins of replication that we have sequenced, localized, and oriented on the wild-type genome (de Zamaroczy *et al.*, 1979; 1981; Goursot *et al.*, 1980; Bernardi *et al.*, 1980; see Figure 1); in this case, *ori*$^{+}$ petite genomes are produced.

The excision process may, however, also lead to special situations. For instance, the excision may take place between two *ori* sequences; this results in the production of *ori*^{h} petite genomes with a hybrid *ori* sequence (Marotta *et al.*, 1982). In other cases, the excision occurs between two sequences located inside and outside an *ori* sequence, leading to *ori*$^{-}$ petite genomes with a partially deleted *ori* sequence (de Zamaroczy *et al.*, 1981). Finally, the excised segment may contain none of the seven canonical *ori* sequences of the wild-type genome, resulting in the formation of an *ori*° petite genome (de Zamaroczy *et al.*, 1981). We have shown that these different situations are accompanied by different replication rates of the defective genome units relative to that of the resident, parental wild-type units. In turn, these relative replication rates influence the differential segregation of the defective genome and, therefore, its level of transmission to the progeny; the other two factors playing a role here are the rate at which the defective genome was excised and its stability in the cells harboring it (Marotta *et al.*, 1982). When a petite is crossed with a wild-type cell, the replication rate of its genome is the main factor in determining its level of transmission to the progeny. As expected, suppressivity can range from almost 100%, in the case of certain *ori*$^{+}$ petites, which we have called super-suppressive, (de Zamaroczy *et al.*, 1979; Goursot *et al.*, 1980; Bernardi *et al.*, 1980; these petites have been called hyper-suppressive by Blanc and Dujon, 1980) to almost 0% in the case of some *ori*° petites. It is of interest to note that the different replication rates associated with different *ori* sequence situations are paralleled by similar changes in transcription; in particular, *ori*° genomes are transcribed at a minimal level, if at all (Baldacci and Bernardi, in preparation).

In the present work, part of which was presented at the Cold Spring Harbor Meeting on Mitochondrial Genes (May 1981), we have studied eight *ori*° petite genomes, mainly to learn about the sequence organization of these genomes and to identify the surrogate *ori* sequences they use to replicate. We have found that the repeat units of all these *ori*° genomes carry at least one 44-nucleotide long sequence, which we call the *ori*^{s} sequence and which appears to act as a surrogate origin of replication.

Results

Table I presents the basic properties of the *ori*° petites investigated here as well as a simplified nomenclature, which will be used throughout this paper. Except for petite *1*, which was derived from wild-type strain A, they were all derived from wild-type strain B. The genome units of these strains have very similar restriction maps, the main difference being that strain B lacks *ori* 4.

The precise localization of six *ori*° petite genomes, *1* to *6*, on the map of the genome of mitochondria of wild-type yeast cells is shown in Figure 1. This localization was determined by hybridizing the corresponding labelled DNAs on restriction fragments from wild-type genomes and from petite genomes

[1]Present address: Department of Biophysics and Biochemistry, Yale University, New Haven, CT 06510, USA

*To whom reprint requests should be sent.

IRL Press Limited, Oxford, England. 0261-4189/82/0106-0705$2.00/0.

Table I. Properties of *ori°* petite genomes

	Petite strain	Ploidy[a]	Origin	Parental wild-type strain	Repeat unit length (bp)[b]	*ori*^{S} sequences		Suppressivity (%)
						Number	Orientation[a]	
1	a-10/3	h	a-15/4/1	A	954 s	1	←	1
2	b-28/1	h	B	B	1210 cm	2(h)[c]	← ←	20
3	a-10/3/2/B11	d	a-10/3/2xB	B	481 s	1(h)	←	–
4	a-3/1/B4	d	a-3/1xB	B	325 s	1	→	–
5	b-10/7/2	h	B	B	1797 s	3	← ← →	30
6	a-10/3/B12	d	a-10/3xB	B	659 s	1(h)		–
a	a-10/3/B4	d	a-10/3xB	B	6000 pm	3–4	→	–
b	a-10/3/B14	d	a-10/3xB	B	1500 pm	3		–

[a]h and d mean haploid and diploid, respectively.
[b]s, cm, and pm mean sequenced, completely mapped, and partially mapped, respectively.
[c](h) indicates a hybrid *ori*^{S} sequence (see text).
[d]arrows pointing to the right indicate a clockwise orientation of the *ori*^{S} sequence (ATAG–GGAG) on the wild-type genome.

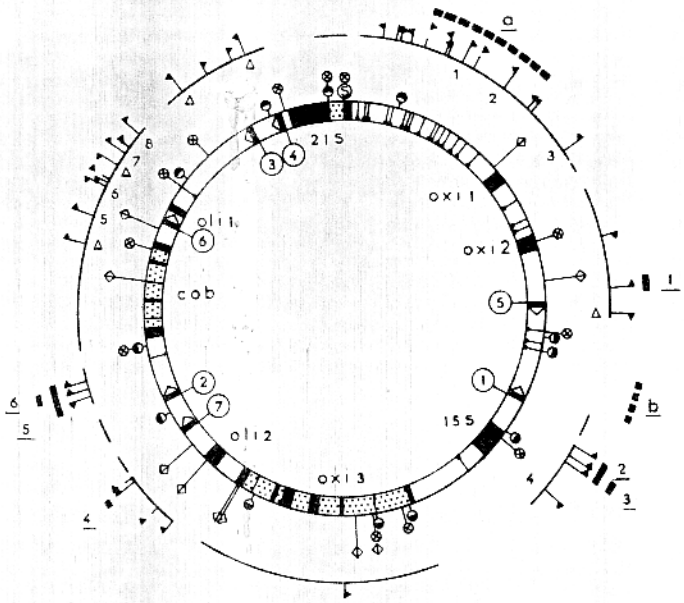

Fig. 1. Map of the mitochondrial genome unit of wild-type yeast strain A (the map of strain B is very similar, but lacks the *ori* 4 sequence; G.Faugeron-Fonty, personal communication). Black and dotted areas correspond to exons and introns, respectively, of mitochondrial genes; thin radial lines indicate tRNA genes; canonical *ori* sequences are indicated by numbered thick bars, and their orientation (cluster C→cluster A) is given. Restriction sites are indicated as in Figure 2; Ⓢ is *Sal*I. Petite repeat units are represented by thick lines when exactly mapped, *1–6*, and by broken lines when not precisely localized, *a* and *b* (see Table I). Flags indicate the position and the orientation (ATAG–GGAG) of the *ori*^{S} sequences, as found on the repeat units of the petites studied here or of other sequences of the *ori*^{S} family as localized on petite genomes investigated in other laboratories (see text and Table II). The outside broken circle carrying the flags indicates the genome segments sequenced so far in several laboratories.

previously localized on the wild-type genome and by comparing the restriction maps (see below). Two additional *ori°* petite genomes *a* and *b* were localized only approximately (Figure 1).

Figure 2 displays the restriction maps of the three regions of the wild-type genome from which six *ori°* petite genomes were excised. Incidentally, this mapping provided information on the excision sequences of some *ori°* petite genomes (de Zamaroczy *et al.*, in preparation).

Partial restriction digests were used to demonstrate the tandem arrangement of the repeat units in all *ori°* genomes investigated. Such an arrangement is the rule for the mitochondrial genomes of all spontaneous petite mutants (Faugeron-Fonty *et al.*, 1979; only one exception was found so far in the hundreds of petite genomes studied in our laboratory; de Zamaroczy *et al.*, 1981; Mangin *et al.*, in preparation). The ladders of restriction fragments showing mol. wts. equal to entire multiples of that of the repeat unit are shown in Figures 3 and 4e for six genomes; those obtained for the other two, less characterized, genomes *a* and *b* are not shown.

Figure 4 presents electrophoretic patterns of restriction digests of two *ori°* petite genomes as well as the corresponding hybridization patterns obtained with an *ori* probe. This experiment shows that under conditions where <0.01 *ori* sequence/genome unit of 50×10^6 could be detected on the control DNA, no *ori* sequence was seen in any of the two *ori°* petite genomes. Similar experiments (not shown) provided the same results for the other *ori°* petites. The purpose of this experiment was to rule out the existence of even a single canonical *ori* sequence which might have been inserted in the genome units of the petites under consideration at the time of their excision from the wild-type genome.

The primary structure of the repeat units of 5 *ori°* mitochondrial genomes is given in Figure 5. The most striking feature is the presence of one, (or three in one case), common GC cluster, 44-nucleotides long, which can be folded as shown in Figure 6a and which we will designate, henceforth, as the *ori*^{S} sequence. The main features of the *ori*^{S} sequence are: the sequences ATAG and GGAG, inserted in AT spacers, two AT base pairs just following them, a GC stem (broken in the middle and, in most cases also near the base, by non-paired nucleotides), and a terminal loop. Other GC clusters are present in three out of five *ori°* genomes. The *ori*^{S} sequences are characterized by several interesting features: (a) with only two exceptions, in each of which a single base change was found (Figure 6a), the sequence is perfectly conserved; (b) the sequence may be present in either orientation on the wild-type genome map (Figures 1 and 5); (c) the sequence is present in intergenic regions; and (d) tandem *ori*^{S} sequences may be used as excision sequences, with the consequence that the *ori*^{S} sequence carried by the resulting petite genome is a hybrid *ori*^{S} sequence (de Zamaroczy *et al.*, in preparation) which will be called *ori*^{Sh}; such is the case of petite genomes *2*, *3*, and *6* (Figure 2).

We have found eight additional sequences extremely similar to the *ori*^{S} sequence in published primary structures

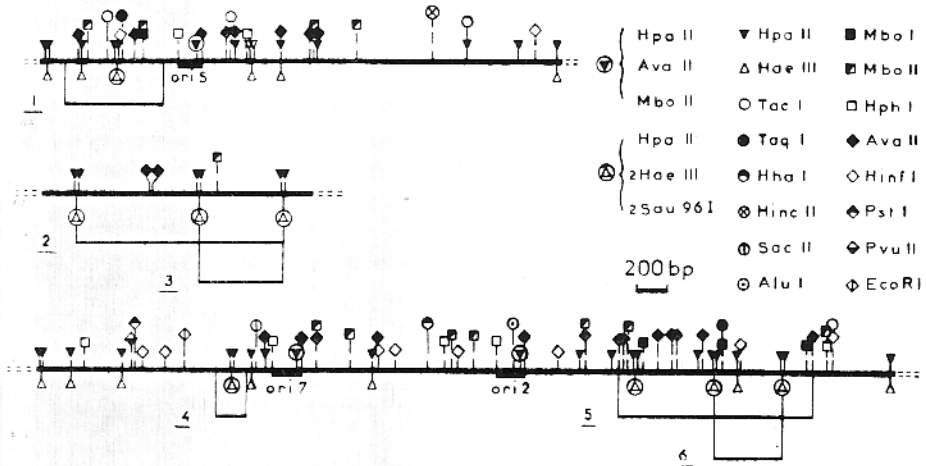

Fig. 2. Restriction enzyme maps of the three regions of the wild-type mitochondrial genome from which the *ori*° petite genomes *1*–*6* were excised. The map of petite a-15/4/1 (Mangin *et al.*, in preparation), was used to precisely localize *ori*° petite *1*. The maps of *ori*° petite genome *2* and *3* were used to reconstruct the wild-type genome region from which these *ori*° genomes were derived. Finally, the map of petite b17 (Marotta *et al.*, 1982) was used to localize *ori*° genomes *4*–*6*. Vertical lines indicate the excision sites of *ori*° petite genomes (de Zamaroczy *et al.*, in preparation). All maps are given in a clockwise orientation on the wild-type genome. Of the two site clusters, one, ●, corresponds to cluster B of *ori* sequences; the other one, ○, to the *ori*$^{\delta}$ sequence (see text).

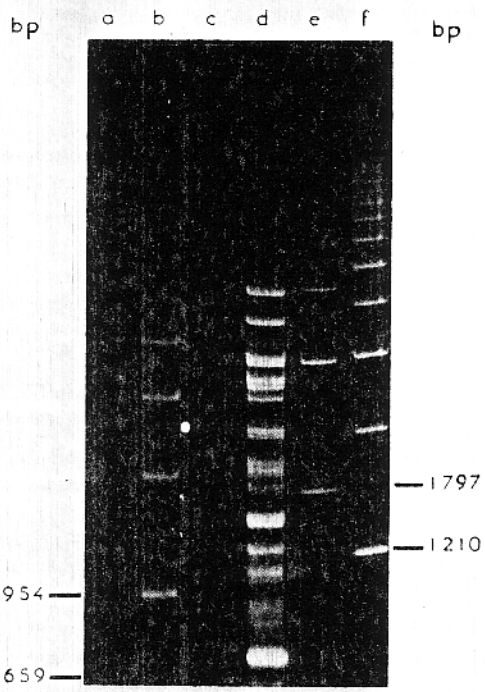

Fig. 3. Electrophoretic pattern of partial restriction digests of mitochondrial DNAs from *ori*° petite mutants *6* (*Hinf*I; **lane a**); *1* (*Hinf*I; **lane b**); *3* (*Mbo*II; **lane c**); *5* (*Hinf*I; **lane e**); *2* (*Mbo*II; **lane f**). A total *Hae*III digest of DNA from wild strain B is shown on **lane d**. Mol. wts. are given for the monomeric fragments.

(Tzagoloff *et al.*, 1979; Bonitz and Tzagoloff, 1980; Nobrega and Tzagoloff, 1980; Sor and Fukuhara, 1980; Coruzzi *et al.*, 1981); these sequences (Figure 6b) share all the main features of the *ori*$^{\delta}$ sequence; we will call them *ori*$^{\delta}$-like. Four other sequences, still sharing the ATAG and GGAG sequences and the contiguous sequences, but apparently missing 5–9 nucleotides, have also been found; these sequences will be indicated as *ori*$^{\delta}$-like, partially deleted. It should be pointed out that the partial deletion seen in the center of the sequence might not be real, but due to the loss of a very small restriction fragment between two *Hae*III sites during the preparation of terminally labelled fragments for the sequencing experiments. Five more sequences, whose central part was not investigated, and which will be called *ori*$^{\delta}$-like, incomplete, may belong in either one of the three classes just mentioned. Finally, yet another class of GC clusters, the *ori*$^{\delta}$-related sequences, was found; this class shared the ATAG and GGAG sequences but none of the other features of *ori*$^{\delta}$ sequences. Table II presents a summary of these classes of *ori*$^{\delta}$ and related sequences, and Figure 1 displays their localization on the sequenced regions of the wild-type genome map.

In order to assess the replication efficiency of the *ori*° petites investigated here, crosses were made of the haploid ones with a tester wild-type strains (A or B). This revealed suppressivities, namely levels of transmission of the parental petite genome in the progeny (de Zamaroczy *et al.*, 1981), ranging from 1% for petite *1*, to 20% for petite *2*, and to 30% for petite *5* (Table I).

As in the case of *ori*$^{+}$ petites, namely of petites carrying at least one canonical origin of replication, crosses of haploid *ori*° petites *2* and *5* with *1* led to the results one would expect on the basis of their suppressivities, namely that the more suppressive one preferentially transmitted its genome to the progeny. Indeed, 21 out of 21 diploids issued from a *1* x *2* cross carried the genome of petite *2*; and 14 out of 14 diploids issued from a *1* x *5* cross carried the genome of petite *5*.

Discussion

GC-rich sequences in the mitochondrial genome of yeast have a long history. Our original work on the melting transitions of the mitochondrial DNA from wild-type yeast strains, (Bernardi *et al.*, 1970), revealed the presence of sequences melting at extremely high temperatures, a finding particularly striking for a DNA having a very low GC content (18%). The presence among pyrimidine isostichs of C_3, C_3T, and C_4 in large excess over statistical expectations (Ehrlich *et al.*, 1972) and the isolation of very G + C-rich (>60%) fragments after micrococcal nuclease degradation (Prunell and Bernardi, 1974) both pointed to a GC clustering in the mitochondrial genome of yeast. A detailed study of *Hae*III and *Hpa*III

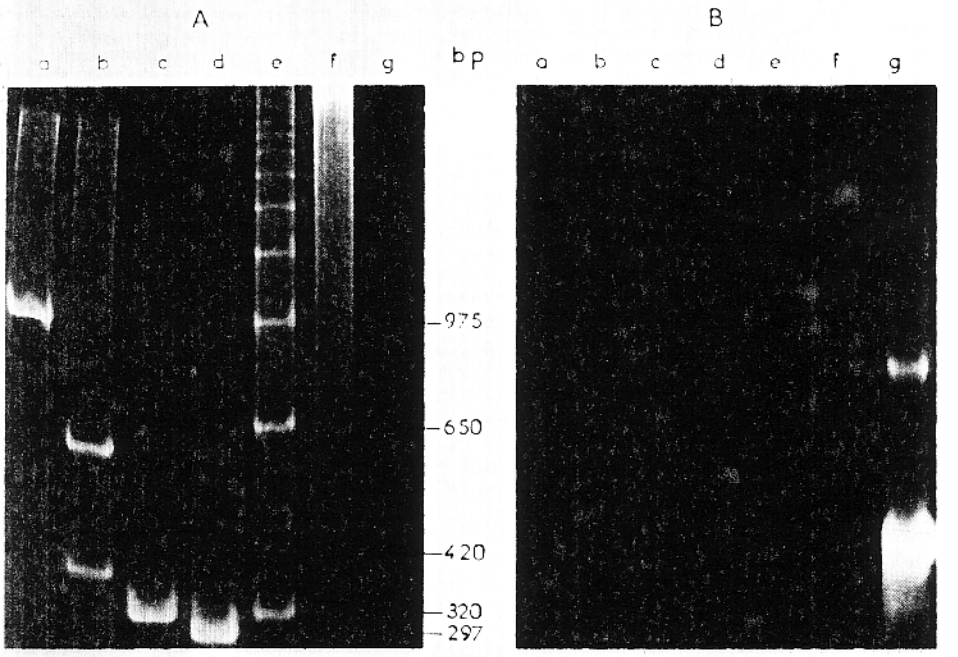

Fig. 4. (A) Electrophoretic pattern of restriction digests of mitochondrial DNAs from *ori°* petite mutants: *1* (*Mbo*I; **lane a**; *Hae*III; **lane b**); *4* (*Hae*III; **lane c**; *Hpa*II; **lane d**; partial *Hae*III; **lane e**; undigested **lane f**); a-1/1R/Z1 (*Mbo*I; **lane g**). DNA amounts loaded were 1 μg **(lanes a,b)**; 2.5 μg **(lanes c – f)**; 25 μg **(lane g)**. **(B)** Hybridization pattern with ^{32}P-labelled mitochondrial DNA from petite a-1/1R/Z1, used as *ori* sequence probe.

digests (Prunell and Bernardi, 1977) led to the discovery of GC clusters, present in large numbers in the genome and some containing *Hae*III and *Hpa*II sites. This was later confirmed by sequence work (Cosson and Tzagoloff, 1979; Gaillard and Bernardi, 1979). On the basis of a previous hypothesis on the symmetry relationships in protein-DNA interactions (Bernardi, 1968), we suggested, at that time, that the symmetry and homology properties of the clusters made them good candidates for both regulatory roles (promoters, operators, replicators) and recombination. The presence of 3 – 5 GC clusters in each of the seven canonical *ori* sequences, which appear to fulfill both a replicator (de Zamaroczy *et al.*, 1981) and a promoter role (Baldacci and Bernardi, in preparation), and the use of GC clusters as sequences involved in the excision of petite genomes by an internal crossing-over mechanism (de Zamaroczy *et al.*, in preparation) confirm the validity of the suggestion just mentioned. More recently, the homology found to exist between the GC clusters (and the AT spacers) on one hand and the *ori* sequences on the other was explained as due to the fact that the former arose from the latter by an expansion mechanism (Bernardi, 1982a).

The findings of the present work are of interest from two viewpoints: (a) because they account for the replication of the mitochondrial genome of *ori°* petites; and (b) because they shed light on several of the points mentioned above. We will consider here the main conclusions arrived at.

1. All *ori°* spontaneous petite genomes investigated here contain one or more *ori*^{S} sequences. This has been shown by full sequencing of the repeat units in five cases (Figure 5 and Table I), by complete mapping in one case (Figure 2 and Table I), and by partial mapping in two cases (Table I). In the latter three cases, sequencing is needed to assess the level of homology of the mapped *ori*^{S} sequences with those already sequenced (Figures 5 and 6a). At the same time, the *ori°* genomes do not contain any canonical *ori* sequence. This latter point is not only demonstrated by the fact that all *ori°* petite genomes are exclusively formed by tandem repeat units mapping outside *ori* sequences (Figure 1), but also by the actual lack of any *ori* probe hybridization under conditions which allow detection of 0.01 *ori* sequence/genome units of 50 x 10^6. The possibility that even only one such sequence is present per *ori°* genome unit, *via* a process of translocation onto the nascent petite genome is, therefore, ruled out.

2. *Ori*^{S} sequences appear to act as surrogate origins of DNA replication. This is indicated by their constant presence in the repeat units of all *ori°* petite genomes we have explored so far and also by the correlation (Table I) between their density in the *ori°* genomes and the suppressivity of the corresponding petites. Indeed, while petite *1* (one *ori*^{S} sequence/954 bp) had a suppressivity of 1%, petites *2* (two *ori*^{S} sequence/1210 bp), and *5* (three *ori*^{S} sequences/1800 bp) have suppressivities of 20% and 30%, respectively. A similar correlation between *ori* sequence density and suppressivity was previously found for *ori*$^{+}$ petites (de Zamaroczy *et al.*, 1981; see point 3 below). It cannot be ruled out that additional GC clusters, different from the *ori*^{S} sequences, such as those present in petite genome *5* (Figure 5), may play a positive or negative subsidiary role. The presence of a single *ori*^{S} sequence in repeat units otherwise only formed by AT spacers (as in petite genomes *3* and *4*) is, however, sufficient for DNA replication.

3. The poor replicative competitivity of *ori°* petite genomes is not only witnessed by their low or very low suppressivities (Table I), but also by the way they were isolated (see Table I and Materials and methods). One of them, *1*, derived from the only spontaneous petite genome, a-15/4/1, found so far to contain a rearranged sequence with two close *ori* 5 sequences in inverted orientations within repeat units which are themselves in both tandem and inverted orientation (de Zamaroczy *et al.*, 1981; Mangin *et al.*, in preparation), a situation which appears to be responsible for a very low sup-

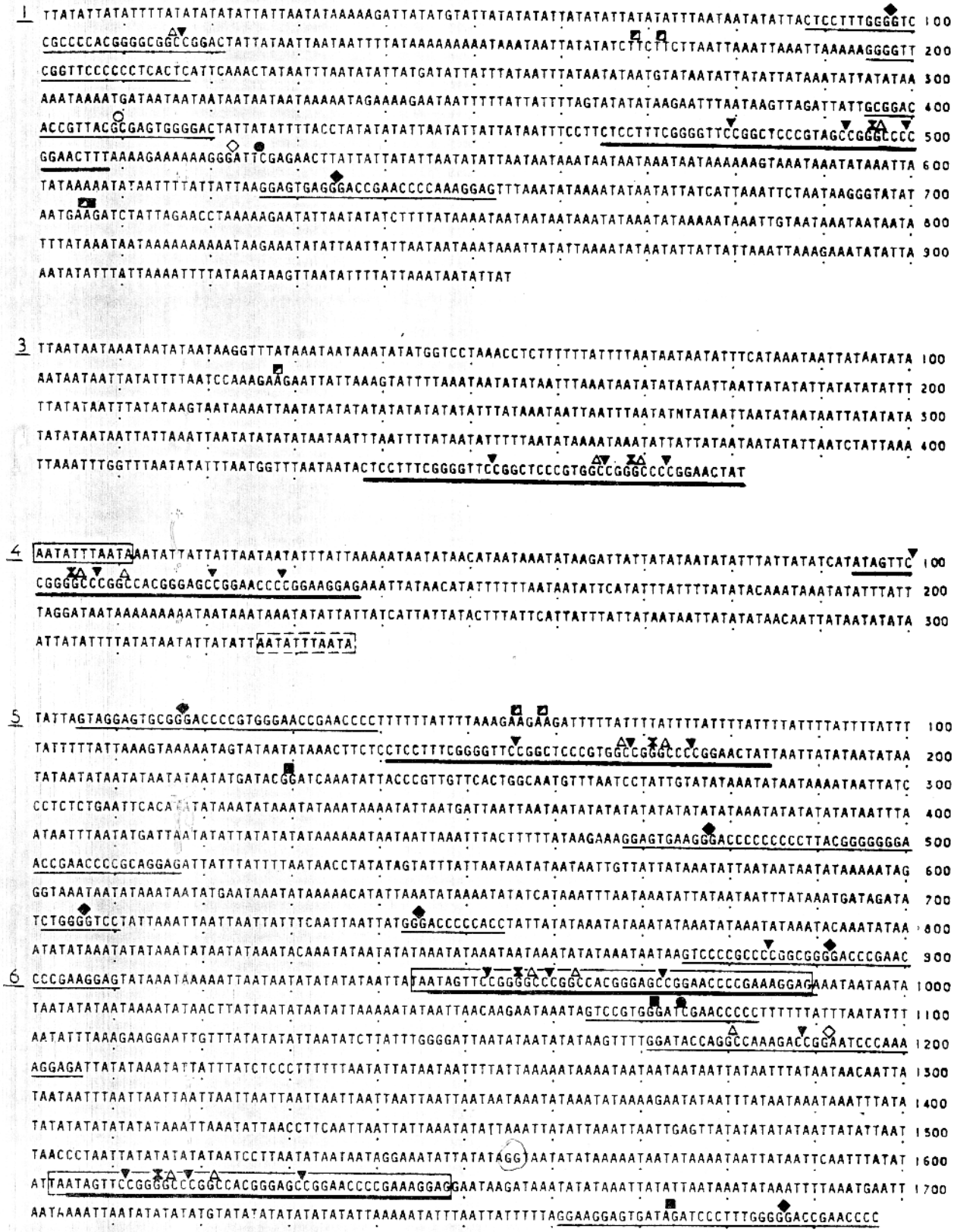

Fig. 5. Primary structure of the repeat units of mitochondrial genomes of *ori*° petites. The sequence corresponding to the "non-transcribed" strand is shown. The *ori* sequences are underlined by thick lines, other GC clusters by thin lines. Boxed sequences are excision sequences (de Zamaroczy *et al.*, in preparation). Restriction sites are indicated by the symbols of Figure 2. ⧗ indicates two *Sau*96 I sites.

pressivity, <5%. It is obvious that the genome of petite *1* (1% suppressivity) had more chances not to be immediately competed out by its low-suppressivity parental petite genome than by a highly suppressive petite genome or a wild-type genome. Four other *ori*° petites were obtained by crossing petite *1*, with wild-type strain B. This procedure clearly favours the survival of poorly replicating mitochondrial genome units originated from the wild-type genome since

they have a chance of co-segregating into the buds with the equally poor replicating petite genome used in the cross. Finally, two *ori*° petite genomes originated directly from wild-type genome B; they were found after extensive screening and, very significantly, had relatively high suppressivities (20% and 30%, respectively). All these findings point to the fact the *ori*° petites are rare, not so much because the excision events from which these genomes originate are rare, but simply because their mitochondrial genomes are usually competed out by the parental genomes.

4. *Ori*$^{\delta}$ sequences are significantly homologous with the GC clusters of *ori* sequences (de Zamaroczy *et al.*, in preparation). In fact, not only is there primary structure homology between the *ori*$^{\delta}$ sequences and the GC clusters of the *ori* sequences, but also their potential secondary structure recalls that of the cluster A-cluster B fold of *ori* sequences; in this case, the total length of the stem is about the same, a non-paired loop is present in the middle of it, and the sequences, GTGG and GGAT inserted into the flanking AT spacers (de Zamaroczy *et al.*, 1981) are somewhat similar to those of *ori*$^{\delta}$ sequences, ATAG and GGAG. Furthermore, like *ori* sequences, *ori*$^{\delta}$ sequences are present in both orientations on the wild-type genome, are localized in intergenic regions of the wild-type genome, and can be used as excision sequences when tandemly oriented.

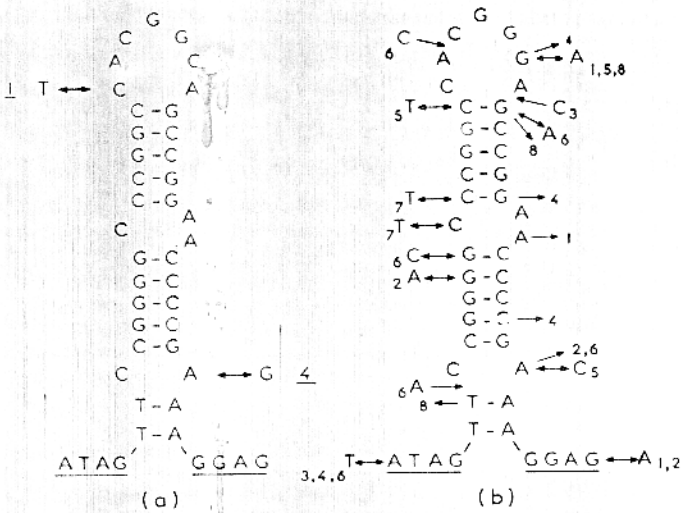

Fig. 6. Potential secondary structure of **(a)** the *ori*$^{\delta}$ sequences of Figure 5; **(b)** the *ori*$^{\delta}$-like sequences 1 – 8 (see Figure 1 and Table II). All sequences have been drawn in the same orientation ATAG→GGAG. Double-headed arrows indicate base exchanges in *ori*$^{\delta}$ or *ori*$^{\delta}$-like sequences; arrows pointing towards, or away from, the structure indicate insertions and deletions respectively. Numbers indicate the *ori*$^{\delta}$ or *ori*$^{\delta}$-like sequences presenting these changes.

5. *Ori*$^{\delta}$ sequences are highly homologous with at least another eight sequences, the *ori*$^{\delta}$-like sequences (Figures 1 and 6b; Table II). Seven of them are localized in intergenic regions, like *ori*$^{\delta}$ sequences. The only exception is localized in the AT-region of the 15S RNA gene (Sor and Fukuhara, 1980); interestingly, though transcribed, this region is, in fact, an insertion, present in some strains and not in others (Sor and Fukuhara, 1982; Martin *et al.*, 1982), which shares all the sequence features of intergenic regions. As for other sequences of the *ori*$^{\delta}$ family, it should be noted that they are all localized in intergenic sequences, with only two exceptions which are localized in the closed reading frames of the first *oxi* 3 intron and the last *cob* intron; in this connection, it has already been stressed (Bernardi, 1982a) that closed reading frames in introns have sequence features indistinguishable from those of intergenic regions. Another point which should be made is that since none of these sequences have been found in *ori*° petites so far, it is not possible to know how the base pair changes they display relative to the *ori*$^{\delta}$ sequences affect the replicative ability of the petite genomes potentially harboring them. It is conceivable that these sequences, as well as other ones, may support initiation of DNA replication, possibly at even lower efficiencies than *ori*$^{\delta}$ sequences, particularly in ethidium-induced petites. A final conclusion along this line must, however, wait for detailed information not only about the primary structure, but also about the organization of the repeat units in these genomes. Indeed, it is well known (Lewin *et al.*, 1978) that the mitochondrial genomes of ethidium-induced petites (in contrast to those of spontaneous petites) are very often rearranged in complex ways and may include DNA segments originating from different regions of the wild-type genome and possibly encompassing canonical or surrogate origins of replication.

6. As a more general conclusion, it should be pointed out that the results obtained in the present work do not say any-

Table 2. *Ori*$^{\delta}$ and related sequences

Sequence classes	Symbol (Figure 1)	Sequences	Ref.
(a) *ori*$^{\delta}$	*1–6,a,b*	A T A G T T C C G G G G C C C G G C C A C G G G A G C C G G A A C C C C G A A A G G A G	(1)
(b) *ori*$^{\delta}$-like	1 – 8	A T A G ——— See Figure 6b ——— G G A G	(2)
(c) *ori*$^{\delta}$-like part-deleted	Δ	A T A G T T C C G G G G . . . . . C C A C G G G T G C C G G A C C C C C G A A G G G A	(3)
		A T A G T A C C G G . . . . . C C G G G G A G . C G G A A C C C C C G A A A G G A G	(4)
		A T A G T T C C G G . . . . . C C G G G A G C C G G A A C C C C G A A A G G A G	(5)
		A T A G T T C C G G G G . . . . . C C A C G G G A G C C G G A A C C C C G G A A G G A G	(3)
		A T A G T T C C G G G G . . . . . C C A C G G G A G C C G G A A C C C C G G A A G G A G	(3)
(d) *ori*$^{\delta}$-like incomplete		A T A G T . C C G G . . . . . C C C C . A A A G G A G	(6)
		A T A A G . C C G G . . . . . C C C C A C A A G G A G	(6)
		A T A G T T . . . . . C C G G A A C C C C G C A A G G A G	(7)
		A T A G T T C C . . . . . G G A G	(5)
(e) *ori*$^{\delta}$-related		A T A G ——— G G A G	(2)

(1) This work (see Figures 5 and 6); (2) see text; (3) de Zamaroczy *et al.* (1982); (4) Bonitz *et al.* (1980); (5) Tzagoloff *et al.* (1980); (6) Bonitz and Tzagoloff (1980); (7) Macino and Tzagoloff (1980).

thing about the physiological function(s) of *ori*^{S} and related sequences. It is most unlikely that these sequences are involved in the replication of the wild-type genome, since obviously the canonical *ori* sequences have a much higher affinity for the replication enzyme complex. They may, however, play other regulatory and/or recombinational roles, as already suggested (Prunell and Bernardi, 1977; Tzagoloff *et al.*, 1979). In any case, these sequences contribute to the tremendous instability of the mitochondrial genome of wild-type yeast cells, since they can be used as excision sequences. Such instability would have already led to the loss of the mitochondrial genome, which is dispensable in yeast, were it not for compensatory selective advantages associated with the physiological function of these sequences. Such an argument, which also applies to the canonical *ori* sequences themselves (Marotta *et al.*, 1982), has been developed elsewhere (Bernardi, 1982a).

7. Another general conclusion is that the present work provides precise examples of how physiological replication origins can be replaced by sequences which, though much less efficient, are still capable of insuring replication. It is very likely that sequences of the *ori*^{S} type also account for the replication as an extrachromosomal element in yeast of a hybrid plasmid containing a petite repeat unit devoid of any *ori* sequences (Hyman *et al.*, 1982), but derived from a region rich in sequences of the *ori*^{S} family (de Zamaroczy *et al.*, in preparation). As for the role of *ori*^{S} sequences as surrogate origins of replication, it should be stressed (Bernardi, 1982a) that the expansion (by replication slippage and unequal crossing-overs) of the duplicated and translocated *ori* sequences to form the intergenic sequences results in partial copies of *ori* sequence segments, still capable of interacting with the replication complex, being spread all over the genome. In this connection, it should be noted (see also Sor and Fukuhara, 1982) that the common ATAG and GGAG sequences suggest that such spreading has occurred by insertion into AT spacers. The overall primary and secondary sequence similarities, which can be seen between *ori*^{S} and *ori* sequences, are limited enough to suggest a great flexibility of the replication complex insofar as its interaction with the origin sequences is concerned. This conclusion is confirmed by an increasing number of experimental findings on DNA replication in heterologous host cells.

Materials and methods

Yeast strains

Among the *ori°* petites studied here (a) one, a-10/3, derived from a petite, a-15/4/1, containing two inverted *ori* 5 sequences in its repeat unit (de Zamaroczy *et al.*, 1981; Mangin *et al.*, in preparation); in turn, a-15/4/1 derived from wild-type strain A (Faugeron-Fonty *et al.*, 1979); (b) seven derived from wild-type strain B (Faugeron-Fonty *et al.*, 1979) either directly (three) or after crosses with a-10/3 (three) or with a-3/1 (one), a petite strain described elsewhere (Faugeron-Fonty *et al.*, 1979). Culture media were as in Marotta *et al.* (1982).

Methods

Mitochondrial DNA preparations, restriction enzyme degradations, gel electrophoresis, ^{32}P-labeling of DNA, and hybridizations, were as in Marotta *et al.* (1982). DNA sequencing after secondary cleavage or strand separation was performed according to Maxam and Gilbert (1980).

Acknowledgements

We wish to thank our colleagues Miklos de Zamaroczy and Claire Gaillard for their contribution to the sequencing of the repeat units of petite *1* and *4*, respectively.

References

Bernardi,G. (1968) in Nord,F.F. (ed.), *Advances in Enzymology*, vol. **31**, Interscience, NY, pp. 1-49.
Bernardi,G. (1979) *Trends Biochem. Sci.*, **4**, 197-201.
Bernardi,G. (1982a) in Slonimski,P.P., Borst,P., and Attardi,G. (eds.), *Mitochondrial Genes*, Cold Spring Harbor Laboratory Press, NY, in press.
Bernardi,G. (1982b) *Trends Biochem. Sci.*, in press.
Bernardi,G., Faurès,M., Piperno,G., and Slonimski,P.P. (1970) *J. Mol. Biol.*, **48**, 23-42.
Bernardi,G., Baldacci,G., Bernardi,G., Faugeron-Fonty,G., Gaillard,C., Goursot,R., Huyard,A., Mangin,M., Marotta,R., and de Zamaroczy,M. (1980) in Kroon,A.M., and Saccone,C. (eds.), *The Organization and Expression of the Mitochondrial Genome*, Elsevier/North Holland, Amsterdam, pp. 21-31.
Blanc,H., and Dujon,B. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3942-3946.
Bonitz,G.S., Coruzzi,G., Thalenfeld,B.E., and Tzagoloff,A. (1980) *J. Biol. Chem.*, **255**, 11927-11941.
Bonitz,S.G., and Tzagoloff,A. (1980) *J. Biol. Chem.*, **255**, 9075-9081.
Coruzzi,G., Bonitz,S.G., Thalenfeld,B.E., and Tzagoloff,A. (1981) *J. Biol. Chem.*, **256**, 12780-12787.
Cosson,J., and Tzagoloff,A. (1979) *J. Biol. Chem.*, **48**, 43-52.
Ehrlich,S.D., Thiéry,J.P., and Bernardi,G. (1972) *J. Mol. Biol.*, **65**, 207-212.
Faugeron-Fonty,G., Culard,F., Baldacci,G., Goursot,R., Prunell,A., and Bernardi,G. (1979) *J. Mol. Biol.*, **134**, 493-537.
Hyman,B.C., Cramer,J.H., and Rownd,R.H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1578-1582.
Gaillard,C., and Bernardi,G. (1979) *Mol. Gen. Genet.*, **174**, 335-337.
Goursot,R., de Zamaroczy,M., Baldacci,G., and Bernardi,G. (1980) *Curr. Genet.*, **1**, 173-176.
Lewin,A., Morimoto,R., Rabinowitz,M., and Fukuhara,H. (1978) *Mol. Gen. Genet.*, **163**, 257-275.
Macino,G., and Tzagoloff,A. (1980) *Cell*, **20**, 507-511.
Marotta,R., Colin,Y., Goursot,R., and Bernardi,G. (1982) *EMBO J.*, in press.
Martin,R.P., Bordonné,R., and Dirheimer,G. (1982) *FEBS Symposium*, in press.
Maxam,A.M., and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.
Nobrega,F.G., and Tzagoloff,A. (1980) *J. Biol. Chem.*, **255**, 9828-9837.
Prunell,A., and Bernardi,G. (1974) *J. Mol. Biol.*, **86**, 825-841.
Prunell,A., and Bernardi,G. (1977) *J. Mol. Biol.*, **110**, 53-74.
Sor,F., and Fukuhara,H. (1980) *C.R. Hebd. Seances Acad. Sci., Paris*, **291**, 933-936.
Sor,F., and Fukuhara,H. (1982) *Nucleic Acids Res.*, **10**, 1625-1633.
Tzagoloff,A., Macino,G., Nobrega,M.P., and Li,M. (1979) in Cummings D.J. *et al.* (eds.) *Extrachromosomal DNA*, Academic Press, NY, pp. 339-355.
de Zamaroczy,M., Baldacci,G., and Bernardi,G. (1979) *FEBS Lett.*, **108**, 429-432.
de Zamaroczy,M., Marotta,R., Faugeron-Fonty,G., Goursot,R., Mangin, M., Baldacci,G., and Bernardi,G. (1981) *Nature*, **292**, 75-78.