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Lack of site-specific recombination between mitochondrial genomes of petite mutants of yeast

(DNA; mitochondria; replication; suppressivity)

Edda Rayko, Regina Goursot and Giorgio Bernardi

Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 75005 Paris, France

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SUMMARY

Previous work from our laboratory showed that mitochondrial (mt) genomes, with tandem repeat units, from spontaneous, cytoplasmic petite mutants of *Saccharomyces cerevisiae* do not exhibit site-specific recombination in petite × petite crosses [Rayko et al., Gene 63 (1988) 213–226]. Here, we have extended and confirmed these observations by studying other crosses of petites with tandem repeat units, as well as crosses in which one of the parents was, instead, an unstable petite, a-15/4/1, having a palindromic mt genome. In no case was site-specific recombination of the parental mt genomes observed. Progeny cells harbored mt genomes derived from either one or both of the two parents, as shown by analysis of restriction fragments. In the case of biparental inheritance, extensive subcloning of the diploids showed that this was due to a persistent heteroplasmic state and not to intermolecular recombination. The 'new' restriction fragments present in the mt DNA from some diploids were shown to be derived from the unstable parental genome, a-15/4/1, by a secondary excision process. Lack of site-specific recombination is, therefore, not only a feature of crosses involving petite genomes made up of tandem repeat units, but also of crosses in which one parental genome consists of inverted repeats and frequently originates secondary petite genomes formed by tandem repeats. Previous reports of mt recombination in petite mutants are discussed in light of these results.

INTRODUCTION

Recombination of mt DNA in respiratory-proficient (ρ^+), wt yeast cells was demonstrated both genetically (Thomas and Wilkie, 1968) and physically (Fonty et al., 1978). Both 'legitimate' (homologous) and 'illegitimate' (site-specific) recombination phenomena may be involved in the process. The latter events can also lead to the formation of the defective mt genomes of spontaneous respiratory-deficient, cytoplasmic petite (ρ^-) mutants. Indeed, the mt genomes of such mutants are formed by an excision process involving short, tandemly oriented sequences located in the abundant intergenic sequences of the wt genomes (Gaillard et al., 1980; Marotta et al., 1982; de Zamaroczy et al., 1983), as previously postulated (Piperno et al., 1972; see also Bernardi, 1979, for a review).

Information at the molecular level on recombination between ρ^+ and ρ^- genomes is limited. It is known, however, that GC clusters (de Zamaroczy and Bernardi, 1986a) may be involved (Dieckmann and Gandy, 1987; Clark-Walker, 1989), as they very frequently are in the excision of petite genomes (de Zamaroczy et al., 1983).

As far as petite × petite crosses are concerned, site-specific recombination was claimed (see Discussion), but could not be detected using restriction mapping and

Correspondence to: Dr. E. Rayko, Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France. Tel. (33-1) 44 27 51 12; Fax (33-1)) 44 27 79 77; e-mail: BERNARDI@Arthuz.CitiZ.FR.

Abbreviations : bp, base pair(s); EtdBr, ethidium bromide; GC, % G+C; kb, kilobase(s) or 1000 bp; mt, mitochondrial; nt, nucleotide(s); *ori*, origin of mt DNA replication; *PIF*, nuclear gene controlling petite integration frequency; *S.*, *Saccharomyces*; wt, mt wild type.

sequencing of mt DNA after extensive sub-cloning of diploids arising from crosses between spontaneous, suppressive, *ori*$^+$ petite mutants with mt genomes consisting of tandem repeat units (Rayko et al., 1988). Indeed, 'new' (junction) restriction fragments different from the parental ones could not be observed. Such fragments (as obtained with appropriate restriction enzymes; see Discussion) are inevitably produced as a consequence of the formation of junctions between non-homologous repeat units arising from different parts of the mt genome of wt cells. The only apparently 'new' fragments that were seen were shown to be the result of secondary excisions in the parental genomes.

The aim of the present work was to study the outcome of crosses involving the palindromic mt genome of petite a-15/4/1 (Faugeron-Fonty et al., 1983; Mangin et al., 1983). This choice was prompted by the finding that palindromic petite genomes recombine more frequently than tandemly arranged petite genomes in $\rho^+ \times \rho^-$ crosses (Michel et al., 1979; Foury and Kolodynski, 1983; see also section **f**). Moreover, petite a-15/4/1 presents a certain degree of instability and, as a consequence, of heterogeneity, and, is therefore, somehow similar to petites used in some experiments purported to demonstrate mt recombination (see section **f**).

The results obtained show that while intragenomic recombination leading to secondary mt genomes is very active in petite a-15/4/1, intergenomic recombination involving this mt genome, as present in one parent, and an *ori*$^+$ mt genome consisting of tandem repeat units in the other parent, cannot be detected.

RESULTS AND DISCUSSION

(a) The mt genome of petite a-15/4/1

The mt genome of the palindromic petite mutant a-15/4/1 was characterized by the presence of repeat units containing an inverted duplication comprising the *ori*5 sequence (Faugeron-Fonty et al., 1983; Mangin et al., 1983; see Fig. 1). The detailed organization of this genome, as well as those of a-60 and a-61 derived from it (see Fig. 1), has been studied further and will be presented elsewhere (E.R., R.G. and G.B., in preparation).

(b) Analysis of the haploid petite strains used in crosses

The haploid petite strains used as the Mat **α** parent in the crosses studied in this work (b1, b11 and b13/1) were derived from our wt strain B (see Rayko et al., 1988). Those used as the Mat **a** parent were a-15/4/1 and petites obtained in the process of mutagenizing a-15/4/1 with nitrous acid (Lawrence, 1982) in order to obtain additional auxotrophic nuclear mutations (since the *ade* 1 marker of strain a-15/4/1 was leaky, making the selection of diploids in crosses difficult). Eight such mutants were obtained (Table I).

None of these eight nuclear mutants exhibited the original mt petite genome, a-15/4/1, which had a 4700-bp repeat unit. Their mt genomes were formed by repeat units of different, shorter sizes (Table I and Fig. 1). Four types of mt genomes containing only a single *ori*5 sequence in their repeat unit were found. Three of them, represented by a-41, a-42 and a-43 contained the direct *ori*5 sequence (the 'left' one in Fig. 1), whereas the repeat unit of a-43 contained the 'inverted' *ori*5 sequence of the original petite (the right one in Fig. 1).

The high frequency of secondary petites derived from a-15/4/1 in this experiment is not surprising, because subcloning of haploid petite a-15/4/1 previously carried out in our laboratory, showed that its genome was indeed very unstable (Mangin et al., 1983). The suppressivity (namely, the percentage of diploid petites resulting from a cross of the parental haploid petite with a wt strain) of the petites derived from a-15/4/1 was much higher than that of the original petite. Mutants a-38, a-39, a-40 and a-43 (Table I) were characterized by a high suppressivity (or supersuppressivity; Goursot et al., 1980; or hypersuppressivity; Blanc and Dujon, 1980), as is the rule for *ori*$^+$ petites characterized by tandem, short repeat units (de Zamaroczy et al., 1981). Mutants a-35, a-41 and a-42 showed a moderate suppressivity (Table I) which may be due (*i*) to the heterogeneity of mt genomes resulting from an unstable parental petite mt DNA (Mangin et al., 1983); (*ii*) to intrinsic properties of the mt genomes (de Zamaroczy et al., 1981; Goursot et al., 1982; Mangin et al., 1983; Faugeron-Fonty et al., 1984; Fangman and Dujon, 1984); (*iii*) to an abundance of ρ^0 diploids; or (*iv*) in some cases, to nuclear mutations (Zweifel and Fangman, 1991). Obviously (Table I), the case of strain b11 corresponds to (*i*), those of strains b1 and a-41 to (*ii*) and (*iii*) respectively, while strain a-42 might correspond to (*iv*), since a mitochondrially identical petite a-10/2 (Mangin et al., 1983) exhibited a different suppressivity, 85%.

(c) Crosses of a-15/4/1, a-41 and a-43 with other *ori*$^+$ petites

Petites a-15/4/1, a-41 and a-43 were crossed with three other *ori*$^+$ petites, b1, b11 and b13/1, in order to compare the crosses involving the palindromic mt genome of a-15/4/1 with those involving the tandemly arranged *ori*$^+$ mt genomes of a-41 and a-43 derived from it (Tables I and II, see also Fig. 2). It should be noted that, while b1 and b11 harbored mt genomes containing *ori*1* and *ori*2, respectively, b13/1 contained *ori*5 and shared therefore a large mt DNA region with a-15/4/1. Analysis of the mt DNA of the zygotic clones obtained in these crosses

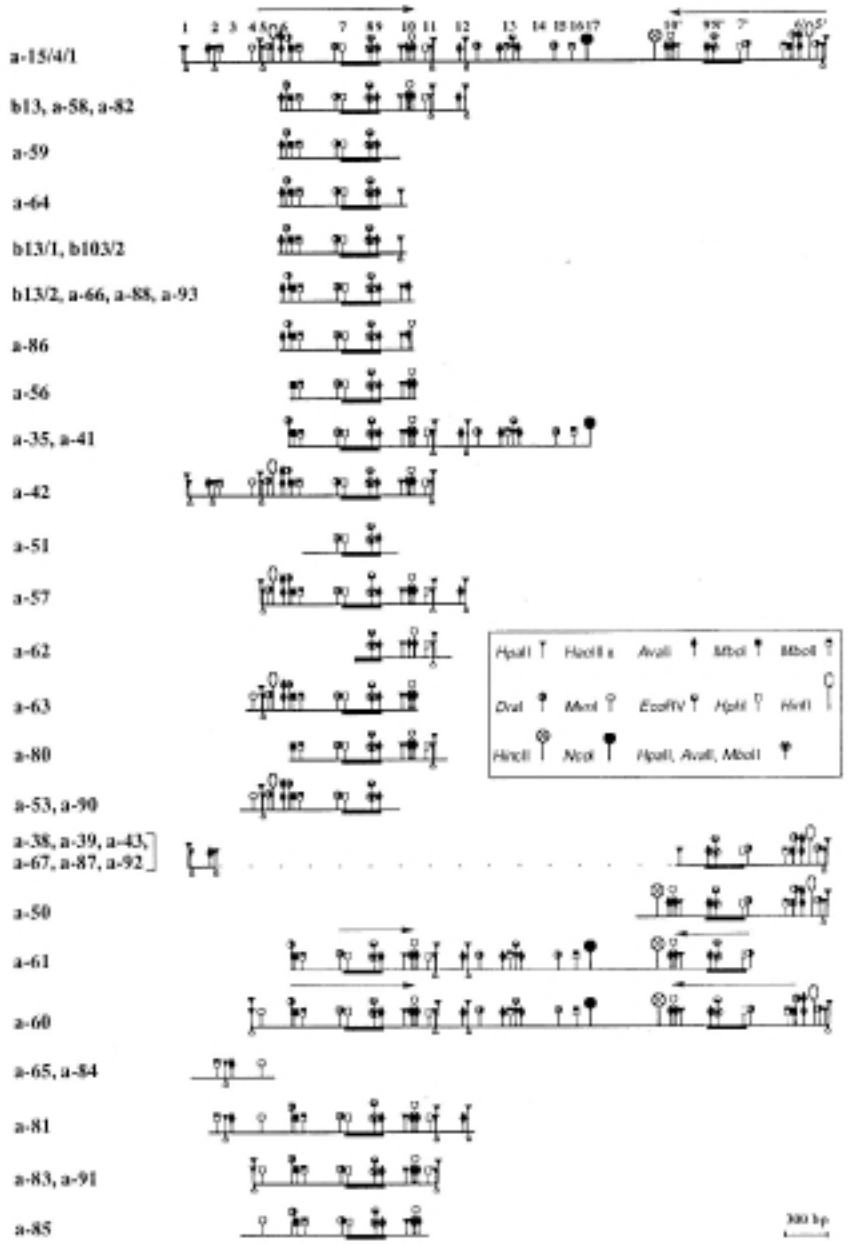

Fig. 1. Restriction maps of the repeat unit of mt genomes of a-15/4/1 (from Faugeron-Fonty et al., 1983) and secondary petites derived from it, and from the mt genomes of petites b13, b13/1, b13/2 and b103/2, derived from wt strain B (Rayko et al., 1988). GC clusters are numbered from 1 to 17 in the case of a-15/4/1. GC clusters 1–11 and 13–17 correspond to GC clusters 36b-46 and 47–51, respectively, in the mt genome of *S. cerevisiae* (de Zamaroczy and Bernardi, 1986b). Arrows indicate inverted duplications in the repeat unit of a-15/4/1. Inverted GC clusters are numbered from 5' to 10'. The *ori*5 (and *ori*5') sequences are shown as thick lines. The dotted line represents deleted sequences in the case of a-38 and identical genomes. The order of presentation of restriction maps was chosen to facilitate comparisons. The mt genomes of petites a-60, a-65 (a-84), a-81, a-83 (a-91) and a-85 will be discussed in detail elsewhere (E.R., R.G. and G.B., in preparation).

showed that the electrophoretic patterns produced by *Hpa*II or *Ava*II consisted of fragments originating from the DNA of one and/or the other parent and of 'new' fragments, namely fragments that were not produced by either parent. In some cases, ρ° zygotic clones were also observed (Table II and Fig. 2).

The predominance of the genome from petite b13/1 over those from a-41 and a-43 (Table II) appears to be simply correlated with the shorter repeat unit size of the former, following a rule already seen to hold in other crosses between suppressive *ori*$^+$ petites (Rayko et al., 1988). Similarly, the mt genomes of b1 (*ori*1*) and b13/1 (*ori*5) clearly predominated over that of a-15/4/1 in the diploids, as expected on the base of their suppressivity, the latter genome being, however, also present in the progeny of these crosses.

Surprisingly, in the cross with b11 (*ori*2), the genome of a-15/4/1 was even the predominant one (Table II). Analysis of 13 subclones of b11 showed, however, that nine of them were ρ° clones, two harbored a very suppressive mt genome with a 700-bp repeat unit, and only two subclones harbored the original mt genome b11 (Table I).

TABLE I

Properties of haploid strains

Strain[a]	*ori* sequence[b]	Repeat unit size (bp)[c]	Suppressivity (%)[d]	ρ^0 subclones[e]	Phenotype (nuclear)[f]
a-15/4/1	5+5'	4700	5	1/26	Mat **a** Ade$^-$
a-40	n.d.	n.d.	85	n.d.	Mat **a** Ade$^-$ Lys$^-$ Arg$^-$
a-41[g]	5	2270	50–60	3/16	Mat **a** Ade$^-$ Ura$^-$
a-42	5	1840	60	0/8	Mat **a** Ade$^-$ X$^-$
a-43[h]	5	1265	92	n.d.	Mat **a** Ade$^-$ Lys$^-$
b13/1[i]	5	915	95	n.d.	Mat α His$^-$ Trp$^-$
b1[i]	1*	1360	50	0/12	Mat α His$^-$ Trp$^-$
b11[i]	2	1855	80	9/13[k]	Mat α His$^-$ Trp$^-$

[a] All strains with exception of a-40 were used in crosses. All were tandemly arranged, except a-15/4/1.

[b] The *ori* sequences are numbered as in de Zamaroczy et al. (1984). The *ori*5' sequence is an inverted *ori*5 sequence. The very particular *ori*1* sequence was described in detail by Faugeron-Fonty et al. (1984).

[c] The repeat unit size of the palindromic mt genome a-15/4/1 is longer than the conserved wt sequence, since it comprises a partial inverted duplication (see Fig. 1).

[d] Suppressivity was determined as in Rayko et al. (1988).

[e] This is the proportion of ρ^0 cells within total petite subclones analyzed.

[f] X$^-$ indicates auxotrophy for a non-identified metabolite.

[g] Same mt genome as a-35, but with a different nuclear phenotype (Mat **a** Ade$^-$ Try$^-$).

[h] Same mt genome as a-38 and a-39, except that a-38 carried an additional nuclear mutation, met$^-$.

[i] These petites are derived from our laboratory strain B (see Rayko et al., 1988).

[k] Two subclones (suppressivity: 95%) contained a deleted genome b11/1 with a repeat unit size of 700 bp, and only two subclones (suppressivity: 95%) harbored the original mt genome.

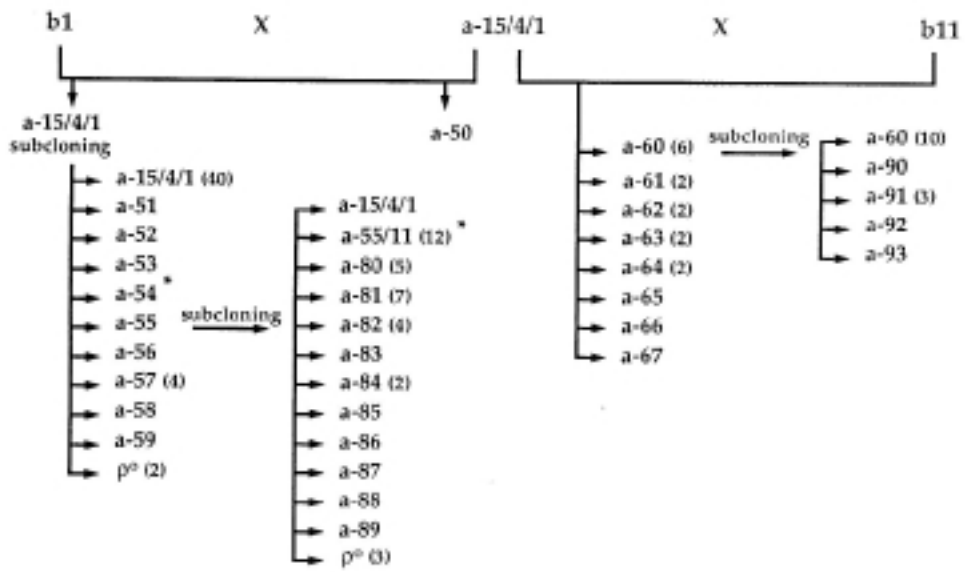

Fig. 2. Origin of the diploid petite genomes derived from crosses involving a-15/4/1. The haploid strain a-15/4/1 was crossed (×) to b1 (left) or to b11 (right) (see Table II), giving rise to two zygotic clones, a-15/4/1 and a-50, in cross with b1, and to 17 zygotic clones of eight types in the cross with b11. The heterogeneous subclone a-55 derived from the pure diploid a-15/4/1, and one of the zygotic clones a-60 were subcloned again, as shown here and described in section **d**. Values in parentheses indicate the number of identical clones isolated.

* These subclones were heterogeneous, as judged from their restriction patterns (not shown).

Overall, we studied 145 zygotic clones: 18 out of 91 clones derived from crosses involving a-15/4/1 contained 'new' fragments which could be the result of recombination between the parental mt genomes (see footnote c, Table II). Restriction mapping (Fig. 1) after subcloning showed, however, that all these 'new' fragments were due to secondary petite genomes (a-50 and a-60 to a-67, Table III and Fig. 2) excised from the mt genome of the parental petite a-15/4/1.

We also analysed the diploid zygotic clone harboring the mt genome of parental petite a-15/4/1 (see footnote d, Table II). Analysis of the mt genomes of 62 subclones of this diploid showed that, again, nine genomes (14.5%) resulted from secondary excisions of the original mt

TABLE II

The mt genomes present in zygotic clones

Cross[a]			The mt genomes in diploids[b]			
type 1	×	type 2	type 1	type 2	types 1+2	ρ°
a-41	×	b13/1	1	15	0	0
a-43	×	b13/1	0	15	7	0
a-15/4/1	×	b13/1	1	28	0	2
a-15/4/1	×	b11	10+13[c]	2	5+4[c]	7
a-41	×	b11	5	2	2	7
a-15/4/1	×	b1	1[d]	6	11+1[e]	0

[a]For each cross, parental strains are designated arbitrarily type 1 or type 2.
[b]Only parental mt genomes of either (or both) types were found.
[c]The second numbers concern genomes containing apparently 'new' fragments. These were shown (Table III; see also Figs. 1 and 2) to correspond to secondary excision products from the unstable parental genome of a-15/4/1.
[d]This pure zygotic clone was further analyzed by subcloning, (see Fig. 2 and Table III).

genome (Table III and Figs. 1,2) confirming that the mt genome of a-15/4/1 was very unstable, whether present in haploid or diploid cells.

(d) The mt genomes of diploid petites derived from crosses of a-15/4/1 with petites b1 and b11

The restriction patterns of the mt genomes of two petites, a-54 and a-55 suggested a heterogeneity which was confirmed by subcloning in the case of a-55. Thus, out of 40 subclones of a-55, only one was the parental petite a-15/4/1; 12 were still heterogeneous; 24 presented ten types of mt genomes with different sizes of the repeat units (a-80 to a-89); and three were ρ° petites (Table III and Fig. 2). Out of 16 subclones of petite a-60 which contains a palindromic mt genome (see below, this section), ten harbored this genome and six subclones harbored four types of mt genomes, a-90 to a-93, identical to a-53, a-83, a-43 and a-66, respectively (Fig. 1).

Restriction mapping of all secondary petites derived from crosses of a-15/4/1 with petites b1 and b11 showed that their mt genomes were formed by repeat units of different sizes, ranging from 550 bp (a-65, a-84) to 4220 bp (a-60); 16 types of diploid mt genomes were the result of excisions leading to shorter repeat units organized in tandem and containing a single *ori*5 sequence. Fourteen of them harbored the 'left' part of the parental repeat unit of the mt genome of a-15/4/1, i.e., the 'direct' *ori*5 sequence (Fig. 1). The presence of the indicative *Hin*cII site on the repeat unit of the mt DNA of strain a-50 showed that in this petite the 'inverted' *ori*5 sequence, i.e., the 'right' part of the repeat unit of a-15/4/1 was conserved (Table III, Fig. 1). Petites a-67, a-87 and a-92 also contained the

TABLE III

Properties of the apparently 'new' diploid petite genomes isolated from crosses involving a-15/4/1

Petite genome[a]	Repeat unit size (bp)[b]	*ori*5 sequences[c]	
		direct (left)	inverted (right)
a-50	1395	−	+
a-51	760	+	−
a-52	1560	n.d.[d]	n.d.[d]
a-53, a-90	1190	+	−
a-54	heterogeneous		
a-55	heterogeneous		
a-56	905	+	−
a-57	1450	+	−
a-58, a-82[e]	1340	+	−
a-59	965	+	−
a-60	4220	+	+
a-61	3370	+	+
a-62	820	+	−
a-63	1370	+	−
a-64	910	+	−
a-65, a-84	550	−	−
a-66, a-88,a-93[f]	915	+	−
a-67, a-87,a-92[g]	1265	−	+
a-80	1140	+	−
a-81	1920	+	−
a-83, a-91	1350	+	−
a-85	1350	+	−
a-86	915	+	−
a-89	1870	+	−

[a]See also Figs. 1 and 2. All genomes were tandemly arranged, except for a-60 and a-61.
[b]See footnote b, Table I for the palindromic petites a-60 and a-61.
[c]This concerns the *ori*5 sequence conserved on the repeat unit, as derived from the original petite a-15/4/1, see also Fig. 1.
[d]Not determined.
[e]Same mt genome as haploid b13 (see also legend to Fig. 2).
[f]Same mt genome as haploid b13/2 (see also legend to Fig. 2).
[g]Same mt genome as haploid a-43 (see also Fig. 1).

'inverted' *ori*5 sequence, their mt genome being identical to that of haploid petite a-43 described above (section **b**).

The mt genome of petite a-62 (Table III, Fig. 1) contained a partially deleted *ori*5⁻ sequence (de Zamaroczy et al., 1981), whereas the mt genomes of diploid secondary petites a-65 and a-84 did not contain any *ori* sequence, i.e., they were *ori*° petites (Goursot et al., 1982). The additional rearrangements present in the repeat units of these genomes, as well as in those of a-60, a-81, a-83, a-85 and a-91, will be discussed in a paper dealing with the palindromic organization of the mt genome of strains a-15/4/1, a-60, and a-61 (E.R., R.G. and G.B., in preparation).

(e) Excision sequences of the mt genome of a-15/4/1 and of secondary petites derived from it

Mapping with restriction enzymes, and in particular with *Hpa*II, *Hae*III and *Ava*II (splitting the sequences

CCGG, GGCC and GGWCC, respectively; W = A or T), showed that the excision sites of most of the primary and secondary petite genomes studied were localized in GC clusters, although sequence studies are needed to define them precisely. The GC clusters of the repeat unit of a-15/4/1 were numbered from 1 to 17, as shown in Fig. 1. All of them, except cluster 12 which was localized in an unsequenced part of this region, were present in the mt DNA sequence compiled by de Zamaroczy and Bernardi (1986b) and corresponded to their clusters 36b to 51. In some cases (a-86, a-41, a-42), the direct repeats used in the excision could easily be deduced by scanning the primary sequence of the wt genome. In other cases, the precise excision site was not obvious. It was, however, clear, that some GC clusters, especially clusters 6 and 10 (Fig. 1), were preferentially involved in the intramolecular recombination process, although they only exhibited patchy homology. The fact that in these cases a relatively low level of homology was sufficient for recombination might be related to topological reasons.

(f) Conclusions

(1) Lack of site-specific mt recombination in diploids from petite crosses

The first conclusion of these investigations, namely the lack of site-specific recombination between petite mt genomes consisting of tandem repeat units, was already drawn by Rayko et al. (1988) in a study of mt genomes from diploids obtained in 26 crosses between 17 cytoplasmic, spontaneous, suppressive, *ori*$^+$ petite mutants. The present results confirm and extend those data.

The second conclusion is that such absence of site-specific recombination also holds for crosses in which one parental petite genome consisted of tandem repeat units and the other parental petite genome was a palindromic genome consisting of inverted repeat units. The case investigated in the present work is particularly striking in that the latter genome (a-15/4/1) was very highly unstable, leading to the formation of a large number of secondary petite genomes, a feature also presented by petite genomes claimed to exhibit intergenomic site-specific recombination (see the following subsection). Incidentally, the characterization of these genomes confirmed the overwhelming role played by GC clusters, and more specifically by some of them, in the spontaneous excision events (de Zamaroczy et al., 1983).

Both conclusions rest on the absence of 'new' (junction) fragments in restriction digests obtained with the mt DNA of the progeny of the crosses. Indeed, the apparently 'new' restriction fragments exhibited by some diploids were shown to derive from one parental genome, 15/4/1, by a secondary excision process (see Fig. 1). It would be stressed here again that recombination of mt genomes formed by repeat units derived from different regions of the mt genome of wt cells inevitably leads to the formation of junctions which are, in turn, responsible for the appearance of restriction fragments (as obtained with appropriate restriction enzymes) different from the parental ones. Appropriate restriction enzymes are those that do not cut exactly at the recombination sites, in which case restriction fragments of only parental type would be produced.

The fact that no 'intergenomic' site-specific recombination is detected while site-specific 'intragenomic' recombination very frequently occurs in the mitochondria of a-15/4/1, both in haploid or diploid cells requires an explanation because both phenomena rely on the same molecular process. The first possibility to consider would be that the tertiary folding of mt genome units (see the specific tertiary folding of the *ori* sequences proposed by de Zamaroczy et al., 1984) might favor intragenomic site-specific recombination. Other explanations are, however, possible. For instance, site-specific intergenomic recombination might never occur in petites because their mitochondria (which are known to be different in membrane structure from wt mitochondria; Yotsuyanagi, 1962) cannot undergo the fusion mechanism which allows physical contact between the two parental genomes whereas intragenomic recombination leading to the defective genomes of petite mutants can occur. It should be noted that the two above explanations have very different implications. Indeed, while the first one implies that the lack of site-specific intergenomic recombination is due to topological reasons and does not rule out intergenomic recombination altogether, the second, less likely, explanation, does.

In any event, the mitochondrially encoded subunit of a heterodimeric endonuclease involved in the recombination of mt DNA (Nakagawa et al., 1992) clearly is not needed for the recombination events responsible for the secondary excisions of petite genomes. The latter is obviously associated with a nuclearly encoded enzyme since it takes place in the absence of mt protein synthesis. In this connection, it should be recalled that, in the case of animal mt genomes, excisions can take place, in the absence of repair and recombination events (for a review, see Poulton, 1992).

(2) Lack of evidence for recombination between petite genomes

Three series of papers reported recombination in diploids from crosses of petite genomes. None of them provided, however, solid evidence for it. We will mention these reports here, and we will comment on their interpretation in the following section. A more detailed discus-

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The restriction patterns of the mt genomes of two petites, a-54 and a-55 suggested a heterogeneity which was confirmed by subcloning in the case of a-55. Thus, out of 40 subclones of a-55, only one was the parental petite a-15/4/1; 12 were still heterogeneous; 24 presented ten types of mt genomes with different sizes of the repeat units (a-80 to a-89); and three were ρ° petites (Table III and Fig. 2). Out of 16 subclones of petite a-60 which contains a palindromic mt genome (see below, this section), ten harbored this genome and six subclones harbored four types of mt genomes, a-90 to a-93, identical to a-53, a-83, a-43 and a-66, respectively (Fig. 1).

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a-60	4220	+	+
a-61	3370	+	+
a-62	820	+	−
a-63	1370	+	−
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a-66, a-88,a-93[f]	915	+	−
a-67, a-87,a-92[g]	1265	−	+
a-80	1140	+	−
a-81	1920	+	−
a-83, a-91	1350	+	−
a-85	1350	+	−
a-86	915	+	−
a-89	1870	+	−

[a]See also Figs. 1 and 2. All genomes were tandemly arranged, except for a-60 and a-61.

[b]See footnote b, Table I for the palindromic petites a-60 and a-61.

[c]This concerns the *ori*5 sequence conserved on the repeat unit, as derived from the original petite a-15/4/1, see also Fig. 1.

[d]Not determined.

[e]Same mt genome as haploid b13 (see also legend to Fig. 2).

[f]Same mt genome as haploid b13/2 (see also legend to Fig. 2).

[g]Same mt genome as haploid a-43 (see also Fig. 1).

The mt genome of petite a-62 (Table III, Fig. 1) contained a partially deleted *ori*5⁻ sequence (de Zamaroczy et al., 1981), whereas the mt genomes of diploid secondary petites a-65 and a-84 did not contain any *ori* sequence, i.e., they were *ori*⁻ petites (Goursot et al., 1982). The additional rearrangements present in the repeat units of these genomes, as well as in those of a-60, a-81, a-83, a-85 and a-91, will be discussed in a paper dealing with the palindromic organization of the mt genome of strains a-15/4/1, a-60, and a-61 (E.R., R.G. and G.B., in preparation).

(e) Excision sequences of the mt genome of a-15/4/1 and of secondary petites derived from it

Mapping with restriction enzymes, and in particular with *Hpa*II, *Hae*III and *Ava*II (splitting the sequences

sion of this problem will be presented elsewhere (R.G., E.R. and G.B., in preparation).

(*i*) Michaelis et al. (1973, 1976a, b) reported that about 80% of the progeny from crosses between chloramphenicol-resistant by erythromycin-resistant petites carried recombinant genomes harboring both resistance markers. Some of these genomes were visualized by electron microscopy of partially denatured DNA molecules as made up of repeat units from both parental genomes illegitimately recombined end-to-end (Lazowska and Slonimski, 1977). However, these latter results were never substantiated by restriction mapping and/or sequencing of 'recombinant' mitochondrial genomes.

(*ii*) A specialized recombination was reported to occur in petite × petite crosses in which an allele lacking a sequence acquired it by unidirectional gene conversion. This concerns the optional 1145-bp omega intron located within the 21S rRNA gene (Jacquier and Dujon, 1985) and an optional 46-bp GC cluster located within the coding region of the *var1* gene (Zinn et al., 1988). In both cases, while the demonstration for this specialized mt recombination was satisfactory for wt × wt crosses, the evidence was not compelling for recombination between petite genomes and other interpretations were possible.

(*iii*) Recombination was also reported in the special case of nascent spontaneous petites by Evans et al. (1985) and Evans and Clark-Walker (1985).

(3) Comments on recombination in petite genomes

The fact that no valid proof was provided so far for the mt recombination between petite mutants does not rule out per se the possibility of mt recombination in petites. We will, therefore, consider such a possibility in the three cases discussed in the previous section.

(*i*) If the 'topological' explanation for the lack of intergenomic site-specific recombination and for the presence of intragenomic recombination is correct, one should consider the possibility that site-specific recombination of petite genomes requires that both parental genomes consist of inverted repeat units. In fact, Foury and Kolodynski (1983) showed that mutations in a nuclear *PIF* gene ('petite integration frequency') prevent the recombination of ρ^+ genomes with ρ^- genomes having tandem, but not inverted, repeat units. *PIF*-dependent recombinogenic signals exist in the mt DNA of yeast, and these signals are related to the topology of the ρ^- DNA. Indeed, denatured sites in the double helix or cruciform structures elicited by local negative supercoiling might be the preferred sites for the initiation of recombination (Foury and Van Dyck, 1985). Cloning and sequencing of the *PIF* gene was reported (Foury and Lahaye, 1987). Its product is a single-stranded DNA-dependent ATPase and a DNA helicase which unwinds partial DNA duplexes (Lahaye et al., 1991), required for the repair of mt DNA after UV light and EtdBr treatments (Foury and Kolodynski, 1983) and for the maintenance of mt DNA at elevated temperatures (Foury, 1990). The possibility under consideration here will be investigated as soon as appropriate parental strains become available. It should be recalled here the petites used in the work of Michaelis et al. (1973) and Lazowska and Slonimski (1977) were believed to be palindromic, even if this organization of some of them was not confirmed by Michel (1982).

(*ii*) If the specialized recombination described by Jacquier and Dujon (1985) and Zinn et al. (1988) was proven to be correct, it would concern homologous sequences which only differ because of the presence or absence of an internal sequence. In other words, it would disprove the explanation that recombination between petite genomes does not take place altogether (as under the hypothesis, because of lack of mt fusion), but would not infirm the 'topological' explanation.

(*iii*) The third case is probably similar to what happens in wt × wt crosses (Fonty et al., 1978) or in wt × petite crosses (Dieckman and Gandy, 1987; Clark-Walker, 1989) in that it concerns large mt genomes endowed with extensive regions of homology.

In conclusion, we do not rule out the possibility of recombination in the case of nascent petites, nor in the case of specialized recombination involving homologous sequences, although solid evidence for the latter is still lacking. As far as site-specific recombination is concerned, the only possibility that remains open is that it can exclusively occur between petites both containing palindromic genomes.

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