Gene, 24 (1983) 73–81
Elsevier

GEN 00818

The *ori*^{r} to *ori*$^+$ mutation in spontaneous yeast petites is accompanied by a drastic change in mitochondrial genome replication

(*Saccharomyces cerevisiae*; DNA replication origins; suppressivity)

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(Received April 1st, 1983)
(Revision received May 12th, 1983)
(Accepted May 16th, 1983)

SUMMARY

The *ori*^{r} petite mutants of *Saccharomyces cerevisiae* show a very low level of suppressivity (5–12%; suppressivity is the percentage of diploid petites issued from a cross of the parental haploid petite with a wild-type cell), indicating a poor replication efficiency of their mitochondrial genome. The latter is made up of repeat units containing two inverted *ori* sequences and arranged as tandem pairs in inverted orientation relative to their nearest neighbors. After subcloning *ori*^{r} petites or crossing with wild-type cells a large number of *ori*$^+$ petites are found in the progeny. In contrast to the *ori*^{r} petites, from which they are derived, these *ori*$^+$ petites are characterized by high suppressivity levels (approx. 90%) and contain mitochondrial genomes made up of tandem repeat units containing single *ori* sequences. The structural changes underlying the *ori*^{r} to *ori*$^+$ mutation are therefore accompanied by a dramatic increase in suppressivity, indicating that the elimination of inverted *ori* sequences causes a drastic change from very poor to very good replicative efficiency in the mitochondrial genome. Finally, crosses of *ori*^{0} petites with wild-type cells were also studied; the results obtained have clarified the reasons for the high frequency of petites having genomes similar to those of *ori*^{r} petites after mutagenesis with ethidium bromide.

INTRODUCTION

The study of the progeny of crosses of wild-type *S. cerevisiae* cells with spontaneous petite mutants harboring well-characterized mitochondrial genomes has led us to an understanding of suppressivity (Bernardi et al., 1980; Goursot et al., 1980; de Zamaroczy et al., 1981; Bernardi, 1982). In such crosses, neutral petites (which can be equated with ρ^0 petites deprived of mitochondrial DNA) yield wild-type progeny only, whereas suppressive petites (or ρ^- petites containing a defective

Abbreviations: bp, base pairs; *ori*, origin of replication; *ori*$^+$, *ori*$^-$, *ori*^{0}, *ori*^{r}, *ori*^{s}, ρ^-, ρ^0, see INTRODUCTION; wt, wild type; YEPD medium, see MATERIALS AND METHODS, section **b**.

mitochondrial genome) yield both wild-type cells and petites in proportions essentially dependent upon the particular petite used (Ephrussi et al., 1955); as a first approximation, suppressivity can be defined as the percentage of petites in the progeny. Our work has shown that suppressivity corresponds to the level of transmission of the parental mitochondrial petite genome to the progeny. In turn, this level depends upon the replication efficiency and the stability of the mitochondrial petite genome relative to the wild-type genome. While both properties are influenced by the length of the repeat units of the petite genome, replication efficiency also very largely depends upon the particular sequence used as the origin of DNA replication, and generally decreases when going from *ori*$^+$ petites, containing one of the seven canonical *ori* sequences from the wild-type genome (de Zamaroczy et al., 1981), to *ori*$^-$ and *ori*^{0} petites, in which the canonical *ori* sequence is partially deleted or absent and replaced (Goursot et al., 1982) by a surrogate origin of replication (*ori*^{s} sequence).

Here we have studied the suppressivity of spontaneous *ori*^{r} petites and of *ori*$^+$ petites derived from them. The repeat units of *ori*^{r} petites contain two inverted canonical *ori* sequences, and are arranged as tandem pairs in inverted orientation relative to their nearest neighbors (Faugeron-Fonty et al., 1983), whereas those of the derived *ori*$^+$ petites are tandemly arranged and only contain one canonical *ori* sequence. Such a study was considered to be of particular interest in view of the demonstration (Faugeron-Fonty et al., 1983) that such an organization was similar to that of mitochondrial genomes with inverted repeat units harbored by petite mutants induced by mutagens. This is one of the two main classes of induced petites, the other one harboring, like spontaneous *ori*$^+$ petites, tandem repeat units (Locker et al., 1974a, b; 1979; Locker and Rabinowitz, 1976; Lewin et al., 1978; Bos et al., 1978; 1980; Heyting et al., 1979). The results obtained, therefore, clarify the issue of suppressivity not only in spontaneous *ori*^{r} petites, but also in a whole class of induced petites. Crosses of *ori*^{0} petites with wild-type cells were then used to understand the reasons for the different frequencies of occurrence of *ori*^{r} petites among spontaneous petites and of petites with inverted repeat units among induced petites.

MATERIALS AND METHODS

(a) Yeast strains

The wild-type *S. cerevisiae* strains used were strains D-243-2B-R1 and C-982-19d (called here, as in our previous papers, strains A and B, respectively; see Faugeron-Fonty et al., 1979 for the properties of these strains). All petite strains were spontaneous respiratory-deficient petite mutants, derived from these wild-type strains. Growth-media and culture conditions were as described by Faugeron-Fonty et al. (1979).

(b) Suppressivity of the petite strains

This was determined as follows: 1 ml each of overnight cultures of the petite strain and the wild-type tester strain B in YEPD medium (1% yeast extract, 1% bacto-peptone and 2% glucose) were added to 10 ml of fresh YEPD medium and incubated at 28°C. After 4 h, samples were plated on minimal medium with glucose (0.67% yeast nitrogen base, 2% glucose), allowing selection of diploids. Petite colonies were identified by their inability to grow on glycerol (0.67% yeast nitrogen base, 3% glycerol) after replica plating. Suppressivity is expressed as the percentage of petite colonies among at least 500 diploid progeny clones.

(c) Mitochondrial DNA

This was purified as already described (Faugeron-Fonty et al., 1983). Alternatively a micro-scale method for rapid mitochondrial DNA purification was used, essentially as described by Dujon and Blanc (1980). All other methods were as described by Faugeron-Fonty et al. (1983).

RESULTS

(a) Properties of haploid subclones of *ori*$^+$ and *ori*^{r} petite mutants

Restriction maps of the repeat units of the mitochondrial genomes of the reference *ori*$^+$ petite, a-15/3/2, and of the *ori*^{r} petite, a-15/4/1, are

TABLE I

Properties of petites a-15/3/2 (*ori*$^+$), a-15/4/1 (*ori*^{r}) and their subclones [a]

Petite strain [b]	Repeat unit length (bp)	*ori* sequence	Suppressivity [c] (%)	Transmission of parental petite genomes [d]
a-15/3/2	4200	*ori*5	50	12:0
24 subclones (par.)	4200	*ori*5	50–60	n.d.
a-15/3/2/5 (ρ^0)	–			
a-15/4/1	4450	*ori*5 + *ori*5	5	4:11
21 subclones (par.)	4450	*ori*5	5–8	n.d.
a—4	1210	*ori*5	90	12:0
a—1	1560	*ori*5	85	12:0
a—23	3950+4210	*ori*5 + *ori*5	12	1:11
16 subclones (par.)	3950+4210	*ori*5 + *ori*5	10–13	n.d.
a—23/3	750	*ori*5	95	12:0
a—23/5	586	*ori*5	90	12:0
a—23/14	1330	*ori*5	85	12:0
a—23/8 (ρ^0)	–			
a—10	heterogeneous	*ori*5	30	11:0
a—10/4 (same as a-15/4/1)	4450	*ori*5 + *ori*5	5	n.d.
a—10/1; 11 subclones	1750	*ori*5	90	12:0
a—10/2; 6 subclones	1810	*ori*5	85	12:0
a—10/3	954	*ori*^{s}	1	5:24
23 subclones (par.)	954	*ori*^{s}	1	n.d.
a—10/3/2 (ρ^0)	–			
a—14 (ρ^0)	–			

[a] All petite mitochondrial genomes were mapped with restriction enzymes.

[b] Subclones of a-15/4/1 are indicated by a—(see RESULTS, section **a**); (par.) indicates parental-type genomes; ρ^0 petites do not contain any mitochondrial DNA.

[c] The frequency of spontaneous petites generated from the wildtype strain B being very low (approx. 0.5%), it does not modify the value of suppressivity of petite strains used in the cross.

[d] Crosses of petites were made with wild-type strain B. The ratio given is that of diploids having the repeat units of the parental petite to diploids having different repeat units; the latter were investigated (see RESULTS, section **b** and Tables II and III; n.d., not determined).

given in Fig. 1; both petite genomes are derived from the same *ori*5 region of the genome of wild-type strain A and have been studied elsewhere (Faugeron-Fonty et al., 1983). Table I presents a complete series of results obtained when subcloning these two petites, in order to provide an unbiased representation of the outcome of the experiment.

In the case of a-15/3/2, 24 out of 25 subclones harbored the parental petite genome and exhibited the same suppressivity, 50–60%; one subclone was a ρ^0 petite, namely a petite deprived of mitochondrial DNA. When a-15/3/2 was crossed with wild-type strain B, 12 out of 12 petite diploids exhibited the parental petite genome (Table I).

The results obtained with a-15/4/1 were quite different, in that only 21 out of 26 subclones harbored the parental petite genome and exhibited the same suppressivity, 5–8%. Of the other five subclones of a-15/4/1, a—14 [subclones of a-15/4/1 will be indicated henceforth as a—(long dash)] was a ρ^0 petite, and a—10 exhibited a strong heterogeneity in its mitochondrial genome, as shown by the nonstoichiometry of the restriction fragments (see Faugeron-Fonty et al., 1979) and by the results of further subcloning (Table I;

TABLE II

Results of crosses of a-15/4/1 and two of its subclones with wild-type cells [a]

Cross	Petite diploids [b]	Repeat unit length (bp)	*ori* sequence
a-15/4/1 × B	a—/B1; 4 diploids of parental type	4450	*ori*5 + *ori*5
	11 diploids of different type:		
	a—/B2	1280	*ori*5
	a—/B3	1170	*ori*5
	a—/B8	2120	*ori*5
	a—/B10	1100	*ori*5
	a—/B11 same as a—10/1	1750	*ori*5
	a—/B12; 6 diploids; same as a—10/2	1810	*ori*5
a—23 × B	a—23/B3; diploid of parental type	3950 + 4210	*ori*5 + *ori*5
	11 diploids of different type:		
	a—23/B1	2220	*ori*5
	a—23/B2	2130	*ori*5
	a—23/B6	1480	*ori*5
	a—23/B7	1200	*ori*5
	a—23/B8	2440	*ori*5
	a—23/B9; 2 diploids	1220	*ori*5
	a—23/B10; 4 diploids; same as a—23/14	1330	*ori*5
a—10 × B	a—10/B1 same as a—15/4/1	4450	*ori*5 + *ori*5
	a—10/B2; 8 diploids; same as a—10/1	1750	*ori*5
	a—10/B3; 2 diploids; same as a—10/2	1810	*ori*5

[a] All petite genomes were mapped with restriction enzymes.
[b] See footnote b in Table I.

see also below). Another subclone, a—4, had a genome formed by a repeat unit only 1210 bp long and containing a single *ori*5 sequence. Restriction mapping (Fig. 1) showed that this repeat unit was homologous to a segment of a-15/4/1. In contrast to the latter petite, however, a—4 had a suppressivity of 90%. Furthermore, its genome was found in all 12 petites produced by a cross with wild-type cells, whereas only four out of 15 petites produced by a-15/4/1 with wild-type cells carried the parental petite genome (Tables I and II). The case of subclone a—1 was very similar to the previous one, except for a deletion and a terminal non-duplicated inversion (Faugeron-Fonty et al., 1983; see Fig. 1). As in the case of a—4, the repeat units of a—1 were tandemly arranged. Finally, subclone a—23 had a genome formed by repeat units which still contained the two inverted *ori*5 sequences of the parental petite genome, but which had undergone some additional rearrangements (Faugeron-Fonty et al., 1983; see Fig. 1). The suppressivity of a—23 was low, 10–13%; transmission of its mitochondrial genome to the progeny of crosses with wild-type cells was poor; only one out of 12 diploid petites harbored the parental genome; the other 11 contained different secondary genomes derived from a—23 (Tables I and II).

Further subcloning of a—23 led to results similar to those reported for a-15/4/1. Out of 20 subclones, 16 were identical in their genomes and suppressivities to the parental one. One was a ρ^0 petite and three were the result of excisions leading to short tandem repeat units containing only one *ori*5 sequence (see Fig. 1) and to very high suppressivity and transmission of the parental petite genome in crosses.

Subcloning of a—10, the petite with a heterogeneous genome and an average suppressivity of 30%, led to the following results. All the subclones examined contained mitochondrial genomes already present in the heterogeneous parental one. Out of 19 subclones, one was identical to a-15/4/1 in its genome and suppressivity, and 17 were petites with genomes having short tandem repeat units,

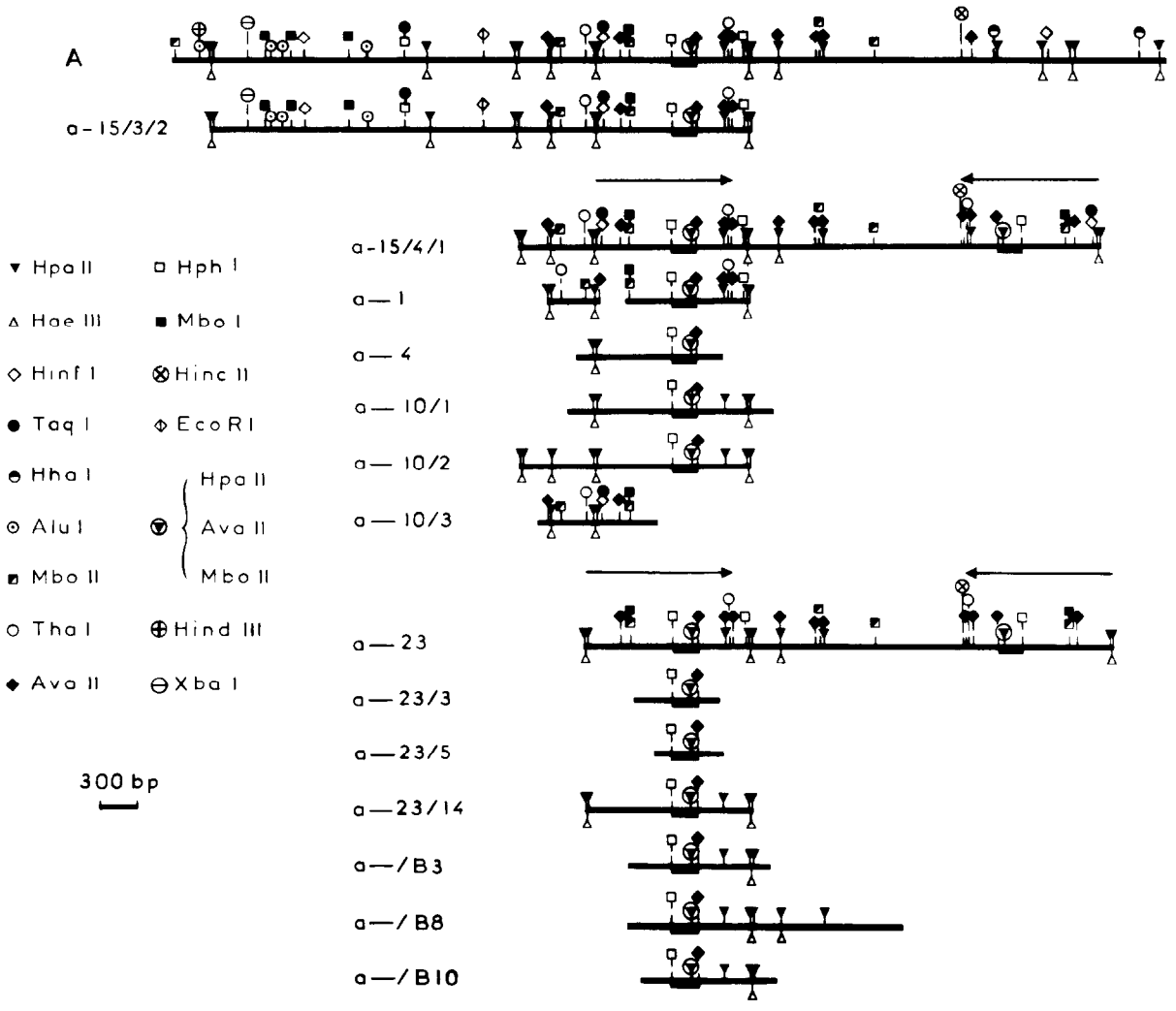

Fig. 1. Restriction maps of the repeat units of petite mitochondrial genomes derived from the *ori*5 region and investigated in this work. Most of the repeat units from diploid petites are not shown since restriction mapping was limited in these cases to *Hpa*II and *Hae*III sites (see also Tables I and II). Not all restriction sites mapped are indicated on the repeat units of petite genomes. The *ori*5 sequence is underlined (double thickness line). Arrows indicate inverted segments on the repeat units of a-15/4/1 and a—23. A class 2 repeat unit of a—23 is shown and the deleted segment of the repeat unit of a—1 is represented by a gap (see Faugeron-Fonty et al., 1983). The restriction map of the corresponding region of the genome of the parental wild-type strain A is also presented. This map is derived from our results and also from data of Thalenfeld and Tzagoloff (1980) for its left end and Martin et al. (1982) for its right end. Not all restriction sites investigated have been mapped.

containing one *ori*5 sequence only, and very high suppressivity and transmission. These petites belonged in two classes; the repeat units of the first class were 1750 bp long, those of the second one 1810 bp long (see Fig. 1). The last subclone, a—10/3 (see Fig. 1), was an *ori*^{0} petite (as initially shown by the lack of hybridization of an *ori* probe), having a suppressivity of 1% and showing very poor transmission in crosses. Its repeat unit has been fully sequenced (Goursot et al., 1982); 23 subclones of a—10/3 were identical to a—10/3 in their genomes and suppressivity; one was a ρ^0 petite.

(b) Properties of the diploid petite progeny of a cross between a wild-type strain and *ori* mutants

Table II lists the properties of petites produced by crosses of wild-type strain B with petites a-15/4/1 and a—23, the two *ori*^{r} petites, and of

petite a—10. Differences between the data of Tables I and II are related to the fact that while in subcloning petite genomes undergo a competition with the poorly replicating parental petite genome, in crosses they are exposed, in addition, to competition with the well-replicating wild-type genome. Again, as in Table I, a complete set of data is given for an unbiased representation of the results.

Crosses of a-15/4/1 and a—23 with wild-type strain B produced diploid petites, a minority of which were identical to the parental ones, whereas the majority had genomes with short repeat units containing one *ori*5 sequence only (see Fig. 1). Some of these independent petites had identical repeat units or had repeat units already found in highly suppressive haploid subclones from the same parental petites. Diploid petites from crosses of a—10 had the same genomes found in subclones of this heterogeneous petite (Table II), but the very low suppressivity ori^0 genome of a—10/3 was not found.

Crosses of ori^0 petite a—10/3 with wild-type strain B were also studied to provide additional information (see DISCUSSION). The results (Table III) were quite different from those just described, in that only 5 diploid petites contained the parental petite genome, whereas 24 petite genomes were derived from the parental wild-type genome. Among the latter, the most frequent one corresponded to b7, a petite whose repeat unit is the result of an excision between *ori*2 and *ori*7 (Marotta et al., 1982), and contains therefore a ori^h (*ori* hybrid) sequence. Others contained *ori*2 + *ori*7, *ori*5, *ori*2 or *ori*3; three were different ori^0 petites, containing at least one ori^s sequence; and five were ori^0 petites with genomes having a very low G + C level and no detectable sites for a large number of restriction enzymes.

DISCUSSION

The data of Table I demonstrate that the structure and organization of the mitochondrial genomes of ori^r petites is associated, in 40 cases out of 40, with a very low suppressivity, 5–12%; the 40 cases are those of a-15/4/1 and a—23, of 37

TABLE III

Results of crosses of ori^0 with wild-type cells [a]

Cross	Petite diploids	Repeat unit length (bp)	*ori* sequence
a—10/3 × B (ori^0 × wt)	a—10/3/B6; 5 diploids of parental type	954	$ori^{s\,c}$
	24 diploids of different type:		
	a—10/3/B1	4500	*ori*2 + *ori*7
	a—10/3/B2	1230	*ori*5
	a—10/3/B3	1860	*ori*2
	a—10/3/B4; 2 diploids	6000	$ori^{s\,c}$
	a—10/3/B5	1760	*ori*3
	a—10/3/B12	659	$ori^{s\,c}$
	a—10/3/B13	380	n.d.
	a—10/3/B14	1500	$ori^{s\,c}$
	a—10/3/B16; 2 diploids	6450	*ori*2 + *ori*7
	a—10/3/B17	4650	*ori*5
	a—10/3/B18; 7 diploids; same as b7 [c]	2200	$ori^{h\,c}$
	a—10/3/B19; 5 diploids	n.d. [b]	

[a] All petite genomes were mapped with restriction enzymes, and hybridized with restriction fragments of the wild-type strain B genome to study their localizations.

[b] These petite repeat units contain no restriction sites, thus preventing the determination of their length. Their GC content is extremely low as estimated by their low buoyant density in CsCl gradient; n.d., not determined.

[c] These petite genomes have been investigated in detail elsewhere; ori^h is a hybrid *ori*2-*ori*7 sequence; ori^s is a surrogate origin of replication (Goursot et al., 1982; Marotta et al., 1982).

subclones derived from them and of subclone a—10/4. In sharp contrast, the other 5 subclones of a-15/4/1 and a—23, and 17 subclones of a—10, which harbor mitochondrial genomes consisting of tandem repeat units excised from the parental *ori*^{r} genomes and carrying single *ori*5 sequences, are characterized by very high suppressivities, 85–90%. In other words, the secondary mutation leading from *ori*^{r} to *ori*$^+$ petites is accompanied by a dramatic increase in suppressivity. These results provide an additional argument in favor of our previous conclusion (Bernardi et al., 1980; de Zamaroczy et al., 1981) that *ori* sequences are responsible for the initiation of DNA replication, since the very poor replication efficiency of *ori*^{r} genomes is clearly due to the inverted orientation of subsequent *ori* sequences. Indeed, repeat units of petite genomes containing two tandemly oriented *ori* sequences (*ori*7 and 2; *ori*3 and 4), separated by about the same distance as the two inverted *ori*5 sequences, have been found to replicate efficiently (de Zamaroczy et al., 1981).

A comparison of the subclones of a-15/3/2 with those of a-15/4/1 and a—23 is of great interest. The *ori*$^+$ petite a-15/3/2 yielded 24 subclones out of 25 (one of them being a ρ^0 petite) carrying an intact parental genome. In contrast, the two *ori*^{r} petites yielded a large percentage (over 10%) of subclones harboring *ori*$^+$ genomes derived from the parental ones by excision. At first sight, one might be inclined to attribute the differences in these subcloning results to a much greater instability of the *ori*^{r} petites relative to the *ori*$^+$ petites. This explanation is, however, challenged by the observation that most secondary petites derived from *ori*^{r} petites arise from the repeat unit region also present in a-15/3/2 (see Fig. 1). Even if for some secondary petites it is not possible to decide whether their origin is from the neighborhood of the *ori*5 sequence or from that of its duplication, no secondary petite appears to arise from excisions involving sequence features only present in *ori*^{r} petites, like sequences located near *ori*5 and near its duplication, respectively.

The alternative, correct explanation for the results of Table I is that the well-replicating *ori*$^+$ secondary genomes can easily compete out the poorly replicating resident parental *ori*^{r} genomes and have, therefore, excellent chances to be largely present in the progeny, whereas this is not the case for the secondary petite genomes derived from the well-replicating *ori*$^+$ genome of a-15/3/2.

The explanation just presented for the data of Table I is supported by the results obtained in the crosses of Table II. In this case, the *ori*^{r} genomes appear only in a minority of the progeny, since they are competed out by the wild-type genomes. Most of the diploid petites thus carry *ori*$^+$ genomes, derived from the parental *ori*^{r} genomes and able to compete successfully with wild-type parental genomes. These *ori*$^+$ genomes are much more frequent than those (not observed in our experiments) derived from the parental wild-type genomes. The reason for this is, in all likelihood, the higher density of *ori* sequences on the *ori*^{r} genomes compared to wild-type genomes. This obviously increases the probability of producing efficiently replicating petites. Interestingly, many petite diploids of Table II are identical to the petite haploids of Table I, indicating the preferential usage of certain excision sequences.

It should be pointed out that the highly suppressive secondary *ori*$^+$ genomes derived from the *ori*^{r} genomes are very largely responsible for the suppressivity of *ori*^{r} petites, as estimated by the usual criteria, namely by the number of diploid petites in the progeny of the cross. It is clear that if one were to correct for this effect, the "real" suppressivity of a-15/4/1 and a—23 would only be 25% and 10%, respectively, of the values given in Table I, namely about 1%.

The results of a cross of a—10/3 (the *ori*^{0} petite with the extremely low suppressivity of 1%) with wild-type cells (Table III) are very interesting. The sizes of the repeat units and the characterization of the *ori* or *ori*^{s} sequences carried by the petite genomes showed that only 5 out of 29 were of parental type, the others being derived from the parental wild-type genome; 14 of these were *ori*$^+$ or *ori*^{h}, 4 were *ori*^{0} and 5 contained genomes very low in G + C with no detectable restriction sites. The finding of such a high percentage (9 out of 29) of genomes which, in all likelihood, replicate poorly can only be due to the fact that they often segregate into the buds together with the extremely poorly replicating parental petite genome, which they can compete out. This result has both a

general and a practical interest, (i) because it shows in a striking way that many more excision events occur in the wild-type genome than those leading to the overwhelming production of *ori*$^+$ petites; and (ii) because it suggests that for obtaining petites harboring poorly replicating genomes a useful approach is to cross a wild-type cell with a petite like a—10/3 harboring an extremely poorly replicating genome.

Finally, the results reported in the preceding paper (Faugeron-Fonty et al., 1983) and here help in better understanding some points concerning the induced petite mutation. First, the tremendous increase in excision rate caused by mutagens appears to be accompanied by frequent inversions, which are very seldom seen in spontaneous petites. It is likely that ethidium bromide favors internal recombination not only at direct repeats, leading to excisions, but also at inverted repeats, leading to inversions. Second, excisions being so much increased in frequency, the mitochondrial genome of wild-type cells literally disintegrates into excision products, many of which either will not contain a canonical *ori* sequence or will contain inversions. The very abundance of these poorly replicating genomes favors their transmission to the progeny because they will co-segregate into buds and escape competition from the minority of well-replicating genomes. This explanation inspired by the results obtained by *ori*^{0} × wild-type crosses would account for the main differences between the spontaneous and the induced petite mutations, namely the much higher frequency of rearranged genomes in the second case.

ACKNOWLEDGEMENTS

We thank Alain Huyard for his help in some of the experiments reported, Giuseppe Baldacci and Miklos de Zamaroczy for useful discussions, Martine Brient for typing this manuscript and Philippe Breton for the art work.

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Communicated by J. Carbon.