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The origins of replication of the yeast mitochondrial genome and the phenomenon of suppressivity

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The 'petite colonie' mutation of *Saccharomyces cerevisiae*[1,2] is characterized by an irreversible loss of respiration and by an extraordinarily high spontaneous mutation rate[3,4]. Crosses of wild-type cells with petite mutants exhibit a non-mendelian segregation of the mutation, yielding either wild-type progeny only[1,2], or both wild-type and petite mutants in proportions essentially dependent on the particular petite used[3]. In the first case, the petites entering the cross are called neutral, in the second one suppressive[3]. While the molecular basis of the spontaneous petite mutation is now understood[4-12], suppressivity has remained an elusive phenomenon for the past 25 yr. We report here that the mitochondrial genome of most spontaneous petites (which is exclusively made up by the tandem repetition of a DNA segment excised from the genome of parental wild-type cells[5-8]) carries at least one of the *ori* sequences of the parental wild-type genome. These are long homologous DNA stretches showing striking similarities with the origins of replication of mitochondrial DNAs from mammalian cells. The properties (intact or altered primary structure, high or low number) of the *ori* sequences of petite genomes seem to determine suppressivity—the level of transmission of petite genomes to the progeny of crosses with wild-type cells. These results indicate that *ori* sequences are indeed origins of DNA replication and that suppressivity depends on the relative replication efficiencies of petite and wild-type genomes.

We previously proposed that the mitochondrial genome of wild-type yeast cells contains several origins of replication and that at least one of them is carried by the excised segment which will become the repeat unit of a spontaneous petite[13]. We tentatively localized such an *ori* sequence within the common central stretch of the genome of two spontaneous petites excised from the same region of the wild-type genome[6,7]. This stretch is characterized (refs 7, 12 and Figs 1, 2, 4) by two short GC clusters, A and B, flanking a 23-nucleotide AT palindrome, *p*, and a short sequence, *s*; and by one long GC cluster, C, separated from B by long AT sequence, *l*. We then found a common 80-base pair sequence centred on cluster B, and more recently, the whole *ori* sequence in petite genomes derived from five regions of the wild-type genome[10-12,14,15].

Screening of the mitochondrial genomes of ~400 spontaneous petites derived from wild-type strain A^5 revealed that all except two contained an *ori* sequence, as judged from the restriction sites located on clusters A, B and C (Fig. 1; Table 1). Restriction mapping showed that the *ori* sequences of different petite genomes were surrounded by different flanking regions and indicated the existence of at least seven *ori* sequences, *ori* 1–7 (Fig. 1), on the wild-type genome. This conclusion was confirmed by hybridization experiments in which labelled mitochondrial DNAs from petite genomes carrying different *ori* sequences showed seven common hybridization bands on *Hae*III fragments from the parental wild-type genome; these fragments had the same sizes as *Hae*III fragments carrying the different *ori* sequences on the petite genomes of Fig. 1. An eighth hybridization band might indicate the existence of yet another origin.

Figure 1 also shows that (1) three petite genomes, a-3/1/5/B1, b17 and a-3/1, contained two *ori* sequences each; (2) two petite genomes, a-3/1/5 and a-3/1/33, had an *ori* sequence lacking cluster C, and two petite genomes, a-1/1R/14 and a-1/1R/1/26, had an *ori* sequence lacking cluster A (*ori*$^-$ petites, having a partially deleted *ori* sequence); (3) petite a-15/4/1 contained two *ori* sequences in opposite orientations—the only rearranged repeat unit found so far among spontaneous petites (this sort of petite will be indicated as *ori*^{r}); (4) petite a-15/4/1/10/3, derived by subcloning from the previous one, lacked an *ori* sequence altogether; another *ori*^{0} petite, a-3/1/B4, was a diploid petite issued from a cross of a-3/1 with wild-type strain B and derived from the 15S-*oxi* 3 region of the B genome; these *ori*^{0} petite genomes will be discussed in detail elsewhere.

Figure 2 presents *ori* sequences 1–5 as determined on the repeat units of 11 different spontaneous petites (see Table 1). The *ori* 6 sequence and the right end of *ori* 7 were found in the published sequences of petites DS-400/A12 and DS-14 (refs 16, 17). Homology in the primary structure of different petite genomes carrying *ori* 1 or 3 was nearly perfect, the very rare differences being indicated by asterisks in Fig. 2. Comparison of different *ori* sequences indicated perfect homology in clusters A, B and C (some differences in clusters A and B of *ori* 6 being probably due to sequence uncertainties[16] and several differences in region *l* between clusters B and C.

The stretch shared by all *ori* sequences is 265 base pairs long, its left border at nucleotide 69, and its right border at nucleotide 334 of Fig. 2. Note, however, that at the left of cluster A *ori* 6 presents a very large GC cluster, α, absent in at least *ori* 1–5; (2) *ori* 4 and 6 contain two additional GC clusters, β and γ, identical in both sequence and location; β is located between clusters B and C, γ at the right of cluster C; at least cluster γ is also shared by *ori* 7; (3) *ori* 4–7 are homologous for 30–40 nucleotides at the right of the position of cluster γ; (4) some sequence homology is present on the left of the *ori* sequences, particularly in *ori* 1 and 2; (5) the overall homology between *ori* 4, 6 and possibly 7 may comprise 370 nucleotides; (6) *ori* 1 and 2 show homologies for at least 30 base pairs on the right of the *ori* sequence; and (7) the full sequence of the repeat units of *ori*$^-$ petites (ref. 12 and paper in preparation) indicated that these are the result of excisions involving two direct repeats, one of which is within the *ori* sequence.

Hybridization of labelled petite mitochondrial DNAs containing different *ori* sequences on restriction fragments from wild-type DNAs has been performed and restriction maps of both sets of DNAs have been compared with maps from other laboratories[16-26]. Using these results, it was possible to localize and orient (Fig. 3) *ori* 1–7 on the mitochondrial genome of wild-type cells and show that the inverted repetition of *ori* 5 on a-15/4/1 was not present on the wild-type genome. The distribution of the *ori* sequences is very uneven with five sequences on one-third of the map, the region most often represented among the genomes of spontaneous petites[27]. Note that *ori* sequences do not have the same orientation (Fig. 3) and that *ori* 3 and 4, and *ori* 2 and 7, share a very similar tandem arrangement.

Three independent lines of evidence indicate that the *ori* sequences are indeed origins of replication. (1) The region between clusters A and B inclusive can be folded in a way (Fig. 4) very similar to that reported for a region of the replication origin of mammalian mitochondrial DNA[28-30]; all base pair changes found in this region in different genomes are localized in the looped-out sequence *s*. Cluster C is almost identical (Fig. 4) in sequence to a GC cluster, similarly located relative to the A–B region, in mammalian mitochondrial DNAs[28-30]. (2) *Ori* sequences are present in the repeat units of most spontaneous petites. (3) A clear correlation exists between the properties of the *ori* sequences and suppressivity, as indicated by the following.

We reported previously that crosses of two spontaneous petites (a-1/1R/1 and b) with wild-type cells produced diploids only harbouring the unaltered mitochondrial genome of the

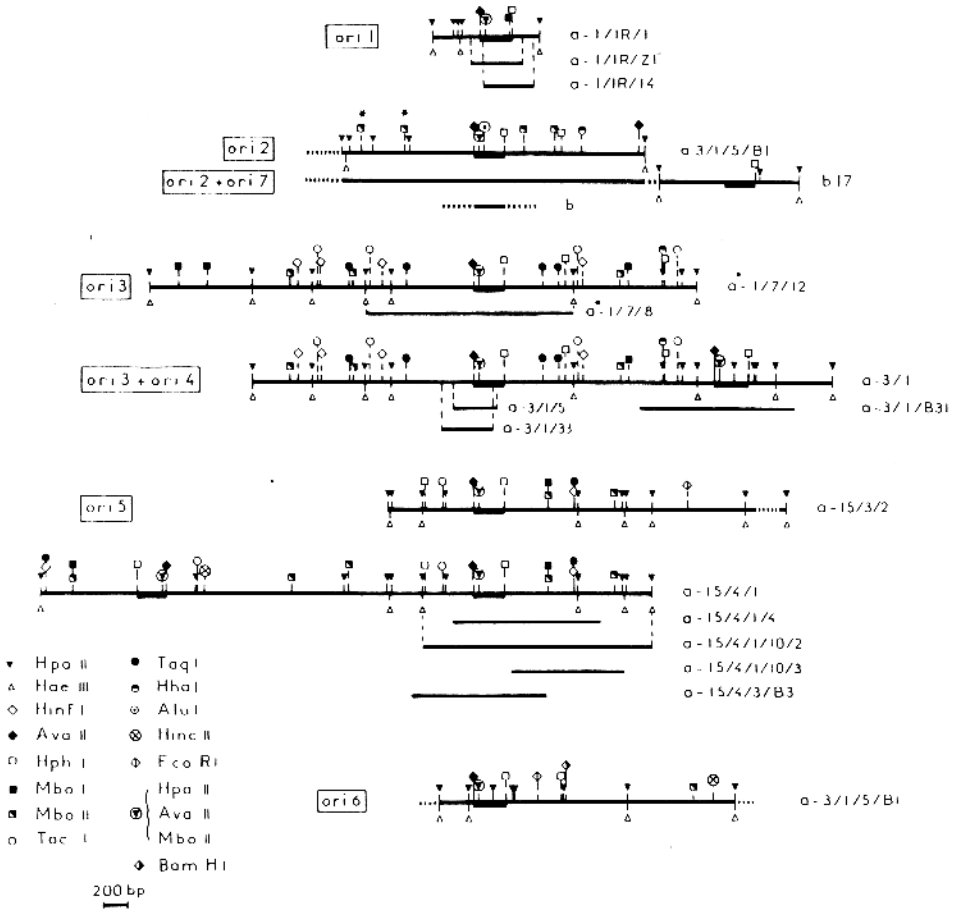

Fig. 1 Restriction enzyme maps of the repeat units of mitochondrial genomes of spontaneous petite mutants. These arose from wild-type strain A, except for a-3/1/5/B1, b17 and b, which derived from a region (*oli* 1–2) of wild-type strain B in which this genome has the same map as A. Some maps present additions and corrections compared with ref. 5. The map of b17 is a preliminary one; that of b contains an internal deletion in its repeat unit (in preparation). Restriction sites are indicated on some repeat units only and not all *Ava*II sites are shown. Asterisks indicate restriction sites whose locations need to be confirmed. *Ori* sequences are underlined: cluster A corresponds to an *Ava*II site, cluster B to *Hpa*II, *Ava*II, *Mbo*II site clusters, cluster C to a *Hph*I and a *Mnl*I site. All maps are centred on cluster B and oriented with clusters A, B, C, from left to right. Vertical broken lines indicate excision sites where precisely known. bp, Base pairs.

parental petite used in the cross[10–12,14,15]. Both petites were very highly suppressive (that is, the percentage of petites in the progeny was >95%) and had mitochondrial genomes characterized by very short repeat units (~1% of the wild-type genome). We called these petites, which have also been reported from other laboratories[31,32], supersuppressive, and suggested that their mitochondrial genomes were only ones found in the progeny because they contained multiple copies of the origin of replication due to tandem amplification of their repeat units and they replicated more efficiently than the wild-type genome, which they could compete out[10–12,14,15]. We have now investigated the general case of petites having any degree of suppressivity. The results are presented in Table 1 and lead to the following conclusions.

(1) Most of the 200 petite diploids had restriction maps identical to those of the parental petites, regardless of the

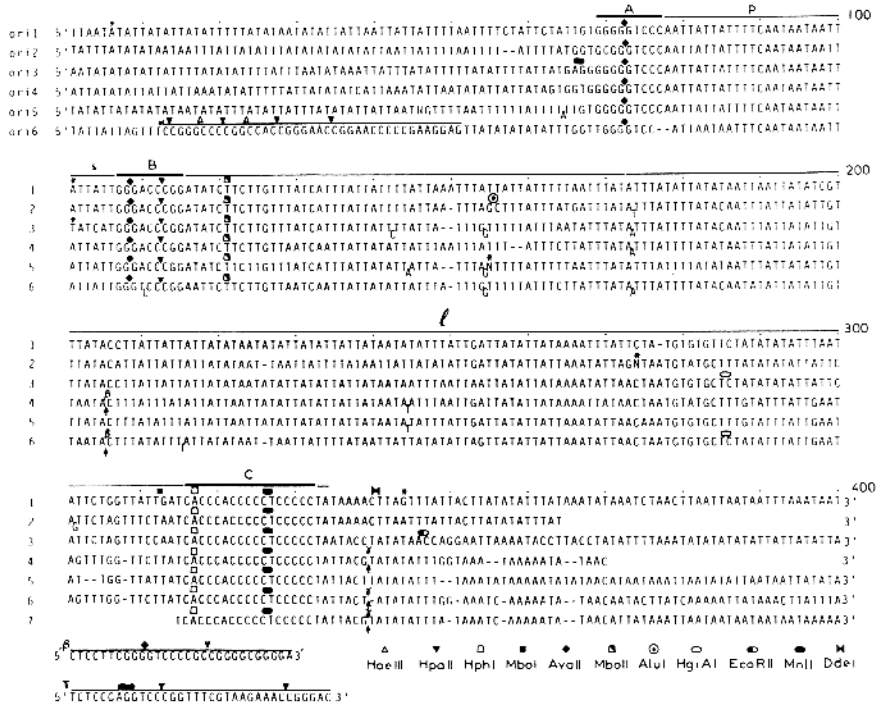

Fig. 2 Primary structure of the *ori* sequences and their flanking regions, as determined by the method of Maxam and Gilbert[43] on the repeat units of the mitochondrial genomes from petites a-1/1R/1 (*ori* 1; position 1 corresponds to position 301 on the repeat unit of the reference sequence of a-1/1R/1)[7], b (*ori* 2), a-3/1 (*ori* 3), a-3/1/B31 (*ori* 4), a-15/4/1/3/B3 (*ori* 5), DS-400/A12 (*ori* 6; from ref. 16) and DS-14 (*ori* 7; from ref. 17). The sequence on the left of *ori* 5 is from HS 1948 (ref. 31). *Ori* sequences were also determined on the repeat units of petites a-1/1R/Z1, a-1/1R/14 (*ori* 1) a-1/1R/26, a*-1/7/12, a*-1/7/8, a-3/1/5, a-3/1/33 (*ori* 3). Asterisks indicate the positions of modified nucleotides (indicated as N) or of differences in the same *ori* sequence as determined on different petites. Regions of *ori* sequences are indicated by thick lines for GC clusters A, B and C and thin lines for AT regions *p*, *s* and *l*. The positions of clusters β and γ in *ori* 4, 6 and 7 are given as well as the sequences of these clusters (bottom lines). Restriction sites are indicated by the symbols shown. The sequence of *ori* 3 corrects some mistakes[14] in the 10 nucleotides at the left of cluster A, and in one nucleotide at the right of cluster B.

Table 1 The mitochondrial genomes of spontaneous petites: properties of *ori* sequences and suppressivity

Petite strain	Repeat unit length (base pairs)	Primary structure	*Ori* sequence	%Suppressivity	Transmission of petite genomes
a-1/1R/1	884	w	*ori* 1	>95	8:4
a-1/1R/Z1	416	w	*ori* 1	>95	
a-1/1R/40	606	m	*ori* 1	>95	
a-1/1R/14	380	w	*ori* 1 (A⁻)	80	11:0
a-1/1R/1/26	392	w	*ori* 1 (A⁻)	80	5:0
b	875	o	*ori* 2	>95	10:0
a*-1/7/12	4,500	o	*ori* 3	60–80	36:0
a*-1/7/8	1,760	o	*ori* 3	85	14:0
a-3/1	4,700	o	*ori* 3+4	60–80	28:4
a-3/1/5	345	w	*ori* 3 (C⁻)	<5	5:1
a-3/1/33	400	w	*ori* 3 (C⁻)	<5	
a-3/1/B31*	1,360	o	*ori* 4	—	—
a-15/3/2	4,300	m	*ori* 5	50–60	12:0
a-15/4/1	4,800	m	*ori* 5+5	<5	3:12
a-15/4/1/1	1,560	m	*ori* 5	85	12:0
a-15/4/1/4	1,220	m	*ori* 5	90	12:0
a-15/4/1/10/1	1,780	m	*ori* 5	90	12:0
a-15/4/1/10/2	1,830	m	*ori* 5	85	12:0
a-15/4/1/10/3	970	m	*ori*⁰	~1	5:13
a-15/4/1/B3*	1,170	o	*ori* 5	—	—
a-3/1/B/B1*	16,500	m	*ori* 2+6	—	—
b17	~7,800	—	*ori* 2+7	80	—

w and o indicate that the primary structure is known for the whole repeat unit or for the *ori* sequence and the flanking sequences, respectively. All other repeat units were physically mapped (m). The transmission of petite genomes concerns ~200 diploid petites from crosses of spontaneous petites with wild-type strain B (A in the case of petite b). Mitochondrial DNAs were mapped with restriction enzymes. The ratio presented is that of diploids having the repeat units of the parental petite to diploids having modified repeat units (see text).

* Diploid petites from crosses of spontaneous petites with wild-type cells. The repeat units of a-3/1/B31, a-15/4/1/B3 and a-3/1/5/B1 originated from the genomes of a-3/1, a-15/4/1 and B, respectively. They were used to sequence *ori* 4, *ori* 5 and to map *ori* 6, respectively.

suppressivity of the latter. The few cases where a different situation was found (Table 1) corresponded either to a genome resulting from a new excision process which had occurred in the parental petite genome or, more rarely, in the parental wild-type genome.

(2) Partial or total deletions or rearrangements of *ori* sequences were always accompanied by drastic changes in suppressivity: the total deletion of *ori* 5 in a-15/4/1/10/3 resulted in a suppressivity of ~1% as opposed to 90% for a-15/4/1/4; the deletion of cluster C plus about 100 base pairs on its left in *ori* 3 led to a suppressivity <5% in a-3/1/5 and a-3/1/33, whereas a*-1/7/8 (although having a much larger repeat unit) had a suppressivity of 85%; the deletion of cluster A in *ori* 1 caused a decrease in suppressivity, from >95% in a-1/1R/Z1 to 80% in a-1/1R/14 and a-1/1R/1/26; and the presence of a duplicated inverted *ori* 5 sequence in a-15/4/1 was associated with a suppressivity of ~5%, whereas a-15/3/2, having a comparable repeat length, but only one *ori* 5 sequence, had a suppressivity of 50–60%.

(3) The repeat unit length affects replication efficiency as seen by comparing the suppressivities of a*-1/7/8 and a*-1/7/12, two petites carrying the same *ori* 3 sequence, or of a-15/3/2 and the series a-15/4/1, all containing *ori* 5. In all cases a shorter repeat length, and thus a higher density of *ori* sequences, is accompanied by a higher suppressivity. Similar observations have been reported previously[5].

Two explanations have been put forward to account for suppressivity. The first one proposes a replicative advantage of

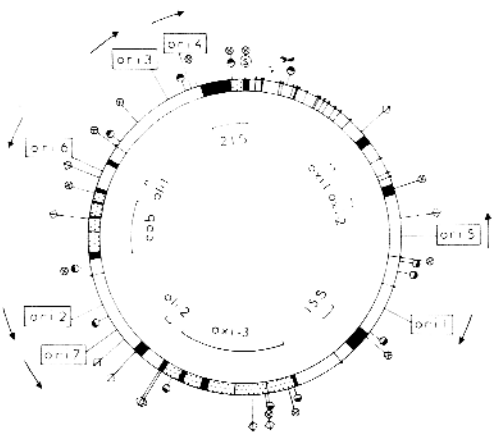

Fig. 3 Localization of *ori* sequences on the physical map of the mitochondrial genome of wild-type strain A. (This map is almost identical to that of strain KL-14-4A[18].) Positions indicated correspond to cluster B. Restriction sites are indicated by the symbols used in Fig. 1; Ⓢ corresponds to the *Sal*I site, used as the map origin. The orientation of *ori* sequences is given by arrows pointing in the direction of cluster C. Black and hatched areas correspond to exons and introns, respectively, of mitochondrial genes. Their localization is based on refs 16–26. The localization of tRNA genes is shown by thin radial lines.

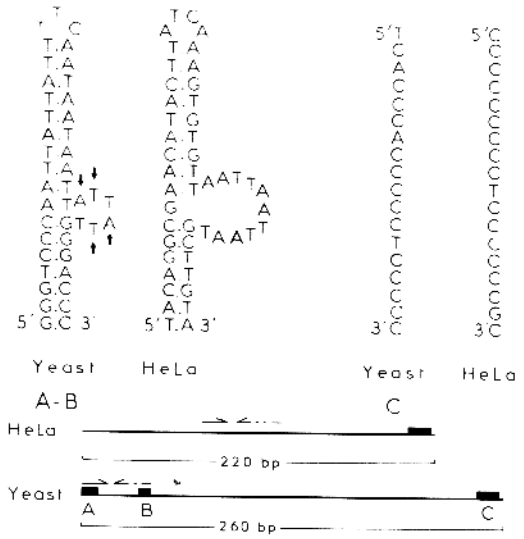

Fig. 4 Comparison of *ori* sequences of mitochondrial genomes from yeast (present work) and HeLa cells[28]. Homology of potential secondary structure is found for the inverted repeats in the A–B region (arrows indicate the base changes found in this region in different petite genomes). Homology of primary structure is found for cluster C. The bottom compares the two *ori* sequences; the arrows indicate the inverted repeats of the A–B region, the broken line corresponding to the looped-out sequence. bp, Base pairs.

the mitochondrial genome of suppressive petites over that of wild-type cells[33–36]. It was directly inspired by the work of Mills *et al.*[37] on the replication of Qβ DNA but was not accompanied by any molecular model. The second one proposes a destructive recombination of the petite genome with the wild-type genome[38–42], and predicts that a number of different petite genomes are formed as the consequence of the increased parental genome instability due to the insertion of the petite genome. The present results contradict this latter explanation because most of the diploid petites studied here had genomes identical to those of the parental petites. Indeed, they provide for the first time a precise molecular basis for the former explanation of the replicative competition.

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