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

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The 'petite colony' mutation of *Saccharomyces cerevisiae* is characterized by an irreversible loss of respiration and by an extraordinarily high spontaneous mutation rate. Crosses of wild-type cells with petite mutants exhibit a non-Mendelian segregation of the mutation, yielding either wild-type progeny only, or both wild-type and petite mutants in proportions essentially dependent on the particular petite used. In the first case, the petites entering the cross are called neutral, in the second one suppressive. While the molecular basis of the spontaneous petite mutation is now understood, 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) 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. 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. This stretch is characterized by short GC clusters, A and B, flanking a 23-nucleotide AT palindrome, p, and a short sequence, s, and by long GC clusters, C, separated from B by long AT sequence, k. We then found a common 80-base pair sequence centered on cluster B, and more recently, the whole *ori* sequence in petite genomes derived from five regions of the wild-type genome.

Screening of the mitochondrial genomes of ~400 spontaneous petites derived from wild-type strain A 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 HaeIII fragments from the parental wild-type genome; these fragments had the same sizes as HaeIII 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-1/1/R/4 and a-1/1/R/26, had an *ori* sequence lacking cluster A (ori.1 petites, having a partially deleted *ori* sequence); (3) petite a-1/5/4/1 contained two *ori* sequences in opposite orientations—the only rearranged repeat unit found so far among spontaneous petites; (4) petite a-15/4/1/10/3, derived by subcloning from the previous one, lacked an *ori* sequence altogether; another *ori* 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-ori.3 region of the B genome; these *ori* 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 of some differences in clusters A and B of *ori* 6 being probably due to sequence uncertainties and several differences in region α 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; *ori* 4 and 6 contain two additional GC clusters, β and γ, identical in both sequence and location; B 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. 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 *ori* 4/*ori* 6 does 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. 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, all base pair changes found in this region in different genomes are localized in the looped-out sequence s. Cluster C is almost identical in *ori* 4 to a GC cluster similarly located in the same region, in mammalian mitochondrial DNAs. (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-1/1/R/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 arise from wild-type strain A, except for a-3/1/5/B1, b17 and b, which derived from a region (ori 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 AaII sites are shown. Asterisks indicate restriction sites whose locations need to be confirmed. On sequences are underlined; cluster A corresponds to an AaII site, cluster B to HpaII. AaII, MboII site clusters, cluster C to a HphI site and a MboII site. All maps are centred on cluster B and oriented with clusters A, B, C, from left to right. Vertical broken lines indicate exonuclease sites where precisely known, bp, Base pairs.

Fig. 2. Primary structure of the ori sequences and their flanking regions, as determined by the method of Maxam and Gilbert on the repeat units of the mitochondrial genomes from pettes 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, 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). On sequences were also determined on the repeat units of pettes a-1/1R/Z1, a-1/1R/14 (ori 1), a-1/1R/36, a-8-1/7/12, a-8-1/7/8, 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 pettes. 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 t. 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 symbols shown. The sequence of ori 3 corrects some mistakes 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 spontaneously peteces: properties of ori sequences and suppressivity

<table>
<thead>
<tr>
<th>Petec strain</th>
<th>Repeat unit length (base pairs)</th>
<th>Primary structure</th>
<th>Ori sequence</th>
<th>% Suppressivity</th>
<th>Transmission of petec genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-1/1R/1</td>
<td>884 w</td>
<td>m</td>
<td>ori 1</td>
<td>&gt;95</td>
<td>8:4</td>
</tr>
<tr>
<td>a-1/1R/21</td>
<td>416 w</td>
<td>m</td>
<td>ori 1</td>
<td>&gt;95</td>
<td>11:0</td>
</tr>
<tr>
<td>a-1/1R/40</td>
<td>606 w</td>
<td>m</td>
<td>ori 1</td>
<td>&gt;95</td>
<td>5:0</td>
</tr>
<tr>
<td>a-1/1R/14</td>
<td>390 w</td>
<td>m</td>
<td>ori 1</td>
<td>&gt;95</td>
<td>28:4</td>
</tr>
<tr>
<td>a-1/1R/1/26</td>
<td>392 w</td>
<td>m</td>
<td>ori 1</td>
<td>&gt;95</td>
<td>36:6</td>
</tr>
<tr>
<td>b</td>
<td>875 o</td>
<td>o</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-1/7/7/2</td>
<td>4,500 o</td>
<td>o</td>
<td>ori 2</td>
<td>&gt;95</td>
<td>88:0</td>
</tr>
<tr>
<td>a-1/7/8</td>
<td>1,700 o</td>
<td>o</td>
<td>ori 3</td>
<td>&gt;95</td>
<td>88:0</td>
</tr>
<tr>
<td>a-3/1</td>
<td>4,700 o</td>
<td>o</td>
<td>ori 3</td>
<td>&gt;95</td>
<td>14:0</td>
</tr>
<tr>
<td>a-3/1/5</td>
<td>345 w</td>
<td>o</td>
<td>ori 3 C1</td>
<td>&gt;95</td>
<td>5:1</td>
</tr>
<tr>
<td>a-3/1/33</td>
<td>490 o</td>
<td>m</td>
<td>ori 3 C1</td>
<td>&gt;95</td>
<td></td>
</tr>
<tr>
<td>a-13/1/81*</td>
<td>1,350 o</td>
<td>m</td>
<td>ori 4</td>
<td>&gt;95</td>
<td>12:0</td>
</tr>
<tr>
<td>a-15/3/2</td>
<td>4,300 o</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td>3:12</td>
</tr>
<tr>
<td>a-15/3/4</td>
<td>4,800 m</td>
<td>m</td>
<td>ori 5 + 5</td>
<td>&lt;5</td>
<td></td>
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<tr>
<td>a-15/3/1</td>
<td>1,550 m</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td></td>
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<tr>
<td>a-15/3/4</td>
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<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
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<tr>
<td>a-15/3/10/1</td>
<td>1,780 m</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td></td>
</tr>
<tr>
<td>a-15/3/10/2</td>
<td>1,830 m</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td>12:0</td>
</tr>
<tr>
<td>a-15/3/10/3</td>
<td>970 m</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td>12:0</td>
</tr>
<tr>
<td>a-15/3/10/3*</td>
<td>1,170 o</td>
<td>m</td>
<td>ori 5</td>
<td>&gt;95</td>
<td>5:15</td>
</tr>
<tr>
<td>a-3/1/8/1*</td>
<td>16,500 m</td>
<td>m</td>
<td>ori 2 - 6</td>
<td>&gt;95</td>
<td></td>
</tr>
<tr>
<td>a-3/1/8/1/26</td>
<td>7,800 m</td>
<td>m</td>
<td>ori 2 - 7</td>
<td>&gt;95</td>
<td>80:0</td>
</tr>
</tbody>
</table>

w and o indicate that the primary structure is known for the whole repeat unit or for the ori sequence and the flanking sequence, respectively. All other repeat units were physically mapped. The transmission of petec genomes concerns ~200 petec petes from crosses of spontaneously petee with wild-type strain BIA (in the case of petee). Mitochondrial DNAs were mapped with restriction enzymes. The ratio presented is that of diploids having the repeat units of the parental petee to diploids having modified repeat units (see text). *Diploids petee from crosses of spontaneously petees with wild-type cells. The repeat units of a-1/1/121, a-15/4/1/133 and a-3/1/3/81 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 petee 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-1/7/7/8 (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/12 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-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-1/7/7/8 and a-a-1/7/7/12, two petees 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.

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-14-44A<sup>15</sup>). Positions indicated correspond to cluster B. Restriction sites are indicated by the symbols used in Fig. 1. 5 corresponds to the SphI 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<sup>16</sup>. 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 petee 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. It was directly inspired by the work of Mills et al. on the replication of QB 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, 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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