Conversion at Large Intergenic Regions of Mitochondrial DNA in Saccharomyces cerevisiae

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Saccharomyces cerevisiae mitochondrial DNA deletion mutants have been used to examine whether base-biased intergenic regions of the genome influence mitochondrial biogenesis. One strain (ΔS.0) lacks a 5-kilobase (kb) segment extending from the proline tRNA gene to the small rRNA gene that includes oriI, while a second strain (ΔS.7) is missing a 3.7-kb region between the genes for ATPase subunit 6 and glutamic acid tRNA that encompasses ori7 plus ori2. Growth of these strains on both fermentable and nonfermentable substrates does not differ from growth of the wild-type strain, indicating that the deletable regions of the genome do not play a direct role in the expression of mitochondrial genes. Examination of whether the 5- or 3.7-kb regions influence mitochondrial DNA transmission was undertaken by crossing strains and examining mitochondrial genotypes in zygotic colonies. In a cross between strain ΔS.5, harboring three active ori elements (ori2, ori3, and ori5), and strain ΔS.7, containing only two active ori elements (ori3 and ori5), there is a preferential recovery of the genome containing two active ori elements (37% of progeny) over that containing three active elements (20%). This unexpected result, suggesting that active ori elements do not influence transmission of respiratory-competent genomes, is interpreted to reflect a preferential conversion of the ΔS.0 genome to the wild type (41% of progeny). Supporting evidence for conversion over biased transmission is shown by preferential recovery of a nonparental genome in the progeny of a heterozygous cross in which both parental molecules can be identified by size polymorphisms.

An unresolved question concerning the structure of yeast mitochondrial DNA (mtDNA) is whether intergenic base-biased sequences play a vital role in mitochondrial biogenesis. More than half the 81-kilobase (kb) mtDNA of Saccharomyces cerevisiae has extreme bias in base composition. Long segments, up to 7 kb in one instance, are more than 90% A+T, while as many as 200 small (20 to 50 base pairs [bp]) G+C-rich clusters are interspersed in these regions (8, 10, 30). Furthermore, a complex of three G+C-rich clusters, separated by A+T stretches and extending for 270 to 300 bp, has been found in seven or eight regions, depending on the strain. These structures have been proposed as origins of replication (oriIrep, henceforth referred to as ori), and are found in mtDNA from highly suppressive petite mutants (1, 2, 13). In addition, five open reading frames (ORFs) have been found in A+T-rich regions, but as yet no functions have been reported for them (see reference 28, however).

Recent developments in manipulating the mitochondrial genome have enabled us to generate strains deleted for large segments of base-biased sequences (3, 4). This has allowed us to examine whether some of these regions influence mitochondrial biogenesis. A comparison between wild-type strains and strains lacking segments from two different regions of the mitochondrial genome (described in detail below) has not revealed detectable differences in growth rates on fermentable and nonfermentable substrates. This result suggests that these particular base-biased regions do not play a direct role in the expression of mitochondrial genes. However, a question remains as to whether specific base-biased segments influence other parameters of mitochondrial biogenesis such as replication, recombination, or transmission of mtDNA.

To examine this question, we employed strains lacking either a 5-kb segment, extending from the proline tRNA gene to the small rRNA gene and encompassing oriI, or a 3.7-kb region between genes for ATPase subunit 6 and glutamic acid tRNA and including ori2 plus ori7 (Fig. 1). With these strains, we were particularly interested to see whether the loss of an active ori region changed any of the processes mentioned above. ori elements can be divided into either active or inactive types. Active ori elements contain an intact consensus transcription initiation sequence and are found in highly suppressive petite mutants (19). Inactive ori elements, on the other hand, contain an insert in the consensus sequence that disrupts the function of this locus. On the basis of observations from a number of different strains (12, 18), we expected ori2 to be active in our strain, while ori1 and ori7 would be inactive. Hence, by analogy to highly suppressive petite mutants, the presence of ori2 in a respiratory-competent mitochondrial genome should confer on molecules containing this element an advantage over those lacking such a sequence. Crossing experiments designed to examine this proposal have shown that larger genomes (generated, we propose, by conversion) are preferentially recovered in zygotic colonies and that this effect is independent of ori sequences.

MATERIALS AND METHODS

Yeast strains. All strains used in this study are listed in Table 1 and were derived from the parental strains D13.1A and T3/3 (17). Strains with large deletions in the 81-kb mitochondrial genome of S. cerevisiae were obtained via a series of in vivo steps. The initial step involved crossing nascent spontaneous respiratory-deficient petite mutants that lack large regions of the mitochondrial genome. Recombination between the defective petite mtDNAs within the zygote can generate a novel DNA molecule containing a direct duplication (16). These zygotes have a restored respi-
and sizes of fragments generated by CfoI-PvuII digestion: ——, positions of genes; 2—, positions of the 3.7- and 5.0-kb deletions.

Abbreviations: CO1, CO2, and CO3. Subunits 1 to 3 of cytochrome oxidase: P, PuvII sites. SrRNA and LrRNA, small and large subunits of rRNA, respectively; A6 and A9. ATPase subunits 6 and 9; CYB, apocytochrome b; V, variant 1 protein. Also shown are positions of orirep sequences (O1 to O8) and tRNAs GLU and PRO.

The sequence of strain ∆5.0 designated ∆5.0/a', contains an approximately 50-bp mini-insertion in the 2.15-kb CfoI fragment adjacent to the 5.0-kb deleted region. The precise nature of this insertion is presently under investigation.

Crossing experiments (see below) involving ∆5.0 and ∆3.7 strains have generated a strain whose mtDNA lacks both the 5.0-kb fragment and the 3.7-kb fragment. A total of 8.7 kb is missing in the mtDNA of this doubly deleted strain which is designated 2Δ8.7.

Media. GYP contains 2% glucose, 0.5% yeast extract, and 1% Bacto-Peptone. GlyYP is GYP with 2% glycerol in place of glucose. GGYP is GlyYP with 0.2% glucose. Glucose minimal medium (GMM) contains 2% glucose and 0.67% yeast nitrogen base (Difco Laboratories, Detroit, Mich.). Sporulation medium contains 0.5% potassium acetate. All media were solidified with 1.5% agar. Methylene blue plates consist of GYP containing 0.03 mg of methylene blue per ml.

Preparation of haploids. AscI were digested with Zymolyase (80 μg/ml of water) for 15 min at room temperature. Spores were released by mild sonication, and haploids were identified on methylene blue plates by increased intensity of staining.

Growth rates. Growth rates of strains in GYP or GlyYP were determined by periodically measuring the A640 of cultures incubated at 30°C with shaking.

Petite frequency determination. Culture petite frequency was determined by growing strains for six generations in GlyYP before plating them on GGYP. The ratio of petite-mutant to wild-type colonies was counted after 4 days at 30°C.

mtDNA preparation. Spheroplasts, produced by Zymolyase, were broken with a French press, and mtDNA was isolated by dye-buoyant density centrifugation with bisbenzamide and CsCl (5). Restriction endonuclease digestion and electrophoretic separation of fragments on 0.8% agarose gels were carried out as previously described (17), except that potassium glutamate buffer was used for all digestions (25).

Cloning and sequencing. A 4.8-kb PuvII-CfoI fragment from wild-type mtDNA, containing the ATPase subunit 6 gene (Fig. 2), was recovered from an agarose gel with Geneclean (BIO 101 Inc., San Diego, Calif.) and digested with EcoRI. The resulting 2.5-kb EcoRI-CfoI fragment was blunt ended with Klenow DNA polymerase I and cloned into the Smal site of pTZ18 (Bio-Rad Laboratories, Richmond, Calif.) to form pSCM17 by standard techniques (23). To determine the entire sequence from the EcoRI site through ori7 without recloning, a nested set of deletions beginning at the EcoRI site and extending towards ori7 was generated by the exonuclease III method of Henikoff (21). All sequencing was undertaken by the dyeoxy-chain termination procedure (27) with [35S]dATP and Sequasene (U.S. Biochemicals, Cleveland, Ohio). The sequence of the region designated gap 12 (Fig. 2) was achieved by first cloning the 1.6-kb EcoR fragment encompassing the region. This fragment was isolated from a gel and cloned into the Smal site of pTZ18 to form pSCM30. The 0.5-kb Apal-EcoRV portion of the plasmid was eliminated by digesting it with Apal-BamHI, separating the fragments on a gel, and isolating, blunt-ending, and religating the larger fragment. The subclone generated by this procedure was designated pSCM30a. Sequencing into the gap was undertaken from the Apal site. By digesting pSCM30a with Rsal, an 0.8-kb Rsal fragment was isolated and cloned in both orientations into the Smal site of pTZ18 to generate clones pSCM30b and pSCM30c. A further portion of gap 12 was sequenced beginning at the Rsal site within the gap. The clone containing the 0.8-kb Rsal fragment in the opposite orientation was treated with exonuclease-

<table>
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<td>a arg4-16 trpl</td>
<td>∆3.7 kb</td>
<td>This study</td>
</tr>
<tr>
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<td>a adel his3-532</td>
<td>∆5.0 kb</td>
<td>This study</td>
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<tr>
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<td>a adel his3-532</td>
<td>∆5.0 kb + mini insert</td>
<td>This study</td>
</tr>
<tr>
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<td>a adel arg4-16</td>
<td>∆8.7 kb</td>
<td>This study</td>
</tr>
<tr>
<td>2Δ8.7(ii)</td>
<td>a his3-532 trpl</td>
<td>∆8.7 kb</td>
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* i and ii refer to different nuclear backgrounds used in crosses to obtain diploid prototrophs.
ase III to generate clones with progressively deleted inserts. These were used to obtain part of the gap sequence on the opposite strand.

To sequence across the novel junction of strain Δ3.7, mtDNA was digested with EcoRI-HinPI. HinPI recognizes the same restriction sites as CfoI but produces different staggered ends. The fragments were separated on an agarose gel, and the 3.3-kb EcoRI-HinPI novel junction fragment (Fig. 2) was isolated and cloned into the EcoRI-AccI sites of pTZ18 to produce pSCM15. A 2.7-kb Apal-HinPI fragment was subsequently removed from this plasmid to form pSCM15a. Sequencing across the novel junction was undertaken starting from the Apal site. To sequence the opposite strand, the 3.3-kb EcoRI-HinPI insert was reversed by using HindIII-EcoRI digestion to obtain the fragment which was blunt ended and inserted into the SalI site of pTZ18. Exonuclease III digestions were undertaken to extend the sequence of this strand beyond the segment of gap 12 remaining in mutant Δ3.7.

mtDNA fragments containing selected ori elements were isolated from agarose gels and cloned into the SalI site of pTZ18. A 1.4-kb fragment containing ori7 and a 0.6-kb fragment containing ori2 were cloned and designated pSCM48 and pSCM24, respectively. ori7 is present on pSCM17.

Investigation of ori activity in a wild-type strain. A wild-type culture, grown overnight in GlyYP, was plated on GYP. After 72 h, petite mutants were identified by their smallness and paleness. Sampled colonies were grown overnight in 1.5-ml of GYP, treated with Zymolyase (0.2 mg/ml of water) for 15 min, lysed in 0.5 M NaOH, and dotted onto a nylon membrane. The membrane was baked for 2 h at 80°C in a vacuum oven and hybridized with the ori-containing probe BB5 (24) at 58°C, as described previously (6), such that all ori sequences cross hybridized with the ori probe. Selected petite mutants were then tested for suppressiveness by crossing them to wild-type strains (15). The ori element retained in hypersuppressive (≥90%) mutants was determined with isolated mtDNA from petite mutants that was labeled and hybridized to separated CfoI-PvuII fragments of wild-type and Δ3.7 mtDNAs. This combination of enzymes enabled the identification of ori-containing mtDNA in petite mutants, because all eight ori elements were separated onto different fragments.

Recombination and transmission analyses. Recombination and transmission experiments were undertaken by growing strains overnight in GYP and then mixing 5 μl of each culture in 2 ml of fresh GYP for 16 h. Cells were then washed in GMM, grown overnight in GMM, and plated on GMM. Total cellular DNA was isolated from colonies chosen at random.

FIG. 2. Detailed maps of wild-type (top) and strain Δ3.7 (bottom) mtDNAs showing positions of cloned fragments and sequencing strategy used to determine the location of the 3.7-kb deletion (— — —). Positions of landmark genes (□) are identified as explained in the legend to Fig. 1. Key restriction sites are marked by the appropriate endonucleases. Other symbols: ⧫, position of GAP 12; □, cloned fragments; →<−, extent and direction of determined sequences; ⧫, ⧫, and ⧫, sequences in Fig. 5.

FIG. 3. CfoI digestion patterns of mtDNAs from wild-type and deletion mutants Δ3.7, Δ5.0, and Δ8.7. Arrows indicate the positions of diagnostic restriction fragments. Lane M is an EcoRI digest of bacteriophage Sppl DNA showing size markers of (from top) 7.8, 7.0, 5.9, 4.7, 3.4, 2.7, 1.9, 1.8, 1.5, 1.3, 1.1, 0.9, and 0.7 kb. WT, Wild type.
by treating 1.5 ml of overnight GYP cultures with Zymolyase as described above for mtDNA isolation. Pelleted protoplasts were lysed by the addition of a 0.5 ml solution of 50 mM EDTA (pH 8.5), 0.2% sodium dodecyl sulfate, and 1.5 μl of diethylpyrocarbonate and incubation at 70°C for 15 min. The dodecyl sulfate was precipitated by adding 50 μl of 5 M potassium acetate, incubating the suspension at 4°C for 1 h, and centrifuging it in a microfuge for 5 min. The supernatant was extracted with phenol and chloroform, and DNA was ethanol precipitated. Examination of mtDNA was performed on CfoI digests of total-cell DNA that had been electrophoretically separated in 0.8% agarose, transferred to nylon, and hybridized with 32P-labeled wild-type mtDNA. Some crosses were performed with GlyYP in place of GYP, as described above. No difference in the outcome of crosses under the two conditions was noted, and the results represent data pooled from experiments performed under both crossing conditions.

RESULTS

Characterization of deletions from mtDNA. Characteristic changes in CfoI fragments from deleted strains relative to those of the wild type are shown in Fig. 3. With mtDNA from strain Δ3.7, the 4.35- and 14.4-kb fragments are missing, while a novel 15.1-kb fragment is present. This novel fragment is generated by a 3.7-kb deletion spanning a CfoI site and containing ori7, ORF5, ori2, and flanking sequences (see below). In strain Δ5.0, a 5.5-kb fragment containing ori7 is missing, whereas a novel 0.54-kb fragment was formed (results not shown). Subsequent sequence analysis confirms that a deletion removed 5.0 kb of sequence from within the 5.5-kb CfoI fragment (see below). mtDNA from strain Δ8.7 has both sets of changes in the singly deleted strains, and its size (72.4 kb) makes it 11% smaller than the mtDNA of the wild-type strain (81.1 kb).

Sequence analysis was undertaken to define the two deleted regions and the sequences at the deletion sites. In the case of strain Δ3.7, mapping studies and a preliminary sequence analysis indicated that one of the deletion endpoints forming the novel junction is situated in a part of the wild-type mtDNA that had not been sequenced previously. This segment has been designated gap 12 (8, 10). Consequently, the sequence from gap 12 was determined (Fig. 4).

The sequence from the novel junction region of Δ3.7 and the two wild-type segments involved in the generation of this junction is shown in Fig. 5. It can be seen that a short direct repeat of 12 nucleotides is involved in deletion formation. The location of these deletion endpoints in wild-type mtDNA is shown in Fig. 2.

The sequence at the novel junction of strain Δ5.0 has been reported elsewhere (26), and the location of the deletion is shown in Fig. 1. Two similar small repeats (ACCCCTTCGGG and ACTCCTTCGGG) are involved in this deletion.

Growth of strains and stability of the mitochondrial genome. The growth rate of Δ8.7 is indistinguishable from that of the wild-type strain under glucose-repressing conditions (0.26 generations per h) and derepressing conditions (0.46 generations per h). The frequency of the petite mutation was 0.78% for strain Δ8.7 (of 29,954 colonies examined, 233

FIG. 4. Nucleotide sequence of gap 12 and flanking sequences (underlined). EMBL accession number, X15185GAP 12. The direction of the Glu tRNA gene is indicated. Arrows and dots bound GC clusters.

FIG. 5. Wild-type sequences from the upstream and downstream regions (1 and 2, respectively) involved in deletion of the 3.7-kb segment encompassing ori7 and ori2 (Fig. 2, boxed numbers). Uppercase letters show the sequence (Fig. 2, box 3) at the novel junction of strain Δ3.7. The 12-bp direct repeats are enclosed by a rectangle. Arrows bound a 60-bp segment that is missing from the mtDNA of a second yeast strain (22).
were petite mutants), compared with 0.96% for the wild-type strain (of 6,675 colonies counted, 64 were petite mutants). Recombination and transmission of mitochondrial genomes.

Mitochondrial genomes in zygotic colonies from a series of crosses were examined by hybridization of labeled wild-type mtDNA to digests of whole-cell DNA (Fig. 6), and the results of these analyses are recorded in Table 2.

In the first instance, we were interested to know whether the genome with an active ori element (ori2; see below), Δ5.0, had an advantage over the Δ3.7 molecule that lacks this element. To our surprise, the opposite result was obtained, since we found that 37% of progeny lacked ori2 (i.e., Δ3.7) and the recovery ratio of this genome to Δ5.0 mtDNA was close to 2:1. Interestingly, a greater bias was found in the recovery of reciprocal recombintant products (Table 2), of which 41% of progeny had wild-type mtDNA, whereas only 3% (five isolates) had genomes lacking both sectors of mtDNA. Note that recovery of wild-type mtDNA in zygotic colonies resulted from recombination and does not indicate the presence of a mixture of the two deleted genomes. If the latter situation arose, we would detect a substoichiometric amount of the 15.1-kb fragment, but this was not observed.

When an experiment for the opposite configuration was performed, by crossing strains with wild-type and doubly deleted mitochondrial genomes, we again observed a bias in favor of the wild-type strain (79% of progeny). In this cross, both reciprocal recombinants were recovered in such small numbers that it is unclear whether any significance should be attached to the differences in recovery of these genomes. Crosses involving the strain containing the doubly deleted genome with either of the singly deleted mtDNA strains again show a strong bias toward recovery of the larger genomes (Tables 2 and 3). These results can be explained either on the basis of conversion (analogous to gene conversion at genetic loci) of smaller to larger molecules or by the preferential replication (or transmission) of the larger molecules.

To assess the importance of conversion over preferential recovery of larger genomes, a spontaneous variant of strain Δ5.0 was used. This variant (designated Δ5.0/a⁺) was crossed with strain Δ68.7/a⁻. Progeny from this cross are shown in Fig. 7, and the overall results are given in Table 3. Once again, a strong bias toward recovery of the larger genome (Δ5.0, comprising Δ5.0/a⁺ plus Δ5.0/a⁻ = 21 + 39 = 60%) was observed. Of the Δ5.0 genomes recovered, the Δ5.0/a⁺ recombinant was seen almost twice as frequently as the parental Δ5.0/a⁻.

Investigation of ori activity in wild-type strain. Most laboratory strains of S. cerevisiae have eight ori elements, with ori2, ori3, and ori5 being active and ori1, ori4, ori6, ori7, and ori8 being inactive (18, 19). However, results from the crosses performed in this study could be interpreted to mean that ori1 is active in our wild-type strain. Hence, it was necessary to investigate the activity of ori elements in the

![FIG. 6. Autoradiogram showing hybridization of 32P-labeled wild-type (wt) mtDNA to CfoI digests of total DNA from zygotic colonies arising from a cross between strains containing the Δ3.7 and Δ5.0 mitochondrial genomes. Patterns from parental types (Δ3.7 and Δ5.0) and recombinants (Δ68.7 and wild type) are shown. Arrows identify diagnostic CfoI fragments.](http://mcb.asm.org/)

![FIG. 7. Autoradiogram showing hybridization of 32P-labeled wild-type mtDNA to CfoI digests of total DNA from zygotic colonies arising from a cross between strains containing the Δ5.0/a⁺ and Δ68.7/a⁻ mitochondrial genomes. Patterns from parental types (Δ5.0/a⁺ and Δ68.7/a⁻) and the recombinant Δ5.0/a⁻ are shown. Arrows identify diagnostic CfoI fragments.](http://mcb.asm.org/)
FIG. 8. Autoradiograms showing hybridization of 32P-labeled mtDNA from hypersuppressive petite mutants containing ori5, ori2, and ori3 to Cfo-PvuII digests of wild-type (WT) and Δ3.7 mtDNAs. An ethidium bromide-stained gel showing CfoI-PvuII-digested wild-type and Δ3.7 mtDNA is at the left. Arrows indicate fragments showing strong hybridization. Because of homology between ori elements, many fragments showed weak hybridization.

mitochondrial genome of our strain. The status of an ori element is determined both by its sequence and by the suppressiveness of petite mutants containing that ori. To assess the status of ori elements in wild-type mtDNA by suppressiveness determinations, a total of 892 spontaneous petite mutants from strain D13.1A were analyzed by dot blot hybridization to the ori probe BB5. From this analysis, we obtained 37 strains showing stronger hybridization than that of the wild-type. Crossing these strains to wild-type strain T3/3 showed that 17 of the 37 strains were more than 90% suppressive. mtDNA was isolated from the 17 strains, labeled, and hybridized to CfoI-PvuII diagnostic digests of wild-type and deletion mutant strain Δ3.7 mtDNAs (Fig. 8). Hybridization patterns revealed that seven petite mutants contained an ori5 element, five contained an ori3 element, four contained an ori2 element, and one had ori2 plus ori5 sequences. None of the suppressive petite strains tested in this experiment contained an ori1 element.

As a further test of the status of ori elements in the deletable regions of mtDNA, we isolated and sequenced the three elements. This analysis revealed that both ori1 and ori7 contain insertions in the consensus transcription initiation sequence located adjacent to G+C-rich cluster C of the ori element, whereas ori2 does not (Fig. 9). This confirms that ori2, in agreement with suppressiveness determinations, is active, while ori1 and ori7 are inactive.

DISCUSSION

In crosses of strains with mtDNAs that contain different amounts of intergenic regions, biased recovery in favor of larger genomes may be due to conversion, whereby deleted molecules are preferentially converted to the wild type. Alternatively, biased recovery may represent the preferential replication or transmission of the larger molecules. This could occur if the larger molecules contain sequence elements (like ori) that enhance their replication-transmission over those of other molecules lacking such elements. As a basis for this assessment and to characterize the deletion mutants, it is necessary to ascertain which sequences have been lost from the Δ3.7 and Δ5.0 mitochondrial genomes. The precise location of the two deleted regions has been obtained by sequence analysis. In the first place, this determination has shown that deletion endpoints occur in short direct repeats of 10 to 11 and 12 bp. In both cases, these repeats comprise part of larger homologous GC clusters belonging to the a3 category (9). This result contrasts with those of another study in which it was found that GC clusters of the a1 category are involved in the generation of different deletions (3). However, both sets of findings confirm the recombinogenic nature of GC clusters and indicate that mitochondrial recombinases involved in deletion exhibit some flexibility with regard to sequence specificity. The involvement of short repeated sequences in the formation of deletions in yeast mtDNA is well documented (11, 14, 20, 33).

Sequence analysis also reveals that ORF4 is missing in our wild-type strain and that a G+C-rich sequence at the upstream site of the 3.7-kb deletion is not present in a second yeast strain whose sequence in this region is known (22) (Fig. 5). Thus, a 60-bp G+C-rich region (Fig. 5) is replaced in the second yeast strain by the 11-mer TTTAAAAGGCAA, demonstrating the polymorphic nature of the mitochondrial genome of S. cerevisiae, particularly with regard to GC clusters.

Deletion endpoint determination has also defined sequences lacking in the Δ3.7 and Δ5.0 mtDNAs. In the case of Δ3.7, both ori7 and ori2 are missing, as are ORF5 and 16 GC clusters (numbers 103 and 105 to 119, inclusive) (10). Some of these clusters comprise part of the ori elements. In addition, the GC clusters of the sequences given in Fig. 5, which are involved in the formation of the mutant strain, have been disrupted in Δ3.7. Finally, a 41-bp GC cluster found in gap 12 is absent from Δ3.7. Deletion in the Δ5.0 strain has removed ori7 and 16 known GC clusters (numbers 58b to 68) (10). However, since sequences for only approximately 3 kb of the regions flanking ori7 have been reported, we cannot estimate the total number of GC clusters that have been lost in the Δ5.0 mtDNA.

Strain 2Δ8.7, as a composite of strains Δ3.7 and Δ5.0, lacks a minimum of 32 GC clusters as well as ori1, ori2, ori7, ORF5, and surrounding A+T-rich sequences. As the growth of 2Δ8.7 is indistinguishable from that of the wild type, we

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FIG. 9. Sequences of the consensus transcription initiation site (underlined) and flanking regions of ori2, ori1, and ori7. In ori7 and ori1, the consensus transcription initiation site is interrupted by the insertion of a G+C-rich sequence (lowercase letters). The sequence designated cluster c is common to all ori elements. Boldface type indicates base differences in these sequences relative to cognate regions of other strains.
conclude that the deleted base-biased sequences are dispensable and that mitochondrial gene expression has not been affected by these deletions. However, the stability of the mitochondrial genome has increased somewhat as a result of the deletions, since the frequency of the petite mutation of strand $2\Delta 8.7$ is 20% lower than that of the wild type. This suggests that some spontaneous petite mutations in the wild-type strain arise from excisions involving repeated sequences in the dispensable 8.7 kb.

Sequence analysis, together with characterization of mtDNA segments retained in highly suppressive petite mutants, has confirmed that mtDNA in our strain contains an active ori2 element, while ori1 and ori7 are inactive. These observations highlight the surprising nature of results from crosses between the $\Delta 3.7$ and $\Delta 5.0$ strains in terms of ori activity. Instead of supporting the concept that genomes containing more active ori elements would have a competitive advantage over those with fewer elements, we found that the $\Delta 3.7$ genome, lacking ori2, was preferentially recovered. In other words, any effect of ori elements was overridden by other factors that influenced recovery of respiratory-competent mitochondrial genomes.

One explanation for the greater recovery of $\Delta 3.7$ genomes over those of strain $\Delta 5.0$ is that the latter genomes were preferentially removed by conversion to the wild type. This conclusion is supported by the recovery of large numbers of progeny containing wild-type molecules. Moreover, the wild-type genome is found in substantially greater number than the $2\Delta 8.7$ genome in the $\Delta 5.0$ and $\Delta 3.7$ cross. An alternative explanation for this result is that wild-type and $2\Delta 8.7$ molecules are formed in equal numbers as reciprocal recombinants and that a mechanism for preferential segregation of the larger wild-type genome exists. Although our results do not exclude the possibility that sequence elements other than active ori elements are present in the 5.0-kb regions and to a lesser extent in the 3.7-kb regions that may enhance transmission, it seems unlikely that such elements would exist in these two regions and be absent from the remaining long segments of intergenic base-biased sequences that still exist in the $2\Delta 8.7$ genome.

The preferential recovery of singly deleted genomes when either strand $\Delta 3.7$ or strain $\Delta 5.0$ is crossed to the doubly deleted strain is similarly explained by the conversion of the $2\Delta 8.7$ molecule, when the singly deleted molecule is used as a template. Support for this concept comes from the results of crosses between strain $2\Delta 8.7a^-$ with either strain $\Delta 3.0$ or the variant $\Delta 5.0a^-$. A preferential recovery of the $\Delta 5.0$ molecules in the progeny of these crosses is seen in both cases. In the case of the $\Delta 5.0a^-$ strain with $2\Delta 8.7a^-$ cross, enhanced recovery is clearly not due to the preferential transmission of the parental $\Delta 5.0a^-$ molecule, since most progeny contain the recombinant $\Delta 5.0a^-$ genome. This suggests that the bias towards recovery of the $\Delta 5.0$ genome is mainly the result of the conversion of the $2\Delta 8.7a^-$ genome to $\Delta 5.0a^-$. In view of the consistent preferential recovery of larger genomes in all crosses, we favor the notion of conversion over loss of the mini-insertion from the $\Delta 5.0a^-$ genome.

Support for asymmetrical conversion from smaller to larger mitochondrial genomes comes from results of other studies of different loci (the variant 1 protein gene, the omega locus in the large rRNA gene, and the a14 intron in the cos1 gene). In crosses between strains carrying a 46-bp G+C-rich element inserted into the var1 gene and strains lacking this insert, the progeny preferentially contain the insert (29, 33). Likewise, at the omega locus, strains carrying a 1,143-bp insertion in the large rRNA gene are preferentially recovered from crosses between strains possessing and lacking this element (32). Similarly, the a14 intron is transmitted in crosses to strains lacking it (31). However, it is unlikely that our results can be explained by the mechanisms of gene conversion at the omega locus and at the a14 intron which involve the action of nucleases encoded by the insertion sequences (7, 31). It is suggested that the conversions in our examples and at the var1 locus share a common mechanism that must involve a nuclease encoded by the nucleus that is capable of recognizing a mismatched structure rather than a specific sequence in the mtDNA.

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