Novel hybrid maturases in unstable pseudorevertants of maturaseless mutants of yeast mitochondrial DNA

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ABSTRACT

Unstable pseudorevertants of mitochondrial mutants of *Saccharomyces cerevisiae* lacking the maturase function encoded by the fourth intron of the cytochrome *b* gene (bI4) were isolated. They were found to be heteroplasmic cells owing their regained ability to respire (and grow on glycerol medium) to the presence of a rearranged (rho$^-$) mtDNA that contains an in-frame fusion of the reading frames of the group I introns bI4 and intron 4α of the *coxI* gene encoding subunit I of cytochrome *c* oxidase (aI4α). The products of those gene fusions suppress the bI4 maturase deficiency still present in those heteroplasmic cells. Similar heteroplasmic pseudorevertants of a group II maturaseless mutant of the first intron of the *coxI* gene were characterized; they result from partial deletion of the *coxI* gene that fuses the reading frames of introns 1 and 2. These heteroplasms provide independent support for the existence of RNA maturases encoded by group I and group II introns. Also, since the *petite/mit*$^-$ heteroplasms arise spontaneously at very high frequencies they provide a system that can be used to obtain mutants unable to form or maintain heteroplasmic cells.

INTRODUCTION

Pseudorevertants of respiration-deficient mutants of *Saccharomyces cerevisiae* have proven useful in defining interand intragenic interactions (e.g., 1–3; reviewed in 4). Some examples of vegetatively unstable pseudorevertants of nuclear *pet*$^-$ mutations have been described (5, 6). Those nuclear mutants affect the translation or stability of a mitochondrially encoded mRNA. Suppression of that phenotype resulted from the relatively rare and spontaneous formation of a rearranged mitochondrial genome in which a chimeric form of the affected mitochondrial gene contains the 5′ untranslated portion of a different mitochondrial gene. Since the rearranged genome is necessary but not sufficient for respiratory growth, only heteroplasmic cells containing it together with wild-type mitochondrial DNA (mtDNA) can respire and grow persistently on glycerol medium. Even when grown on a non-fermentable substrate, some progeny having only one or the other genome result during each culture doubling so that the pseudorevertants are unstable for respiratory growth. More recently, rare unstable pseudorevertants of a mitochondrial tRNA gene mutation were reported (7); in that case the mutant tRNA gene is evidently amplified so that its expression at a high level suppresses the original phenotypic defect.

During our studies of a group of mitochondrial point mutations affecting the maturase encoded by the open reading frame of the fourth intron of the cytochrome *b* (*cob*) gene, we noted that such mutants yield *numerous* unstable pseudorevertants, as well as more rare stable revertants. In this paper we characterize examples of such pseudorevertants. These prove to be heteroplasmic cells capable of respiratory growth only when two differently defective mitochondrial genomes co-exist in a common cytoplasm. In this instance, the maturaseless phenotype is apparently suppressed by the expression of a hybrid intron reading frame formed by one of a number of possible crossovers between the closely related fourth introns of the *cob* and cytochrome oxidase subunit I (*coxI*) gene. A similar situation is also defined for pseudorevertants of a maturaseless mutant of the first intron of the *coxI* gene; in that case, however, two *mit*$^-$ genomes interact rather than *mit*$^-$ and *petite* genomes.

MATERIALS AND METHODS

Strains and growth conditions

Strain ID41-6/161 was the parent of all mitochondrial mutants used here. *Cob* intron 4 (bI4) mutants PZ16 and A103 and all *petite* mutants used to characterize them were reported previously (8, 9). Mutant C1036 is a *trans*-recessive mutation of *coxI* intron 1 (aI1) (10); it contains six point mutations in the latter third of the intron open reading frame (ORF) at least one of which is responsible for blocking the maturase function encoded by that intron ORF (in preparation). Strains used to characterize C1036 and intron 1/2 fusion mutations derived from it (this paper) are described in ref. 10 and 11. Cytoduction experiments were carried out using published methods (12) using strains carrying the nuclear *kar1* mutation (13). Standard media were used.

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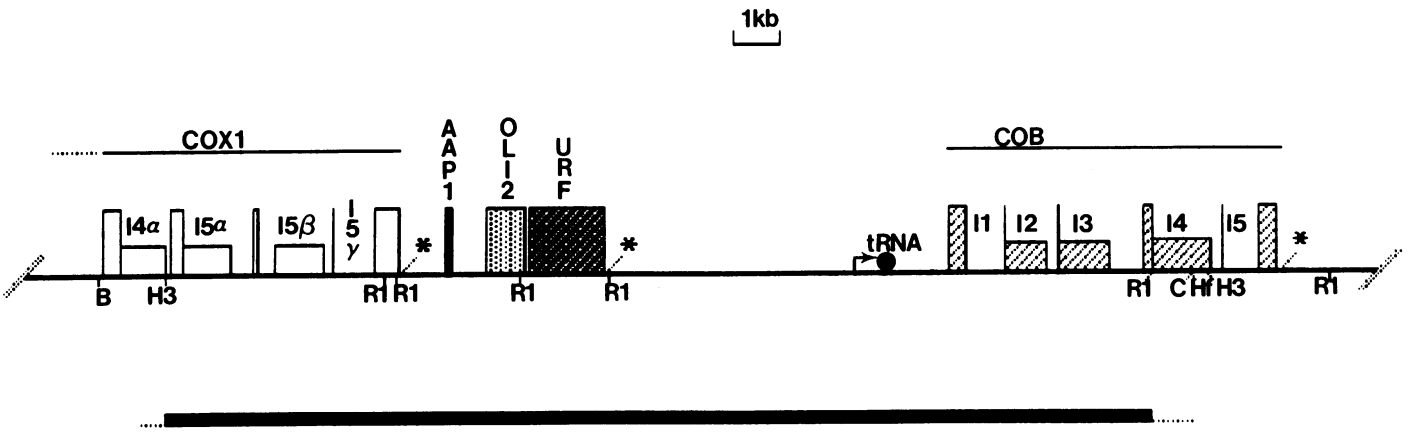

Figure 1. *Diagram of the portion of yeast mtDNA containing the coxI and cob genes.* Genes and key restriction sites within a roughly 30 kb portion of yeast mtDNA germane to this study are shown; abbreviations for restriction enzymes are B = Bam HI, C = Cla I, Hf = Hinf I, N3 = Hind III and R1 = Eco RI. The promoter of the *cob* gene is indicated by an arrow and the three dodecamer (3′ processing) sites in this region are denoted by asterisks (*). All of the *petite* genomes isolated from heteroplasmic pseudorevertants of *cob* intron 4 mutants PZ16 and A103 reported here contain the continuous region between the Hind III site of *coxI* intron 4α and the Eco RI site of *cob* exon 4 (indicated by the solid line below the map). The dotted lines indicate that additional sequences are present that differ in each case analyzed (see Figures 3 and 4).

Biochemical methods

Mitochondrial DNA was isolated from yeast cells according to ref. 14. Restriction fragment patterns of mtDNA samples were obtained on 0.7% agarose gels. Restriction enzyme digests were carried out according to technical information provided by the supplier (BRL). The fragments of mtDNAs analyzed in detail were cloned into plasmid or M13 vectors indicated in Figure legends. DNA sequencing was carried out using Sequenase reagents (United States Biochemicals) using α-^{32}P-dATP (ICN).

RESULTS

Initial observations

The *cob* intron 4 (bI4) mutation PZ16 is a typical 'box7' mutation that blocks the splicing of bI4 and *coxI* intron 4α (aI4α) (8, 9). It is a −1 frameshift that truncates the maturase reading frame so that a non-functional, roughly 14 kd, fragment of the bI4 maturase accumulates. When cells of a haploid strain containing that mutation are plated on rich glucose medium, large colonies containing no obvious revertants are evident after three days incubation. After incubation for a total of ten days, however, revertant foci are evident as papillae (bumps) on the surface of the colonies. The unexpected finding was that *each* colony becomes literally covered with bumps, each of which contains cells capable of sustained growth on glycerol medium. In contrast, when spontaneous revertants are sought from known *cis*-dominant point mutants in bI4 or elsewhere in the genome, few bumps are present after incubation for ten days and individual mutant colonies rarely have more than a single revertant sector. Since reversion frequencies are difficult to measure for mitochondrial mutants, the relative frequencies of bumps on individual colonies are a reliable, semi-quantitative, means of characterizing reversion events as rare or frequent.

A simple subcloning procedure was used to show that these high frequency revertants of mutant PZ16 are vegetatively unstable for their ability to sustain growth on rich glycerol medium. Papillae were suspended in sterile water and streaked onto glycerol medium. After four days incubation, a number of the resulting colonies were picked, suspended and plated, after appropriate dilution, on glucose and glycerol media. Surprisingly, only about 1% of the cells viable on glucose medium yield colonies on glycerol medium (6 experiments each sampling roughly 1000 cells on glucose medium and ten-times that on glycerol medium). When the same experiment was repeated using the gly$^+$ cells from the first cycle of this procedure, the same low percentage of gly$^+$ cells was observed. The subclones on glucose plates were tested for their content of cells capable of growth on glycerol by replica-plating and virtually all had lost the respiration ability present in the previous passage. These plating experiments include heteroplasms containing the *petite* genomes characterized below as well as randomly chosen newly arisen heteroplasms; no indication was obtained that the degree of instability varies from isolate to isolate.

Respiration deficient segregants were sampled from the glucose plates, subcloned again on the same medium and individual subclones analyzed genetically. Most of the segregants proved to be indistinguishable from the original PZ16 strain based on test crosses such as were used earlier to distinguish that mutation from other bI4 mutants (9). Those gly$^-$ segregants retain the ability to yield high frequency revertants. These same results were obtained even using cells that had been subcloned repeatedly on glycerol medium prior to isolation of gly$^-$ cells. Thus, these frequent pseudorevertants retain the original mutant mtDNA.

A minority of segregants proved to be *petite* mutants. Based on test crosses with *mit*$^-$ mutants scattered around the mitochondrial genome, most of the *petites* were found to retain a similar portion of the genome, including the 5′ end of the *cob* gene and the 3′ end of the *coxI* gene. Reconstruction experiments showed that these *petites* genomes are responsible for the unstable gly$^+$ phenotype. This was shown first by mating individual *petite* isolates with a derivative of PZ16 having the opposite mating type. The mated cells were streaked on glycerol medium and gly$^+$ 'recombinants' isolated. Since the PZ16 strain used here contains the *kar1* (karyogamy deficient) nuclear mutation (13), some of the resulting respiration sufficient cells were found to be haploids having the nuclear genome of the *petite* parent. Those haploids were unstable for respiratory growth as defined above. Therefore, the unstable pseudorevertants yield two different kinds of respiration deficient segregants that can be

isolated and used to reconstitute the original heteroplasm. In control experiments, in which the PZ16 strain was mated to strains lacking mtDNA or containing the PZ16 mutant genome, no gly$^+$ progeny were obtained under comparable conditions.

Unstable pseudoreversion is not a special feature of the PZ16 mutation. The same phenomenon was observed when similar experiments were carried out using another maturaseless mutant of bI4, A103. That mutant contains a −1 frameshift of the intron reading frame 280 bp downstream from the PZ16 mutant site (8; indicated in Figure 4). Colonies of mutant A103 yield numerous papillae that exhibit the same growth and subcloning behavior as do those of PZ16. *Petite* segregants from A103 papillae can be used to reconstitute the unstable gly$^+$ state when crossed back to an A103 strain. Also, *petite* segregants from A103 can unstably suppress the PZ16 mutation and *vice versa*. Unstable reversion does not depend on the configuration of introns present in the *cob* and *coxI* genes of strain ID41−6/161 (5 introns in *cob* and 7 introns in *coxI*); strain D273/A103 (constructed and characterized in ref. 15) contains only two introns in its *cob* gene and only five in its *coxI* gene plus the same frameshift mutation as in the original A103 mutant of strain ID41-6/161 and also has the same bumpy (*bmp*$^+$) phenotype. And finally, the *bmp*$^+$ phenotype is not restricted to the nuclear background of strain ID41−6/161 used here; while the exact colony morphology obtained varies somewhat among other strains carrying the PZ16 or A103 mtDNAs we have not yet encountered a strain that is naturally *bmp*$^-$.

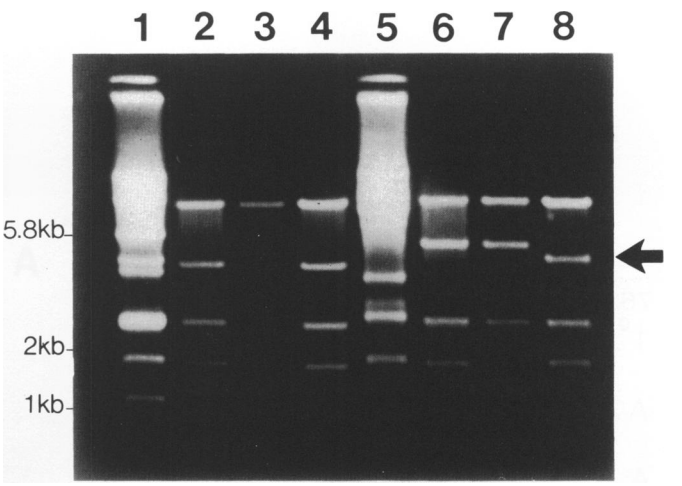

Figure 2. *Restriction fragment patterns of mtDNA from strain ID41-6/161 and petite segregants of heteroplasms.* Mitochondrial DNAs were isolated and analyzed on 0.7% agarose gels from wild-type strain ID41−6/161 (lanes 1 and 5) and *petites* A103−1 (lanes 2 and 6), PZ16-A12B10 (lanes 3 and 7) and A103−3 (lanes 4 and 8). Samples in lanes 1−4 were cleaved with Eco RI plus Hind III and those in lanes 5−8 were cleaved with Eco RI plus Cla I. The arrow points to the junction fragment of *petite* A103−3 containing a Cla I site absent from the other *petite* genomes. The small fragment cleaved by Cla I from the Eco RI junction fragment of *petite* A103−3 comigrates with a fragment in the wild-type (not shown).

Analysis of the mtDNA of *petite* segregants from heteroplasms

The mtDNAs of three independently isolated *petite* segregants of gly$^+$ heteroplasms were purified and analyzed by cleavage with various restriction endonucleases. Two were obtained from A103 papillae (*petites* A103-1 and A103-3) and the other was obtained from PZ16 (*petite* PZ16-A12B10). Figure 1 is a partial restriction site map of mtDNA from the parent strain showing the region of the genome relevant to this analysis. The three *petite* genomes yielded indistinguishable fragment patterns in a variety of digests (see Figure 2, lanes 2−4 for data using Eco RI plus Hind III). When the fragment patterns of the *petite* mutant mtDNAs are compared with comparable digests of wild-type mtDNA (lane 1) and the restriction site map of Figure 1, it is clear that each retains a single, continuous portion of the wild-type genome beginning near the Hind III site near the end of aI4α through the Eco RI site of *cob* exon 4. All of the fragments of the *petite* genomes comigrate with fragments of the wild-type mtDNA with the exception of a single 1.1 kb junction fragment. All three *petite* genomes contain the same parental fragments and the same size junction fragment.

Further restriction site mapping and DNA sequence analysis (see below) of the junction fragment revealed that it contains 5′ sequences of bI4 joined to 3′ sequences of aI4α. As shown in Figure 2, lanes 6−8, one of those *petites* contains the Cla I site of bI4 (see Figures 1 and 4) while the other two lack that site. In total, three *petite* segregants from independently isolated pseudorevertants of PZ16 were analyzed in this way and all lacked the Cla I site. Nine other *petites* from A103 papillae were also analyzed; five contained the Cla I site and the others lacked it. Among the *petites* containing the Cla I site, some also retain the nearby Hinf I site of bI4 (see Figures 1 and 4) while others (such as A103-3) lack it. All of the *petites* analyzed retain the Hpa II site of bI4. Thus, in each case, the Eco RI-Hind III junction fragment contains *cob* sequences from the Eco RI site in exon 4 through the Hpa II site of the intron and terminates within *coxI* intron 4α sequences. Furthermore, the data shown illustrate that while all of the genomes are the same length, the precise boundaries of the retained sequences can vary somewhat.

The Eco RI-Hind III junction fragment from each of the three *petites* shown in Figure 2 was cloned and sequenced. Figure 3 shows the sequence of the junction between *cob* and *coxI* sequences that, in each case, confirms the inferences from the restriction site mapping experiments. Each sequence begins with nucleotides unambiguously identified as bI4 and ends in known aI4α sequence. At the boundary between each unique sequence is a short sequence that is present in both introns and each boundary is different.

The fourth introns of the *cob* and *coxI* genes contain an extended region of very high sequence homology (reported first for strain D273-10B in ref. 11 and confirmed with minor strain-specific differences in ref. 8 and 16 for strain ID41−6/161, used here). As shown schematically in Figure 4, that region of the two introns contains thirteen strings of 11 to 28 bp of identical sequence at which crossovers yielding *cob-coxI* gene fusions could occur. The junction in *petite* A103-1 occurs at the third site; that in *petite* PZ16-A12B10 at the sixth; and that in *petite* A103-3 at the eighth. As summarized in Figure 7, each of these sites is different and there is no obvious sequence homology among them; thus, it appears that the recombination events responsible for these *petite* deletions are not site-specific, nor are they limited to sites having a particular base composition. The hybrid intron formed in each case contains an intron reading frame that lacks the *mit*$^-$ mutation of the parent strain.

Heteroplasms containing two *mit*$^-$ genomes

The only other pair of related introns in yeast mtDNA are the group II introns, *coxI* I1 and I2 (aI1 and aI2). Both encode maturase functions (10, 17) and contain long colinear reading frames having roughly 50% base sequence homology (11).

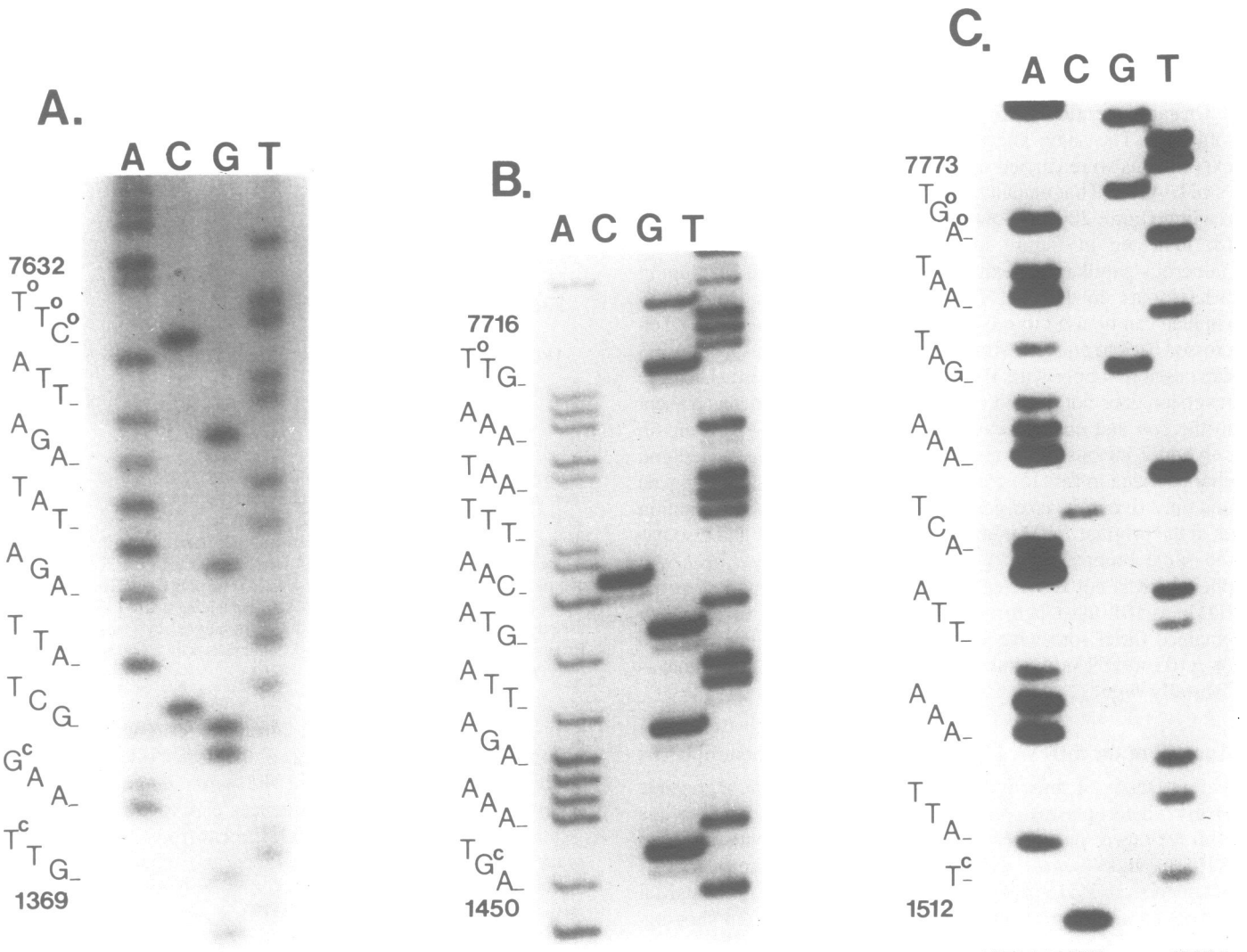

Figure 3. *DNA sequence of bI4-aI4α* fusion sites. The Eco RI Hind III junction fragment of each *petite* genome shown in Figure 2 (diagrammed in Figure 4) was cloned and sequenced. The portion of each sequence containing the actual site of fusion between the reading frames of aI4α and bI4 is shown. Numbers alongside of each figure denote nucleotide positions of the original published sequence (11). 1368, 1450 and 1512 are positions in *cob* intron 4 of the first nucleotide listed at the bottom of each panel. Where the sequence shown differs from that cited, it is due to known sequence difference between strains D273-10B and ID41-6/161, the parent of these *petite* mutants (8). The numbers assigned to the top nucleotides in each panel, 7634, 7718 and 7795, are positions in the *coxI* gene sequence (11). Nucleotides lacking any superscript are identical in both introns; those with a superscript 'c' are unique to the *cob* intron; while those with a superscript 'o' are unique to the *coxI* intron. Panel A: *petite* A103-1. Panel B: *petite* PZ16-A12B10. Panel C: *petite* A103-3.

Mutant C1036 is a maturaseless mutant of aI1 that contains six single base changes in the last third of the intron reading frame (indicated in Figure 5). It yields few papillae on glucose plates but some of them proved to be unstable.

Unstable gly$^+$ cells were grown on glycerol medium and then allowed to segregate on glucose medium. Two different types of respiration deficient segregants are discerned using appropriate test crosses (see Materials and Methods). Roughly half are genetically indistinguishable from the original mutant strain; the remainder are *mit*$^-$ mutants having a different mutation in the 5′ region of the *coxI* gene. Those were shown to be capable of suppressing the C1036 mutation in reconstruction experiments analogous to those described above for *mit*$^-$/*petite* heteroplasms. Two of those reconstructed heteroplasms (involving the mtDNA of strains 5B and AD1, see below) were analyzed by subcloning; again, roughly 1% of cells in glycerol colonies retain the ability to yield a colony when plated on glycerol medium.

Five *mit*$^-$ strains that suppress the C1036 mutation were isolated and characterized. Each contains a shortened form of the *coxI* gene, lacking about 2,500 bp. Restriction site mapping showed each genome to contain a fusion of the upstream portion of aI1 to the downstream portion of aI2 (shown schematically in Figure 5). At least two different types of events were detected; in one, the Eco RI site of aI2 is retained while in the other it is absent. In both, the Cla I site of aI1 and the Hha I sites at the 5′ boundary of aI2 are missing.

Mutants 5B and AD1, representing each type of deletion, were characterized at the DNA sequence level (Figure 6). Each deletion appears to have resulted from a crossover between short conserved sequences present in each intron. In both cases the hybrid intron contains an intact reading frame lacking all of the

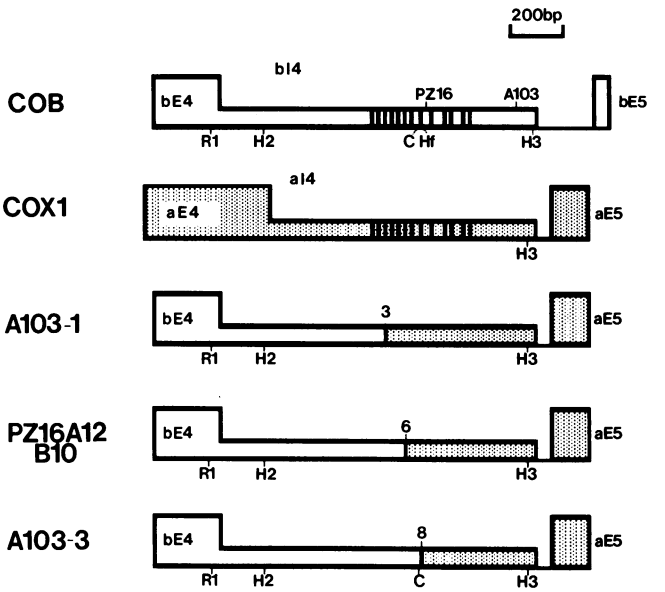

Figure 4. *Diagram of bI4-aI4α* reading frame fusions. The first two lines depict bI4 and aI4α plus flanking exon sequences. Restriction sites and the locations of bI4 point mutations relevant to the analysis are noted; all abbreviations are as in Figure 1. Vertical bars within the aI4α and bI4 reading frames indicate the locations of 13 strings of sequence identity 11−27 bp long in the region of the introns having the highest homology. *CoxI* sequences are shaded. The last three lines depict the structure of the fused intron in each of the *petites* analyzed. The number at each fusion site define the sequence identity block at which that event occurred.

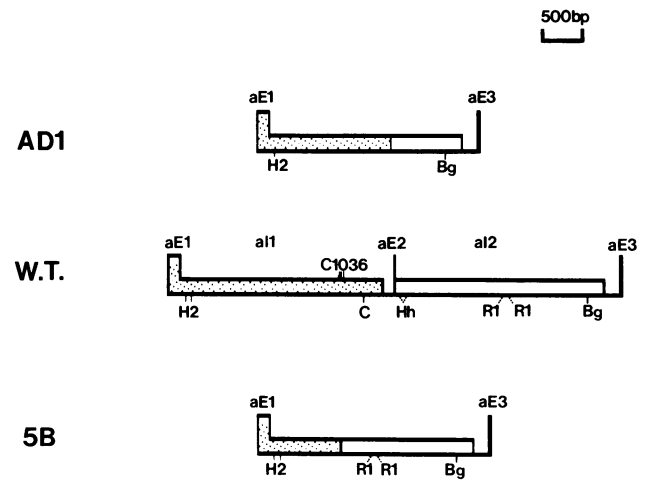

Figure 5. *Diagram of aI1-aI2 fusions.* The line labeled w.t. (wild-type) is a diagram of the first 5100 bp of the *coxI* gene (c.f., 11). The locations of the six base changes in mutant C1036 are indicated; the changes are T to A at nt 1815, G to A at nt 1898, C to T at nt 1907, C to T at nt 1936, T to A at nt 2128 (forming a stop codon) and C to A at nt 2164. Restriction sites relevant to the analysis are indicated; Hh = Hha I; BG = Bgl II; H2 = Hinc II; other sites as in Figure 1. The structures of the intron fusion in strains AD1 and 5B (derived spontaneously from mutant C1036) are indicated above and below the wild-type diagram, respectively (see Figure 6 for the sequence of each junction).

base changes of the C1036 mutation (see Figure 5); thus, it follows that no stable recombinants are obtained in crosses between C1036 and either of the deletion strains. Each hybrid reading frame can encode a protein related to both the aI1 and

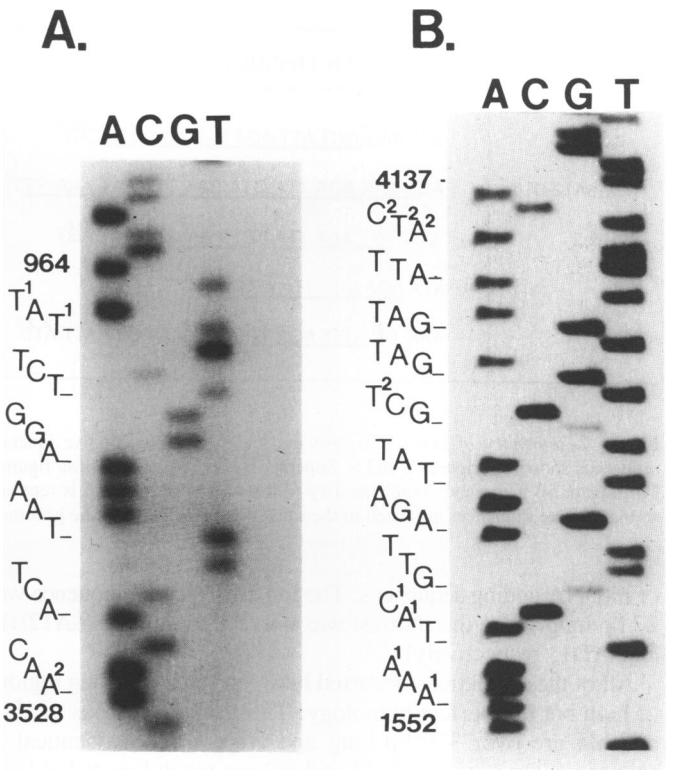

Figure 6. *DNA sequence of junctions between aI1 and aI2.* Panel A: Junction sequence of mutant 5B. The Eco RI to Hinc II fragment containing the junction of mutant 5B was cloned in M13mp18 and sequenced using a commercial oligonucleotide primer. The sequence shown begins at position 3536 of aI2, includes a 14 bp sequence present in both introns, and ends at position 964 of aI1 (11). As in Figure 3, superscripts denote nucleotides unique to one intron or the other while nucleotides lacking superscripts are identical in both. The strand shown is the complement of the published sequence. Panel B: Junction sequence of mutant AD1. The Hae III fragment containing the junction of mutant AD1 was cloned and sequenced as in Panel A. The sequence shown begins at position 1555 of aI1, includes an 11 bp sequence present in both introns, and ends at position 4139 of aI2. The strand shown is the same as the published one.

aI2 maturases and, clearly, each hybrid intron ORF retains aI1 maturase function.

DISCUSSION

This paper describes unstable pseudorevertants in which a mitochondrial mutation of one genome is suppressed by the presence of a second, defective, mitochondrial genome. Heteroplasmic pseudorevertants containing the original *mit*$^-$ genome plus either a *petite* or a different *mit*$^-$ genome were characterized. In both types, the suppressing genome is derived from the original mutant genome and apparently arose as an intramolecular deletion. Each deletion results in a fusion which lacks the wild-type allele of the original mitochondrial mutant. As such, stable recombinants are not obtained even after prolonged maintenance of the heteroplasm.

Each deletion evidently resulted from a crossover between short, tandemly repeated sequences present in the original mitochondrial genome. Five different events are reported here and the sequences involved are summarized in Figure 7. Since each sequence is different, it is clear that these recombination events are not 'site-specific'. Also, while G+C-rich sequences that punctuate yeast mtDNA are often sites of deletion events, these sites average 79% A+T, a base composition quite typical

MUTANT	JUNCTION FRAGMENT
A103-1	GTT^{c}AAG^{c}GCT ATT AGA TAT AGA TTA CCT^{o}
PZ16A12-B10	AG^{c}T AAA AGA TTA GTA CAA TTT AAT AAA GTT^{o}
A103-3	CCT^{c}ATT AAA TTA ACT AAA GAT AAT A^{o}G^{o}T
5B	A^{1}TA^{1} AGA CCT TTA AGT GTT^{2}
AD1	A^{1}AA^{1} TA^{1}C^{1} GTT AGA TAT GCT^{2}GAT GAT ATT A^{2}T^{2}C^{2}

Figure 7. *Summary of junction sequences.* The figure lists all five junction sequences shown in Figures 3 and 6. Superscripts are as defined those figures. For mutant 5B the strand complementary to that shown in Figure 6 is reported so that all five sequences are listed in the same polarity relative to the genome.

of mtDNA coding sequences. The longest repeated sequence was 27 bp long while the shortest was only 11 bp (in PZ16-A12B10 and AD1, respectively).

All of these deletions occurred between relatively long regions of high but not perfect homology. The relevant regions of aI4α and bI4 are over 370 bp long and are over 85% identical in sequence. The regions of aI1 and aI2 are much longer but have only about 50% homology with relatively rare strings longer than 11 bp. These factors may explain why heteroplasms of PZ16 are much more frequent than are those of C1036. However, it is also possible that repeated sequences farther apart than those in aI1 and aI2 may be better sites for these events; alternatively, this kind of recombination may be disfavored in the *coxI* region of mtDNA for some reason.

The *petite* genomes derived from bI4 maturaseless mutants suppress both aspects of the original mutant phenotype, namely, the inability to splice bI4 and aI4α. *Petite* mutants are known to be transcriptionally active and, at least for the case of the 14S and 21S rRNA genes, are known to utilize the same promoters as in wild-type (18, 19). In *petites* retaining other genes (e.g., *var1* which has been studied in particular detail (20) transcripts having the same processed 5′ and 3′ ends as are seen in wild-type mitochondria accumulate even when the natural promoter is deleted from the *petite* genome. In the present case, each *petite* contains a hybrid gene flanked by the *cob* gene promoter and the 3′ processing site of the *coxI* gene. Some intron splicing is required for translation of the hybrid intron ORF since the transcript using the *cob* gene promoter contains three introns before the hybrid one, two of which depend on maturases for their splicing. In the heteroplasmic state, trans-acting splicing factors provided by the *mit*$^{-}$ genome probably splice those introns in the transcript of the *petite* genome to yield an mRNA encoding the hybrid maturase.

Evidence defining maturases as *trans*-acting splicing factors in yeast mitochondria has been accumulating since 1980 (21, reviewed in ref. 4). Results of transient complementation experiments in zygotes play a key role in distinguishing maturaseless intron mutants from those that alter *cis*-acting splicing signals within introns (8, 17, 21–23). Some reservations may remain concerning that assay since recombination between the mutant genomes used could provide some of the activity detected. Here, however, the two interacting genomes do not yield wild-type recombinants and continued growth requires co-existence of the two mutant genomes. Thus, our studies of these heteroplasms provide independent demonstration that a group I and a group II intron encode a *trans*-acting splicing factor.

The wild-type bI4 maturase acts at two different, but related, introns (8, 15, 24). There is ample evidence that the probable translation product of the aI4α ORF is normally inactive as a maturase (25, 26). Here we report three different hybrid intron ORFs, each of which promotes the splicing of both target introns. It is not known whether the hybrid intron is capable of splicing in heteroplasmic cells. If it does, then the hybrid maturase protein would be expressed at a low level and would have to be a rather effective accessory to splicing. Alternatively, if the hybrid intron does not splice, then the hybrid protein would be expressed at high levels so that even a weakly active protein would permit heteroplasmic cells to grow. We have recently reported that the aI4α-encoded protein is a potent site-specific DNA endonuclease involved in intron mobility (gene conversion) (16). We are currently evaluating several strategies for testing whether proteins encoded by those hybrid intron ORFs have endonuclease activity.

The second group of unstable pseudorevertants, heteroplasmic cells containing two different defective *coxI* genes, have similar implications for studying maturase function. It should be noted that the maturases of group II introns are substantially different from those of group I introns. In particular, they lack the conserved LAGLI-DADG peptides typical of the latter (27) and contain a clear homology to reverse transcriptase (28) plus two zinc finger motifs (29), both absent from the former.

The high incidence of papillae on colonies of PZ16 and A103 apparentally depends on intramolecular recombination events that create gene fusions that provide an alternate source of maturase protein. The sustained respiratory growth of such heteroplasms depends on the relative replication and segregation rates of the two interacting genomes. Thus, mutations that modify the size and/or number of papillae may prove useful in studying mitochondrial recombination, replication and/or segregation events. Isolation and initial studies of mutants of nuclear genes that block unstable pseudoreversion of mutant PZ16 ('bump^{-} mutants') will be presented elsewhere.

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