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# A 5 kb intergenic region containing *ori1* in the mitochondrial DNA of *Saccharomyces cerevisiae* is dispensable for expression of the respiratory phenotype

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A mutant mitochondrial DNA of *Saccharomyces cerevisiae* strain R 0.54 has been shown to have a 5 kb deletion which removes the *ori1* sequence and its surrounding intergenic sequences between the pro tRNA and 15 S rRNA genes. The deleted DNA has been sequenced and is found to be flanked by two 11 bp direct repeats. The deletion mutant displays a normal phenotype in non-fermentable media. This implies that the intergenic sequence is not required for expression of the respiratory phenotype.

Mitochondrial DNA; Intergenic sequence; Recombination; (Yeast)

## 1. INTRODUCTION

The mitochondrial DNA (mtDNA) of *Saccharomyces cerevisiae* is characterized by coding regions interspersed with intergenic regions. The intergenic regions consist of long AT spacers in which various GC clusters are embedded [1]. Eight highly conserved *ori* sequences are found in the intergenic regions. The *ori* sequences consist of three GC rich clusters and four AT rich spacers and can be found with slight variations in all wild type (*wt*) strains [2,3]. One such *ori* sequence, *ori1* is located between the pro tRNA and 15 S rRNA genes [4].

A great diversity can be observed between mtDNA intergenic regions of various yeast species. For instance in the yeast *Torulopsis glabrata* the intergenic regions consist of a dozen simple short AT spacers [5].

The possible biological roles of yeast mtDNA intergenic sequences are (i) that they are 'selfish DNA' or (ii) that they participate together with coding parts in expression of the respiratory phenotype and stability of mtDNA [6]. In this paper I report the results of a study of a 5 kb long deletion of the intergenic region located between the pro tRNA and 15 S rRNA genes.

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## 2. MATERIALS AND METHODS

### 2.1. *Yeast strains*

Strain R 0.54 (*a, rho*$^+$, *ade1, his*-3-532) was from a collection of stable *rho*$^+$ revertants isolated from the high frequency petite (*hfp*) strain 13.10s (see section 3).

A restriction map of 13.10s and its parent, the *wt* strain D13.1A (*α, rho*$^+$, *his*-3-532, *trp1*) can be found in [7,8].

### 2.2. *Growth rate of the strains*

The growth rates of the *wt* strain D13.1A and R 0.54 were measured by absorbance (650 nm) in a glycerol medium containing 2% glycerol, 0.1% yeast extract and 1% bactopeptone.

### 2.3. *Cloning and sequencing*

Mitochondrial DNA of the R 0.54 strain was isolated according to [8] and digested with restriction enzyme *Hin*PI which has the same recognition site as *Hha*I. The 0.54 kb *Hin*PI fragment containing the deletion was electroeluted from a 0.6% agarose gel (ultrapure agarose from IBI). After chloroform-phenol extraction and ethanol precipitation it was cloned into the *Acc*I site of the pUC13 plasmid. The insertion fragment was subcloned in the *Bam*HI/*Hin*dIII site of the M13 phage (mp18

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and mp19) and sequenced from both ends by the method in [9], as described in [10]. The sequencing overlap is between the 134 bp site and the 405 bp site (fig.2B).

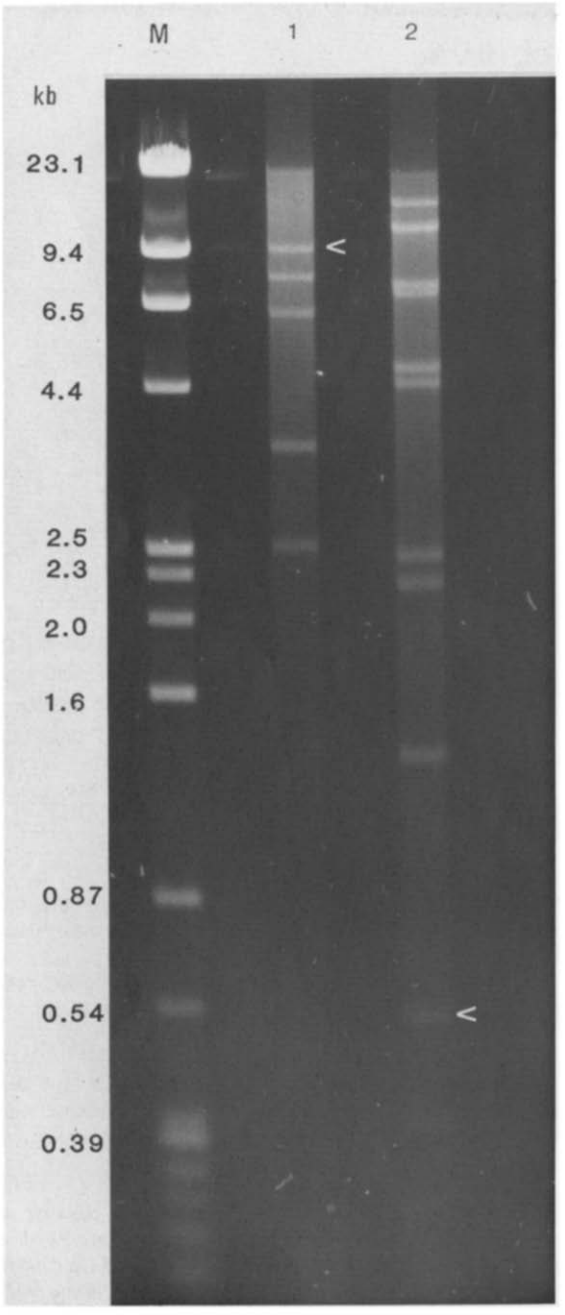


Fig.1. Electrophoresis in 1% agarose gel of mtDNA from R 0.54 digested with *HpaI* (1) and *Cfo* (2). The novel junction fragment is arrowed. Marker fragments (in kb) are in the lane labeled M.

## 3. RESULTS AND DISCUSSION

High frequency petite (*hfp*) producing mutants of *Saccharmyces cerevisiae* with sectored colony morphology are characterized by a directly oriented duplication in the mitochondrial genome [7]. *hfp* mtDNA genomes can (spontaneously or by induction with ethidium-bromide) generate deletions of duplicated regions that result in stable revertant strains with smooth unsectored colony morphology. Revertant mtDNAs can have the normal gene order and are of a smaller size than *wt* mtDNA [5]. The strain was obtained from a collection of revertant strains kindly provided by Dr G.D. Clark-Walker.

The restriction pattern of *HhaI* and *HpaI* digest of D13.1A and R 0.54 mtDNAs are shown in fig.1 and table 1. In R 0.54 mtDNA the 14.05 kb *HpaI* fragment of *wt* mtDNA is only 9.05 kb due to the deletion of a 5 kb sequence. Digestion with *HhaI* localised the deletion entirely within a *wt* 5.5 kb *HhaI* fragment which is 0.54 kb in R 0.54. The *wt* 5.5 kb *HhaI* fragment contains the pro tRNA and 15 S rRNA genes and ori1 (not shown). There are no other detectable differences between the D13.1A and R 0.54 mtDNAs, indicating the R 0.54 mtDNA has a normal gene order.

The 0.54 kb *HhaI* fragment of R 0.54 mtDNA is represented in fig.2A and its sequence is shown in

Table 1

Sizes (in kb) of *HhaI* and *HpaI* fragments from mtDNA of the D13.1A and R 0.54 strains

| *HhaI* | | *HpaI* | |
|---|---|---|---|
| D13.1A | R 0.54 | D13.1A | R 0.54 |
| 21.30 | 21.30 | 25.15 | 25.15 |
| 14.40 | 14.40 | 22.50 | 22.50 |
| 10.85 | 10.85 | 14.0 | 9.05* |
| 7.05 | 7.05 | 7.40 | 7.40 |
| 6.70 | 6.70 | 6.00 | 6.00 |
| 5.50 | – | 3.50 | 3.50 |
| 4.65 | 4.65 | 2.40 | 2.40 |
| 4.35 | 4.35 | | |
| 2.35 | 2.35 | | |
| 2.15 | 2.15 | | |
| 1.30 | 1.30 | | |
| – | 0.54* | | |
| 0.40 | 0.40 | | |

A fragment with a deletion is indicated by a symbol (*)

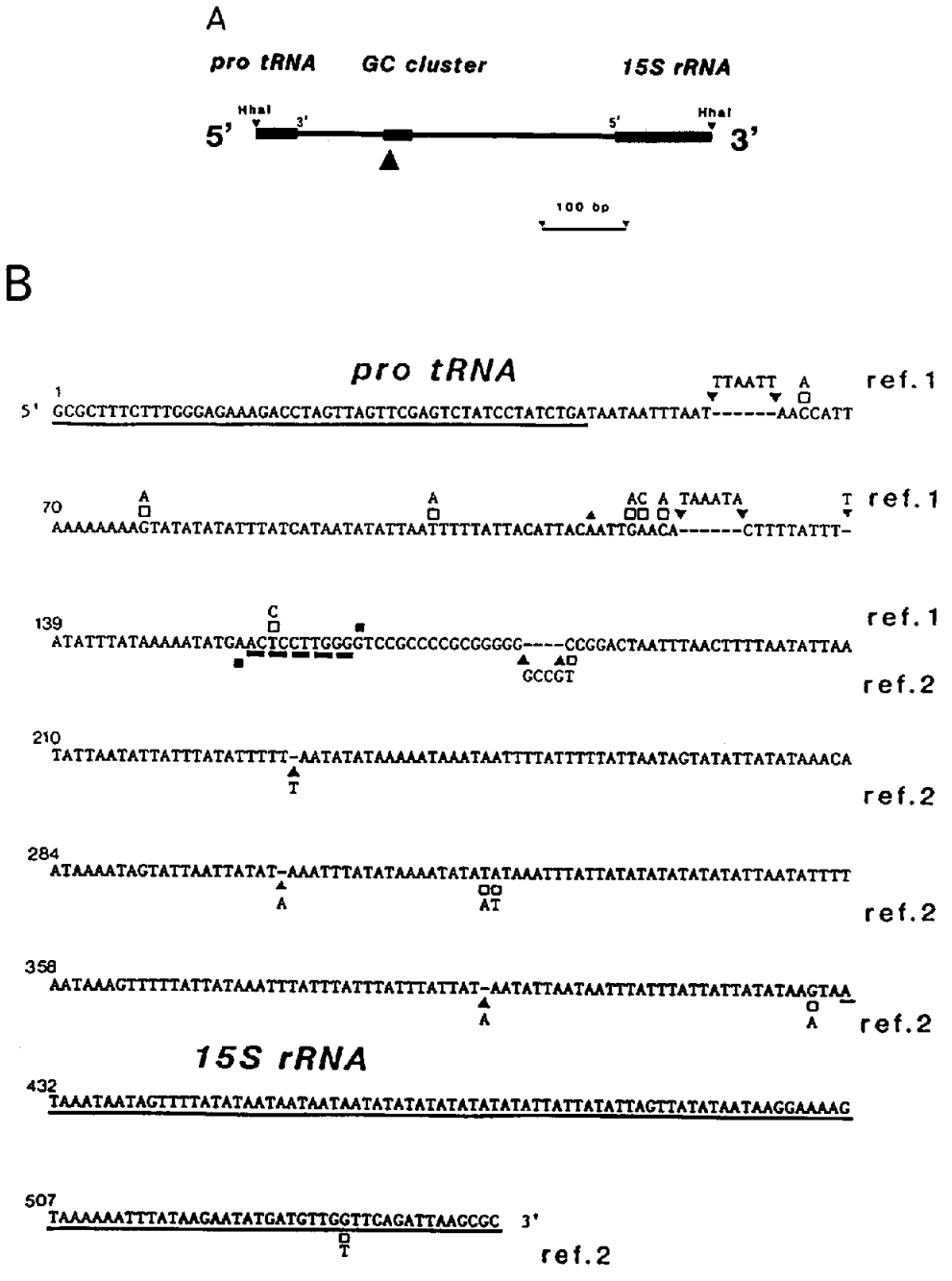


Fig.2. Diagram and sequence of the 0.54 kb *HhaI* fragment from the R 0.54 strain. (A) Diagram. Fragments of two genes extending into the fragment and a GC cluster are indicated by thick bars. The location of the novel junction sequence which is the result of deletion is indicated by a black triangle. (B) Sequence. The changes relative to a previously published sequence around the pro tRNA gene that extends 179 bp from the 5'-end (ref.1 sequence from [11]) are indicated above the R 0.54 sequence. The changes in the region adjacent to the 3'-end of the fragment from *wt* mtDNA (ref.2 sequence from [12]) are indicated below the sequence. Base substitutions are indicated by (□). Deletions are indicated by (▲) and insertions by (▼). The coding regions of both genes extending into the sequence are underlined. A direct repeat of 11 bp which is present in all three sequences and is a possible site of recombination events is underlined by a broken line. (■) A lack of homology between the sequence and each of the referential sequences (ref.1 and ref.2) as soon as it extends beyond the repeat.

fig.2B. Coding regions of the pro tRNA and 15 S rRNA genes extend into the fragment at both ends. The published sequence extends 179 bp from the 5′-end [11] and 537 bp from the 3′-end [12]. Comparison of the R 0.54 sequence with these sequences indicates that the ends of the R 0.54 deletion are approx. 160 bp and 390 bp from the left and right ends of the *Hha*I fragment, respectively, as shown in fig.2B. At these sites the known sequences from *wt* mtDNA match with each other over an 11 bp long sequence (5′-AC$^{C}_{T}$CCTTC-GGG-3′). This short sequence was presumably involved in the processes which resulted in the loss of the duplication from 13.10s mtDNA when R 0.54 mtDNA was generated. Fig.3 shows how site-specific recombination involving the short direct repeats could have caused this.

The only marked differences from the previously reported sequences (fig.2B) are a 6 bp deletion located 62 bp downstream from the 5′-end, another 6 bp deletion located 129 bp downstream from the 5′-end and a 4 bp deletion located 366 bp upstream from the 3′-end of our sequence. As the sequences derive from different *wt* strains, the observed differences could all be natural polymorphisms. Similar differences were observed between the sequences reported for the 3′-end of the 5.5 kb *Hha*I fragment [12,13].

As a result of the deletion, two genes (pro tRNA and 15 S rRNA) separated in *wt* mtDNA by an approx. 5.3 kb intergenic sequence [7] are separated in R 0.54 mtDNA by only a 0.38 kb long AT rich DNA sequence containing one GC cluster (fig.2A). Apart from the previously mentioned sequenced ends, only a short sequence containing *ori1* from the middle of the *wt* 5.5 kb *Hha*I fragment has been reported [1,4].

To ascertain whether the 5 kb region deleted from R 0.54 mtDNA contains a sequence essential for growth on non-fermentable substrates, growth rates were determined. The growth rate in glycerol medium of the mutant R 0.54 was 0.23 generations per hour compared with 0.22 generations per hour for its *wt* parent D13.1A. These results indicate that the deletion in R 0.54, which removes much of the region between pro tRNA and 15 S rRNA together with *ori1*, does not affect the respiratory phenotype.

The function of intergenic sequences in the replication and stability of the yeast mitochondrial genome is still speculative. However these results show that revertants of *hfp* strains can be useful tools in further experiments concerning the role of intergenic sequences.

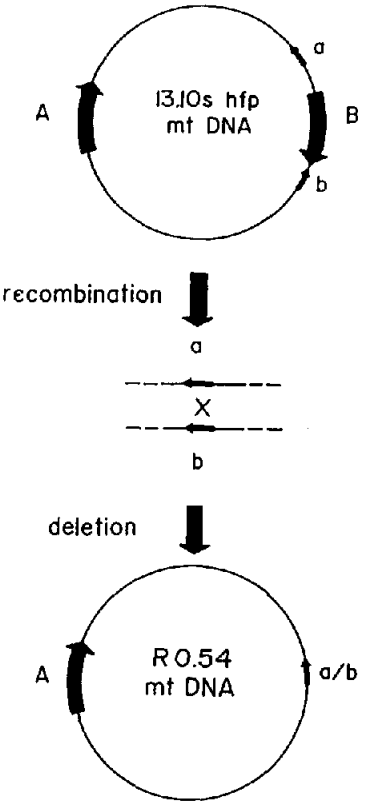


Fig.3. The 13.10s mitochondrial genome, from which R 0.54 is derived, contains a like-oriented duplication (A and B). The duplication B which undergoes deletion is surrounded with the two (a and b) direct repeats (5′-AC$^{C}_{T}$CCTTCGGG-3′). The excision-deletion intramolecular process leads to the generation of R 0.54 mtDNA. The duplication B has been deleted together with some single copy regions adjacent to the duplication. Only one direct repeat (a/b) has remained in the novel mtDNA.

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