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Additional copies of the mitochondrial Ef-Tu and aspartyl-tRNA synthetase genes can compensate for a mutation affecting the maturation of the mitochondrial tRNA^{Asp}

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Abstract In an attempt to identify new nuclear genes involved in the synthesis and processing of mitochondrial tRNAs, we utilized a multicopy nuclear library to suppress the heat-sensitive phenotype of a *Saccharomyces cerevisiae* mitochondrial mutant strain. This strain (Ts 932) is defective in the 3′-end processing of the mitochondrial tRNA^{Asp} transcript. The nuclear genes coding for the mitochondrial elongation factor Tuf M and for the mitochondrial aspartyl-tRNA synthetase have been found to restore the temperature-resistant phenotype and to correct the RNA processing defect. Suppression was effective even when the genes were present on a centromeric plasmid.

Key words Mitochondrial tRNA · RNA processing · Tuf M · AspRS M

Introduction

The mitochondrial genome of yeast carries one copy of each necessary tRNA gene and mitochondrial tRNA biogenesis requires the presence of several nuclearly encoded factors and of one mitochondrial gene product. These facts open the way to a genetic analysis of tRNAs and tRNA-interacting components. We have taken advantage of this to study a mutation of tRNA^{Asp} which is defective in 3′-end maturation (Zennaro et al. 1989). It is known that mitochondrial tRNAs are released from primary transcripts by processing at the 5′ and 3′ ends. 5′-end processing requires mitochondrial RNAse P (Dang and Martin 1993) composed of a nuclear protein and a mitochondrial RNA component, while 3′-end processing is catalyzed by an endonuclease which has been only partially purified (Chen and Martin 1988). The *Saccharomyces cerevisiae* Ts 932 mutant exhibits defective growth on glycerol at 36°C which can be ascribed to a C to T transition at position 61 of the tRNA^{Asp} gene, resulting in defective processing of the 3′ end of this tRNA at the non-permissive temperature. In an attempt to better understand the molecular mechanism of the deficiency, we searched for multicopy suppressors which could (at least partially) restore normal growth. The genes which encode the mitochondrial elongation factor Ef-Tu and the cognate tRNA synthetase have been found to restore the temperature-resistant phenotype and to correct the RNA-processing defect.

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Materials and methods

YPG (1% bactopeptone, 1% yeast extract and 2% glucose), YPGLY (1% bactopeptone, 1% yeast extract and 2% glycerol), YPGAL (1% bactopeptone, 1% yeast extract and 2% galactose), were used as rich culture media for yeast. WO (0.17% yeast nitrogen base, 0.5% ammonium sulphate and 2% glucose) was utilized as a minimal medium. All media were supplemented with 2.3% bacto agar (Difco) for solid media, and WO was supplemented with the appropriate nutritional requirements according with the phenotype of the yeast strains. As bacterial culture media we used LBA (0.5% yeast extract, 1% bacto-tryptone, 0.5% sodium chloride and 100 μg/ml of ampicillin) or LBAC, which is LBA medium with 10 μg/ml of chloramphenicol. The yeast strain used to perform the experiments described in this paper is Ts 932/2.12 (*Mat* **a**, *his3*, *ade2*, *leu2*, *ura3*) which possesses a heat-sensitive phenotype at 36°C on glycerol medium. The wild-type nuclear library was constructed in the YEp13 multicopy plasmid. Transformation was obtained by the lithium-chloride procedure of Ito et al. (1983). Mitochondrial RNAs were prepared as described in Baldacci and Zennaro (1982) from wild-type and mutant cells grown on YPGAL medium at 36°C. The transformed strain was grown on YPGLY medium at 36°C. Electrophoresis of mitochondrial RNAs was performed in a 1.5% agarose/6 M urea (par-

tially denaturing) gel as described in Locker (1979). About 10 μg of RNA were loaded in each slot. Mini-Mu analysis was performed as described in Castilho et al. (1984).

Results and discussion

Strain Ts 932/2.12, carrying the Ts 932 mitochondrial point mutation, was transformed with a wild-type nuclear library constructed in Yep13. Five transformants (out of 1500) grew on glycerol at 36°C, the growth phenotype being associated with the presence of plasmids which were shown by preliminary restriction analysis to belong to three different classes.

We used mini-Mu mutagenesis to localize rapidly the sequences responsible for suppression (Daignan-Fournier and Bolotin-Fukuhara 1988). When a modified mini-Mu transposon is inserted into these sequences, the suppressor gene is no longer functional and the strain that carries such a modified plasmid cannot grow on glycerol at 36°C. Moreover, through the use of oligonucleotides complementary to the mini-Mu extremities, this procedure allows one to rapidly obtain the sequences adjacent to the point of insertion. In this way we identified the multicopy suppressor gene present in pYE70 as the gene coding for the mitochondrial aspartyl-tRNA synthetase (GenEMBL accession number M24418) and the suppressor gene present in plasmids pYE66, pYE90 and pYE45 as the gene coding for the mitochondrial elongation factor Tuf M (GenEMBL accession number K00428).

An additional plasmid (pYE60) was shown to carry an unknown gene which we sequenced and called SMM1 (GenEMBL accession number X91816). This gene was subsequently localized on chromosome XIV as ORF YNR015w (Yeast Sequence Project). Suppression by the SMM1 gene product was very weak and its effect could be indirect. In order to verify the multicopy effect, a 3000-bp *Cla*I-*Sst*I fragment from pYE70 (containing the mitochondrial aspartyl tRNA synthetase gene) and a 2700-bp fragment from pYE66 containing the Tuf M gene were inserted into two plasmids: the high-copy number plasmid YEpLac181 and the centromeric plasmid YCpLac111 (Gietz and Sugino 1988). The resulting constructions were transformed into strain Ts 932/2.12. Growth at 36°C was obtained for all four combinations.

Our results show that even a limited number of additional copies of the above factors can suppress the defect due to faulty $tRNA^{Asp}$ processing. Both the Tuf M and the mitochondrial aspartyl tRNA synthetase genes should be highly expressed (Tuf M more than the synthetase) according to what is known in other systems. This might explain why their presence on centromeric plasmids (usually 1–3 copies) is sufficient to correct the defective phenotype.

Mitochondrial aspartyl tRNA synthetase charges $tRNA^{Asp}$ with aspartic acid while Tuf M promotes the binding of aminoacyl-tRNAs to the ribosome-A site via a ternary complex (in this case aspartyl-$tRNA^{Asp}$-Tuf M-GTP). It is highly improbable that each of these two proteins can directly replace the 3′-processing endonuclease and restore correct processing. We propose two alternative mechanisms that could explain growth.

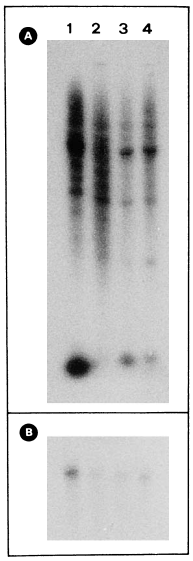

Fig. 1A Northern-blot analysis of total mitochondrial RNAs extracted from the wild-type haploid strain FF1210-6C (*lane 1*), from the mutant strain Ts 932 (*lane 2*), and from the same mutant strain transformed with the plasmids pYE70 (AspRS M gene, *lane 3*) or pYE66 (Tuf M gene, *lane 4*). The probe utilized is the $tRNA^{Asp}$ gene and the gel is a partially denaturing gel (see Materials and methods). The faster-migrating bands correspond to 4*s* RNA. Their position is slightly higher in *lanes 3 and 4* due to the mutation (see Rinaldi et al. 1994). The slower-migrating smear is mainly due to the partially processed multigenic transcripts containing the $tRNA^{Asp}$ gene. **B** the same blot was hybridized with a mitochondrial *COX 3* probe in order to quantify the amount of mitochondrial RNAs

The suppression effect might be due to stabilization, by these factors, of the pre-tRNA tertiary structure altered by the mutation, which will result in increased processing at the non-permissive temperature. Alternatively, the increased amounts of the above factors could provide the additional capacity to select and provide the limited number of processed molecules which exist at the non-permissive temperature.

The correct maturation of the $tRNA^{Asp}$ gene in the transformants was assessed by Northern analysis. Total mitochondrial RNA was extracted from the Ts 932/2.12 strain containing the Tuf M or AspRS M genes on centromeric plasmids, grown at 36°C, and a Northern blot was hybridized with a specific $tRNA^{Asp}$ probe. The results, shown in Fig. 1, indicate that mature $tRNA^{Asp}$ is present in the strain carrying the AspRS M gene and, in lower amounts, in the one carrying Tuf M. This result favours the first hypothesis and indicates an effect of these molecules in restoring the structure of mitochondrial $tRNA^{Asp}$ altered by the mutation at position 61. The experimental analysis of the points of interaction between the proteins and $tRNA^{Asp}$ should yield informative results.

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