

TWO GENES FOR MITOCHONDRIAL TYROSINE TRANSFER RNA IN YEAST

Localization and expression

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1. Introduction

In all mitochondrial systems studied so far, the number of transfer RNA (tRNA) genes is unusually small. In yeast, a total of 24 mitochondrial tRNA genes has been identified [1–6] and this number may represent the complete set of mitochondrial tRNA genes [7]. According to the mitochondrial coding rules proposed in [7–9] the codons belonging to the 'mixed families' of the code table, and having a pyrimidine at the wobble position, are read by a single anticodon carrying a guanine at the corresponding position. Thus the tyrosine codons UAC should be recognized by a single GUA anticodon. In fact, we have identified a single tyrosine tRNA gene [1–3] of which the anticodon is not known but is supposed to be GUA [7].

Here, we show that one of the mitochondrial iso-accepting tRNAs for tyrosine is the product of a second gene for tRNA_{tyr}, which is not, or scarcely, expressed in some strains and had therefore not been detected in previous studies. The existence of the second tyrosine tRNA gene, which is not homologous in sequence to the first one and maps in a different position, raises some questions as to the nature of its anticodon and the regulation of its expression.

2. Materials and methods

Three respiratory sufficient strains of *Saccharomyces cerevisiae* were used: TR3-15A (*a* his₁ trp, rho⁺ ω⁻ C^RE^RO₁^RP^R), MH41-7B (*a* ade₂ his₁, rho⁺ ω⁺ C^RE^RO₁^RP^R) and FF2201-7C (*α* leu₂lys₂trp₁met₈-

tyr₆aro₁). The latter strain was obtained by crossing MH41-7B to a rho⁰ (no mitochondrial DNA) strain and it is therefore isomitochondrial with MH41-7B. All strains were grown aerobically to the late log-phase in galactose complete medium. Preparation of mitochondrial DNA (mtDNA) and tRNA as well as the procedures for aminoacylation, RPC5 chromatography and hybridization have been described in [2,4]. The origin and genetic structure of the 5 rho⁻ deletion numbers, CEP2, R53, O₁P2, BS52 and ME121, have been described [3,10]. Their deletion structures are illustrated in fig.1.

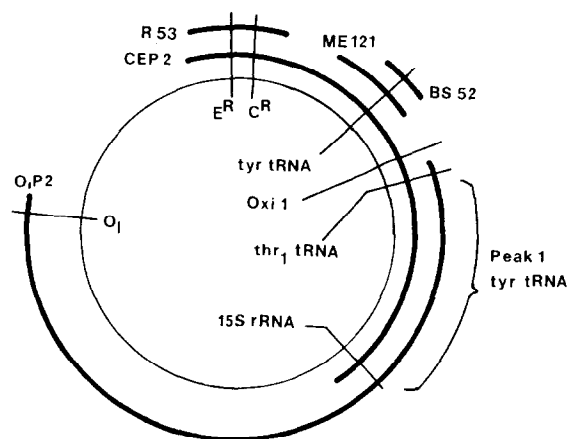


Fig.1. Deletion structure of some rho⁻ mutants used for tyrosine tRNA gene localization. The central circle stands for the complete wild-type mtDNA. Each heavy arc represents a rho⁻ genome as indicated. C^R, E^R, O₁^R and Oxi 1 are genetic markers [2]. The genes for 15 S rRNA, thr₁tRNA and the classical tyrosine tRNA are indicated according to [3,10].

3. Results

3.1. Multiplicity of mitochondrial tyrosine tRNA

Mitochondrial tRNA from the strain TR3-15A was acylated with L-[2,3,5,6- ^3H]tyrosine by mitochondrial enzymes and chromatographed on a RCP5 column (fig.2a). Six species of tyrosine tRNA were observed, including one cytoplasmic contaminant (marked C). All other 5 peaks were mitochondrial gene products as established by hybridization with mtDNA. Peak 5 was constantly the major species. The minor peak 3 has not been studied below because it is only available in small amounts.

3.2. Presence of two distinct tyrosine tRNA genes in mtDNA

The individual tyrosine isoacceptors were collected and hybridized to the mtDNAs from various *rho*⁻ deletion mutants. Each of the mutant genomes repre-

sents a defined segment of the complete wild-type genome [3,10]. The previously identified tyrosine tRNA gene is located within the segment BS52. Therefore, tRNA species transcribed from this gene should hybridize to the mtDNA of CEP2, BS52 and ME121 and not to O₁P2 and R53 (see fig.1). This is the case for the major species, peak 5. Species 2 and 4 may also be interpreted as different forms of the same gene product (table 1). Unexpectedly, peak 1 hybridized to CEP2 and O₁P2 and not to others. This result indicates that peak 1 is the product of a second gene located at the overlapping segment of CEP2 and O₁P2 genomes, that is between 15 S rRNA gene and threonine₁ tRNA gene. The sequences of the 2 genes are different since the hybridization distinguishes them without ambiguity.

3.3. The new tyrosine tRNA is not always expressed

In most of the previous mapping studies, the mito-

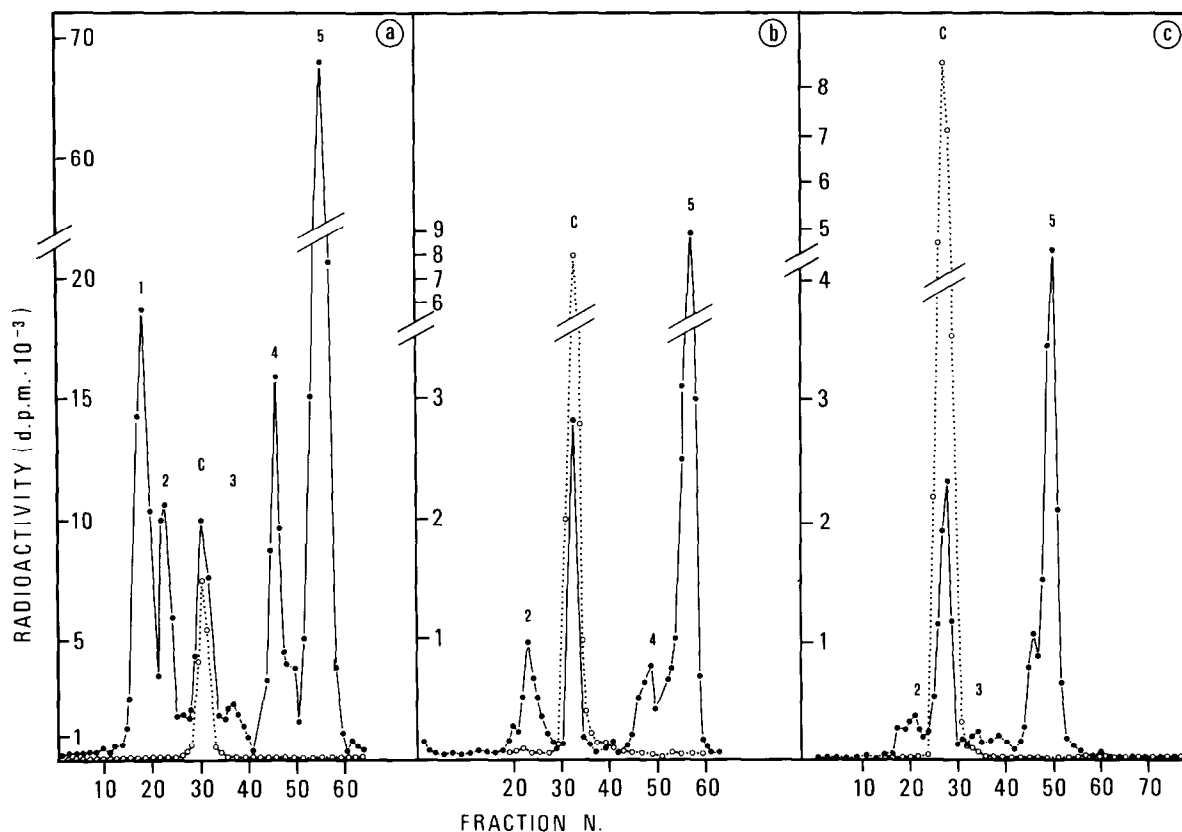


Fig.2. RPC5 chromatography of mitochondrial tyrosine tRNA. Mitochondrial tRNA was isolated from (a) TR3-15A, (b) MH41-7B and (c) FF2201-7C. The tRNA was acylated with [^3H]tyrosine and chromatographed on an RPC5 column. In each run, [^{14}C]-tyrosyl tRNA from the cytoplasm of a *rho*⁰ strain was included as reference (marked C). (—●—) dpm ^3H ; (—○—) dpm ^{14}C .

Table 1
Hybridization of mitochondrial [^3H]tyrosyl tRNA species to
mtDNA of various *rho*⁻ mutants

mtDNA	[^3H]tyrosyl tRNA hybridized (dpm)				
	Peak 1	Peak 2	Peak C	Peak 4	Peak 5
CEP2	1020	n.d.	21	n.d.	920
R53	0	0	n.d.	5	0
BSS2	145	n.d.	49	3000	5340
ME121	30	1580	60	4210	3296
O ₁ P2	1835	80	n.d.	0	0

Each [^3H]tyrosine tRNA species was concentrated from the RPC5 eluates and hybridized to the indicated *rho*⁻ mtDNA (20 μg) immobilized on nitrocellulose filters; n.d., not determined

chondrial tRNA was isolated from strain MH41-7B, and the unfractionated tRNA was acylated with [^3H]tyrosine and used for hybridization. In doing this, we have found a single gene on the BS52 segment and nowhere else. We fractionated the tyrosine tRNA from the MH41-7B strain and found that, in contrast to TR3-15A, peak 1 species was absent (fig.2b). In another strain, FF2201-7C which is iso-mitochondrial with MH41-7B, peak 1 was also missing (fig.2c). These results explain why the previous studies failed to detect the second tyrosine tRNA gene. The absence of peak 1 in MH41-7B genome is not due to the absence of the corresponding gene, since peak 1 hybridizes the CEP2 and O₁P2 which are direct *rho*⁻ derivatives of MH41-7B.

4. Discussion

The presence of two tyrosine tRNA genes in yeast mitochondria raises 2 questions:

- (i) Do they have the same anticodon as expected from the general mitochondrial coding rules? If so, why are there 2 genes which do not seem to result from a duplication (no detectable cross-hybridization)? Alternatively, do they represent 2 different

anticodons, making an exception to the rule? Structural analyses of the gene or the tRNA will resolve the issue.

- (ii) We may ask why the second gene is not detectably expressed in certain strains.

Either there is a regulatory mechanism for this particular tRNA gene, or it is unessential and defective in some strains.

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