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# Peptides from aminoacyl-tRNA synthetases can cure the defects due to mutations in mt tRNA genes

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ABSTRACT

Recent results from several laboratories have confirmed that human and yeast leucyl- and valyl-tRNA synthetases can rescue the respiratory defects due to mutations in mitochondrial tRNA genes. In this report we show that this effect cannot be ascribed to the catalytic activity *per se* and that isolated domains of aminoacyl-tRNA synthetases and even short peptides thereof have suppressing effects.



## 1. Introduction

Human mitochondrial (mt) diseases are a group of disorders due to mutations in nuclear or mt genes affecting mitochondrial functions.

A large proportion of these diseases is due to mutations in mt tRNA genes, resulting in the inhibition of mt protein synthesis and consequent OXPHOS defects, which can have variable severity, and for which at present no treatments exist. This absence is partly due to the scarcity of suitable models and to the fact that it is generally impossible to obtain site specific mitochondrial mutations. This difficulty has been overcome in yeast, in which biolistic mitochondrial transformation is possible (Bonnefoy and Fox, 2007; Fox et al., 1991).

Some years ago, we have established a yeast model of mitochondrial defects by introducing into yeast mt tRNA genes several base substitutions equivalent to those causing diseases in humans, and have observed that the consequent respiratory defects could be alleviated by overexpression of nuclearly encoded protein synthesis factors such as the mt protein elongation factor EF-Tu and the mt aminoacyl-tRNA synthetases (aaRS) (De Luca et al., 2006; De Luca et al., 2009; Feuermann et al., 2003; Montanari et al., 2008; Montanari et al., 2010).

Furthermore, we found that the same suppression of yeast defects could be obtained by the overexpression of the orthologous human factors (Montanari et al., 2010).

Moreover, nuclear factors orthologous to those we had identified in yeast were found to have suppressing activity in patient cell lines and cybrids (Li and Guan, 2010; Park et al., 2008; Rorbach et al., 2008; Sasarman et al., 2008).

AaRS are evolutionarily important enzymes on which the fidelity of protein synthesis is based; to distinguish similar aminoacids some of them have editing domains which allow misacylation to be avoided; these and the other domains involved in aminoacylation and aminoacyl-adenylate formation have been extensively studied (Hsu et al., 2006; Hsu and Martinis, 2008; Tukalo et al., 2005). In fungi, some aaRS domains also have a splicing function on mt introns (Herbert et al., 1988; Paukstelis and Lambowitz, 2008).

Another important observation was that a point mutation in mt LeuRS strongly reducing the catalytic activity did not impair the suppression capability (De Luca et al., 2009). Therefore we hypothesized that the suppressing function of aaRS might be exerted by protein regions involved in the interaction with tRNA, which might restore the correct structure of tRNA altered by the mutation in a sort of chaperon-like effect.

Isolated aaRS domains were subcloned in multicopy yeast vectors which were introduced into yeast mt tRNA mutants and examined for suppression capability.

We report here that the carboxy-terminal (C-terminal) region of human and yeast mt LeuRS has full suppressing activity. This domain is 66 (yeast) or 67 (human) aminoacids in length, so we investigated whether reduced peptides could maintain suppressing activities and hence possible therapeutic applications may be envisaged.

## 2. Materials and methods

### 2.1. Strains, media and growth conditions

*Saccharomyces cerevisiae* strains used are the WT MCC123 (MAT a, ade2, ura3-52, Δleu, kar1-1, Kan$^R$, rho$^+$) (Mulero and Fox, 1993) and

*Abbreviations:* aaRS, aminoacyl-tRNA synthetase(s); bp, base-pair; MELAS, Mitochondrial Encephalomyophathy, Lactic Acidosis, and Stroke-like episodes; mt, mitochondrial; nt, nucleotide; rho$^+$, mt DNA wild-type; WT, wild-type.

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the isogenic *syn*$^{-}$ mt mutants LeuC25T, ValC25T (bearing the same substitution in tRNA$^{Leu}$ and tRNA$^{Val}$ respectively), IleT32C and IleT33C (Feuermann et al., 2003; De Luca et al., 2009; Montanari et al., 2010, and unpublished results). The IleT33C mutant showed no defective phenotype when mutated mitochondria were associated with the MCC123 nuclear context. Therefore we utilized a spore derivative showing a thermosensitive glycerol growth defect and the experiments were performed at the non permissive temperature.

The mutant strains were named with a three-letter code name of the aminoacid indicating the tRNA gene and the position of the base substitution referring to yeast cytoplasmic tRNA$^{Phe}$ (Sprinzl and Vassilenko, 2005). The mutants were obtained by biolistic transformation: the required mutations in tRNA genes were created by site-directed mutagenesis following the procedure described in Rohou et al. (2000) and details can be found in Feuermann et al. (2003) supplementary information.

Standard protocols (Sambrook et al., 1989) were used for *E. coli* and yeast transformations as well as plasmid preparations (see Supplementary material for details).

Strains were grown in YP complete medium (1% yeast extract and 1% peptone from Difco) containing 3% glycerol or glucose (2% or 0.25%). To induce the Gal1 promoter in the suppression experiments, 0.1% galactose was added to YP 3% glycerol plates. Minimal medium was 0.7% yeast nitrogen base (Difco), 5% ammonium sulphate and 2% glucose, supplemented with the necessary auxotrophic requirements. For solid plates, 1.5% agar (Difco) was added to the aforementioned media.

### 2.2. Microscopy

GFP plasmids were used to observe the specific cellular localization of mt LeuRS and its variants. After transformation, the selected GFP clones were grown in 2% galactose supplemented with adenine (45 µg/ml). The late exponential culture was washed in YP liquid complete medium and the pellet suspended in growth media. One aliquot was fixed on glass slide and observed by fluorescent microscopy; another aliquot was treated with 1% formaldehyde for DAPI staining to control colocalization.

## 3. Results and discussion

We have previously used the biolistical methodology to introduce several human equivalent base substitutions into yeast mt tRNA genes. In particular we introduced mutations into tRNA$^{Leu}$ UUR at positions: 14, 25, 60 (MELAS equivalent), 20 (MM/CPEO equivalent) and 29 (MMC equivalent). Mutations were also introduced into tRNA$^{Val}$ at position 25, equivalent to the homoplasmic m. 1624 human mutation causing a syndrome with variable penetrance in the members of the same family (McFarland et al., 2002) and in tRNA$^{Ile}$ at positions 32 and 33 equivalent to human homoplasmic mutations m. 4090 and m. 4091 reported by Limongelli et al., 2004 and by Wilson et al., 2004 respectively.

In all these mutants we have found respiratory defects mirrored by the inability to grow on respiratory carbon sources. In most cases, the severity of defects paralleled that of the corresponding human diseases. The extent of respiratory defects was also studied by oxygen consumption and by northern blot analysis of charged and uncharged tRNAs; mt protein synthesis products were also analyzed by gel electrophoresis (Montanari et al., 2010).

From the whole of these results a rather clear picture emerged concerning the defects of the various mutants; we also could demonstrate that the nuclear genetic context strongly influenced the phenotypes.

The severity of defects was relieved by the overexpression of EF-Tu (Feuermann et al., 2003) and of the cognate aminoacyl-tRNA synthetases (De Luca et al., 2006). Recently, we have demonstrated that suppression also took place when non-cognate aaRS as well as orthologous human enzymes were overexpressed (Montanari et al., 2010).

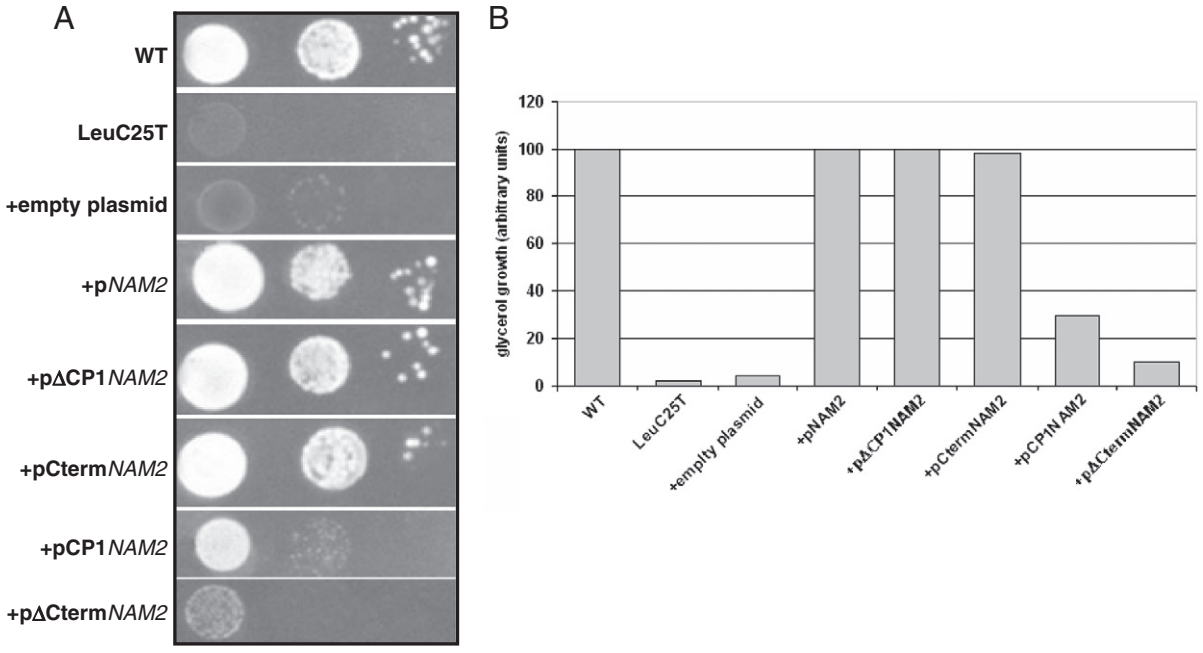


**Fig. 1.** Suppressive effect of the overexpression of *NAM2* and its variants. (A) Serial dilutions of WT (MCC123), of the isogenic LeuC25T mutant and of the same mutant transformed with multicopy empty plasmid and multicopy plasmids bearing the WT *NAM2* gene (coding for *Sc* mt leucyl-tRNA synthetase) or its different variants obtained by deletion or isolation of individual domains as indicated. The tested domains are the CP1 (Connecting Peptide 1 having editing function) and the C-terminal domains. All strains were spotted on YP plate containing 3% glycerol and incubated at 28 °C. The same results were obtained at 37 °C. (B) Graphic representation of the results shown in panel A. Values obtained by Phoretix analysis are calculated as percentage of the WT.