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The yeast model suggests the use of short peptides derived from mt LeuRS for the therapy of diseases due to mutations in several mt tRNAs

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ABSTRACT

We have previously established a yeast model of mitochondrial (mt) diseases. We showed that defective respiratory phenotypes due to point-mutations in mt $tRNA^{Leu(UUR)}$, $tRNA^{Ile}$ and $tRNA^{Val}$ could be relieved by overexpression of both cognate and non-cognate nuclearly encoded mt aminoacyl-tRNA synthetases (aaRS) LeuRS, IleRS and ValRS. More recently, we showed that the isolated carboxy-terminal domain (Cterm) of yeast mt LeuRS, and even short peptides derived from the human Cterm, have the same suppressing abilities as the whole enzymes.
In this work, we extend these results by investigating the activity of a number of mt aaRS from either class I or II towards a panel of mt tRNAs. The Cterm of both human and yeast mt LeuRS has the same spectrum of activity as mt aaRS belonging to class I and subclass a, which is the most extensive among the whole enzymes. Yeast Cterm is demonstrated to be endowed with mt targeting activity.
Importantly, peptide fragments β30_31 and β32_33, derived from the human Cterm, have even higher efficiency as well as wider spectrum of activity, thus opening new avenues for therapeutic intervention. Bind-shifting experiments show that the β30_31 peptide directly interacts with human mt $tRNA^{Leu(UUR)}$ and $tRNA^{Ile}$, suggesting that the rescuing activity of isolated peptide fragments is mediated by a chaperone-like mechanism.
Wide-range suppression appears to be idiosyncratic of LeuRS and its fragments, since it is not shared by Cterminal regions derived from human mt IleRS or ValRS, which are expected to have very different structures and interactions with tRNAs.

1. Introduction

AaRS are evolutionarily important enzymes on which the fidelity of genome decoding is based. All aaRS contain catalytic and anticodon recognition domains to catalyse the aminoacylation reactions specific for their cognate amino acids. Additionally, several aaRS have developed editing activities to hydrolyse mis-activated amino acids or mis-charged tRNAs and prevent insertion of incorrect amino acids during protein synthesis [1]. The canonical functions of aaRS, including aminoacylation and editing activities, are highly conserved throughout the three domains of life, i.e., archaea, bacteria and eukarya. Based on sequence, structure and functional features, including catalytic domain topology and tRNA esterification site, aaRS have been assigned to two different Classes, each divided into subclasses. Class I aaRS are characterized by two structural motifs – HIGH and KMSKS – and have a Rossmann fold dinucleotide-binding domain. This catalyses ATP dependent activation of amino acids and transfer of activated aminoacyl–adenylates to the 2′-OH of the CCA sequence at the tRNA 3′-end. Enzymes with a Class I catalytic domain include those active on amino acids Leu, Ile, Val, Met, Cys, Arg, Glu, Gln, Tyr, and Trp. Class II aaRS employ three degenerate motifs in an anti-parallel core of β strands to coordinate ATP binding, and aminoacylate the 3′-OH of their cognate tRNAs. The classification of aaRS is conserved in evolution, with only a few exceptions [2]. However, during evolution from prokaryotes to vertebrates, including mammals, certain aaRS have acquired other domains, unrelated to aminoacylation and generally localized at the amino- or carboxy-terminus [3]. The function of these additional domains include splicing of structured RNAs, translation control, transcription regulation, signal transduction, cell migration and others. In particular splicing of mt

Abbreviations: mt, mitochondrial; bp, base-pair; WT, wild type; MELAS, mitochondrial encephalomyophathy, lactic acidosis and stroke-like episodes; aa, amino acid(s); aaRS, aminoacyl-tRNA synthetase(s) (specificity is indicated by the name of the amino acid, abbreviated in the three-letter code); rho^+, mtDNA wild-type; rho°, mtDNA absent; MTS, Mitochondrial Targeting Sequence

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introns has been shown in *Saccharomyces cerevisiae* for mt LeuRS [4] and in *Neurospora crassa* for mt TyrRS [5]. This activity has been shown to be conserved in human mt LeuRS although human mt introns are not present [6].

It has been shown that the overexpression of genes coding for mt LeuRS, ValRS or IleRS, all belonging to Class I and subclass a, were able to suppress the pathological phenotypes associated with mutated mt tRNAs both in human and in yeast cells [7–13]. Further, we have observed that human mt aaRS were able to suppress the defective phenotypes of yeast mt tRNA mutants even more efficiently than the orthologous yeast enzymes [7]. Moreover we have demonstrated that the suppression activity does not require the catalytic activity of the enzyme [14]. The three aforementioned aaRS bind very similar amino acids, which only differ for the presence or position of a methyl group. It has been reported that this similarity can result in mis-adenylation, followed by mis-acylation by aaRS. Such mistakes are then corrected by the editing domain that is present in mt LeuRS, IleRS and ValRS [15].

More recently, two novel aspects of the suppressing ability of aaRS have been observed, namely the possibility to rescue the defective phenotypes of mutants having substitutions in mt $tRNA^{Leu(UUR)}$, $tRNA^{Ile}$ and $tRNA^{Val}$ by overexpressing i) non-cognate mt LeuRS, IleRS or ValRS (cross-suppression activity); ii) a 67 aa Carboxy-terminal sequence derived from mt LeuRS (Cterm suppression activity). The same properties were demonstrated to be present in human mt aaRS sequences in studies using cybrids, thus opening the way to new therapeutic perspectives. To this end, it is important to note that the Cterm peptide, even though lacking a canonical N-terminal mt targeting sequence (MTS), enters the mitochondria of human cybrids and interacts with mt tRNAs in vitro [9]. Subsequently, we have shown that two isolated peptides derived from the human mt LeuRS Cterm, named β30_31 (15 amino acids) and β32_33 (16 amino acids), which are in contact with the cognate $tRNA^{Leu}$ in known three-dimensional (3D) structures, are endowed with suppressing activity comparable to the whole Cterm towards mt $tRNA^{Leu(UUR)}$, $tRNA^{Val}$ and $tRNA^{Ile}$ mutants [7]. In view of potential therapeutic applications, some relevant issues need to be addressed. In this work, we aim at establishing: i) the extent of cross-suppression among different mt aaRS; ii) whether the Cterminal regions of human mt IleRS and ValRS are endowed with suppressing and cross-suppressing abilities analogous to the Cterm region of LeuRS; iii) whether the short β30_31 and β32_33 peptides derived from human mt LeuRS Cterm are able to rescue the defective phenotypes due to mutations in different mt tRNAs, as well as the previously studied mt $tRNA^{Leu(UUR)}$, $tRNA^{Ile}$ and $tRNA^{Val}$. Additionally, to investigate the molecular mechanism of the suppressing activity we tested the interaction between human mt tRNAs (leu, ile and gly) and the β30_31 and β32_33 peptides; demonstrated that the yeast mt LeuRS Cterm is endowed with mt targeting activity; and analysed the available 3D structures of aaRS–tRNA complexes to highlight the details of molecular recognition.

2. Material and methods

2.1. Strains, media and growth conditions

S. cerevisiae strains are the WT MCC123 *MATa*, *ade2-1*, *ura3-52*, *leu2*, *kar1-1* rho^+ [16] and the isogenic mt tRNA mutants listed in Tables 1 and 2, which were originated by cytoduction crosses as previously described [14]. Mutants are named with the three-letter code name of the amino acid indicating the tRNA gene and the base substitution. The original LeuA14G, LeuC25T, ValC25T and IleT32C mutants were obtained by biolistic transformation in order to obtain substitutions equivalent to pathological mutations in the yeast model [14,17,18]. The procedure is described in Rohou et al. [19] and details can be found in Feuermann et al. 2003 [17], supplementary information. The original mutants GlyG30T, PheC2T, PheC62T, AspC61T and the GlnC6T, and the HisG51A mutants were obtained by $MnCl_2$ random mutagenesis by M. Bolotin Fukuhara and A. Tzagoloff, respectively, as previously described [19–22].

The TUF1 null strain (MCC123ΔTUF1) was obtained as previously described [23]. Strains were grown in YP complete medium (1% yeast extract and 1% peptone from Difco) containing 3% glycerol, or 2% glucose. 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 above media. The glycerol growth capability of WT, mutant and transformant cells was investigated by serial dilutions from concentrated suspensions (5×10^6 cell/ml) prepared from fresh single colony spotted onto a unique plate.

2.2. Plasmids and cloning

Standard protocols [24] were used for *Escherichia coli* and yeast transformations as well as plasmid preparations.

The vectors containing mt aaRS sequences are:

pNAM2 (kindly provided by Prof. C. Herbert) in which the yeast mt LeuRS gene with its own promoter is cloned into the multi-copy vector pFL44S [25];

pCtermNAM2 in which the sequence of the Carboxy-terminal domain of *NAM2* gene (from aa 829 to 894) is cloned in multi-copy

Table 1
Suppression capability of Class I and Class II aaRS on mt tRNA mutants aminoacylated by Class I mt aaRS.

			Glycerol growth of transformant strains							
			Class I aaRS							**Class II aaRS**
tRNA Mutant	tRNA defect	Glycerol growth 28 °C 37 °C	**LeuRS** Sc (*NAM2*) Ia	Hs (*LARS2*) Ia	**ValRS** Sc (*VAS1*) Ia	Hs (*VARS2*) Ia	**IleRS** (*ISM1*) Ia	**GluRS** (*MSE1*) or Cyt **GlnRS** (*GLN4*) Ib	**TyrRS** (*MSY1*) Ic	**GlyRS, HisRS, AspRS, LysRS, PheRS**
VAL C25T [14]	Low amount of $tRNA^{Val}$	+/− +/−	+ [29] +	+ [29] +	+ [14,29] +	+ [29] +	+ +	+/− +/−	+ +	+/− +/−
LEU A14G [17]	Aminoacylation defect	− −	+ [38] +	+ +	+ +	+ +	+ +	− −	− −	− −
LEU C25T [17]	No tRNAs	− −	+ [14,29,38] +	+ [29] +	+ [29] +	+ [29] +	+ +	− −	− −	− −
ILE T32C [18]	No tRNAs	− −	+ [18] +	+ +	+ [18] +	+ +	+ [18] +	− −	− −	− −
GLN C6T [40]	Structural and aminoacylation defects	+/− −	+ +	+ −	+/− −	+/− −	+/− −	+/− −	+/− −	+/− −

+ indicates growth similar to wild type; − indicates absence of growth; +/− indicates partial growth.