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

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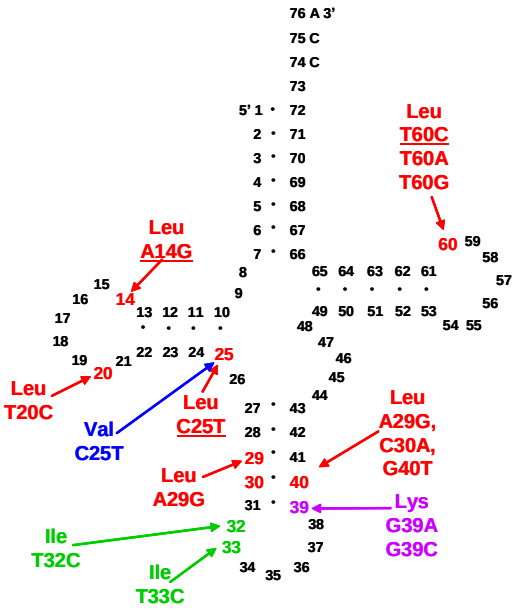
A large proportion of mitochondrial (mt) diseases are due to base substitutions in mt tRNA genes resulting in impairment of mt protein synthesis and consequent OXPHOS defects. Notwithstanding the widespread effort of many laboratories, no treatment has been found up to now, mostly due to incomplete knowledge of molecular mechanisms and to the absence of suitable models in which mt transformation can be achieved.

We have used the yeast *Saccharomyces cerevisiae* as a model to analyse the molecular and cellular aspects of the mt defects due to mutations equivalent to human pathogenic substitutions in mt tRNA genes (see Figure).

We performed mutant phenotypic analyses measuring their glycerol growth capability and oxygen consumption and we observed phenotypic variability dependent on the nuclear context of mutant cells. By Northern blot on high resolution gels we could study whether the defect might be related to the amount or to aminoacylation defects of mutated tRNA.

In the present contribution we will highlight the following points:

- Human equivalent mutations produce in yeast respiratory defects mirroring the severity of defects observed un human pathologies. Defects are highly dependent from the genetic context.
- Defects can be relieved by overexpression of some nuclear genes encoding mt protein synthesis factors such as the mt protein synthesis Elongation Factor (EF-Tu) and the aminoacyl-tRNA synthetases (aaRS).
- Several laboratories have shown the orthologous human factors can have the same rescuing effects in human cell lines and cybrids.
- Suppression can be obtained when non-cognate aaRS as well as the orthologous human enzymes are overexpressed (at least as far as the three similar aminoacids leucine, valine and isoleucine are concerned).
- The suppression by mt aaRS is probably not related to the enzyme activities *per se*, and may be due to a stabilizing chaperon-like effect of the synthetase molecules on the tRNA structure altered by the mutation.
- Suppression can be obtained by transforming mutants with multicopy plasmids, bearing the carboxiterminal domain sequences of leucyl- ,valyl- and isolecyl-tRNA synthetase.
- The human mt leuRS carboxiterminal domain can also restore glycerol growth (respiratory competence) of all yeast defective mutants in mt $tRNA^{Leu}$, in $tRNA^{Val}$ and $tRNA^{Ile}$ we have tested.
- Structure analysis of yeast mt leucyl-tRNA synthetase (Hsu and Martinis, 2006; Tukalo et al, 2005) shows that the carboxyterminal domain contacts the ”elbow” of the L-shaped tRNA structure. This might exert a sort of stabilizing chaperon function on the tRNA molecule.
- Cloned short sequences from the carboxyterminal of human mt leuRS gene corresponding to the β-strands contacting the mutant tRNA have full suppressing activity.
- Fluorescence microscopy experiments with GFP fusion plasmids show that the tested peptides are imported into mitochondria.



Secondary structure of tRNAs. The mutations introduced in yeast mtDNA by biolistic procedures and the equivalent human substitutions are indicated by arrows. Colours indicate the specific tRNA gene. Red: $tRNA^{Leu}$(UUR) mutants; blue: $tRNA^{Val}$ mutant; violet: $tRNA^{Lys}$ mutants; green: $tRNA^{Ile}$ mutants.