Bioinformatics and experimental characterisation of yeast mitochondrial tRNA mutants responsible of defective phenotypes mirroring human pathologies

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Motivation

Mutations in human mitochondrial (mt) tRNA genes correlate with a number of important pathologies, such as Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS). However, in vivo studies are complicated by heteroplasmy and variable nuclear genetic background, and limited by the availability of biopsy material. We have previously shown that yeast can be a suitable model system to study the effect of mitochondrial tRNA mutations associated with MELAS pathologies in human (Feuermann et al., 2003, EMBO Rep. 4:53).

To investigate the pathological effect of a panel of human mt tRNA mutations, we introduced equivalent mutations into yeast mt DNA. We studied the effects of these targeted mutations on yeast growth and respiratory phenotypes, as well as on the conformation and acylation properties of the mutated tRNAs. Analyses of yeast tRNA sequences and three-dimensional tRNA structures were performed to rationalise the experimentally determined effects of the mutations.

Methods

Growth and respiratory phenotypes of mutant and wild-type tRNAs were characterised on different carbon sources and temperatures: glucose, galactose and glycerol at 28 and 37 centigrade degrees. By Northern blot experiments we examined the effect of the mutation on the transcription, stability and correct maturation of the mutated tRNA, as well as the presence of conformational or acylation defects. Total tRNAs were extracted from purified mitochondria and fractionated in partially denaturing 10% polyacrylammide urea acidic sequencing gels. Sequences of yeast tRNAs were downloaded from the 'Compilation of tRNA sequences and sequences of tRNA genes' (Sprinzl and Vassilenko, 2005, Nucleic Acids Res. 33:D139). For each sequence we recorded the length of the stems of the loops and of the connector segments, the number of Watson and Crick base pairs, and the number of mismatches, insertions, and deletions in the stems. Non redundant structures of free tRNAs and tRNA complexes with protein partners determined by X-ray crystallography were collected from the PDB data archive. Structure analyses and modelling of tRNA mutations were performed using the software InsightII.

Results

All the characterised mt tRNA mutations equivalent to those responsible for human pathologies determined various degrees of respiratory deficient phenotypes in yeast, mirroring the human pathologies. Compared to the wild- type molecules, the mutated tRNAs exhibited detectable conformational changes and/or defective acylation properties.

Based on analyses of tRNA structures and of mitochondrial and cytoplasmic yeast tRNA sequences we propose explanations for the observed defects, which can be ascribed to either the loss of identity nucleotides or specific secondary and/or tertiary interactions that are largely conserved in native mitochondrial and cytoplasmic tRNAs.

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