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Mitochondrial Aminoacyl-tRNA Synthetase Single-Nucleotide Polymorphisms That Lead to Defects in Refolding but Not Aminoacylation

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Defects in organellar translation are the underlying cause of a number of mitochondrial diseases, including diabetes, deafness, encephalopathy, and other mitochondrial myopathies. The most common causes of these diseases are mutations in mitochondria-encoded tRNAs. It has recently become apparent that mutations in nuclear-encoded components of the mitochondrial translation machinery, such as aminoacyl-tRNA synthetases (aaRSs), can also lead to disease. In some cases, mutations can be directly linked to losses in enzymatic activity; however, for many, their effect is unknown. To investigate how aaRS mutations impact function without changing enzymatic activity, we chose nonsynonymous single-nucleotide polymorphisms (nsSNPs) that encode residues distal from the active site of human mitochondrial phenylalanyl-tRNA synthetase. The phenylalanyl-tRNA synthetase variants S57C and N280S both displayed wild-type aminoacylation activity and stability with respect to their free energies of unfolding, but were less stable at low pH. Mitochondrial proteins undergo partial unfolding/refolding during import, and both S57C and N280S variants retained less activity than wild type after refolding, consistent with their reduced stability at low pH. To examine possible defects in protein folding in other aaRS nsSNPs, we compared the refolding of the human mitochondrial leucyl-tRNA synthetase variant H324Q to that of wild type. The H324Q variant had normal activity prior to unfolding, but displayed a refolding defect resulting in reduced aminoacylation compared to wild type after renaturation. These data show that nsSNPs can impact mitochondrial translation by changing a biophysical property of a protein (in this case refolding) without affecting the corresponding enzymatic activity.

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Abbreviations used: aaRS, aminoacyl-tRNA synthetase; nsSNP, nonsynonymous single-nucleotide polymorphism; hmtPheRS, human mitochondrial phenylalanyl-tRNA synthetase; ANS, 1,8-anilino naphthyl sulfonate; hmtLeuRS, human mitochondrial leucyl-tRNA synthetase; PDB, Protein Data Bank; RMSF, root-mean-square fluctuation.

Introduction

In eukaryotes, ATP synthesis by oxidative phosphorylation occurs in mitochondria. Mitochondria maintain a small genome separate from that found in the nucleus, as exemplified by the human mitochondrial genome that encodes 13 proteins, 2 rRNAs, and 22 tRNAs. Polypeptides encoded by the human mitochondrial genome are subunits of respiratory chain complexes and are essential for mitochondrial function. To maintain viability, mitochondria must import numerous components of their translational machinery from the cytosol, including proteins and some RNAs.[1–3] Numerous proteins are imported into mitochondria, including nuclear-encoded organelle-specific aminoacyl-tRNA synthetases (aaRSs[4]), whose role is to correctly pair amino acids with their cognate tRNAs during translation. Mitochondrial aaRSs vary in structure; some correspond to splice variants of their cytosolic counterparts,[5] while others are of bacterial ancestry and are only distantly related to the corresponding cytosolic enzymes.[6,7]

Mitochondria are responsible for ATP synthesis during aerobic respiration, and different mutations that compromise this essential function have been linked to a wide range of human diseases, including MELAS (*m*itochondrial myopathy, *e*ncephalopathy, *l*actic *a*cidosis, and *s*troke-like episodes), MERRF (*m*yoclonic *e*pilepsy with *r*agged *r*ed *f*ibers), diabetes, and deafness.[8] The comparative ease with which the human mitochondrial genome can be sequenced has allowed the identification and characterization of numerous mutations that can be directly linked to particular diseases. The most commonly reported class of mitochondrial genome mutations associated with diseases is that found in genes encoding tRNAs.[9] Mutations in tRNAs can compromise mitochondrial translation in a number of ways,[10,11] either by directly impairing protein synthesis[8,12–15] or by disrupting biogenesis or folding of tRNA.[16–19] Recent reports have shown that mitochondrial aminoacylation of tRNA may also be impaired in some diseases as a result of mutations in nuclear genes that encode mitochondrially targeted aaRSs. For mitochondrial aspartyl-tRNA synthetase (encoded by *DARS2*) and arginyl-tRNA synthetase (*RARS2*), loss of exons, premature termination, and missense mutations lead to aaRS variants with severe reductions in aminoacylation activity, leading to diseases such as leukoencephalopathy[20] and hypoplasia.[21] Another class of *DARS2* pathogenic mutations linked to leukoencephalopathy, where a single amino acid substitution does not impair aminoacylation but instead prevents mitochondrial import of aspartyl-tRNA synthetase, was also recently reported.[22] In other instances, the molecular basis by which changes to an organelle aaRS lead to mitochondrial dysfunction are unclear as, for example, in the case of a mutation in the *LARS2* gene (encoding leucyl-tRNA synthetase), which may play a role in susceptibility to type 2 diabetes.[23,24]

Most mitochondrial matrix proteins, such as the aaRSs, are synthesized in the cytosol with an N-terminal targeting sequence that facilitates import. During their subsequent import across the mitochondrial membranes, cytosolically synthesized matrix proteins are either unfolded or partially unfolded, so that they adopt a molten-globule-like conformation.[25–29] Following import, mitochondrial proteins such as aaRSs must then be refolded correctly within the organelle matrix to ensure proper function. Defects in the refolding of matrix proteins could be expected to lead to mitochondrial dysfunction, perhaps by triggering an unfolded protein response or by limiting levels of stable folded active protein.[30] One example of the latter has been reported for a common mutant of mitochondrial medium-chain acyl-CoA dehydrogenase found in patients deficient in mitochondrial medium-chain acyl-CoA dehydrogenase, where a single amino acid substitution decreases protein folding and assembly in mitochondria.[31]

Despite the growing number of disease-related nonsynonymous single-nucleotide polymorphisms (nsSNPs) in both cytosolic and mitochondrial aaRS-encoding genes,[32] in most cases, the molecular basis underlying loss of activity is still unknown.[33] Nonsynonymous changes account for approximately half of the characterized genetic diseases in humans and are documented by two databases containing disease-causing variants: Online Mendelian Inheritance in Man[34] and the Human Gene Mutation Database.[35] The human population is estimated to have 67,000–200,000 nsSNPs, and each person is thought to be heterozygous for 24,000–40,000 nsSNPs.[36] nsSNPs that affect aaRS activity, alter splice sites, and limit subcellular localization have each been described, but whether there are also variants that specifically impact stability and folding is unknown. To investigate the possible effects of mutations on the folding of mitochondrial aaRSs, we studied nsSNPs of human mitochondrial phenylalanyl-tRNA synthetase (hmtPheRS), a monomeric enzyme for which a high-resolution crystal structure is available.[37] Using the National Center for Biotechnology Information single-nucleotide polymorphism database, we selected two nsSNPs for *FARS2* (encoding hmtPheRS), both of which correspond to amino acid changes distal to the catalytic and tRNA binding sites of the enzyme. The hmtPheRS nsSNP variants and an nsSNP of *LARS2* all retained wild-type aminoacylation activity but displayed reduced stability and refolding defects, suggesting a mechanism by which aaRS mutations that do not directly impact enzymatic activity can still lead to loss of function.