# Chapter 3
# Translation in Mammalian Mitochondria: Order and Disorder Linked to tRNAs and Aminoacyl-tRNA Synthetases

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**Abstract** Transfer RNAs (tRNAs) and aminoacyl-tRNA synthetases (aaRSs) are key actors in all translation machineries. AaRSs aminoacylate cognate tRNAs with a specific amino acid that is transferred to the growing protein chain on the ribosome. Mammalian mitochondria possess their own translation machinery for the synthesis of 13 proteins only, all subunits of the respiratory chain complexes involved in the synthesis of ATP. While 22 tRNAs and two ribosomal RNAs are also coded by the mitochondrial genome, aaRSs are nuclear encoded and become imported. The fact that the two cellular genomes, nuclear and mitochondrial,

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evolve at different rates raises numerous questions as to the co-evolution of partner macromolecules. Herein we review the present state-of-the-art on structural, biophysical, and functional peculiarities of mammalian mitochondrial tRNAs and aaRSs, and of their partnership in their wild-type state. Then, we oppose this mitochondrial "order" to the "disorder" generated by the presence of a variety of mutations occurring in the corresponding human genes that have been correlated to an increasing number of diseases. So far, more than 230 mutations in mitochondrial tRNA genes and a rapidly growing number of mutations in mitochondrial aaRS genes have been reported as causative of a large variety of pathologies. The molecular incidence of mutations on structural, biophysical and functional properties of the related macromolecules will be summarized. Mutations in mitochondrial tRNA genes lead to complex mosaic effects with a major impact on tRNA structure. Some mutations affecting mitochondrial aaRS genes do not interfere with the housekeeping aminoacylation activity, suggesting that mitochondrial aaRSs, alike cytosolic aaRSs are involved in other processes than translation. This opens new research lines.

## 3.1 Introduction

Mammalian mitochondria possess a small circular genome, coding for 13 polypeptide chains only. These are subunits of the respiratory chain complexes involved in the oxidative phosphorylation of ADP into ATP. The synthesis of these 13 subunits requires a complete mitochondrial translation machinery. Interestingly, the RNA components of this machinery are encoded by the mitochondrial genome. This is the case of 11 mRNAs (leading to the 13 proteins), 2 rRNA, and 22 tRNAs representing the minimal and sufficient set to read all codons (Anderson et al. 1981). All protein components are coded by the nuclear genome, synthesized in the cytosol, and imported into the organelle. They include the full sets of ribosomal proteins, as well as tRNA maturation and modification enzymes, aminoacyl-tRNA synthetases (aaRSs), and also translation initiation, elongation, and termination factors. The dual genetic origin of the macromolecules constituting the translation machinery has raised numerous questions as to their properties, mechanism, and specificities of their partnerships, regulation of expression and mechanisms of co-evolution. Indeed, the mitochondrial genome evolves 15–20 times more rapidly than the nuclear genome (Brown et al. 1979; Castellana et al. 2011), generating highly variable sequences so that the RNAs coded by the mammalian mitochondrial genomes are peculiar. All have lost some information as compared to their bacterial homologs. For instance, mRNAs miss $3'$ and $5'$ untranslated regions, ribosomal RNAs are significantly shorter than bacterial counterparts and tRNAs present a range of peculiarities, from the absence of a few nucleotide signature motifs to the absence of full structural domains (Willkomm and Hartmann 2006).

Herein we first focus on human mitochondrial tRNAs (mt-tRNAs) and aaRSs (mt-aaRSs) and on their partnerships, to illustrate the present knowledge on

peculiar structural, biophysical, and functional properties of two partner macromolecules encoded by two different genomes. Peculiarities of human mt-tRNA genes as highlighted by recent powerful bioinformatics approaches will be summarized and compared to mitochondrial tRNA genes in metazoan. Analysis of tRNAs will illustrate large sequence and structural variability, low thermodynamic stability, and high structural flexibility of this family of RNAs. Current knowledge on human mt-aaRSs will be summarized from genes to structural and functional properties of the corresponding proteins. The partnerships of aaRSs with their substrates will be described not only in terms of aminoacylation properties but also in terms of thermodynamics of substrate binding, a parameter that allowed for clear distinction from bacterial aminoacylation systems. As will be highlighted, the enlarged plasticity of the mitochondrial enzymes as compared to that of aaRS from other origins might be the result of an evolutionary adaptation of the nuclear-encoded protein to the rapidly evolving mitochondria-encoded RNAs. The human mitochondrial aspartylation system, studied in our laboratory, will serve as case study all along the review. After this first part, illustrating the status of wild-type macromolecules and thus referring to "order" in mitochondrial translation, "disorder" will be considered as well as a molecular perturbation as well as a mitochondrial pathology.

In the last two decades, mt-tRNA genes were recurrently reported as hosting point mutations linked to a variety of neuromuscular and neurodegenerative disorders. More recently, nuclear genes coding for mt-aaRSs also became the center of attention, with mutations causative of further disorders. The present understanding of their impact on the molecular level of "disorder" induced within tRNAs and aaRSs molecules will be summarized. While many mutations do interfere with the housekeeping aminoacylation reaction, several others have no detectable effect on it. This is in favor of the existence of additional mt-tRNA and mt-aaRS functions or properties that are altered by these mutations. These supplementary functions and properties need still to be determined. New research lines in this direction will be suggested.

## 3.2 Mammalian Mitochondrial tRNAs

### *3.2.1 Structural Properties of Mammalian Mitochondrial tRNAs*

The gene content of the human mitochondrial genome is similar to what found in other metazoan mitochondria. It encodes for 22 mt-tRNAs (Anderson et al. 1981), one for each of the 20 amino acid specificities, and two additional ones for leucine and serine, respectively. This minimal set of tRNAs is sufficient for reading of all codons despite the genetic code is different in mitochondria from nuclear genomes. Already at the stage of bovine and human mitochondrial DNA sequencing (Anderson et al. 1981) peculiar structural properties of tRNAs were noticed,

marking significant differences with so-called canonical tRNAs present in bacteria or in the cytosol of eukaryotic cells. Most striking was the absence of a possible full cloverleaf structure for some of the expressed gene products, noticeably the absence of a complete structural domain for tRNASer1 (the D-arm of the cloverleaf), and, despite the presence of a potential cloverleaf for the other tRNAs, the absence of typical signature motifs in many sequences, motifs so far known as being conserved in all tRNAs. Mammalian mt-tRNAs fall into two groups according to the location of the corresponding gene on either of the mt-DNA coding strand. "Heavy" tRNAs (eight cases) are G-rich, while "Light" tRNAs (14 cases) are A, U, and C rich, leading to a series of typical structural characteristics such as biased base-pair content (Helm et al. 2000). Further, the full set of mammalian (and metazoan) mt-tRNAs do have a short variable region (Fig. 3.1) while canonical tRNAs also include structures with large variable regions (Giegé et al. 2012). Bioinformatic alignments to additional mammalian mt-tRNA genes confirmed and extended these properties and allowed for a fine-tuned description of detailed structural characteristics of each of the 22 tRNA families (Helm et al. 2000). As an outcome, mammalian mt-tRNAs structural properties range from canonical to highly degenerated types. Figure 3.1a indicates the most common degenerations of the classical cloverleaf structure in human mitochondrial sequences, particularly the loss of D/T loop interactions. Mt-tRNAs are expressed in cells to very low levels as compared to their cytosolic counterparts (estimated as 1 mt-tRNA per 160 cytosolic tRNA), rendering access for biochemical investigation of these RNAs very limited (Enriquez and Attardi 1996). Detailed experimental secondary structures were however established on in vitro transcripts and revealed large structural flexibility, with alternative folds to the cloverleaf co-existing in equilibrium (Bonnefond et al. 2005b; Helm et al. 1998; Sohm et al. 2003). This is due to a severe bias in nucleotide content (A, U, and C-rich, and G-poor) especially for the 14 tRNAs coded by the light DNA strand, leading to very low numbers of stable G-C base-pairs, and at opposite, to high levels of weaker A-U and G-U pairs. To be noticed also that the thermodynamic stabilities calculated for the cloverleaf folds are twice as weak as compared to those of canonical tRNAs (Fender et al. 2012). Post-transcriptional modifications (only characterized in a limited number of sequences; Suzuki et al. 2011) were found to be the triggers for stabilization of the cloverleaf fold as demonstrated with the case study highlighting the role of m1A9 modification in human mt-tRNA$^{Lys}$ (Helm and Attardi 2004; Helm et al. 1998; Motorin and Helm 2010). This modification hinders base pairing of residue 9 (and of neighboring nucleotides 8 and 10) in the 3′-end domain of the tRNA with residues 64 (and neighboring residues 65 and 63, respectively) in the T-stem. Post-transcriptional modifications in mammalian mt-tRNAs (only characterized in a limited number of sequences; Suzuki et al. 2011) remain however quantitatively far more limited as compared to other tRNAs (7 % of modified nucleotides as compared to 13 %) suggesting that they play crucial roles (Helm et al. 1999; Suzuki et al. 2011). Interestingly, a number of specific modifications, such as taurine-dependent modifications of anticodon nucleotides, were reported to be key actors in mitochondrial codon reading (Kirino et al. 2005).

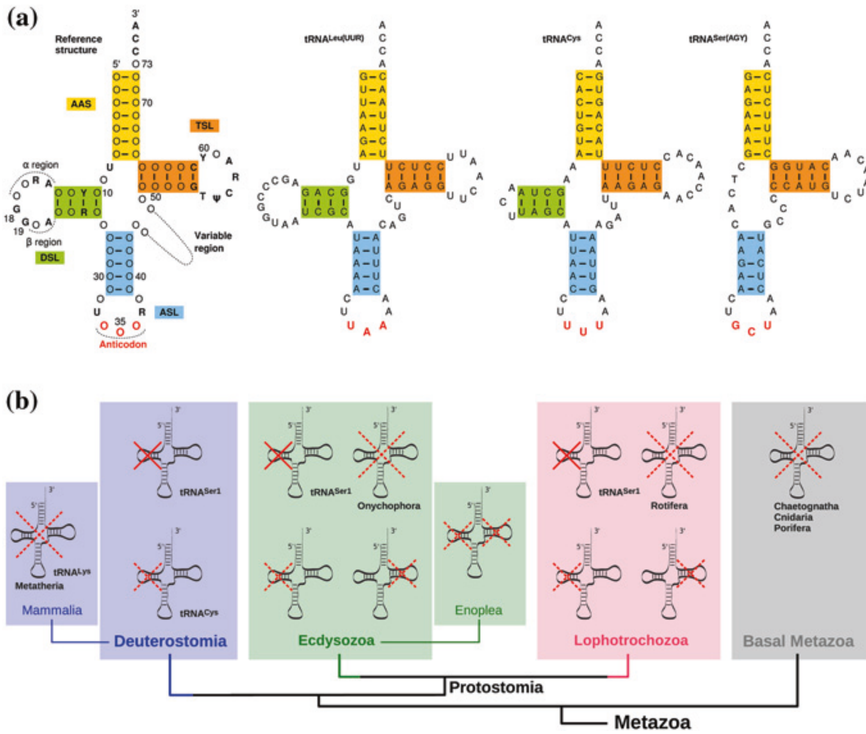


**Fig. 3.1** Structural diversity and evolution of human mitochondrial tRNAs. **a**. Cloverleaf structures of three typical human mt-tRNA secondary structures as compared to the canonical cloverleaf reference structure (*left*). *AAS* amino acid acceptor stem, *DSL* D-stem and loop, *ASL* anticodon stem and loop, *TSL* T-stem and loop. The three examples presented, support the large range of structural profiles covered. tRNA$^{Leu(UUR)}$ is of classical type, with the full cloverleaf domains as well as the full set of conserved nucleotides involved in tertiary folding (see, in particular, the presence of residues G18, G19 in the D-loop, and of residues T54T55C56 in the T-loop, allowing for interaction between the corresponding domains). tRNA$^{Lys}$ illustrates the family of mt-tRNAs still presenting the four domains of the cloverleaf, but with serious variations in the size of the D- and T-loops and their nucleotide content, missing the conserved elements, as well as the unusual nucleotides in the short connector between the acceptor and the D-stems. Finally, tRNA$^{Ser(AGY)}$ is the typical tRNA missing a full structural domain, namely the D-arm. **b**. Simplified view on the evolution of tRNA sets and tRNA secondary structures in Metazoa. Each panel highlights the most striking and representative structural deviation present within the considered evolutionay group. Accordingly, basal metazoan lost the full set of mt-tRNA genes and mammals, lost the gene for tRNA$^{Lys}$ in methateria

The question as to the 3D fold of mammalian mt-tRNAs has retained the attention of many investigators. Indeed, whatever the secondary structural properties of these RNAs, it is expected that their 3D structures allow for the recognition by partner macromolecules, in particular the cognate aaRS for aminoacylation and the ribosome for codon reading and protein synthesis. An L-shaped structure based on an acceptor branch (T-arm and acceptor arm) and an anticodon branch (D-arm and anticodon

arm) is thus expected as for tRNAs of all kingdoms of life (Giegé et al. 2012). This is considered despite the general absence of signature nucleotides (the so-called conserved and semi-conserved nucleotides) known to support such a fold in canonical tRNAs. Birefringence measurements, NMR, and chemical structure probing in solution were the leading approaches confirming the existence of L-shape-like folds for mt-tRNAs, bringing the two functional extremities, namely the acceptor 3′-end and the anticodon, at the expected distance from each other (reviewed in Giegé et al. 2012). The 3D structure of canonical tRNAs is based on a set of tertiary interactions between nucleotides at distance in the secondary structure, in particular on a network of interactions defining and stabilizing the "central core" of the L-shaped structure. Fine-tuned chemical structural probing on two case studies, human mt-tRNA$^{Asp}$ and bovine mt-tRNA$^{Phe}$ confirmed the existence of these networks (Messmer et al. 2009; Wakita et al. 1994). It remains to be verified if such networks can take place in all mt-tRNAs, an hypothesis that could not be supported by nucleotide conservation, but that will need a more detailed consideration of nucleotide partnership rules as tackled by Leontis and Westhof (Leontis et al. 2002). Initial data are available suggesting that at least all mammalian mt-tRNA$^{Asp}$ would benefit from the set of tertiary network interactions despite nonconservation of involved nucleotides (Messmer et al. 2009). Extension of the analysis to the full set of mammalian mt-tRNAs is in progress. In regard of the elbow of the L, no hydrogen bonds could be detected between the D and T-loops, so that there may be no stabilization of the global L at this level. Accordingly, the angle formed by the two branches of the L may vary according to the tRNA, as also demonstrated by birefringence methods (Friederich and Hagerman 1997), and likely may vary according to its functional state, allowing for various structural adaptations to partner macromolecules (reviewed in Giegé et al. 2012). The role of post-transcriptional modifications in the global flexibility of the tRNA remains to be determined.

In summary, mammalian mt-tRNAs present a large diversity of structural types, ranging from canonical stable type for a few tRNA families only, to highly "bizarre" versions, characterized by an unusually high flexibility and plasticity of the full macromolecule, along an L-shaped-like global fold.

### *3.2.2 Comparison with Mitochondrial tRNA Genes from Other Metazoan*

Due to their peculiarities, metazoan mt-tRNA genes are difficult to detect with the available search programs such as tRNAscan-SE (Lowe and Eddy 1997) and ARWEN (Laslett and Canbäck 2008), so that an approach using INFERNAL and covariance models was developed and implemented (Nawroki et al. 2009). A first systematic overview on nearly 2,000 metazoan RefSeq genomes (Pruitt et al. 2007) and their tRNA gene content allowed for interesting insights on the evolution of mt-tRNA genes (Jühling et al. 2012a). An overview on tRNA genes deprived of information coding for a full structural domain of the expressed RNA (D- or T-arm), and

on loss of full mt-tRNA genes is illustrated in Fig. 3.1b. While mt-tRNA genes are encoded by mt-genomes over all metazoan clades, some organisms miss mt-tRNA genes and form exceptions. The loss of mt-tRNA genes was shown to be related with the loss of the corresponding aaRS genes in Cnidarians (e.g., jellyfish) (Haen et al. 2010). Members of Ceractinomorpha (sponges) (Wang and Lavrov 2008), Chaetognatha (arrow worms) (Miyamoto et al. 2010), and Rotifera (freshwater zooplankton) (Suga et al. 2008) also lost mt-tRNA genes. To be mentioned is the specific loss of $tRNA^{Lys}$ genes in Metatheria (e.g., marsupials) where only a pseudo-gene remains (Dörner et al. 2001). It is assumed that in these organisms, by nuclear tRNAs (Alfonzo and Söll 2009; Duchêne et al. 2009).

Highly truncated tRNA gene sequences are known for Onychophora (velvet worms) (Braband et al. 2010), where unusual editing mechanisms are assumed to repair the tRNAs (Segovia et al. 2011) while other genes are completely missing. Other mt-tRNA genes lost only small sequence stretches so that the canonical cloverleaf of the tRNA can form without the need of repairing mechanisms. However, more than 90 % of mt-tRNAs share a four-arm cloverleaf structure, and the 10 % remaining mostly lost either the T-or the D-stem and developed replacement loops. The corresponding genes are found throughout all metazoan clades but are locally conserved. Hotspots of organisms where the full set of mt-tRNA genes have lost a complete domain are Ecdysozoa (arthropoda and nematoda) and Lophotrochozoa (molluscs and worms). In contrast, in Lepidosauria (turtles, snakes, and lizards) only the genes for mt-$tRNA^{Cys}$ seem to spontaneously have lost the D-stem coding region along several parallel events (Macey et al. 1997; Seutin et al. 1994). Another well-known example of truncated mt-tRNA genes concerns Nematoda, where all mt-tRNAs lost their T-stem, except those of both $tRNA^{Ser}$, which instead lost their D-stem (Wolstenholme et al. 1994). While these truncated secondary structures in *C. elegans* are conserved within all other members of its family (round worms), a recent study on their sister group Enoplea detected even genes for “armless” tRNAs, namely sequences without both D- and T-stems (Jühling et al. 2012b). These sequences are conserved and are thereby the shortest functional tRNA sequences known so far (Jühling et al. 2012b). Compared to other metazoan mt-tRNA genes, mammalian genes lead to nearly canonical cloverleaf structures, and encode the full gene content with the exception of $tRNA^{Lys}$, which is missing in marsupials. However, hotspots of gene losses and of genes of truncated structures are distributed in other parts of the metazoan tree. Only the $tRNA^{Ser1}$ gene, coding for a tRNA deprived of a D-domain, shows equal distribution all along the metazoans (Arcari and Brownlee 1980; de Bruijn et al. 1980).

## 3.3 Mitochondrial aaRSs

### *3.3.1 Genes and Proteins*

Present-day mitochondrial genomes do not code for aaRSs, so that the full set of enzymes is necessarily nuclear encoded and that the expressed proteins become

imported into mitochondria. A set of specific genes for mammalian mt-aaRSs has been recorded (Bonnefond et al. 2005a). This set distinguishes from the set of cytosolic synthetases for most enzymes, with the sole exceptions of LysRS and GlyRS, coded by a same gene but with distinguishing features allowing for dual location of the protein. The gene for LysRS undergoes alternative splicing so that the two final mature proteins distinguish by a few N-terminal amino acids only (Tolkunova et al. 2000). GlyRSs are generated from two translation initiation sites on the same gene, so that the two mature proteins are the same, but differ by the presence or the absence of a mitochondrial targeting sequence (MTS) (Mudge et al. 1998; Shiba et al. 1994). A further exception concerns mt-GlnRS for which no specific gene was found so far. As an alternative to the existence of a specific mt-GlnRS, the possible import of the cytosolic enzyme was proposed (Rinehart et al. 2005) as well as the existence of an indirect pathway based on misaminoacylation of mt-tRNA$^{Gln}$ with mt-GluRS, followed by transamidation of the charged glutamic acid into glutamine. Such a pathway exists both in yeast (Frechin et al. 2009a) and human mitochondria (Nagao et al. 2009). In yeast, the dual localization of GluRS is controlled by binding to Arc1p, a tRNA nuclear export cofactor that behaves as a cytosolic anchoring platform. When the metabolism of the yeast cell switches from fermentation to respiration, the expression of Arc1p is down-regulated and this increases the import of GluRS to satisfy a higher demand of mt glutaminyl-tRNA$^{Gln}$ for mitochondrial protein synthesis (Frechin et al. 2009b).

Almost all mammalian mt-aaRSs have about the same size (once the MTS is removed upon import into mitochondria) and the same structural 2D organization as their homologs from the three kingdoms of life (Bonnefond et al. 2005a). The only exception is PheRS which is classically a tetramer ($\alpha 2\beta 2$) but only a monomer in mitochondria (Klipcan et al. 2008). Also, all mt-aaRSs contain the expected signature motifs for amino acid specificity and signature motifs of either class I or class II of aaRSs (Bonnefond et al. 2005a; Brindefalk et al. 2007; Sissler et al. 2005; Woese et al. 2000). The evolutionary origin of the different genes however remains intriguing. Sequence alignments have indeed not identified sequence stretches or signature motifs that could originate from alpha-proteobacterial ancestors, favoring various horizontal gene transfers events along evolution (Brindefalk et al. 2007).

During import into the mitochondria, the MTS is cleaved enzymatically. The detailed process is still under exploration for aaRSs but the trend for other proteins follows a two-step process. Once the polypeptide chain has crossed the mitochondrial membranes and entered the mitochondrion, a first cleavage occurs. This cleavage might be definite. Alternatively, the partially matured protein then localizes either in the matrix or anchors into (or at the surface of) the inner mitochondrial membrane before another part of the MTS is cut off. Interestingly, ribosomes and elongation factors have been found close to the inner membrane suggesting that probably the entire machinery required for protein biosynthesis is located there, and accordingly aaRSs too. A particularity of MTSs is their nonconserved length, sequence and amino acid compositions (e.g., Chacinska et al. 2009). So far, no reliable consensus sequence was found that could be used to accurately

predict the positions of maturation. This was highlighted by a wrongly predicted cleavage site of human mt-LeuRS, which led to a poor expression of a tentative mature protein in *E. coli* while a variant, shortened by further 39 N-terminal amino acids overproduced well in *E. coli* and was purified as an active enzyme while the one deprived of only 21 amino acids was insoluble (Bullard et al. 2000; Yao et al. 2003). Another well-documented example is human mt-AspRS. The predicted mature protein had a very low solubility when overexpressed in *E. coli* cells. Dynamic light scattering analyses revealed that aggregation proceeded during purification. A comparative analysis of a set of variants differing by their N-terminal sequences revealed that expression of the protein was actually enhanced when the N-terminus was extended by seven natural amino acids of the predicted mature N-terminus (Gaudry et al. 2012). The redesigned protein was highly soluble, monodisperse and functionally active in tRNA aminoacylation. It yielded crystals that were suitable for structure determination (Gaudry et al. 2012; Neuenfeldt et al. 2013). These results suggest that additional criteria should be taken into account for the prediction of the correct MTS cleavage sites and that the definition of the precise N-terminus of mature mt-aaRS should be determined experimentally.

### *3.3.2 Crystallographic Structures*

As already highlighted, despite various evolutionary origins of the genes coding for mammalian mt-aaRSs, several enzymes have a same modular organization than their bacterial homologs. This is illustrated in Fig. 3.2 with three of the four crystal structures that have been determined so far for mt-aaRSs for exclusive mitochondrial location (additional crystallographic structures are available for human GlyRS (Cader et al. 2007; Xie et al. 2006) and for LysRS (Guo et al. 2008), aaRSs of dual cytosolic and mitochondrial location). Bovine mt-SerRS, human mt-TyrRS, and human mt-AspRS show an overall architecture close to that of their prokaryotic homologs (Bonnefond et al. 2007; Chimnaronk et al. 2005; Neuenfeldt et al. 2013). Mt-PheRS is again the exception. Instead of forming complex heterodimeric assemblies as bacterial, archaeal, and cytosolic enzymes, it forms a two-domain monomer, which only maintains the catalytic domain characteristic of class II. This human mitochondrial version is the smallest known aaRS (Yadavalli et al. 2009).

Along with the reduction of the tRNA pool (22 in human mitochondria) and the simplification of identity rules, several of these enzymes have adapted the way they recognize their tRNA substrate, especially when the latter display a non canonical 2D fold leading to higher flexibility. Positively charged patches at the surface of mt-SerRS were redistributed to bind tRNAs lacking an extended variable region, the hallmark and major identity element of prokaryotic, eukaryotic, and archaeal $tRNAs^{Ser}$ (Chimnaronk et al. 2005). The three other mt-aaRSs display a more electropositive tRNA-binding interface, which may favor interactions

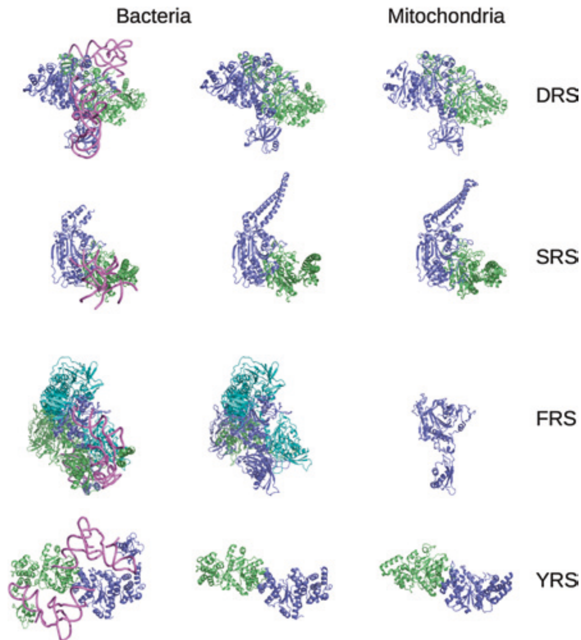


**Fig. 3.2** Cyrstal structures of mammalian mitochondrial aaRSs (bovine or human) and of their bacterial homologs (*E. coli -Eco-* or *Thermus thermophiles -Tth-*). On the left, bacterial complexes (PDBids: 1C0A for *Eco*DRS/tRNA, 1SRS for *Tth*SRS/tRNA, 2IY5 for *Tth*FRS/tRNA and 1H3E for *Tth*YRS/tRNA) are shown with monomers A in blue, monomers B in green and tRNAs in pink, indicating the binding site of the cognate substrate. In the case of tetrameric bacterial FRS, monomers C and D are depicted in cyan. On the right, free forms of bacterial aaRSs (1EQR for *Eco*DRS, 1SRY for *Tth*SRS, 1B7Y for *Tth*FRS, 1H3F for *Tth*YRS without its C-terminal domain) and mitochondrial enzymes (4AH6 for *Hsa*DRS2, 1WLE for *Bta*SRS2, 3TUP for *Hsa*FRS2, and 2PID for *Hsa*YRS2) are represented in the same orientation and same color code. Except *Hsa*FRS which exhibits a totally different structural organization (monomer instead of heterotetramer), mitochondrial aaRSs have retained the overall architecture of their bacterial relatives. Names of organisms are abbreviated in a three-letter code (e.g., *Homo sapiens*: *Hsa*). Mitochondrial enzymes are referred to by the addition of the number 2

with the sugar-phosphate backbone of the substrate to compensate for a reduction of specific contacts with identity elements (Neuenfeldt et al. 2013). The ability of mt-TyrRS, mt-PheRS, and mt-AspRS to aminoacylate heterologuous tRNAs (Bonnefond et al. 2005a; Klipcan et al. 2012; Neuenfeldt et al. 2013) indicates a much higher substrate tolerance, which may be linked to an increased structural plasticity. For instance, mt-PheRS undergoes a large movement of its anticodon-binding domain upon tRNA binding, switching from a closed to an open conformation (Klipcan et al. 2012). The higher thermal sensitivity of human mt-AspRS as compared *E. coli* AspRS, its more open catalytic groove in the absence of tRNA and the amplitude of thermodynamic terms associated with tRNA binding are also in favor of a more dynamic structure (Neuenfeldt et al. 2013). Altogether,

it appears that mt-aaRS properties have evolved to accompany the sequence and structure drift of mt-tRNAs. Enlarged intrinsic plasticity within a conserved architectural framework is one striking feature along this line. The underlying mechanisms enabling the crosstalk between nuclear and mitochondrial genomes remain to be explored.

## 3.4 tRNA/aminoacyl-tRNA Synthetase Partnerships in Mammalian Mitochondria

### *3.4.1 Aminoacylation of tRNAs, the Housekeeping Function of aaRSs*

The partnership of tRNAs and aaRSs is a key event in translation. Each synthetase recognizes specifically its tRNA or family of isoaccepting tRNAs, and esterifies its 3′-CCA end with the specific amino acid. The charged tRNA enables delivery of the amino acid to the ribosome where translation takes place. The aminoacylation reaction involves a two-step process including first the activation of the specific amino acid into an adenylate in the presence of ATP, and second, the specific recognition of the cognate tRNA followed by transfer of the activated amino acid (Ibba et al. 2005). Deciphering the detailed mechanisms of these steps for bacterial, archeal, and eukaryotic cytosolic aminoacylation has retained the attention of a large number of research groups over several decades (Ibba et al. 2005). Analysis of mammalian mitochondrial aminoacylation systems is only at initial stages. Due to the dual origin of the two partner macromolecules and to the diverging structural properties of mt-tRNAs, the mechanisms of reciprocal recognition and of co-evolution of these macromolecules deserve much attention.

### *3.4.2 Mammalian Mitochondrial Synthetases have Low Catalytic Activities*

Only a limited number of recombinant mammalian aaRSs have been obtained so far, allowing for biochemical and enzymatic characterization in vitro. As already reviewed elsewhere (Florentz et al. 2003; Suzuki et al. 2011) these enzymes present a 20- to 400- fold lower catalytic activity than their cytoplasmic and bacterial homologs. In the specific case of human mt-AspRS as compared to *E. coli* AspRS, the affinity for the substrate tRNA is higher by an order of magnitude as measured by isothermal titration calorimetry (ITC) while the affinity for an analog of the activated amino acid is of same level. However, the catalytic rate kcat for aminoacylation is 40-fold lower for the mt-aaRS (Neuenfeldt et al. 2013). The molecular reasons explaining the lower rate remain however elusive. Indeed,

overimposition of the catalytic sites in the crystallographic structures of both enzymes, lead to an important overlap (less than 2 Å rmsd) not allowing to pinpoint intrinsic differences inline with a different catalytic activity (Fender et al. 2006; Neuenfeldt et al. 2013).

### *3.4.3 Identity Elements in Mitochondrial tRNAs are Limited*

Specific recognition of tRNAs by aaRSs is driven by identity elements present in the tRNA (Giegé 2008; Giegé et al. 1998). These elements have been searched by mutagenic approaches on in vitro transcripts for a few mammalian mt-tRNAs (Florentz et al. 2003; Suzuki et al. 2011). Interestingly, while these sets are generally conserved along different organisms and even along kingdoms for a given amino acid specificity, they were found distinct in mt-tRNAs. A striking example concerns identity elements for aspartylation, one or the rare systems so far investigated. Major identity elements (elements for which strongest effects are observed upon mutation) are conserved all along evolution, as residues G73 (the so-called discriminator residue near the 3′- acceptor end), residue G10 in the D-stem, and residues G34, U35, and C36 forming the anticodon triplet. Transfer of this set of residues into host tRNAs of different specificities, converts these tRNAs into aspartic acid accepting species (Giegé et al. 1996). A mutagenic analysis performed on human mt-tRNA$^{Asp}$ revealed that only residues U35 and G36 are important elements for specific recognition and aspartylation by mt-AspRS while the other elements can be replaced by any other nucleotide without influencing the efficiency of tRNA recognition and aspartylation (Fender et al. 2006). Figure 3.3 illustrates this point. The striking non-importance of residue 73, otherwise highly conserved as G in tRNA$^{Asp}$ over all kingdoms of life, is a signature of mt-tRNA degeneration, and at the same time, of evolutionary adaptation of the synthetase. Deep insight into the structural environment of residue 73 in the catalytic site of human mt-AspRS reveals an enlarged space as compared to *E. coli* AspRS and other AspRS, allowing the fit of any of the four nucleotides, rather than the exclusive fit of a G residue at this position. A mutagenic analysis of the enzyme has confirmed this view (Fender et al. 2006). Another example of the peculiarity of identity elements in a mitochondrial system concerns human mt-tRNA$^{Tyr}$ (Bonnefond et al. 2005b). Base-pair G1-C72, forms an important identity element in archaeal and eukaryal tRNA$^{Tyr}$ (Bonnefond et al. 2005b). The mitochondrial tyrosine identity disobeys this rule, since mt-TyrRS is able to aminoacylate as well a tRNA with the G1-C72 pair as the opposite pair C1-G72. Other examples have been reviewed previously (Florentz et al. 2003) and will not be further discussed herein. They indicate that sequence analysis of mammalian mt-tRNAs lead to the conclusion that only a limited number of identity elements known for non mitochondrial aminoacylation systems are present (Florentz et al. 2003).

Despite the limited number of aminoacylation identity elements in mt-tRNAs, translation in mitochondria needs to be accurate so that the 13 synthesized proteins

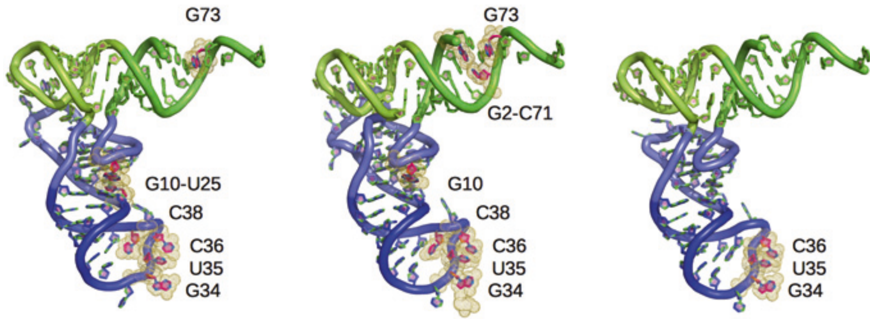


**Fig. 3.3** Evolution of $tRNA^{Asp}$ structure and identity elements. From the left to the right: $tRNA^{Asp}$ from the yeast *Saccharomyces cerevisiae* (conformation in the complex with AspRS, PDBid: 1ASY), from *E. coli* (conformation in the complex with AspRS, PDBid: 1C0A), from human mitochondria (homology model—(Messmer et al. 2009)). The three molecules are shown in the same orientation with the acceptor stem in green, the D stem loop in light green, the anticodon stem loop in dark blue, the variable loop and the T stem loop in light blue, respectively. Nucleotides defining the aspartate identity (Fender et al. 2012; Giegé et al. 1996) in each system are depicted in pink with a dotted surface. The human mitochondrial $tRNA^{Asp}$ is characterized by a shortening of D and T loops, leading to an absence of bases interactions at the corner of the L scaffold, and by a reduced set of identity elements

(all subunits of respiratory chain complexes that are partners of more than 80 nuclear encoded subunits) are prepared without mistakes. It is hypothesized that the small competition created by 22 tRNAs only toward about the same number of aaRSs in the mitochondrial environment, as compared to more complex translation machineries in bacteria or eukaryotic cytosol with several hundreds of tRNAs for 20 aaRSs, can deal with a restricted number of identity elements. Further, selection by the elongation factor EF-Tu of properly charged tRNAs only may represent an additional process toward accurate protein synthesis (Nagao et al. 2007), as is the case in bacteria (LaRiviere et al. 2001).

### *3.4.4 Unprecedented Plasticity of Mitochondrial aaRSs and tRNAs*

The discovery of mt-tRNAs that have lost critical structural information raised the question about how the nuclear-encoded mt-aaRSs have adapted to be able to deal with their partners? As already discussed, first insights were provided by resolution of crystal structures of mt-aaRSs (Bonnefond et al. 2007; Chimnaronk et al. 2005; Klipcan et al. 2008; Klipcan et al. 2012; Neuenfeldt et al. 2013). In the specific case of human mt-AspRS, a typical homodimeric bacterial-type AspRS, the 3D architecture is very close to that of the *E. coli* enzyme of same specificity, with the exception that it has a wider catalytic groove, a more electropositive surface potential, and an alternate interaction network at the subunits interface, a set of properties in line with

support to facilitated tRNA partnership. An additional biophysical property, namely thermostability, illustrates further the originality of the protein. Comparative differential scanning fluorimetry analyses indicated that the mitochondrial protein is far less stable with regard to temperature than its bacterial homolog. It has a 12 °C lower melting point (Neuenfeldt et al. 2013). These properties are summarized in Table 3.1.

The partnership of a mt-aaRS (human mt-AspRS) with its substrates was investigated by ITC, an approach allowing for direct measurement of affinity (Kd) and of the thermodynamic parameters $\Delta H$ (variation in enthalpy), $\Delta S$ (variation in entropy), and $\Delta G$ (variation in free energy) (Neuenfeldt et al. 2013). Comparative analyses between human mt-AspRS and *E. coli* AspRS revealed a one order of magnitude higher affinity of the mitochondrial enzyme for cognate and noncognate tRNAs (cross binding studies of mt-AspRS with *E. coli* tRNA, and of *E. coli* AspRS with mt-tRNA$^{Asp}$), but with highly different entropy and enthalpy contributions (Table 3.1). Binding parameters of the cognate mitochondrial partners requires far larger enthalpic and entropic contributions than binding of the cognate bacterial partners, underlining reciprocal reorganization along complex formation. Such an adaptation is still possible when the mt aaRS meets the bacterial tRNA but is not possible in the opposite situation, namely when the bacterial enzyme and the mt-tRNA face each other. Thermodynamics thus contribute to explain the well-known unilateral aminoacylation of bacterial synthetases for bacterial tRNAs (Kumazawa et al. 1991). Interestingly, ITC measurements of small substrate binding, revealed that both enzymes bind a synthetic analog of the aspartyl-adenylate by a cooperative allosteric mechanism between the two subunits of the dimeric enzymes, but again with different thermodynamic contributions (Neuenfeldt et al. 2013) (Table 3.1).

Altogether, presently available structural, biophysical, and thermodynamic data support the view of so far unsuspected greater flexibility of mt-aaRS with respect to its bacterial homolog albeit a common architecture. This gain in plasticity may represent an evolutionary process that allows the nuclear-encoded proteins to adapt to the structurally degenerated RNAs from organelles. Evolutionary induced changes in intrinsic properties of proteins, may thus represent an alternative to other strategies, such as those reported for the mitochondrial ribosome, where the strong restriction in RNA sizes is compensated by extension of the number and size of the nuclear encoded proteins (Willkomm and Hartmann 2006). If mt-aaRS do have partner proteins that might also contribute to improved recognition of degenerated tRNAs remains an open question.

## 3.5 Human Mitochondrial tRNA and Synthetases in Pathologies

### *3.5.1 Mitochondrial tRNAs and Human Pathologies*

In the last two decades, a large number of human neuromuscular and neurodegenerative disorders have been reported as correlated to point mutations in the mt-DNA encoded genes, with a large prevalence of mutations in tRNA genes

**Table 3.1** Major structural and functional differences between dimeric mt and bacterial aspartyl-tRNA synthetases in favor of a greater plasticity of the organelle enzyme

| Aspartyl tRNA synthetase | | | |
|---|---|---|---|
| Dimer surface | 20 additional basic residues | | (18 Lys + 2 Arg) |
| | Electrostatic potential more positive | | |
| Dimer interface | 70 versus 60 H bonds and 28 versus 20 salt bridges | | |
| | About 25 % less specific interactions per $Å^2$ | | |
| Thermal stability ($T_m$) | Alone | | 37 °C versus 50 °C |
| | Bound to cognate tRNA | | 40 °C versus 50 °C |
| | Bound to AspAMS | | 45 °C versus 55 °C |
| Thermodynamic parameters | | | |
| Cognate $tRNA^{Asp}$ | Kd | 0.26 versus 3.1 | μM |
| | ΔH | −20.3 versus −13.0 | kcal/mol |
| | ΔT | + 11.2 versus + 5.5 | kcal/mol |
| | ΔG | −9.1 versus −7.5 | kcal/mol |
| Noncognate $tRNA^{Asp}$ | Kd | 0.24 versus 22 | μM |
| | ΔH | −14.0 versus −30.8 | kcal/mol |
| | ΔT | + 5.0 versus +24.4 | kcal/mol |
| | ΔG | −9.0 versus −6.4 | kcal/mol |
| AspAMS (monomer 1) | Kd | 129 versus 29 | nM |
| | ΔH | −13.2 versus −5.5 | kcal/mol |
| | ΔT | + 3.9 versus −4.8 | kcal/mol |
| | ΔG | −9.4 versus −10.2 | kcal/mol |
| AspAMS (monomer 2) | Kd | 17 versus 3 | nM |
| | ΔH | −21.8 versus −8.2 | kcal/mol |
| | ΔT | + 10.6 versus −3.5 | kcal/mol |
| | ΔG | −10.6 versus −11.6 | kcal/mol |
| **$tRNA^{Asp}$** | | | |
| Nucleotide content | 90 versus 66 % A, U, and C | | |
| | 16 versus 7 out of 21 A–U and G–U base pairs | | |
| | D and T loops are not classical | | |
| Structural stability | ΔG | −22 versus −42 | kcal/mol |

Both polypeptide chains share 43 % identity between their amino acid sequences
Data compiled from (Fender et al. 2012) and (Neuenfeldt et al. 2013)

(reviewed for example in Florentz and Sissler 2003; Suzuki et al. 2011; Yarham et al. 2010; Ylikallio and Suomalainen 2012). Among the mutations leading to “mitochondrial disorders”, 232 are distributed all over the 22 tRNAs (Fig. 3.4; data from MITOMAP, a human mitochondrial genome database http://www.mitomap.org/MITOMAP). Most striking cases concern $tRNA^{Lys}$, $tRNA^{Leu(UUR)}$, and $tRNA^{Ile}$, which form “hot spots” for mutations. Mutations in these tRNAs are most frequently correlated with Myoclonus Epilepsy with Ragged Red Fibers (MERRF) (Shoffner et al. 1990) and Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes (MELAS) (Goto et al. 1990), respectively. However, this does not indicate a peculiar mutational susceptibility of the three mitochondrial genes, but is more likely due to systematic and intensive

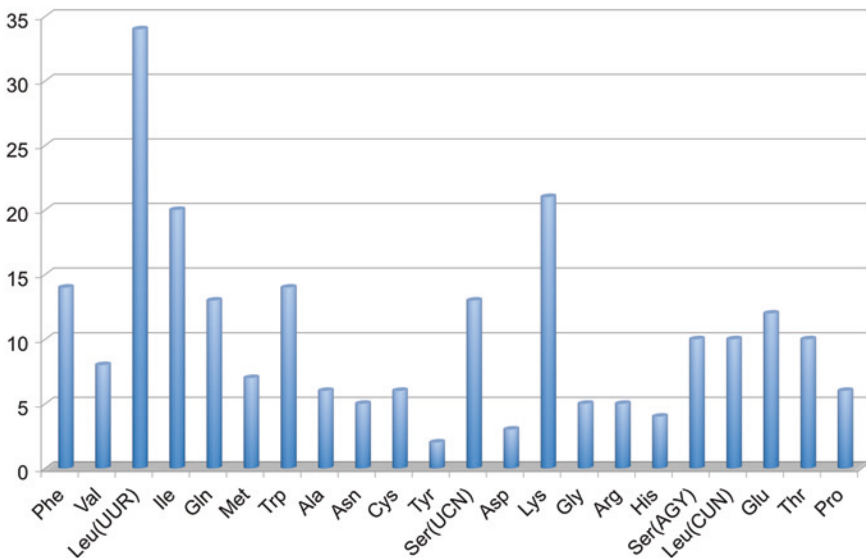


**Fig. 3.4** Pathology-related mutations in the 22 mt- tRNA genes. tRNA genes are indicated by the three-letter code of the corresponding amino acid and are sorted according to their location on mt-DNA. Each column corresponds to the number of different mutations reported so far. To be noticed, three additional mutations are reported in the precursor of $tRNA^{Ser(UCN)}$ and one additional mutation at the junction between $tRNA^{Gln}$ and $tRNA^{Met}$. Data are from MITOMAP, a database for mitochondrial genome mutations (http://www.mitomap.org/MITOMAP)

investigations of theses firstly reported examples of mt-tRNA genes correlated with human mitochondrial disorders.

The exponential rate of discovery of mutations in tRNA genes (3 mutations reported in 1990 and 232 mutations in 2012), together with the key role of tRNA in mitochondrial protein synthesis (linked to its global biology including gene expression, tRNA maturation, specific amino acid transfer, and regulation of these different functions), called for clarification of the molecular mechanisms for their pathogenicity. However, the relationships between genotypes and phenotypes appear very complicated since a given mutation can lead to a large variety of disorders of different severities (ranging from, e.g., limb weakness and exercise intolerance, to diabetes, leukoencephalopathy, encephalomyopathy, or fatal infantile cardiomyopathy, etc.). At the opposite, a given disorder can be linked to a variety of single point mutations in different tRNA genes. Also, each cell may contain hundreds to thousands copies of the mitochondrial genome, in a mixture of wild-type and mutated versions (heteroplasmic status). The variable distribution of affected tissues and the variable heteroplasmy levels lead to remarkable erratic and heterogenous clinical manifestations. Therefore, establishment of a mitochondrial disorder diagnosis can be difficult. It requires an evaluation of the family pedigree, in conjunction with a thorough assessment of clinical, imaging, and muscle biopsy analyses (McFarland et al. 2004). Also, a theoretical comparison of polymorphic

(neutral mutations with no pathogenic manifestations) versus pathogenic mutations remains unsuccessful to identify simple basic features (at the levels of primary and secondary tRNA structures) that would make possible the prediction of pathogenicity of new mutations (Florentz and Sissler 2001; Yarham et al. 2011).

Numerous studies have attempted to unravel the molecular impacts of the mutations on the various properties of the affected tRNAs and lead so far to a mosaïcity of impacts. It is now clear that mutations can affect any step of the tRNA life cycle, either along tRNA biogenesis (maturation of 3′- or 5′- ends within the initial primary transcript, synthesis of the non coded CCA end, post-transcriptional modifications, folding and structure, stability), or tRNA function (aminoacylation, interaction with translation factors). Several reviews summarize the present view (Florentz and Sissler 2003; Rötig 2011; Ylikallio and Suomalainen 2012). In most cases, the effects of mutations are mild and affect either a single step of the tRNA life cycle or a combination of several of them. However, an initial impact is frequently observed on structural properties of affected tRNAs, followed by subsequent cascade effects on downstream functions (Florentz et al. 2003; Levinger et al. 2004; Wittenhagen and Kelley 2003). Therefore, any insight on the precise rules governing secondary and tertiary folding of the full set of human mt-tRNAs remains of high importance in order to comprehend the high sensitivity of these tRNAs to mutations perturbing their structure. Along these lines, pioneered experiments (Helm et al. 1998; Messmer et al. 2009), combined with the implementation of dedicated database (Pütz et al. 2007), and the development of bioinformatics tools (Bernt et al. 2012; Jühling et al. 2012a) are opening the path toward a solid knowledge on mt-tRNA 3D structures.

Finally, the housekeeping function of tRNAs, namely their capacity to become esterified by an amino acid, is not systematically affected in mutated variants, so that alternative functions of mt-tRNAs (Hou and Yang 2013; Mei et al. 2010) or alternative partnerships have to be considered (Jacobs and Holt 2000; Giegé et al. 2012).

### *3.5.2 Mitochondrial aaRSs and Pathologies*

Lately, case-by-case reports linking mutations in nuclear genes coding for mitochondrial translation machinery proteins to pathologies (such as mutations in genes for elongation factor, tRNA modification enzymes, and ribosomal proteins), opened the way to “mitochondrial translation disorders” (Jacobs 2003). A new breakthrough took place in 2007 with the discovery in patients with cerebral white matter abnormalities of unknown origin of a first set of mutations present in *DARS2*, the nuclear gene coding for mt-AspRS (Scheper et al. 2007). These abnormalities were part of childhood-onset disorder called Leukoencephalopathy with Brain stem and Spinal cord involvement and Lactate elevation (LBSL; van der Knaap et al. 2003). Since this first discovery, mutations in eight additional mt-aaRS-encoding genes have been reported. They hit mt-ArgRS (Edvardson et al. 2007), mt-TyrRS (Riley et al. 2010), mt-SerRS (Belostotsky et al. 2011), mt-HisRS (Pierce et al. 2011), mt-AlaRS

(Götz et al. 2011), mt-MetRS (Bayat et al. 2012), mt-GluRS (Steenweg et al. 2012) and mt-PheRS (Elo et al. 2012) (Table 3.2). These recent correlations with human pathologies and the exponential description of reported cases, suggest as evidence that all mt-aaRS genes are likely affected by pathology-related mutations (that remain yet unveil), leading to a new family of disorders named according to the incriminated proteins namely "mt-aaRS disorders".

A detailed description and analysis of the full set of mutations in human mt-aaRS genes and their molecular and phenotypic implications has been reviewed (Konovalova and Tyynismaa 2013; Schwenzer et al. 2013). Here, the general outcomes are summarized. Table 3.2 recalls the main features characterizing the 65 nowadays-reported mutations in mt-aaRS genes. These include the type of pathogenic manifestation, familial pedigree, and affected tissues, as well as the number and types of mutations in each gene, their heterozygous versus homozygous, as well as the molecular impact on the synthetase and the final molecular impact on respiratory chain complexes. As a major outcome, it appears that whatever the mutation, no common combination of molecular steps correlates the mutations with the phenotypic expressions. Interestingly, the molecular impact of the mutations is not necessarily at the level of the housekeeping function of the synthetase, namely aminoacylation. Pathology-related mutations may have either a direct effect on the mitochondrial translation machinery by impacting one or several steps of mt-aaRS biogenesis and/or functioning. They may alternatively have an indirect effect by impacting ensuing steps and/or subsequent products activities [translation of the 13 mt-DNA-encoded subunits of respiratory chain complexes, respiratory chain complexes activities, and ATP synthesis]. Also, despite a dominant effect on brain and neuronal system is observed, sporadic manifestations are as well occurring in skeletal muscle, kidney, lung and/or heart. Along these lines, the selective vulnerability of tracts within the nervous system in case of mutations leading to splicing defects, for instance, is explained by tissue-specific differences in the concentration of the splicing factors (reduced in neural cell) (Edvardson et al. 2007; van Berge et al. 2012). However, the tissue-specificity of disorders remains an intriguing question. It is worth to establish the steady-state levels of various components of the mitochondrial translation machinery in different tissues, and correlate these levels with mitochondrial activity. This approach has been initiated by the evaluation of mRNA levels of the full set of human mt-aaRSs in 20 different human tissues (Fig. 3.5). A striking landscape of mRNA levels is observed highlighting tissue-specific differences by several orders of magnitude. There is no correlation between the various levels of mRNA and the amino acid content of the 13 mt-encoded proteins: leucine content is highest (14.4 %) followed by isoleucine, serine, and threonine (7 %), while arginine, aspartate, cysteine, glutamine, glutamate, and lysine contents is below 3 % (Schwenzer et al. 2013). We suggest that the low levels of aaRS mRNAs in brain, muscle and heart, lead to limiting mt translation activity in these tissues. Even a subtle change in mitochondrial translation efficiency may be detrimental in these tissues of high-energy demand. These data also suggest that mt-aaRS expressed to high levels may be involved in other functions than exclusively translation as is the case for cytosolic synthetases (Park et al. 2008).

**Table 3.2** Human mt-aaRSs involved in mitochondrial disorders

| | mt-AlaRS | mt-ArgRS | mt-AspRS | mt-GluRS | mt-HisRS | mt-MetRS | mt-pheRS | mt-SerRS | mt-TyrRS |
|---|---|---|---|---|---|---|---|---|---|
| Pathogenic manifestation[a] | CMP | PCH | LBSL | LBSL | PS | ARSAL | Encephatopathy | HUPRA | MLASA |
| Consanguinity of the parents | no | yes/no | yes/no | yes/no | no | no | yes/no | yes/no | yes/no |
| Affected tissues | Heart, brain, skeletal muscle | Brain | Brain, spinal cord | Brain | Ovarian sensori-neural system | Brain | Brain ± liver | Kidney, lung, | Blood, skelatal muscle |
| Number of mutations | 2 | 10 | 27 | 15 | 3 | ? | 4 | 1 | 2 |
| Non sense (frameshift/stop) | 0 | 1 | 8 | 3 | 0 | 0 | 0 | 0 | 0 |
| Missense | 2 | 7 | 14 | 11 | 2 | 0 | 4 | 1 | 2 |
| Deletion/insertion | 0 | 2 | 5 | 1 | 1 | Large insertion | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 0 | Gene duplication | 0 | 0 | 0 |
| Genetics compound | Heterozygous | Heterozygous/ homozygous | Heterozygous/ homozygous | Heterozygous/ homozygous | Heterozygous | Heterozygous/ homozygous | Heterozygous/ homozygous | Homozygous | Homozygous |
| *Negativly affected molecular event* | | | | | | | | | |
| AaRS encoding mRNA expression/ processing | nd | yes/no | yes/no | nd | no | no | nd | nd | nd |
| AaRS expression/ stability | nd | yes/no | yes/no | nd | yes/no | yes | nd | nd | yes/no |
| AaRS import/ oligomerziation/ structure | nd | nd | yes/no | nd | yes/no | nd | yes/no | nd | no |

(continued)

**Table 3.2** (continued)

| | mt-AlaRS | mt-ArgRS | mt-AspRS | mt-GluRS | mt-HisRS | mt-MetRS | mt-pheRS | mt-SerRS | mt-TyrRS |
|---|---|---|---|---|---|---|---|---|---|
| Aminoacylation activity | yes(predicated) | yes/no | yes/no | nd | yes | no | yes | yes | yes/no |
| *Impact on the respiratory chain complex* | | | | | | | | | |
| Global impact on translation | no | nd | no | yes | nd | yes | yes | nd | yes |
| Impact on RC activity[b] | yes | yes | yes | yes | nd | yes | yes | yes | yes |

[a] Pathogenic manifestations are presented under the following acronyms. *CMP* Infantile Mitochondrial Cardimyopathy, *PCH* Ponto Cerebellar Hypoplasia, *LBSL* Leukoencephalopathy with Brain stem and Spinal cord involvement and Lactate elevation, *PS* Perrault Syndrome, *ARSAL* Autosomal Recessive Spastic Ataxia with Leukoencephalopathy, *HUPRA* HyperUricemia, Pulmonary hypertensions and Renal failure in infancy and Alkalosis, *MLASA* Myopathy, Lactic Acidosis and Sideroblastic Anemia. Consanguineous state of the parents and affected tissues are recalled. The number and type of mutations as well as their genetic compound are given. Molecular effects and impacts on the respiratory chain complexes are displayed

[b] All possible molecular effects on either aaRS biogenesis and/or function, or on translation and/or activity of the respiratory chain complexes, have not necessarily been investigated for all reported cases. For a more detailed view, please refer to (Schwenzer et al. 2013). For negatively affected molecular events, "yes" means that all tested mutations show this defect; "no" means that all tested mutations show no defect; "yes/no" means that some show a defect some do not. The table displays a mean picture: (i) a defect in mRNA expression and in aminoacylation does not necessarily refer to a same mutation; (ii) impacts on translation and aminoacylation activity can originate from separate mutations; and (iii) RC defects do not refer to tissue specificity: translation defect may correspond to, e.g., fibroblasts while aminoacylation activity was measured in muscle cells. *nd* stands for not determined

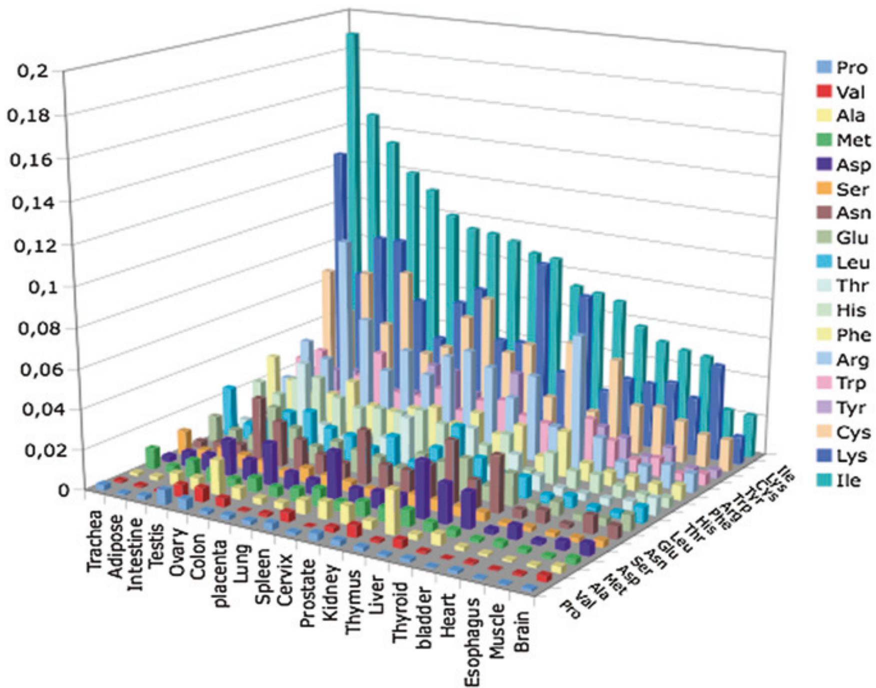


**Fig. 3.5** Dosage of mt-aaRS-encoding mRNAs in different human tissues. The amount of mRNAs coding for 18 mt-aaRSs is determined by quantitative PCR on cDNAs prepared from total mRNA extracted from 20 human tissues. The mRNA for LysRS codes both for the cytosolic and mt enzyme. Results obtained for GlyRS, highly expressed, are missing from the graph (cytosolic and mt GlyRSs are both encoded by a single nuclear gene). No gene for mt-GlnRS has been reported so far. Values are normalized against the standard expression of GAPDH. They are mean values out of at least three independent experiments, from which the standard deviation is close to 50 %

To conclude, links between the activity of a given mt-aaRS along mitochondrial translation on one hand and ATP production on the other hand, involved a number of issues that need to be further explored. Those issues should take also into account the possibility that aminoacylation may turn out to be not the sole function of mt-aaRSs in a living cell and that these enzymes may also participate in other processes and/or be implicated in various fine-tuning mechanisms as is the case for cytosolic aaRSs. Indeed, various bacterial and eukaryal aaRSs were found to have many additional functions (e.g., Guo and Schimmel 2013). It becomes thus necessary to determine all potential interacting components of mt-aaRSs and to study their dynamic location within the organelle. In other works, the functional network of mt-aaRSs and its regulation needs to be tackled. New routes toward understanding of the molecular impacts of point mutations in nuclear mt-aaRS genes outside the frame of mitochondrial translation should become opened along these lines.

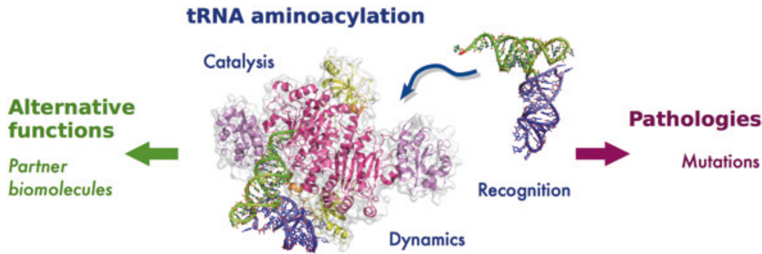


**Fig. 3.6** Mammalian mitochondrial tRNAs and aminoacyl-tRNA synthetases are key macromolecules in mitochondrial translation. They are also hot spots for a growing number of human pathologies, some of which being related to defects in the housekeeping functions, some not. Numerous questions both on fundamental knowledge ("order") and toward understanding of the molecular events underlying pathologies ("disorder") remain open. Both stimulate the development of new research lines outside the strict frame of mitochondrial translation

## 3.6 Conclusion and Perspectives

Mammalian mt-tRNAs and mt-aaRSs are known as key actors of mitochondrial translation, leading to the synthesis of 13 essential mitochondrial inner membrane proteins and subunits of respiratory chain complexes. The fact that these two families of partner macromolecules are coded by either of the two cellular genomes, the nuclear genome coding for the mt-aaRSs, and the mitochondrial genome coding for the mt-tRNAs, highlights different, but however connected, evolutionary pathways between RNAs and partner proteins allowing for tRNA aminoacylation. Despite important structural degeneration of mt-tRNAs linked to the high mutation rate of the mitochondrial genome, the conservation of "canonical" structural properties of mt-aaRSs include subtle but strong molecular adaptation so that the partnership between both macromolecules is maintained for accurate mitochondrial translation (Fig. 3.6). Detailed analysis of mammalian mt-tRNAs revealed new structural rules for RNAs, and bioinformatics compilations confirmed and enlarged the structural diversity of mt-tRNAs to the shortest versions ever discovered, calling for additional structural adaptation of functional RNAs. Investigation of mammalian mt-tRNAs and aaRSs are however still in an initial stage. Only a few systems have been characterized along a limited number of aspects. Open questions include for example, the common structural properties of mt-tRNAs and of synthetases all along metazoan mitochondria. Which are the identity elements in mt-tRNAs for specific aminoacylation by cognate mt-aaRS? Are these conserved or idiosynchratic? How far can a given set be degenerated and still allow for specificity? Post-transcriptional modification patterns of tRNAs and possible post-translational modifications of synthetases remain to become determined on full scale. The importance of these modifications is of crucial interest not only in structural stabilization and in the housekeeping codon reading function of mt-tRNAs, but also in alternative functions of mt-aaRSs. Post-translational modifications are indeed triggers to alternative functions of cytosolic aaRSs (e.g., Kim et al.

2012; Ofir-Birin et al. 2013). The related question of alternate function of mt-aaRSs is of key importance, especially in regard of understanding the molecular mechanisms of related disorders. Such functions, unrelated to aminoacylation, correspond to an emerging field of discoveries for cytosolic aaRSs. Examples include TyrRS involved in receptor-mediated signaling pathways associated with angiogenesis (Wakasugi et al. 2002a), TrpRS activated as an angiostatic factor (Wakasugi et al. 2002b), Glu-ProRS involved in the inflammatory response (Jia et al. 2008), LysRS plays a role in HIV-I packaging (Kleiman and Cen 2004) of GlyRS as anti-tumorigenic agent (Park et al. 2012).

The existence of an always growing panel of human disorders correlated to point mutations in either mt-tRNA genes or mt-aaRS genes, leads to investigations as to the molecular impacts of the mutations (Fig. 3.6). While a number of approaches pin-pointed a mosaicity of impacts on human mt-tRNA structure and function, and more recently on biophysical and functional properties of a small set of mt-aaRS, all linked to the housekeeping activity of both macromolecules in mitochondrial translation, a new field of investigation is emerging. Several of the pathology-related mutations are not located in the catalytic site of the protein, do not affect protein synthesis, and are thus indicative of new properties of mt-tRNAs and mt-aaRSs outside translation. Some hints on alternative functions have already been reported. Any route along this new topic deserves interest and should now be considered, as is currently the case for cytosolic tRNAs and synthetases (Guo and Schimmel 2013; Guo et al. 2010a, 2010b). Importantly, many other actors of the mammalian mitochondrial translation machinery also remain to be explored more systematically, not only for fundamental and evolutionary understanding but also because of their growing implication in human pathologies (e.g., Rötig 2011; Taylor and Turnbull 2005; Watanabe 2010).

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