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Mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are essential components of the mitochondrial translation machinery. The correlation of mitochondrial disorders with mutations in these enzymes has raised the interest of the scientific community over the past several years. Most surprising has been the wide-ranging presentation of clinical manifestations in patients with mt-aaRS mutations, despite the enzymes' common biochemical role. Even among cases where a common physiological system is affected, phenotypes, severity, and age of onset varies depending on which mt-aaRS is mutated. Here, we review work done thus far and propose a categorization of diseases based on tissue specificity that highlights emerging patterns. We further discuss multiple *in vitro* and *in cellulo* efforts to characterize the behavior of WT and mutant mt-aaRSs that have shaped hypotheses about the molecular causes of these pathologies. Much remains to do in order to complete our understanding of these proteins. We expect that futher work is likely to result in the discovery of new roles for the mt-aaRSs in addition to their fundamental function in mitochondrial translation, informing the development of treatment strategies and diagnoses.

Translation in mammalian mitochondria is unusual in many ways compared with the process in the cytosol and even compared with mitochondrial translation in simpler eukaryotes. The organelles, thought to be descendants of alphaproteobacteria, retain a DNA genome distinct from the eukaryotic cells (1), but extant mitochondrial genomes, especially in animals, are considerably smaller than those of bacteria. Mammalian mitochondrial genomes contain just 13 protein-encoding genes, all of which are components of the oxidative phosphorylation pathway (2). Production of even this small number of proteins requires a distinct mitochondrial translation apparatus (Fig. 1). Mammalian mitochondrial genomes encode all of the RNA components of this machinery: 22 mitochondrial tRNAs and two mitochondrial ribosomal RNAs. In contrast, all of the protein components, including tRNA maturation and modification enzymes, initiation and elongation factors, ribosomal proteins, and aminoacyl-tRNA synthetases, are encoded by the nuclear genome, translated in the cytosol, and then imported into the mitochondria (3, 4).
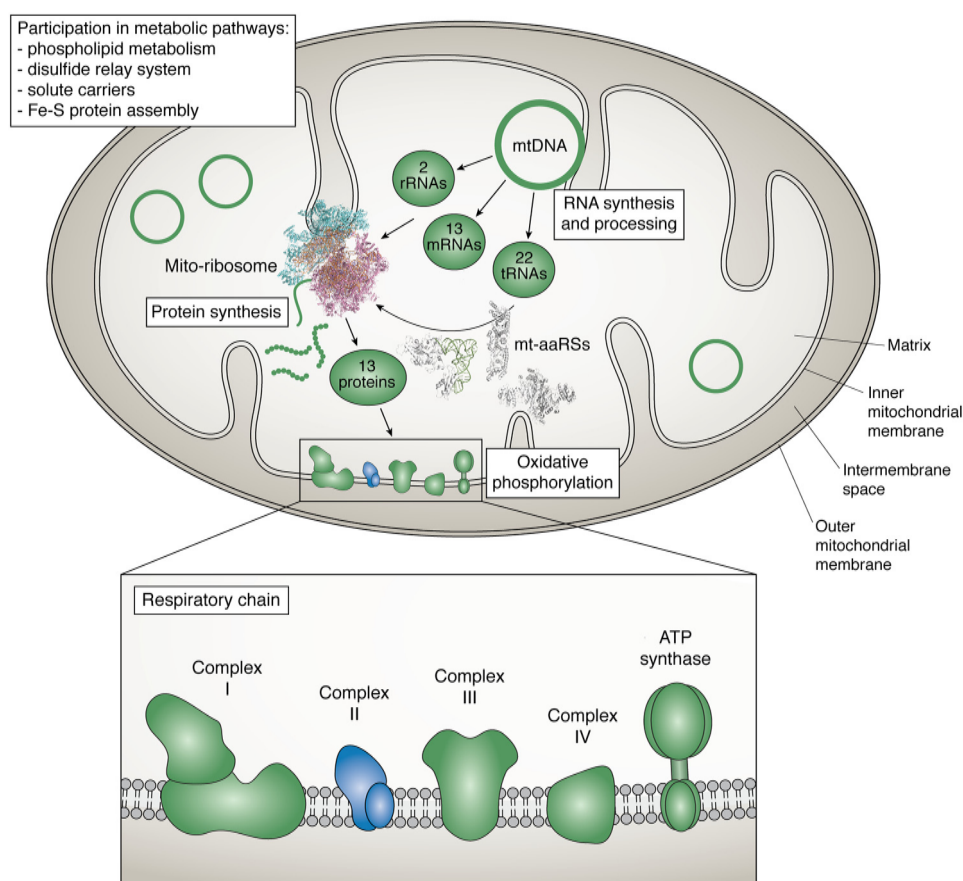
Some of the peculiarities of mitochondrial translation derive from the high mutation rate in the oxidizing mitochondrial environment and the correspondingly high mutation rate of mitochondrial DNA. For example, mammalian mitochondrial ribosomal RNAs are considerably truncated relative to their cytosolic homologs. Apparently to compensate for this change, the mitochondrial ribosomes contain increased numbers of proteins, resulting in a 2:1 protein/RNA ratio, inverted from the ratio typically found in bacteria (5–7). Mammalian mitochondrial tRNAs are also truncated and lack many conserved features typical of tRNAs in the rest of evolution (Fig. 2). In some cases, one of the arms of the classic cloverleaf secondary structure is lost, most frequently in mitochondrial tRNA[Ser] (8, 9). All mammalian mitochondrial RNAs are A:U-rich, probably as a consequence of the relative ease of oxidation of guanine nucleotides.

Given the mitochondria's central role in ATP synthesis via oxidative phosphorylation, it is not surprising that errors in mitochondrial translation have been linked with human disease (10–12). Mutations within mitochondrially encoded molecules of the translational machinery have been identified in patients since the late 1980s, leading to the presently recognized concept of "mitochondrial translation disorders," which include a large spectrum of clinical presentations, particularly muscular and neurological disorders (13–16). Initial work focused on mutations within the mtDNA, in either the rRNAs, the 22 mitochondrial tRNAs, or the 13 mRNAs. Although some correlations between particular mutations and distinct disease states were made (17, 18), tissue specificity and differences in symptoms and time of onset are most readily explained by heteroplasmy, nonhomogeneous mitochondrial populations in cells and tissues. The penetrance of a particular mutation within the multiple copies of mtDNA in any cell can vary from tissue to

Figure 1. Schematic representation of a mitochondrion. Mitochondria host numerous metabolic pathways. They are double-membrane organelles, hosting a distinct genome (mtDNA) and translation machinery, dedicated to the synthesis of 13 proteins, all subunits of the respiratory chain complexes (with representatives in all complexes except complex II). All additional proteins required for mtDNA maintenance and expression are encoded in the nucleus, synthesized in the cytosol, and subsequently imported into the mitochondria. This is, for instance, the case for proteins for the mito-ribosome and for mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs). Represented structures are the large (PBD code 4V19) and small (PBD code 5AJ3) subunits of the *Sus scrofa* mito-ribosome and the human mt-PheRS in complex with tRNA (PBD code 3TUP), mt-TyRS (PDB code 2PID), and mt-AspRS (PDB code 4AH6).

tissue in a random manner, resulting in idiosyncratic phenotypes (14).

More recently, it has been recognized that mutations in nuclearly-encoded mitochondrial proteins involved in translation are also correlated with mitochondrial diseases. In this review, we focus on the aminoacyl-tRNA synthetases (aaRS),[3] which play the crucial role of specifically aminoacylating mitochondrial tRNAs with their cognate amino acid. In humans, mitochondria-specific aaRSs exist for 17 of the 20 proteogenic amino acids (19). Genes for these proteins are generally designated as *ARS2*; for example, the mitochondrial alanyl-tRNA synthetase is designated *AARS2*. Exceptions are the glycyl-tRNA synthetase gene (*GARS*), which uses an alternate start sequence to encode both the cytosolic and mitochondrial enzymes (20, 21), and the lysyl-tRNA synthetase gene (*KARS*), which uses alternate splicing to generate distinct mRNAs (22). In both cases, the cytosolic and mitochondrial enzymes differ mainly in the presence or absence of an N-terminal mitochondrial-targeting sequence. Mitochondrial Gln-tRNA^{Gln} is

formed by transamidation of Glu-tRNA^{Gln} by a tRNA-dependent amidotransferase (23).

Pathogenic mutations in each of the 19 nuclear genes coding for a mitochondrial aaRS have been reported (24–29). Defects in the exclusively mitochondrial enzymes all have either homozygous or compound heterozygous presentations, giving rise to autosomal recessive disorders. Mutations in the dual-localized *GARS* and *KARS* genes have been reported with both recessive and dominant inheritance, giving rise to different clinical presentations. Autosomal dominant mutations in *GARS* and *KARS* affect the peripheral nervous system and are correlated with Charcot-Marie-Tooth disease type 2 (CMT2) (30). Recessive mutations in these genes, however, have been reported to produce phenotypes similar to those reported by mutations in exclusively mt-aaRSs (31, 32). Information about all reported pathogenic mutations in human mt-aaRSs has been compiled in a knowledge base we recently developed (33). The entry page of the website illustrates the apparently random distribution of the disease-related mutations within the different human mt-aaRSs.

Despite the fact that genes for mitochondrial aaRSs are nuclearly encoded and ubiquitously expressed, mutations give rise to a variety of distinct phenotypes (24–29). With a few exceptions detailed below, all mutations in a particular synthe-

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[3] The abbreviations used are: aaRS, aminoacyl-tRNA synthetase; mt-aaRS, mitochondrial aminoacyl-tRNA synthetase; CNS, central nervous system; PDB, Protein Data Bank; LBSL, leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation.

A Canonical tRNAs
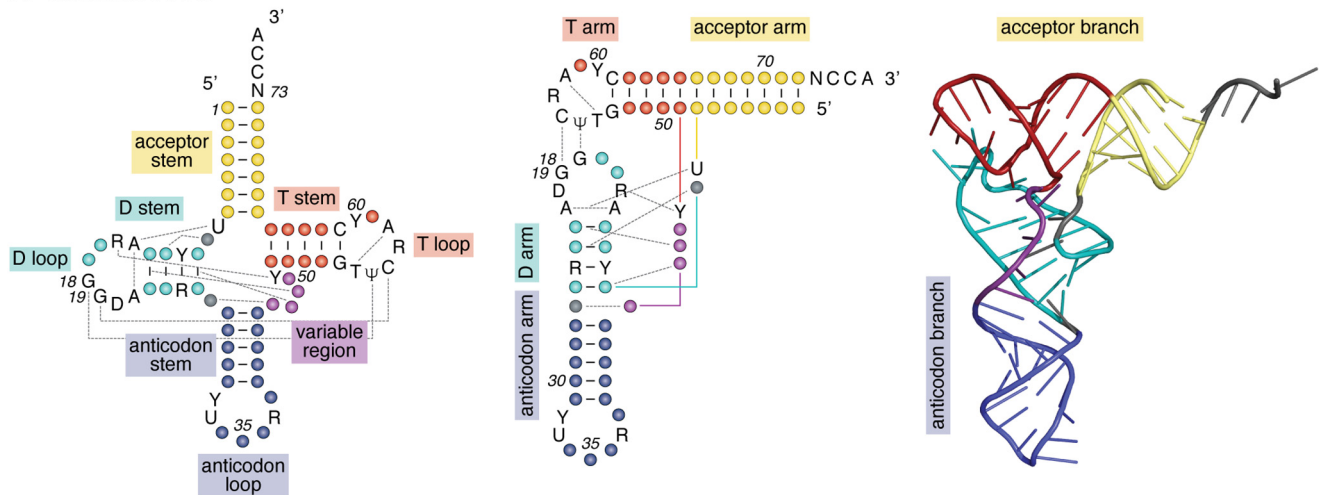
B Human mitochondrial tRNAs
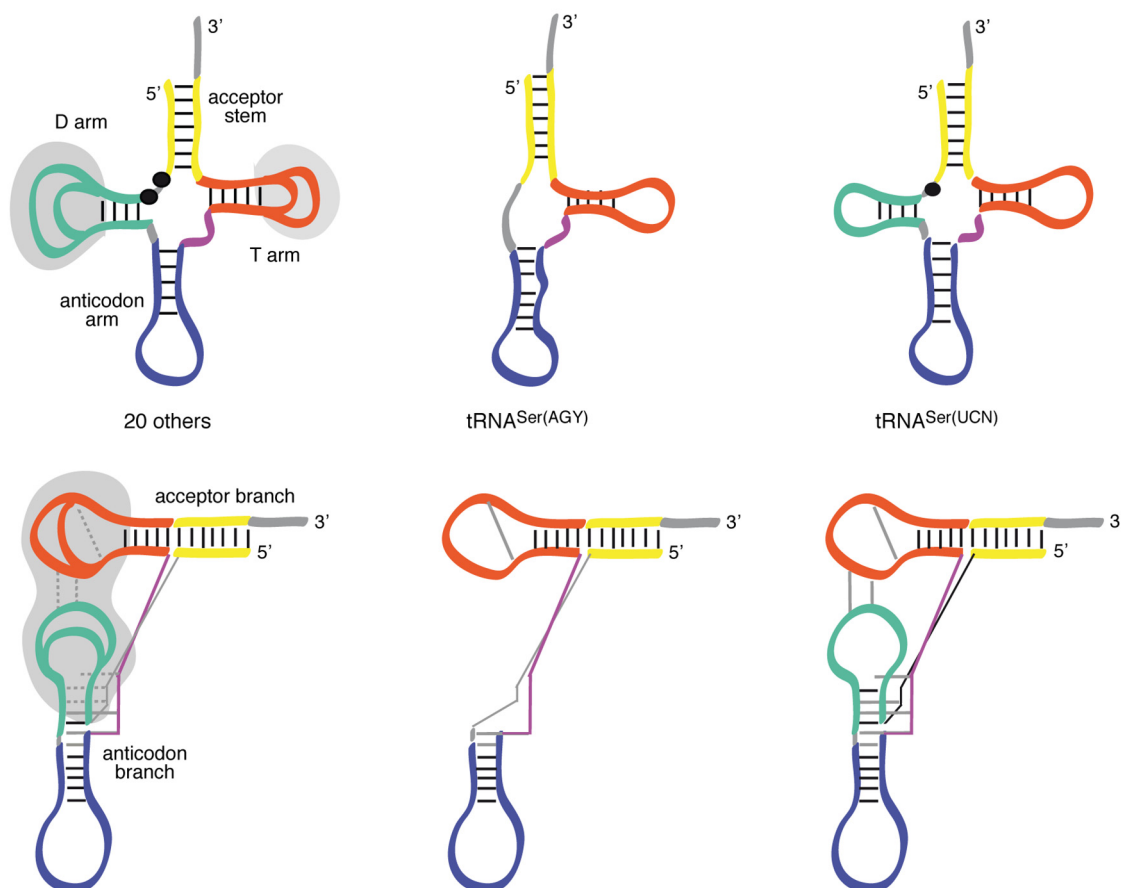
Figure 2. Canonical tRNAs *versus* human mitochondrial tRNAs. *A,* secondary and tertiary structures of canonical tRNAs. The different structural domains are named and *colored*. The network of tertiary interactions at the origin of the three-dimensional folding is represented by *black dashed lines*. The nucleotides indicated in *black* are those conserved in all tRNAs. *Y,* pyrimidine; *R,* purine; *A,* adenosine; *C,* cytosine; *G,* guanosine; *T,* thymine; *U,* uridine; and Ψ, pseudouridine. *Left,* cloverleaf consensus secondary structure of canonical tRNAs; *middle,* two-dimensional representation of tertiary refolding of tRNA; *right,* crystallographic structure of *S. cerevisiae* tRNA^Phe (PBD code 1EHZ). *B,* schematic representations of cloverleaf secondary structures (*upper part*) and 3D structures (*lower part*) of human mt-tRNAs. Schematic representations of the general structure of 20 tRNAs (*left*), of tRNA^Ser(AGY) missing the D-arm (*middle*), and of tRNA^Ser(UCN) displaying a shorter connector between the acceptor stem and the D-arm (*right*). *Dashed lines* correspond to nonstrictly conserved triple interactions. *Gray zones* highlight domains where variations in the number and type of interactions differ from tRNA to tRNA. This figure is adapted from Ref. 9.

tase result in similar disease states. These effects are manifested mostly in the central nervous system but also in a variety of other tissues. The available data present a number of surprising contrasts that complicate simple hypotheses based on the linkage between defects in mitochondrial translation and a reduction in cellular ATP production. Tissue-specific developmental

differences in energy requirements, connections with pathways for mitochondrial homeostasis associated with differences in intraorganellar localization, and alternative functions of the mitochondrial aaRS proteins are among the hypotheses currently under investigation.

Diversity of clinical manifestations

Disorders correlated with mutations in mitochondrial aminoacyl-tRNA synthetases span a broad range, including diseases characterized by defined symptoms and/or neuroradiological features (*e.g.* LBSL), isolated clinical signs (*e.g.* nonsyndromic hearing loss) to described syndromes (*e.g.* Perrault syndrome). Since the first description of a correlation between mutations in mt-aaRS– encoding gene and a human disease (34), the number of reported cases has increased steadily (33). In this section, we categorize mt-aaRSs according to the affected tissues and organ systems, including some details described in the medical reports that introduced these mutations into the literature. This physiological classification is intended to highlight similarities and differences in the pathological phenotypes that are not easily explained at a molecular level.

Four main groups emerge (Table 1 and Fig. 3): mt-aaRSs with mutations leading to clinical manifestations (i) exclusively in the central nervous system (CNS); (ii) in the CNS *and* another system; (iii) in the CNS *or* another system, and (iv) a system other than the CNS.

The first group, containing mt-aaRSs with mutations leading to clinical manifestations in exclusively the CNS, is further subdivided into those causing mainly epileptic encephalopathies and those causing leukoencephalopathies. Epileptic encephalopathies are observed in patients with mutations in *CARS2*, *FARS2*, *NARS2*, *PARS2*, *RARS2,* and *TARS2.* Epilepsy, which can present either as myoclonus, spastic, or focal seizures, is the common clinical manifestation. Leukoencephalopathies are observed in patients with mutations in *DARS2*, *EARS2*, *MARS2*, and *WARS2.* Changes in the white matter are the main hallmark in the diagnosis of the disease. These mutations manifest in patients mostly as ataxia, predominantly in the lower limbs. The appearance of these neurological clinical symptoms may be due to demyelination.

The second group, where defects are observed in both the CNS and another organ system, includes some patients with *AARS2* mutations (those leading to leukodystrophy) and all reported patients with mutations in *HARS2*, *LARS2*, *IARS2*, and *VARS2.* Although this group is clinically distinct, it has been suggested that secondary symptoms are the result of a primary defect in the CNS (28). For example, the ovarian failures in Perrault syndrome (correlated with mutations in *HARS2* and *LARS2*) and the ovarian failure in female patients with *AARS2* mutations correlated with leukodystrophy are likely induced by a primary dysfunction in the pituitary gland, the hormonal center responsible for the correct ovarian function. In the case of patients with mutations in *IARS2,* defects in growth hormone production (by the pituitary gland at the level of the CNS) may cause injuries in the musculoskeletal system, explaining the skeletal dysplasia syndrome (35). In *VARS2*-related patients, cardiomyopathy is proposed to result from an encephalopathy

that primarily produces hypotony (36). We suggest that this hypotonia causes stronger heart contractions, which is the underlying cause of the hypertrophic cardiomyopathy.

The third group, where mutations lead to effects in either the CNS or another system, includes only *SARS2* mutations. Some of the *SARS2*-related patients have the HUPRA syndrome (hyperuricemia, pulmonary hypertension, renal failure, and alkalosis), with injuries in the kidneys that in most cases lead to renal failure (37). Other *SARS2*-related patients manifest with neurological clinical symptoms (not shown in the Fig. 3 because the reported mutations are splicing defects for all those patients), which lead to progressive spastic paresis (increased muscle tone) (38). Interestingly, the two sub-groups of patients do not present overlapping clinical symptoms and have distinct sets of mutations.

The last group includes *YARS2*-related patients and some of the *AARS2*-related patients, with presentations of myopathy and cardiomyopathy, respectively. None of these patients have clinical manifestations in the CNS. Again, cardiomyopathy-related mutations of mt-AlaRS are distinct from the leukodystrophy-related mutations.

As mentioned above, heterogeneity exists within these four groups. For example, among the mutations that affect the CNS, there is a strong correlation between early onset of disease and the severity of the clinical symptoms, illustrated by the contrast between *DARS2*-associated leukoencephalopathies, which present as LBSL disease, and *RARS2*-associated epileptic encephalopathy, which presents as Pontocerebellar hypoplasia type 6 (PCH6). LBSL patients usually develop movement problems during childhood or adolescence, but in some cases, the clinical manifestations do not appear until adulthood. Symptoms presented by individuals with LBSL are mainly spasticity (muscular stiffness) and ataxia (difficulty with coordinating movements). These conditions tend to affect the legs more than the arms. In the most severely affected patients, the use of wheelchair assistance is required (39). In contrast, PCH6 patients manifest the symptoms soon after birth with, in most cases, intractable seizures and recurrent apnea (40). Other neurological signs include generalized hypotonia, microcephaly (unusually small head size, caused by impaired growth of some parts of the brain), lethargy, poor suckling, and poor feeding. The most heavily affected patients live only into infancy or childhood, and they never achieve developmental milestones (41). Patients with *RARS2* mutations usually manifest symptoms soon after birth, with severe seizures that tend to evolve into epileptic status. In contrast, the later the symptoms become present in LBSL patients, the milder the symptoms (*e.g.* weakness in the lower limbs).

This relationship between early onset and severity of symptoms is observed in other cases as well. In patients with *YARS2* mutations that present <u>m</u>itochondrial myopathy, <u>l</u>actic <u>a</u>cidosis, and <u>s</u>ideroblastic <u>a</u>nemia (MLASA) mortality was usually a consequence in patients with early onset. However, some exceptions have been noted; for instance, one *YARS2*-related patient with early onset showed spontaneous improved muscle strength and stamina at the age of 17 years and no longer required blood transfusions (which had previously been given every 6 weeks) (42).