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Opinion

Recent Advances in Mitochondrial Aminoacyl-tRNA Synthetases and Disease

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Dysfunctions in mitochondria – the powerhouses of the cell – lead to several human pathologies. Because mitochondria integrate nuclear and mitochondrial genetic systems, they are richly intertwined with cellular activities. The nucleus-encoded mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are key components of the mitochondrial translation apparatus. Mutations in these enzymes predominantly affect the central nervous system (CNS) but also target other organs. Comparable mutations in mt-aaRSs can lead to vastly diverse diseases, occurring at different stages in life, and within different tissues; this represents a confounding issue. With newer information available, we propose that the pleiotropy and tissue-specificity of mt-aaRS-associated diseases result from the molecular integration of mitochondrial translation events within the cell; namely, through specific crosstalk between the cellular program and the energy demands of the cell. We place particular focus on neuronal cells.

Trends

Mutations in nucleus-encoded mt-aaRSs impact on tissues with high energy demand, notably the CNS.

Disease-associated mutations occur at positions rarely conserved in phylogeny, but some occur at positions that are highly conserved in mammals.

These observations point to complex molecular origins with either direct and/or indirect effects on the efficiency of mitochondrial translation and/or alternative functions played by mt-aaRSs.

We propose that the pleiotropy and tissue-specificity of these disease-associated mutations reflect the integration of mitochondrial translation processes within cellular homeostasis; these may occur through specific crosstalk between the cellular program and cellular energy demands, especially in neuronal cells.

Among the multiple communication pathways between mitochondria and the nucleus, the mitochondrial unfolded protein response may play a role in the integrated response.

Human Mitochondrial Translation: Relevance to Disease

Human mitochondria host numerous essential metabolic pathways, including cellular energy production in forms of ATP via **oxidative phosphorylation** (OXPHOS, see Glossary), carried out by proteins of **respiratory chain complexes**. Thirteen of these proteins are encoded within **mitochondrial DNA** (mt-DNA) and are synthesized via the mitochondrial translation machinery, while 84 additional proteins are encoded by the nuclear genome [1]. This skewed genomic distribution also applies to molecules of the mitochondrial translation machinery, given that all required RNAs (two rRNAs and 22 tRNAs) are encoded by mt-DNA, and all the necessary proteins (ribosomal proteins, tRNA modifying enzymes, translation factors, aminoacyl-tRNA synthetases, etc.) are encoded by the nuclear genome, synthesized within the cytosol, and imported into mitochondria [2,3]. Noteworthy, the nuclear and mitochondrial genomes accumulate mutations at different rates [4,5], which can lead to a large-scale reduction or alteration of mt-DNA-encoded RNA components that still need to be recognized by nucleus-encoded proteins [6]. From the more than 1000 proteins imported within mitochondria, only some are necessary for translation [1]. Presently, the network of interactions between proteins involved in translation with those involved in non-translation is poorly characterized.

Pathologies associated with mitochondrial dysfunctions involve a large number of genetic causes with different inheritance mechanisms – either maternally inherited mutations in RNAs and proteins encoded by mt-DNA, or Mendelian inherited mutations of proteins encoded by the nuclear genome [7–9] (Box 1). Mutations in proteins involved in mitochondrial metabolic processes and directly affecting mitochondrial central functions such as ATP production have been known and studied for some time [10,11]. However, mitochondrial disorders linked to

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Box 1. Genetics of mt-aaRSs and mt-tRNAs Mutations

All mutations reported have been identified by genomic sequencing. Mutations in mt-tRNA occur in mt-DNA and follow the maternal inheritance of mitochondria. The copy number of mitochondria in human cells is tightly regulated and varies enormously depending on the cell type [68]. Mutations in mt-DNA generally affect only a proportion of mt-DNA copies (termed heteroplasmy, in contrast to homoplasmy where all mt-DNA copies are identical [28,29]). The appearance of the disease state depends on the proportion of mutated versus wild-type mt-DNAs and on the amount of available functional gene products (biochemical threshold). The affected organ system(s), in this situation, depend(s) on the random segregation of mutated mt-DNA during mitotic division (mitotic segregation). By contrast, mt-aaRS mutations occur in nuclear DNA and present a Mendelian pattern of inheritance. Up to now, the observed mutations exhibit **recessive inheritance**. Thus, reported mutations lead to autosomal (i.e., affecting any chromosome other than a sex chromosome) recessive disorders. Patients are mainly **compound heterozygotes**, although some have been reported to be **homozygotes**.

Clearly, missense mutations in structurally/functionally key regions of the protein (evolutionary conserved positions; Box 4) are lethal and are not available for study. However, in the heterozygous compound state a disease might be observed when mutations affecting both alleles lead to mild functional defects, or when a 'strong' mutation (e.g., a premature stop) is coupled to a splicing defect in the second allele that leaves some remaining/residual functional activity [30,67]. In short, the cumulative effects of two mutations should maintain sufficient gene products with some degree of activity. The overall threshold level for the degree of activity that must remain so as to be observable may depend on the onset of the disease stage (**early-onset** vs **late-onset disease**) and on the cell type. In addition, the window around the threshold may vary with the mutational effects and the type of mt-aaRS. For example, in LBSL patients, the window starts when the symptoms become detectable, reaching a level that is still compatible with a life-expectancy of >20 years [30]. By contrast, for mt-ArgRS mutations, which cause severe early-infantile epileptic encephalopathy, the window is narrow around the threshold [69].

mutations in various components of the mitochondrial translation machinery potentially affecting ATP production indirectly, are being reported at an increasing rate [12–14]. Mitochondrial tRNAs (mt-tRNAs) and mt-aaRSs constitute two families of molecules that are raising particular interest in disease because of their functional connection (one being a substrate of the other in the **aminoacylation** reaction) [15,16]. In the following we first illustrate the diversity of affected anatomical systems related to mutations within these two types of molecules. We then focus on mt-aaRSs as key players in the mitochondrial translation machinery (Figure 1, Key Figure). An overview of the structure and function of aaRSs is provided in Box 2. The first description of a series of mutations in an mt-aaRS causing a particular **leukodystrophy** dates back to 2007 [17]. In only 10 years, mutations in each of the nuclear genes coding for the 19 mt-aaRSs have now been correlated to human diseases, with pleiotropic clinical manifestations [18–20]. Our stance follows two tracks: first, we propose that the effects of mutations on the translation machinery are subtle and difficult to identify, essentially appearing within a neuronal context in the CNS; second, that alternative biochemical pathways – some of which are yet to be discovered – may be involved in differentially modulating mt-aaRS functions. We also attempt to organize the available data, analyzing reported mt-aaRS mutations in the context of protein architecture and in terms of evolutionary amino acid conservation of affected positions. We aim to delineate the boundaries that define the potential molecular origins of these diseases and suggest directions for future research along several lines that should be integrated to promote possible therapeutic strategies.

Diseases Associated with mt-aaRSs and mt-tRNAs

The diversity of pathologies linked to mutations in mt-aaRSs and mt-tRNAs display a marked bias for the CNS (Figure 2). Indeed, mutations in all mt-tRNAs and in 17/19 mt-aaRSs (the exceptions being mt-TyrRS and GlyRS) are reported to cause CNS damage [21,22]. This apparent convergence does not, however, hold true when considering the clinical manifestations. For example, mutations in mt-AspRS and $tRNA^{Asp}$ involve pathologies linked to the CNS; nevertheless, mutations in mt-AspRS result exclusively in **LBSL disease** [23], while mutations in mt-$tRNA^{Asp}$ lead to **sporadic bilateral optic neuropathy** [21], **mitochondrial myopathy** [24], **myoclonic epilepsy**, and **psychomotor regression** [25].

Glossary

Aminoacylation: an enzymatic reaction involving the attachment of an amino acid to its isoaccepting tRNA carrying an anticodon complementary to the codon specifying that amino acid according to the genetic code. It is a two-step reaction involving amino acid activation and transfer of the activated amino acid to the cognate tRNA(s) catalyzed by an aminoacyl-tRNA synthetase (aaRS).
Catalytic motifs: highly conserved residues necessary for catalysis (aminoacylation) to occur. These motifs are **HIGH** and **KMSKS** (or close derivatives) for class I aaRSs; and motif 1 (. . . **P** . . .), motif 2 (. . . F**R**XE/D . . .) and motif 3 (. . . GXGXGXE**R**FFFFF . . .) for class II aaRSs, where F, X, or **bold** characters stand for hydrophobic, non-conserved, or strictly conserved residues, respectively.
Cardiomyopathy: a group of diseases affecting the heart muscle, stemming from multiple causes, for example mitochondrial cardiomyopathy is caused by mitochondrial dysfunction.
Charcot–Marie–Tooth (CMT) disease: an inherited motor and sensory peripheral neuropathy (peripheral nerves in the nervous system are affected); patients present with muscle loss, weakness, and sensory nerve damage in the hands and feet.
Compound heterozygosity: the two alleles of a gene are not identical at the two chromosomal loci.
Early-onset disease: in the case of mitochondrial dysfunction, a disease that strikes immediately or a few hours after birth.
Homozygosity: alleles for a gene are identical on both chromosomes.
HUPRA syndrome: hyperuricemia, pulmonary hypertension, renal failure in infancy, and alkalosis – a severe mitochondrial autosomal multisystem disorder with early-onset presentation. Patients manifest renal failure leading to metabolic alkalosis (high pH in serum), pulmonary hypertension (high blood pressure that affects the arteries in the lungs and the right side of the heart), hypotonia (decrease in muscle tone), and developmental delay.
Hyperuricemia: excess uric acid levels in the blood.

Box 2. The Aminoacyl-tRNA Synthetases: From Housekeeping to Moonlighting Function

aaRSs are ancillary/housekeeping proteins whose main recognized function is to perform aminoacylation of cognate isoaccepting tRNAs. They generally comprise a catalytic domain and a tRNA anticodon-binding domain, but other functional domains are occasionally added. While the 20 aaRSs differ widely in size and oligomeric state (from monomer to tetramer), they have been partitioned into two classes based on the fold of the catalytic domains (Rossman fold for class I, and an antiparallel β-sheet flanked by α-helices for class II), on the signature **catalytic motifs**, and on the amino acid mode of linkage with either the 2′-hydroxyl or the 3′-hydroxyl of the terminal A of tRNA for class I (ValRS, IleRS, LeuRS, MetRS, CysRS, TyrRS, TrpRS, GluRS, GlnRS, and ArgRS) or class II (SerRS, ThrRS, ProRS, HisRS, AspRS, LysRS, AsnRS, PheRS, AlaRS, and GlyRS) aaRSs, respectively [70,71]. Most class I aaRSs are monomers and class II aaRSs dimers (note that class I TyrRS and TrpRS are dimers and the tRNAs straddle each monomer for recognition). Usually, there is one specific tRNA synthetase for each of the 20 amino acids. However, as in some bacteria (and most archaea), Gln-mt-tRNA^{Gln} is made in human mitochondria by transamidation of Glu-mt-tRNA^{Gln} through the action of an amidotransferase, in agreement with the fact that no gene for mt-GlnRS has been found [72].

In humans, there are two sets of distinct nuclear genes that code for either the cytosolic aaRSs or the mitochondrial aaRSs [16]. The only two exceptions concern GlyRSs and LysRSs, for which cytosolic and mitochondrial forms (differing by the absence or the presence of a mitochondrial targeting sequence, respectively) are encoded by single genes, but are generated either from two translation initiation sites or by alternative mRNA splicing, respectively [73–75]. Interestingly, human cytosolic aaRSs have been recognized to participate in a diversity of alternative (moonlighting) functions beyond translation, connecting them to other cellular activities. Examples include enzymes that operate in pathways linked to, for example, angiogenesis, immune responses, inflammation, tumorigenesis, or neuronal development (reviewed in [43,44]). The functional switch from canonical to non-canonical roles can be achieved by alteration of the polypeptide fold, alteration of surface properties (post-translational modification(s), other), or by atypical cellular organization (different cellular location, relocated in the nucleus or secreted, different macromolecular complex organization, different partnership, etc.) (reviewed in [76,77]). Recently, a first description of an alternative function for a mt-aaRS has been identified: a pro-angiogenic function for rat mt-TrpRS [63]. This suggests that other mt-aaRSs may also play alternative roles in the cell.

Remarkably, the diversity of pathologies is much wider for mutations in mt-tRNAs than in mt-aaRSs. In the CNS-related pathologies, eight mt-aaRSs lead to encephalopathies (mt-ArgRS, mt-AsnRS, mt-CysRS, mt-IleRS, mt-PheRS, mt-ProRS, mt-ThrRS, mt-ValRS), four to **leukodystrophies** (mt-AlaRS, mt-AspRS, mt-GluRS, mt-MetRS), and two to **Perrault syndrome** (mt-HisRS, mt-LeuRS). Mutations also result in isolated pathological conditions such as hearing loss or deafness (mt-MetRS, mt-AsnRS), and intellectual disability (mt-ArgRS, mt-TrpRS). For pathologies not affecting the CNS, three aaRSs provoke **cardiomyopathies** (mt-AlaRS, GlyRS, LysRS), one the **MLASA syndrome** (mt-TyrRS), and one the **HUPRA syndrome** (mt-SerRS). By contrast, mutations in mt-tRNAs are connected with ~50 pathological disorders (and about half are linked to the CNS) displaying a broad clinical spectrum, ranging from mitochondrial myopathy to neurogastrointestinal syndrome, **myoglobinuria**, diabetes mellitus, hypertension, and **Kearns–Sayre syndrome** (see Table S1 in the supplemental material online).

Another outcome is reflected by the contrast between the multiple impacts of mutations in mt-tRNAs versus the singular impact of mutations in mt-aaRSs. Indeed, not only does every mutated mt-tRNA have a broad impact in different major **organ systems**, but each is also linked to more than two diseases [e.g., mutations in mt-tRNA^{Pro} manifest as **MERRF-like disease**, mitochondrial myopathy, or dilated cardiomyopathy) [21]; and, in addition, a mutation in the same tRNA nucleotide can lead to several disorders (e.g., the mutation A5814G in mt-tRNA^{Cys} manifests either as severe encephalopathy or cardiomyopathy) [26,27]. This peculiarity is attributed to the random distribution of heteroplasmic pools of mt-DNA during mitotic segregation [28,29] (Box 1) and, by contrast, is not observed for mt-aaRSs. For 13 of the mt-aaRS enzymes, mutations can lead to a single pathology (e.g., mutations in mt-ProRS lead exclusively to epileptic encephalopathy). There are exceptions where multiple system injuries might be explained by collateral damage, as in the case of mutations in mt-SerRS that are associated with HUPRA syndrome. In this clinical scenario, renal failure (primary injury) might be the cause of **hyperuricemia** and pulmonary hypertension (secondary injuries). When a single mt-aaRS is associated with two unrelated clinical

Isodecoder: tRNA isodecoders share the same anticodon triplet but present sequence differences in other tRNA regions. tRNA isodecoders and tRNA isoacceptors (varying at the anticodon triplet), are charged with the same amino acid. In the human nuclear genome there are more than 270 isodecoder genes (~450 tRNA genes distributed among 49 isoacceptor families).
Kearns–Sayre syndrome: a multisystem disorder mainly affecting the eyes (weakness of the eye muscle and loss of vision, among others). Patients can also present cardiac conduction problems and ataxia.
Late-onset disease: refers to cases where symptoms of a disease strike in childhood or later (early adulthood).
LBSL disease: leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation – a progressive leukodystrophy disorder that affects the white matter of the brain and spinal cord; patients also present elevated lactate levels. Ataxia and spastic paresis are primary clinical symptoms.
Leukodystrophies: a group of disorders affecting white matter in the CNS.
Mitochondrial DNA (mt-DNA): small circular double-stranded DNA; in humans mt-DNA comprises 16 569 bp, coding on both strands for only 37 genes: 13 protein subunits of the respiratory chain complexes, two ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs). The only non-coding region is the displacement loop (D-loop), also called the control region.
MERRF-like disease: myoclonic epilepsy with ragged-red fibers – a mitochondrial disorder with onset in childhood that affects the CNS and skeletal muscle. The main feature is myoclonus, but patients can also manifest other clinical signs including muscle weakness, ataxia, seizures, dementia, degeneration of the optic nerve, hearing loss, and cardiomyopathy, among others.
Mitochondrial myopathy: mitochondrial disorder manifesting in muscular impairment; primarily muscle weakness.
Mitochondrial unfolded protein response (mt-UPR): a signaling pathway between mitochondria and the nucleus that responds to mitochondrial stress in response to

manifestations (e.g., mt-AlaRS with leukodystrophy or cardiomyopathy, GlyRS with cardiomyopathy or peripheral neuropathy, **Charcot–Marie–Tooth (CMT) disease**, and mt-SerRS with HUPRA or **spastic paresis**), it is always due to distinct sets of mutations [22]. This suggests that, in these cases, organ-specific variations in disease may originate from different aaRS functions associated with different sets of mutated residues [22] (Box 3).

Overall, these findings indicate that, although both mt-aaRSs and mt-tRNAs are involved in aminoacylation, the pathological consequences of mutations in either system probably arise from different molecular mechanisms and, consequently, that our understanding and analysis of the two systems must be dissociated.

Box 3. Mutations in the Context of Protein Architecture May Help To Identify the Molecular Mechanisms of Disease

Structurally, the effects of mutation of a protein residue could be due to its presence at or around any of the binding sites necessary for function (Figure I) [78–81]. First, mutations in the binding sites for the amino acid, ATP, or the tRNA terminal -CCA end will lead to an absence of catalysis or impaired catalysis (depending on the level of conservation and the structural role). Second, for specificity and tight binding, accessible residues of the aaRSs bind to the cognate tRNAs at multiple locations on their surface (termed the tRNA identity elements), and residues in the anticodon loop play key roles in binding. Mutations at the binding interfaces may alter the dynamics and folding of protein residues, leading to loss of specificity of binding. In addition, aaRSs frequently form oligomers, and mutations may affect the oligomerization process, thereby leading to inactive particles or misfolded or aggregated proteins. Third, mutations at positions far from known key functional regions can still lead to deleterious effects. Indeed, mutation of a buried amino acid may alter communication between domains (e.g., between the binding of a tRNA identity element and the site of catalysis). Similarly, a mutation occurring in a region where no known substrate binds might still impact, through molecular rearrangements and dynamics, on the binding of a substrate or of an alternative molecular partner at a distance. Finally, the state of oligomerization may vary when residues participating in the oligomerization interface are required for alternative function(s). In such a case, a mutation may prevent the adoption of alternative conformations or may prevent an interaction with a new partner, thereby hindering the alternative function.

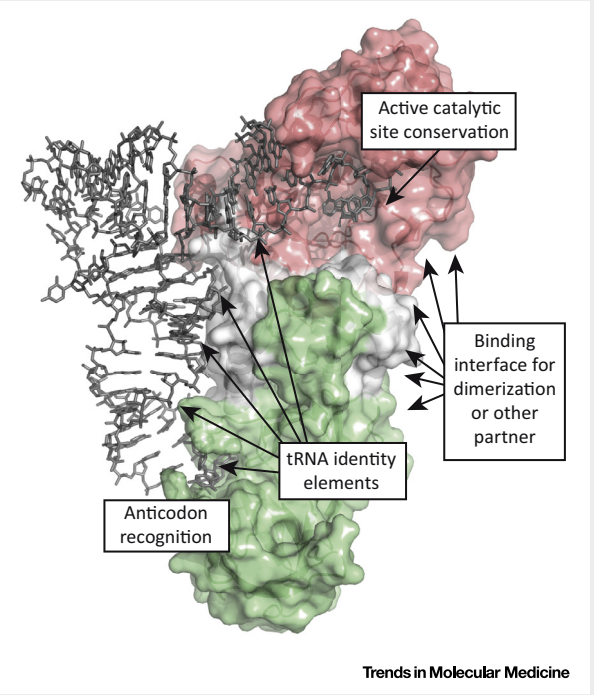

Figure I. Putative Functional Regions of an aaRS and Its Substrates: The tRNA, the Activated Amino Acid, and Other Possible Protein Partners. The cartoon, generated with Pymol [82], is based on the crystallographic structure of the *E. coli* GlnRS complexed with its cognate tRNA (PDB code 1QTQ [83]).

unfolded or misfolded proteins or to an imbalance in mitochondrial homeostasis.
MLASA syndrome: myopathy, lactic acidosis, and sideroblastic anemia – a mitochondrial autosomal recessive disorder that affects the skeletal muscle and bone marrow.
Myoglobinuria: presence of myoglobin in the urine.
Myoclonic epilepsy: an increase in muscle tone which causes sudden, brief spasm movements that can affect the face or limbs.
Organ system: a group of anatomical structures that together perform specific functions in the body.
Oxidative phosphorylation (OXPHOS): in mitochondria, electrons from electron donors are transferred, in redox reactions, to electron acceptors such as oxygen, which then split, take up protons, and form water. During electron transport, proton pumps in complexes I, III, and IV become activated, leading to the expulsion of protons from the mitochondrial matrix to the intramembrane space. The generated proton gradient combined with electron movement lead to the return of protons to the matrix, thus activating ATP synthase by a proton-motive force. ATP synthase links ADP to inorganic phosphate to generate ATP.
Perrault syndrome: a mitochondrial disorder characterized by hearing loss. Affected females also have ovary abnormalities (abnormal morphology or missing ovaries). Both males and females can also present neurological problems such as ataxia and/or intellectual disability.
Psychomotor regression: deterioration of cognitive abilities and reduction of physical movements.
Respiratory chain complexes: consist of five large multiprotein complexes, located in the inner mitochondrial membrane, that host OXPHOS. The complexes are NADH: ubiquinone oxidoreductase (I), succinate-coenzyme Q reductase (II), coenzyme Q-cytochrome c reductase (III), cytochrome *c* oxidase (IV), and ATP synthase (V). The sets of proteins involved are of dual genetic origin. A total of 84 subunits and an additional 28 assembly factors are encoded by the nuclear genome while 13 subunits are encoded by the mitochondrial genome. Complex II comprises

Genetics of Disease-associated Mutations in mt-aaRSs

We recently developed a knowledge-based repository coupled with a web server (http://misynpat.org) that integrates and links clinical, genetic, and structural data on disease-related mutations of human mt-aaRSs [22]. The MiSynPat repository is one of the sources of the data discussed below and contains an exhaustive list of reported mutations and related references. Presently, 208 different disease-related variations have been identified, either in coding sequences (153) or in intronic regions (55) of mt-aaRS genes.

For a variety of diseases associated with mt-aaRSs mutations, patients are mainly **compound heterozygotes** [22]. **Homozygosity** is sometimes reported (26 of 153 missense or nonsense mutations). Although it seems obvious that mutations in essential enzymes would cause disease, one can also expect that most mutations in such enzymes are lethal. Thus, these observations have suggested that, to be compatible with survival, homozygous mutations should not exert too severe an effect on structure and/or on function [20,30] (Box 1). A unique case is the dimeric mt-TyrRS, for which mutations reported so far are homozygous (with one patient with two different variations affecting the same position, i.e., G191D associated with G191V [31]) (Figures 3 and 4) [22]. The only compound heterozygous patient associates a missense mutation (G191D) with a nonsense mutation (R360X) [32], and only the G191D variation is present in the expressed dimeric protein. This indicates that the pathogenicity of mutated mt-TyrRS exclusively relies on homodimeric mutated forms of the enzyme. Although the relevance to the molecular mechanism is unclear, TyrRS is peculiar because $tRNA^{Tyr}$ binds simultaneously to each of the two monomers of the synthetase (the only other case is TrpRS) [33]. However, the mutated positions are not conserved throughout the global phylogeny and are spread over the 3D structure. Of note, the mt-TyrRS-related MLASA syndrome stands apart from other mt-aaRS-associated diseases because it is the only one known to lead to a musculoskeletal primary defect and no CNS primary injury [34].

For amino acids Gly and Lys, single genes encode both the mitochondrial and the cytosolic aaRSs (Box 2) [16]. Reported mutations in those two genes show either **recessive inheritance** or dominant inheritance, and are thus found in both the cytosolic and the mitochondrial forms of these two enzymes [22]. On the one hand, because the dominant mutations of cytosolic aaRSs (cytosolic TyrRS, AlaRS, HisRS, and MetRS) are linked to peripheral neuropathy CMT disease, it has been suggested that the CMT-related dominant mutations of GlyRS and LysRS (both cytosolic and mitochondrial) probably mainly affect the cytosolic function(s) of these enzymes [35,36]. On the other hand, recessive mutations correlated with cardiomyopathies (for GlyRS and LysRS), or with visual impairment, progressive microcephaly, or hearing impairment (for LysRS), probably mainly impinge on their mitochondrial function(s), but this has not been directly tested. The selective impairment of either cytosolic or mitochondrial forms of these enzymes remains to be carefully investigated experimentally before reaching firm conclusions; indeed, CMT has also been observed to result from mutations in the mitochondrial fusion protein MFN2 [19].

Collectively, these observations emphasize a confounding issue in the field, namely that seemingly comparable mutations in different mt-aaRSs lead to diseases that are extremely different, occurring at different stages in life and in different tissues.

Analysis of Mutated Positions in mt-aaRS Architecture and Potential Functional Implications

Presently, 153 mutations impacting on protein sequences (missense, nonsense, insertion, and deletion) have been reported, and these are spread over the constitutive functional domains of mt-aaRSs (Figure 3). Of note, the tRNA-edge binding domain (found in mt-ArgRS, mt-SerRS, and mt-LeuRS) is the only known functional domain without disease-prone mutations [22], which may

exclusively nucleus-encoded proteins.

Recessive inheritance: conditions where both alleles must be affected to manifest disease.

Selective or evolutionary pressure: molecular features which are essential for cell survival are under selective (or evolutionary) pressure because their disappearance or absence of functionality would lead to either death or lack of progeny. Molecular features that are not under selective pressure can evolve with more freedom, and are said to be neutral at the evolutionary level.

Spastic paresis: affected individuals show an increase in muscle tonus and in tendon reflexes.

Sporadic bilateral optic neuropathy: damage to and/or death of neurons in the optic nerve. The main symptom is loss of vision and damage can be unilateral (affecting only one of the two optic nerves) or bilateral (affecting both optic nerves)

Thylakoïds: small structures suspended within the chloroplast that contain sack membranes where chlorophyll is localized and photosynthesis occurs.

Key Figure

The Canonical Role of Mitochondrial Aminoacyl-tRNA Synthetases (mt-aaRSs) in Mitochondrial Translation and the Tissue-Specific Impact of Disease-Associated Mutations

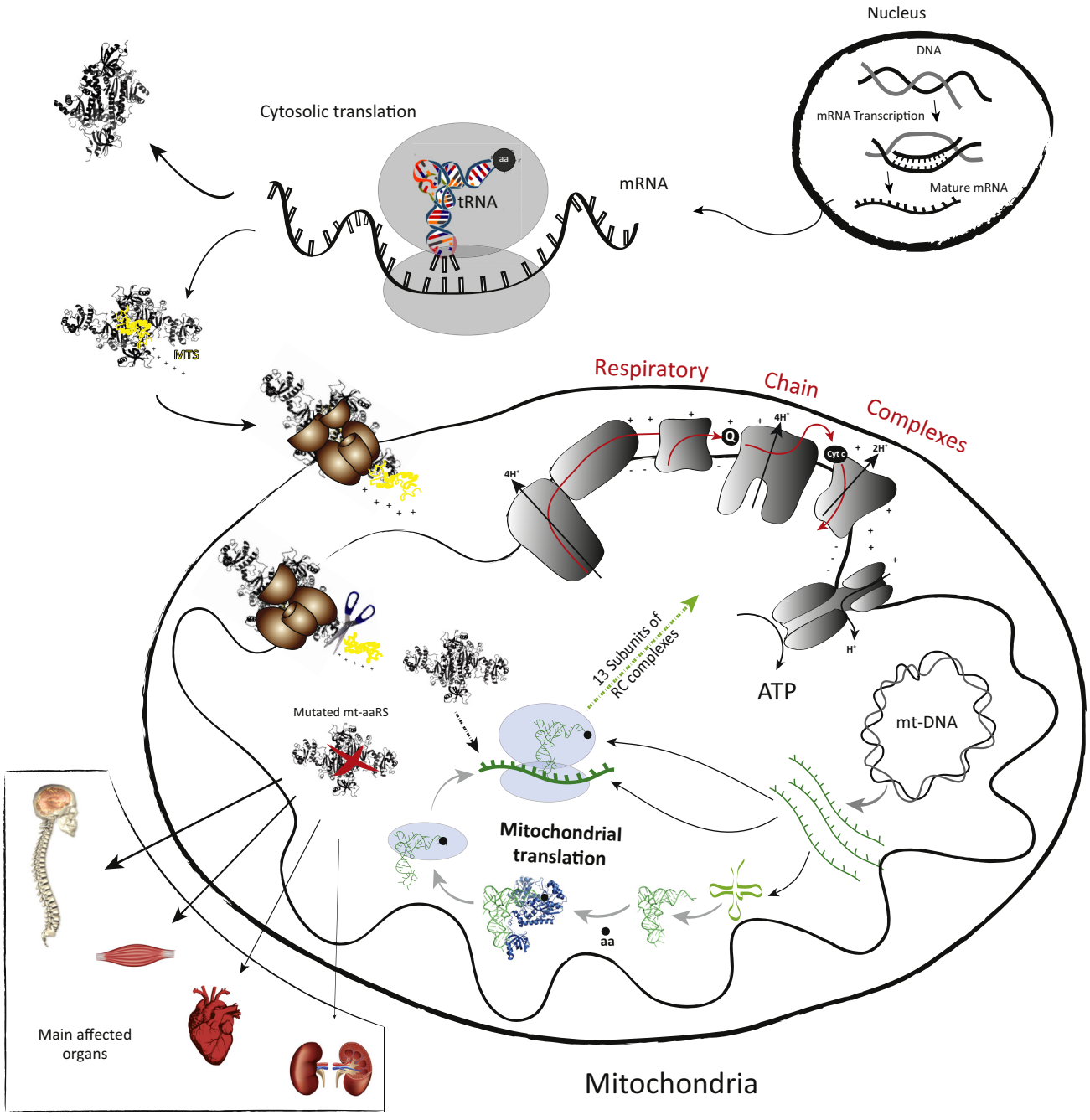

Figure 1. Human mt-aaRSs are encoded in the nucleus, synthesized in the cytosol, and delivered and imported into mitochondria via a mitochondrial targeting sequence (MTS) which is cleaved upon entry into mitochondria. mt-aaRSs, key actors in mitochondrial translation, catalyze the specific attachment of each amino acid

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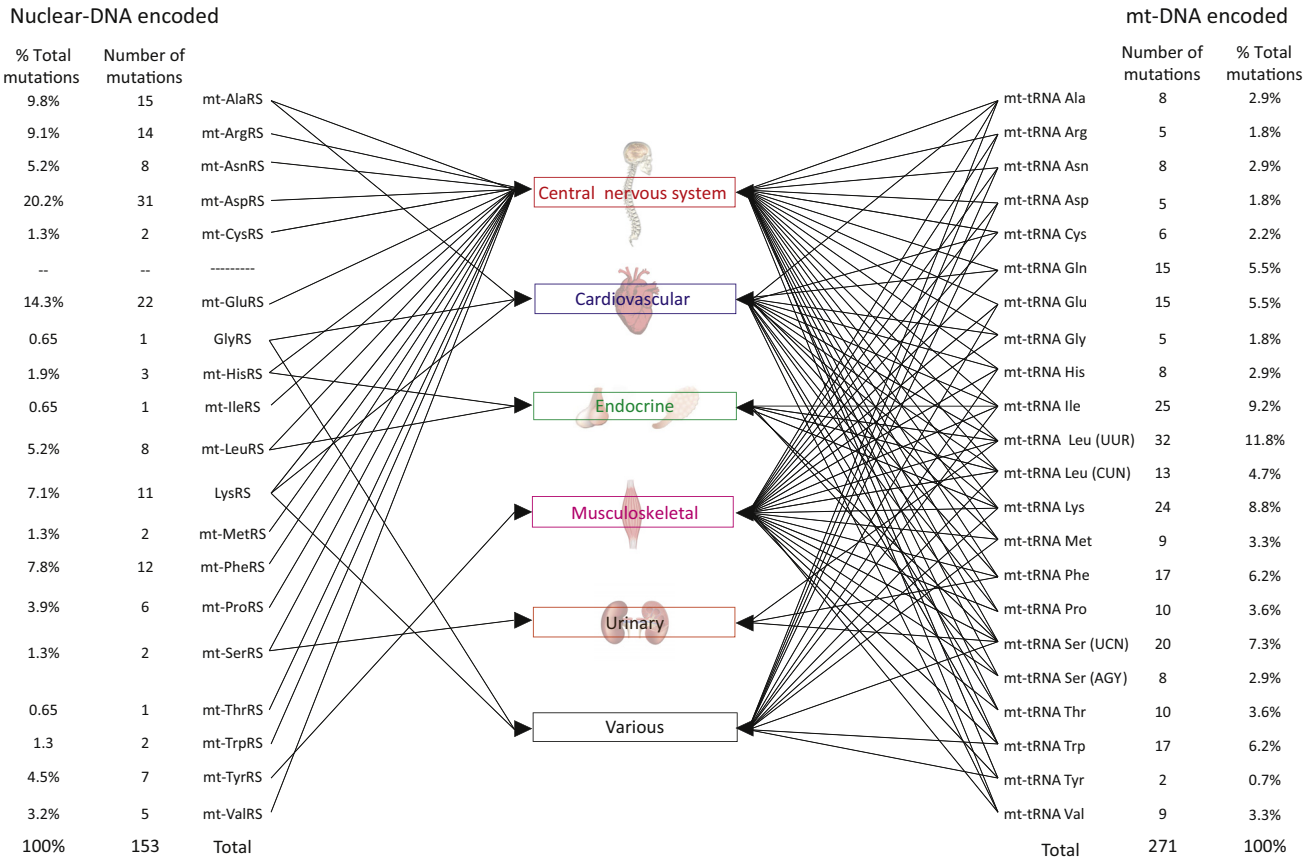

Figure 2. Mitochondrial Aminoacyl-tRNA Synthetase (mt-aaRS) Versus mt-tRNA Mutations and Affected Organ Systems. The diagram shows the total numbers of known reported mutations and their percentages for mt-aaRSs (left) and mt-tRNAs (right). mt-aaRSs are encoded in the nucleus and mt-tRNAs in the mt-DNA, and thus mutations segregate in patients following Mendelian or maternal inheritance, respectively (Box 1). The primary impacts of the mutations are classified according to five major organ systems: central nervous system (CNS), cardiovascular, endocrine, musculoskeletal, and urinary. A further category named 'various' contains reports of less frequent diseases. Data are extracted from the MiSynPat (http://misynpat.org [22]) and MitoMap (www.mitomap.org/MITOMAP [21]) databases for mutations in mt-aaRSs and mt-tRNAs, respectively.

indicate that this domain, in a mitochondrial context, is either non-essential (or neutral) or so essential that its mutations are lethal. Interestingly, a loss of **selective or evolutionary pressure**, reflecting an evolutionary loss of function, has been shown for bacterial insertion domains of mitochondrial AspRSs [37]. Only nonsense mutations have been reported in this domain of human mt-AspRS, and this is interesting because it may be indicative of a lack of specific role for constitutive amino acids, supporting the neutral role of this domain.

We now discuss the effects of mutations on the architecture of mt-aaRSs; this analysis allows the identification of functional domains (Box 3) and thus facilitates the prediction of the potential impact of mutations on these functional domains. Based on an exhaustive list of mutations for these enzymes, in conjunction with 3D models, all gathered in the repository MiSynPat, it is possible to

onto the cognate tRNA(s). Mitochondrial translation synthesizes 13 proteins that, together with 84 additional nucleus-encoded proteins, form the respiratory chain complexes. Thus, mt-aaRSs are central to cellular energy production. Mutations in mt-aaRSs have been identified in diverse human diseases, and the main organs affected are the central nervous system, as well as the musculoskeletal, cardiovascular, and urinary systems (indicated in the inset). Fully functional mitochondria require ~1200 additional nucleus-encoded proteins to guarantee mitochondrial genome maintenance and expression.

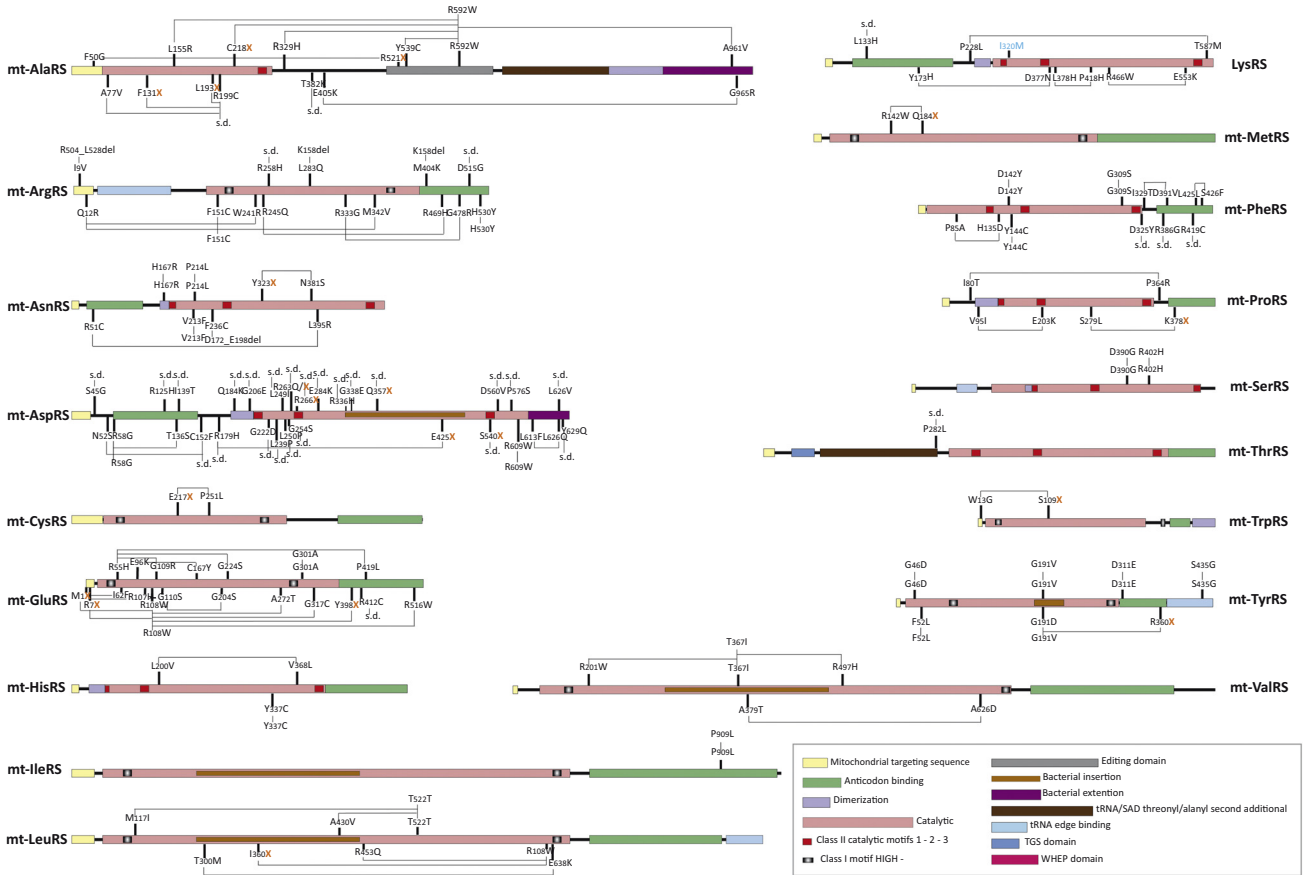

Figure 3. Disease-Associated Mutations of Human Mitochondrial Aminoacyl-tRNA Synthetases (mt-aaRSs). The mt-aaRS mutations are shown to scale; known functional domains are color-coded. Allelic compositions, as identified in patients, are linked through black lines for all reported mutations. Amino acid substitutions due to missense mutations are indicated. The orange X indicates a STOP codon created by a nonsense mutation. The mutation in blue (in LysRS) corresponds to a dominant mutation. Data were extracted from MiSynPat (http://misynpat.org [22]) which contains all related references. The GlyRS is absent because most reported mutations are dominant and lead to Charcot–Marie–Tooth disease, suggesting an impact on the cytosolic activity of the enzyme rather than on the activity of the mitochondrial enzyme. Abbreviation: s.d., splicing defect or defect involving an intronic region.

reach some conclusions [22]. Protein architectures can be extracted either from crystal structures or from models derived by homology (based on crystallographic structures of homologous proteins). Analysis of these structures has revealed that, for most mt-aaRSs (among those with a significant number of records), mutations occur throughout the known 3D structures, including surfaces that do not interact with tRNAs and, furthermore, the mutations do not localize spatially around functional regions that are necessary for aminoacylation (e.g., mt-ArgRS, mt-GluRS, and mt-TyrRS in Figure 4) [22]. These observations preclude simple mechanistic explanations (Box 3). Occasionally, however, cases do occur where mutations concentrate in identified functional regions. For example, in mt-AspRS, most mutations have been identified at the dimeric interface (mt-AspRS in Figure 4; compare with the visually random distribution of mutations in LysRS, noting that these two enzymes have similar folds and organizations) [22]. Regarding mt-PheRS, the pattern of mutations on the free form of the enzyme provides little information. It is known, however, that the binding of $tRNA^{Phe}$ is accompanied by rearrangement involving a ~160° hinge-type rotation from a 'closed' to an 'open' state of PheRS, with global repositioning of the anticodon binding domain upon tRNA binding [38]. When displayed in the 'open' state, mutations occur in the catalytic core, the anticodon-binding region, and the rearrangement zone, strongly suggesting a direct impact on aminoacylation function (Mt-PheRS in Figure 4).

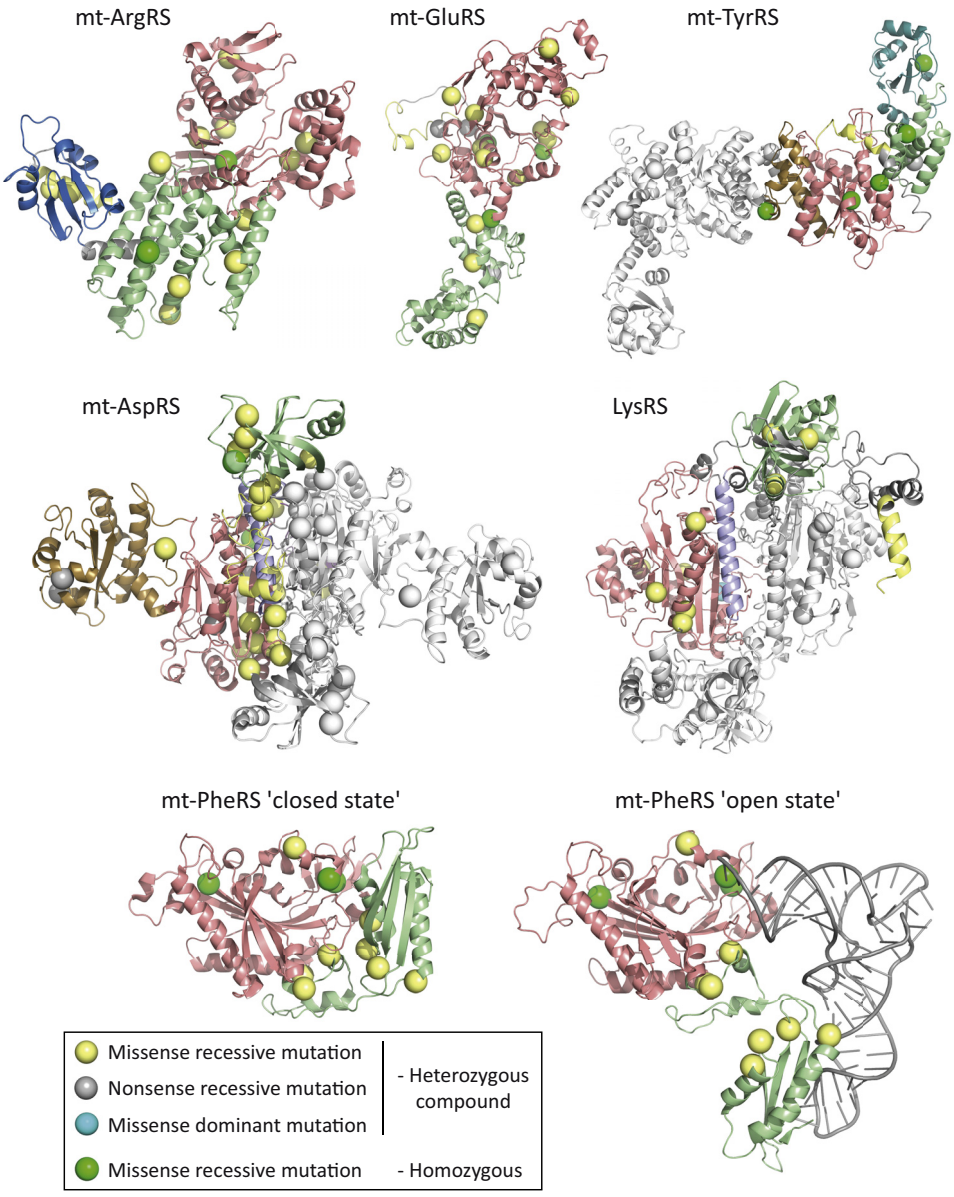

Figure 4. Selected Human Mitochondrial Aminoacyl-tRNA Synthetase (mt-aaRS) Mutations in 3D Structural Representations. Coordinates for the 3D models (which include MTSs) of mt-ArgRS, mt-GluRS, mt-TyrRS, mt-AspRS, and LysRS were uploaded from MiSynPat (http://misynpat.org [22]). Human mt-PheRS is depicted in a 'closed' state in the absence of cognate tRNA (PDB: 3CMQ [38]) or in the 'open' state when complexed with *Thermus thermophilus* $tRNA^{Phe}$ (in grey) (PDB: 2TUP [84]). For all structures, the mitochondrial targeting sequence (MTS) is in yellow, the catalytic domain in red, the anticodon binding domain in green, and the hinge region in grey, and additional domains are in hues of blue (the color code is the same as in Figure 3). Where appropriate, the second dimer is displayed in light grey. The color codes for missense and nonsense mutations, in homozygous or compound heterozygous contexts, are indicated in the inset. Cartoons were generated with Pymol [82].

Therefore, the distribution of mutated positions on structural models covers almost all regions of the architecture. However, in some instances only, mutations occur at positions that are involved in substrate binding, suggesting a putative impairment in the canonical function of aaRSs) [22].

Box 4. Mutations in the Evolutionary Context of Protein Sequences

With the advent of genomics, sequences of homologous proteins can be obtained from a large variety of organisms and organelles. Multiple sequence alignments can help visualize how amino acids are conserved at various positions along the sequence. Sequence alignments are even more powerful when at least one crystal structure is available for one of the sequences. That sequence can then serve as a structural template, and the ensuing structural alignments allow key architectural parameters to be derived for various amino acid positions (e.g., how buried or accessible they are, how close they are to the active site or to some other binding site, whether they occur in a helix or a loop, etc.). The levels of conservation derived from sequence alignments at mutated positions are highly informative for the understanding of the underlying molecular mechanisms. Conservation can be evaluated strictly (always the same type of amino acid) or more broadly, for example whether the variations occur with the maintenance of similar physicochemical properties (charge, size, hydrophopathy).

Conservation can also be analyzed within a single phylum or group of phylae. Residues conserved throughout the whole phylogeny (bacteria, archaea, eukarya, including organelles) are indicative of a **selective pressure** on that position, suggesting either a structural or a functional role related to the central function of the protein (in this case aminoacylation). However, an absence of conservation for an affected position does not call into question the pathogenicity of the mutation. Most importantly, such differentially conserved residues, in other words those that are conserved in only a subgroup or phylum of sequences, reveal loss or gain of selective pressure. Consequently, the impact of mutations at such positions may indicate tissue-specific modulation of translation or a moonlighting (alternative) function (e.g., through the participation in larger molecular complexes). Furthermore, mutation at a non-conserved position (especially with a change in physicochemical properties) may lead to an unstable or misfolded protein *in vivo* (aggregation or degradation) and/or to defective import into mitochondria.

Analysis of the Evolutionary Conservation of Mutated Positions in mt-aaRSs and Potential Functional Implications

The amino acid conservation patterns along the protein sequence can be derived from precise sequence alignments (Box 4). Such patterns convey crucial functional molecular insights on the wild-type protein and potentially further our understanding of the molecular mechanisms of disease (Box 4). An analysis of the amino acid conservation of mutated positions has been recently conducted based on sequence alignments contained and accessible through MiSynPat [22]. Overall, among the observed mutations in mt-aaRSs, 67% occur at positions that are not conserved in phylogeny, 13% are conserved based on physicochemical properties, and only 5% are 100% conserved [22]. For some mt-aaRSs, sufficient data are available to conduct more detailed analyses.

For only two aaRSs, the observed mutations occur at highly conserved positions: in mt-PheRS (leading to encephalopathy) and in mt-AlaRS (but only for mutations leading to leukodystrophy) [22]. For mt-PheRS, in agreement with structural observations (see above), experimental *in vitro* aminoacylation measurements reveal a strong impact of these mutations on aminoacylation levels, potentially leading to impaired translation [39]. Indeed, biochemical characterization of recombinant mutant mt-PheRS proteins has shown that mutations can affect synthetase function in different ways, such as by reducing the binding of ATP, phenylalanyl, or tRNA [39], and/or by affecting synthetase folding and stability [39]. No direct experimental data on aminoacylation are currently available for mt-AlaRS, and therefore a quantitative evaluation of the effects of mutations on translation efficiency cannot be presently made.

For three mt-aaRSs, mutations have been reported at conserved positions for at least one allele: mt-ArgRS, associated with encephalopathy, and mt-LeuRS and mt-HisRS, both leading to Perrault syndrome [22]. In these cases, one can envisage that the canonical structures and functions might be affected by these mutations, suggesting that the amount of active mt-aaRS might reside below a minimum biochemical threshold, but this remains to be tested.

For other mt-aaRSs, there is only low or no conservation of mutated positions: mt-GluRS and mt-AspRS (leukodystrophy), mt-ValRS (encephalopathy), mt-TyrRS (MLASA), and mt-AlaRS (with mutations in the latter leading to cardiomyopathy only) [22]. The available aminoacylation

data reveal an absence (e.g., mt-AspRS) or only a weak (e.g., mt-TyrRS) effect of the mutations on the level of aminoacylation [22]. In the case of mt-AspRS, extended investigations on a subset of LBSL-related mutants revealed an absence of a common impact(s) on the molecular, cellular, and/or structural properties of mt-AspRS [17,23,30,40,41]. Consequently, the pathomechanism of LBSL remains unsolved.

Overall, an analysis of the level of conservation leads to the following possibilities. First, there appears to be no linked segregation between conservation of the affected positions and disease (e.g., leukodystrophy is found in two of the classes listed above and encephalopathy in three). Second, there is presumably no correspondence between the level of conservation and the severity of disease (e.g., 'mild' symptoms for mt-AlaRS/leukodystrophy mutated at positions that are highly conserved, vs 'drastic' symptoms for mt-AlaRS/cardiomyopathy mutated at non-conserved positions). Third, the levels of amino acid conservation might reflect different molecular mechanisms, despite similarities in diseases. As a notable example, leukodystrophies linked to mutations in mt-GluRS or mt-AspRS (at non-conserved positions) are probably due to a molecular mechanism that is different from the mechanism underlying leukodystrophy linked to mt-AlaRS mutations (at highly conserved positions) for which aminoacylation deficiencies are strongly suspected. In mt-AspRS, however, it has been shown that, considering exclusively the subphylum of mammals, all reported mutations affect positions that are highly conserved ([30]; preliminary data suggest that this may also be the case for mt-GluRS, but this remains to be validated). This suggests that, for these two enzymes, there may be a selective pressure, possibly restricted to mammals. Of note, numerous functions beyond translation (i.e., 'moonlighting activities') may have emerged from new selective pressures or from the generation of new architectural domains, principally in vertebrates, as has been described for cytosolic aaRSs [42–45] (Box 2). A hypothesis is thus that alternative, albeit uncharacterized functions may also have emerged for these mt-aaRSs.

In summary, the level of amino acid conservation at the mutated positions ranges from low to high for the same aaRS and for similar diseases, suggesting that, although the canonical function of aminoacylation is affected in some cases, other unknown additional functions are also likely to be affected, and this should provide a future area of research.

Towards a Cellular Integration of Mitochondrial Translation Processes

That mutations in mt-aaRSs generally impact on mitochondrial translation is an inescapable conclusion from the preceding sections but, at the same time, it is also an insufficient one, in the sense that we do not know whether, how, why, or when translation is affected. Further, mutations in mt-aaRSs present striking tissue-specificity (see Outstanding Questions and Figure 1) that cannot be rationalized without considering mitochondrial translation as being fully integrated within the cell and actively participating as an environmental sensor [46]. Douglas C. Wallace has evoked the crucial coupling between mitochondria and the nucleus [46]. In his words: 'The nucleus must "know" that mitochondria can generate the required energy before proceeding with DNA replication and transcription'. We suggest that mitochondrial translation plays a pivotal role as an environmental sensor of the state of the cell. We further suggest that the integration of translation occurs through specific modulations and/or connections with other biological processes, which might be tissue-specific or tissue-dependent and which might involve some or all mt-aaRSs. In recent years, several communication and signaling pathways between the nucleus and mitochondria have been uncovered [47]. Thus, it is pertinent to examine the effects aaRSs and their mutations to achieve a better understanding and holistic integration of mitochondrial translation processes within cells. Indeed, mutations in aaRSs may be able to impact upon many or all translational events and signaling pathways, with unsuspected biological consequences. We thus suggest different possibilities for how molecules from the mitochondrial translation machinery might be integrated within the cellular environment.

Integration Through the Unfolded Protein Response

Among the communication pathways between nucleus and mitochondria are the cytoplasmic and **mitochondrial unfolded protein responses** (UPRs) that are intimately linked to a fully-functional translation process. mRNA translation is the result of a wide range of factors and enzymatic activities that must occur to accurately produce correct polypeptides while simultaneously proceeding at the proper speed for native protein folding. The final product, protein folding, is ultimately an error-prone process. In protein synthesis, the types of proteins synthesized vary widely between cell types and environments and, accordingly, so do the levels of nutrients, metabolites, and energy requirements for proper protein folding [48]. Any slowing down or impairment of translation can influence the folding of a nascent protein, leading to the accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER), leading to ER stress and activation of the UPR [48]. As central cofactors, adequate levels of functional aaRSs (and fully functional tRNAs) are essential to achieve smooth translation. For example, a lack of tRNA modifications in the anticodon triplet has been reported to lead to protein misfolding with activation of the UPR signaling pathway in various cell types [49]. Similarly, we can envisage that a decrease in the amount of fully functional aaRSs might trigger the UPR pathway, especially in stringent environments such as those for neuronal cells in the CNS. For example, it is well known that neuronal cells harbor great morphological diversity and extremely high energy demands. Functional mitochondria must be efficiently transported to distal regions of neuronal cells where specific quality-control mechanisms remove dysfunctional mitochondria [50]. Further, as postmitotic cells, neurons are very sensitive to toxic species such as misfolded proteins [51]. Thus, it cannot be excluded that some mutations might lead to either misfolded mt-aaRSs or misfolded products of mitochondrial translation, thereby triggering the mt-UPR pathway. The mt-UPR pathway is linked to the nucleus through the shuttling of transcription factors that can alter nuclear gene expression [52] upon sensing mitochondrial stress [53]. Moreover, the links between mitochondrial protein synthesis and ER occur architecturally through specialized areas – the mitochondria-associated membranes (MAMs). In terms of tissue-specific UPR, one study has shown that conditional knockout mice for heart or skeletal muscle-specific *Dars2* (the gene coding for mt-AspRS) exhibit severe mitochondrial dysfunction and activation of various stress responses in a tissue-dependent manner [54]. However, the activated UPR is only observed in cardiomyocytes, which suggests that this response may be tissue-specific [54]. In error-prone mutant cytosolic AlaRS (with a mutation in the editing domain), protein aggregation also led to death of cerebellar Purkinje cells [55]. Consequently, both sets of observations implicate the mt-UPR and/or misfolded or aggregated proteins in mt-aaRS-associated pathologies.

Integration Through other Communication and Metabolic Pathways

Several examples lend support to the hypothesis that aaRSs are involved in other communication and signaling pathways, and may be used as sensors for the mitochondrial environment. For instance, mt-MetRS has been shown to physically interact with thioredoxin 2, subsequently contributing to mitochondrial redox regulation and detoxification [56]. Furthermore, in cyanobacteria, the ValRS has been demonstrated to be anchored to the membrane of **thylakoïds** and is connected to ATP synthase, suggesting that this enzyme may play a role as a functional sensor between elements of gene translation and energy production machineries [57]. Preliminary information seems to indicate that some human mt-aaRSs may also be anchored to the mitochondrial membrane, possibly connecting these two cellular processes; however, this remains to be validated.

Another example includes the synthesis of nonessential amino acids, which are included amongst the numerous essential metabolic pathways hosted by mitochondria. These amino acids are synthesized from precursors generated either by glycolysis (cytosolic) or by the Krebs cycle (mitochondria). For instance, aspartate is the most abundant metabolite in mitochondria

[58] and is tightly connected to the respiratory chain complexes (aspartate synthesis is sensitive to changes in the matrix NADH/NAD redox balance), and is also the precursor of *N*-acetyl-L-aspartate, an essential neuron-specific transmitter [59]. One can envisage that mt-aaRSs could monitor amino acid levels, as reported for the cytosolic LeuRS that was shown to be a leucine sensor in the mTOR (mechanistic target of rapamycin) pathway which regulates both metabolism and protein synthesis [60].

One can also consider that alternative, moonlighting, function(s) may be impacted by disease-associated aaRS mutations. Moonlighting functions have been reported for mitochondrial proteins in the nucleus [61] and for cytosolic aaRSs (Box 2 for examples). Indeed, molecular evolution uses and reuses existing folded proteins for several *ad hoc* purposes. AaRSs are ancient and abundant proteins, and it is possible that moonlighting functions have also arisen for mt-aaRSs. This would correlate with the observation that some mutated positions are exclusively conserved within the mammalian subphylum, and in regions that are not primarily associated with the ancillary functions of aaRSs, meaning that these regions might theoretically display alternative function(s) [22] (Box 3). Of note, mRNAs of mt-aaRSs present various splice variants [62]. Some of the resulting shortened proteins are 'catalytic nulls', implying that they may be repurposed towards an alternative function. Moreover, some disease-related mutations present in genomic DNA may be maintained in the splice variants, and others lost. Therefore, one cannot exclude the possibility that mt-aaRS splice variants carrying mutations might be deleterious, potentially executing impaired alternative functions. Although the splice variant hypothesis cannot constitute a global explanation for each mt-aaRS and for each disease, these truncated/modified proteins might play an important role for specific mt-aaRSs and in specific diseases. Further experimental work is clearly required.

Moonlighting functions, or specialized functions that are necessary only in specific differentiated cells, can be easily contemplated as tissue-specific. The first alternative function of a mt-aaRS was only recently described: rat mt-TrpRS was identified as a novel determinant of angiogenesis in the heart and other tissues by acting as an integrator of pro-angiogenic signaling, directing cell motility and division [63]. Moonlighting activity has also been reported for the human mitochondrial ribosomal protein L12 (MRPL12), for which two mature forms, generated by two-step cleavage during import, have been implicated in translation (canonical role) and in transcriptional regulation (alternative role) [64]. Recently, the coexistence of two forms of human mt-AspRS, matured after importation into mitochondria following the same enzymatic cleavages, has also been demonstrated [65]. It is possible that one of the forms of mt-AspRS harbors a moonlighting activity, and also that processing of mt-aaRSs (presently poorly investigated) represents another means by which to generate and increase the reservoir of alternative functions for mt-aaRSs. However, this hypothesis remains to be tested.

Because disease-related mutations in mt-aaRSs do not lead to multisystemic injuries, and the CNS is the most frequently affected organ system, the preceding observations imply that numerous specific mechanisms may affect, exploit, or modulate the mitochondrial translation apparatus in neuronal cells. Indeed, we believe that future research should focus on identifying the properties of mt-aaRSs that may be specific to neuronal cells in the context of health and disease. By analyzing specific diseases, it may be possible to decipher distinct molecular mechanisms underlying mt-aaRS-related pathologies affecting the CNS. Some examples of neuron-specific molecules or mechanisms have been reported. For instance, one **isodecoder** of cytosolic $tRNA^{Arg}$ has been shown to exist only in neuronal tissues; indeed, mutations in this $tRNA^{Arg}$ can lead to neurodegeneration [66]. In addition, cell type-specific splicing efficiency differences in mt-AspRS mRNA have been observed; correct inclusion of exon 3 in this mRNA occurs less efficiently in neural cells than in other cell types (e.g., glia), as evidenced from *in vitro* experiments using LBSL patient-derived cells [67]. It is therefore essential to reach an

Box 5. Clinician's Corner

The nucleus-encoded mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are central components of the mitochondrial translation apparatus; they charge a specific tRNA with the correct amino acid for protein synthesis.

Mutations in mt-aaRSs predominantly affect the CNS, but other organs may be affected.

The term 'mitochondrial disease' covers a large number of different pathologies and disorders. The diversity of onset-ages, symptoms, organ involvement, and clinical presentations constitutes a challenge for the medical community in terms of diagnosis and even more in the development of therapeutic treatments.

Until now, the clinical management of patients is limited to symptomatic treatments and nutritional supplements. CoQ, vitamins A and E, carnitine, creatine, dichloroacetate, lipoic acid, and L-arginine are some of the supplements that are used routinely in patients with mitochondrial diseases. However, such treatments do not cure the diseases. In recent years numerous therapeutic approaches [including gene therapy using adeno-associated viral (AAVs) to correct the mutation or CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) technology for genome editing] have been developed. Some of those experimental approaches have shown efficacy in animal models. The testing of the efficacy of such treatments in humans is eagerly awaited.

The development and testing of any new therapeutic approach requires an understanding of the molecular mechanisms underlying the disease, and for mt-aaRSs these remain unclear. Therefore, integrative collaborations between clinicians and researchers should be encouraged to (i) assist diagnosis, (ii) counsel patients, and (iii) improve and develop novel and efficient therapeutic approaches.

integrated view of mitochondrial translation in a wide range of cellular environments and also under distinct physiological and pathophysiological conditions (Box 5).

Concluding Remarks

The present discussion indicates that mutations in all mt-aaRSs do not lead to pathologies via a single unique molecular mechanism, even though this might be easy to assume because mt-aaRSs constitute necessary and crucial components of the mitochondrial protein synthesis process. Thus, although many questions are to be pursued (see Outstanding Questions), we suggest that distinct mechanisms are at play in the pathogenesis of mt-aaRS-associated diseases and, furthermore, that these mechanisms may not be necessarily the same for each mt-aaRS. Among such putative mechanisms we have mainly considered that mt-aaRs mutations may generate: (i) dysfunction in the translation machinery, potentially triggering the mt-UPR; (ii) the alteration of alternative mt-aaRS function(s); and (iii) loss of activity as environmental sensors or mediators that integrate cellular pathways. The understanding of complex mitochondrial pathologies requires novel fundamental insights into the mechanisms of mitochondrial translation within cells and on the precise roles that mt-aaRSs play in these integrated molecular networks.

Cells have an absolute need for ATP produced by mitochondria. Unsurprisingly, the cell exerts constant surveillance and quality control of mitochondrial processes, meaning that mitochondria are fully integrated within cellular homeostasis. Mitochondrial translation, which is restricted to the synthesis of 13 proteins in humans, constitutes a point of convergence within the cell, and merges the products of nuclear and mitochondrial genomes. It is thus possible that mitochondrial translation may have been maintained through evolution for networking purposes, guaranteeing crosstalk between the cellular program and cellular energy demands, and enabling the surveillance of metabolic activities and stress. It will therefore be exciting to follow future investigations on the divergent pathways of mt-aaRSs in mitochondrial translation and disease.

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Outstanding Questions

How can mutations in ancillary proteins necessary to perform mitochondrial translation lead to a variety of disorders that impact on a restricted number of tissues/organs and with a great temporal diversity?

Is this marked tissue- and cell-specificity solely due to impaired mitochondrial translation in tissues or cells with high energy demands?

Alternatively, does the tissue-specificity result from non-translation functions played by mt-aaRSs that are only crucial in some tissues or cells? Are both phenomena present for some aaRSs?

Can one systematically assign the observed syndromes to results from direct damage to a single molecular process or to consequences from other linked processes?

Do cells exploit still unknown molecular mechanisms involving nucleus-encoded mt-aaRSs to monitor mitochondrial activity and ATP production?

The mitochondrial UPR connects nuclear and mitochondrial genomic expression. Could some imported and misfolded mutated aaRSs participate in this response, either directly or indirectly, because they slow down translation by impairing aminoacylation?

Alternatively, is mitochondrial translation particularly involved with or dependent upon amino acid metabolic pathways?

We should clarify how mitochondrial translation machineries are idiosyncratically integrated within different cells and tissues. We should disentangle the environmental contributions that either modify or modulate mitochondrial translation from those involved in moonlighting activities.

Neuronal cells present a great diversity of morphologies and an extremely high energy demand. Functional mitochondria must thus be efficiently transported to distal regions of neuronal cells where specific quality-control mechanisms can remove dysfunctional mitochondria. What, and how specific, are the pathways of mitochondrial translation within neuronal cells?

Supplemental Information

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