Mixing and matching mitochondrial aminoacyl synthetases and their tRNAs: a new way to treat respiratory chain disorders?

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Mutations in mitochondrial DNA are an important cause of human disease and from a therapeutic standpoint, these disorders are currently untreatable. New studies now show that a non-cognate mitochondrial aminoacyl tRNA synthetase can overcome the respiratory defect caused by an mt-tRNA mutation and that the isolated carboxy-terminal domain of human mt-leucyl tRNA synthetase can ameliorate the pathologic phenotype.

“Even though most mutations in tRNAs affect mitochondrial translation, the resulting clinical phenotypes are remarkably heterogeneous.”

Patogenic mutations in mt-tRNAs typically interfere directly with cellular ATP production, because the tRNAs are part of the organelar translation system devoted to the synthesis of the 13 subunits of the oxidative phosphorylation (OXPHOS) complexes that are encoded by mtDNA. Except for the two ribosomal RNAs (also mtDNA-encoded), all the other factors necessary for mitochondrial protein synthesis are encoded by nuclear genes and are imported into mitochondria from the cytoplasm. These include the protein subunits of the mitoribosome, translation elongation factors, and the aminoacyl-tRNA synthetases (aaRSs). For most amino acids, the cognate aaRS for each mitochondrial tRNA is distinct from the counterpart tRNA synthetase that functions in the cytoplasm.

Although aaRSs have the basic housekeeping function of charging tRNAs with their cognate amino acids in the initiation step of protein synthesis, recent studies have revealed unexpectedly versatile “moonlighting” functions for many of the cytoplasmic synthetases (Guo & Schimmel, 2013), in pathways ranging from angiogenesis to inflammation and apoptosis. Many of these functions are performed by added domains in the aaRS that are dispensable for aminoacylation. For example, a C-terminal “added” domain, UNE-S, in the cytoplasmic SerRS, is essential for vascular development through directing SerRS to the nucleus, where it regulates VEGF-A transcription (Xu et al., 2012). Such domains with “non-canonical” functions have not been described previously for mammalian mitochondrial aaRS’s, but a domain of the mitochondrial leucyl-tRNA synthetase (mtLeuRS) from S. cerevisiae has been shown to have group I intron splicing activity (Labouesse, 1990). Importantly for mitochondrial tRNA disease, this mtLeuRS domain was shown to rescue mutants in yeast mt-tRNAs equivalent to pathological mutations in the corresponding human tRNA (Francisci et al., 2006).

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2011). In addition, several examples exist where the expression level of the cognate aaRS modulates the effect of the mitochondrial tRNA mutation, in both yeast and human cell models: for example, mtLeuRS suppressed a tRNALeu\(^{AUC}\) mutation in human cells (Park et al., 2008) and a female with a pathogenic tRNALeu\(^{UUR}\) mutation remained clinically unaffected because she had a naturally higher expression level of mttLeuRS (Perli et al., 2012). Another recent demonstration of how aaRSs could influence the penetrance of mitochondrial diseases is the work by Meiklejohn et al. (2013) showing incompatibility of mtTyrRS and mt-tRNATyr genotypes modulating mitochondrial protein synthesis in Drosophila. It should also be noted that the first reported mitochondrial tRNA rescue was not by an aaRS, but by a naturally-occurring structurally modified tRNALeu\(^{CUN}\) that suppressed a mutation in tRNALeu\(^{UUR}\) (El Meziane et al. 1998).

Perli et al have now tested the ability of a human C-terminal mtLeuRS fragment to rescue pathogenic tRNA mutations in human patient-derived cytoplasmic hybrid (cybrid) cell models. Their results show that the C-terminal mtLeuRS can be imported in significant amounts into mitochondria (although it lacks a typical mitochondrial localization signal) and is then sufficient to ameliorate the bioenergetic defects of the cybrids. Importantly, rescue by the C-terminal mtLeuRS was demonstrated for the m.3243A>G mutation in tRNALeu\(^{UUR}\), which is associated with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), one of the most common, and devastating, mtDNA disorders (Perli et al., 2014).

While rescue of a tRNA mutation by its cognate aaRS, by whatever mechanism (see below), makes a certain amount of sense, what is more surprising is the finding that overexpression of an aaRS can ameliorate dysfunction caused by mutations in non-cognate tRNAs. The aaRSs are divided into two classes (I and II), based on the architecture of the active site (Ribas de Pouplana & Schimmel, 2001). Hornig-Do et al. provide evidence that the C-terminal mtLeuRS (Class I) binds to a number of mitochondrial tRNAs, but the rescue effect on mutations was demonstrated here only within Class I (Fig 1). The studies focusing on pathogenic mutations in tRNALeu\(^{AUC}\), tRNALeu\(^{AUR}\), and tRNALeu\(^{AGY}\) and their cognate aaRSs showed interchangeability, but it should be noted that these Class I aaRSs are particularly closely related. The generalizability of the results for the other Class I aaRSs and for Class II aaRSs remains to be verified. Considering that there are no rational treatments for mitochondrial tRNA diseases, a universal suppressor molecule for all tRNA mutations would be an outstanding advance, given, of course, all the caveats that attend gene therapy-based approaches to treating human disease.

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ways. Clearly, development of optimal treatment strategies will require a more precise understanding of the exact molecular mechanisms of rescue.

Among the necessary future work is to identify the minimal C-terminal fragment that still retains both mitochondrial localization and rescue ability, and to optimize the delivery method. Delivery of a short peptide could be ideal as a treatment, although obvious hurdles regarding pan-tissue delivery, stability, and potential toxicity present themselves. Even if sufficient amounts of the peptide were to enter the mitochondria, the remainder will stay in the cytoplasm and it is not yet clear whether it might interact with any of the cytoplasmic tRNAs and cause possible side effects. Unfortunately mouse models of targeted mt-tRNA point mutations are not available for testing, because of our inability to directly manipulate the mammalian mitochondrial genome in a stable and heritable way.

The importance of the mtLeuRS C-terminal in understanding the basic biology of mammalian mitochondria is also worth investigating further – for example, does it have an essential stabilizing function for some or all mitochondrial tRNAs through its RNA binding ability? Do other aaRS C-terminals have a similar function? Do those from Class I and Class II aaRS’s behave differently? Finally, mutations in many mitochondrial aaRSs themselves have recently been found to cause mitochondrial diseases (Konovalova & Tyynismaa, 2013). Perhaps the C-terminal mtLeuRS could improve the compromised tRNA stability in some of these patients as well.

In summary, therapeutic options for mitochondrial diseases are scarce and novel ideas for treatment may arise from unexpected directions. Detailed work to understand basic functions of mitochondria and their evolution are the key to many discoveries. The mtLeuRS peptide is still far away from the clinic, but its potential in treating mitochondrial tRNA diseases is clearly worthy of further investigation. The two papers reported here are showing us the way.

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Conflict of interest

The authors declare that they have no conflict of interest.

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