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Research paper

Released selective pressure on a structural domain gives new insights on the functional relaxation of mitochondrial aspartyl-tRNA synthetase

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ABSTRACT

Mammalian mitochondrial aminoacyl-tRNA synthetases are nuclear-encoded enzymes that are essential for mitochondrial protein synthesis. Due to an endosymbiotic origin of the mitochondria, many of them share structural domains with homologous bacterial enzymes of same specificity. This is also the case for human mitochondrial aspartyl-tRNA synthetase (AspRS) that shares the so-called bacterial insertion domain with bacterial homologs. The function of this domain in the mitochondrial proteins is unclear. Here, we show by bioinformatic analyses that the sequences coding for the bacterial insertion domain are less conserved in opisthokont and protist than in bacteria and viridiplantae. The divergence suggests a loss of evolutionary pressure on this domain for non-plant mitochondrial AspRSs. This discovery is further connected with the herein described occurrence of alternatively spliced transcripts of the mRNAs coding for some mammalian mitochondrial AspRSs. Interestingly, the spliced transcripts alternately lack one of the four exons that code for the bacterial insertion domain. Although we showed that the human alternative transcript is present in all tested tissues; co-exists with the full-length form, possesses 5′- and 3′-UTRs, a poly-A tail and is bound to polysomes, we were unable to detect the corresponding protein. The relaxed selective pressure combined with the occurrence of alternative splicing, involving a single structural sub-domain, favors the hypothesis of the loss of function of this domain for AspRSs of mitochondrial location. This evolutionary divergence is in line with other characteristics, established for the human mt-AspRS, that indicate a functional relaxation of non-viridiplantae mt-AspRSs when compared to bacterial and plant ones, despite their common ancestry.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are housekeeping enzymes involved in the essential process of protein biosynthesis, i.e. the translation of the genetic information from mRNA into proteins. In every cell and organelle, each of the 20 aaRSs esterifies specifically its corresponding tRNA(s) with the correct amino acid, which is then transferred to the growing peptide chain on the ribosome. AaRSs have been extensively explored during the past decades to unravel their structural, functional and evolutionary properties (reviewed in e.g. Refs. [1–4]). In human mitochondria, protein biosynthesis is dedicated to the production of 13 proteins, all sub-units of the respiratory chain complexes, which are the seats for energy production (reviewed in Ref. [5]). Human mitochondria possess a specific set of aaRSs, all encoded by the nuclear genome, synthesized within the cytosol and imported into the mitochondria thanks to a mitochondrial (mt) targeting sequence (MTS) [6]. Their genes differ from the ones encoding cytosolic-addressed aaRSs, with solely two exceptions where one single gene encoded both

Abbreviations: aaRS, aminoacyl-tRNA synthetase (specificity is indicated by the name of the amino acid abbreviated in a three-letter code, transferred to the cognate tRNA, e.g. AspRS stands for aspartyl-tRNA synthetase); MTS, mitochondrial targeting sequence; mt, mitochondrial.

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forms (GlyRSs and LysRSs) [7]. Despite the conventional view of the endosymbiotic origin of mitochondria [8], the source of nuclear gene for mt-addressed aaRSs is diverse, reflecting numerous post-endosymbiotic and/or lateral gene transfer events [9]. Nevertheless, many of the mt-aaRSs originate from the bacterial domain. This is also the case for human mt-aspartyl-tRNA synthetase (mt-AspRS) [7].

The human mt-AspRS shares 43% of identical residues, the same secondary structure organization (including the bacterial-type insertion BID and C-terminal extension domains), and a same architecture with *Escherichia coli* AspRS, a representative bacterial homolog [10]. Of note, the BID is a structural module, shared by all bacterial-type AspRSs, and located between motif 2 and motif 3 of the catalytic core of the enzyme (Fig. 1A). This domain is alternatively named GAD in the Pfam annotation, according to its presence in both some **G**lutamyl-tRNA **A**midotransferase and bacterial-type AspRSs (**D**RS). Its function remains, however, unclear. Despite the fact that the two enzymes are likely descendants from a common ancestor, numerous functional idiosyncrasies and discrepancies were reported for human mt-AspRS when compared to *E. coli* AspRS. These concern a reduced catalytic efficiency [6,7], the requirement of a minimal set of determinants within cognate tRNA [11], a higher sensitivity to small substrate analogs [12], the capacity of cross aminoacylation of bacterial $tRNA^{Asp}$ [13], and an increased structural plasticity of the mitochondrial enzyme when compared to its bacterial homolog [10].

To further investigate evolutionary discrepancies, sequence analyses at the genomic and proteic levels were performed and revealed an extensive divergence in the BID. In contrast to its strong conservation in bacteria, we uncovered a high sequence variability of the BID in almost all eukaryotes mitochondrial sequences (with the exception of green plants). In vertebrates, the BID is encoded by exons 11, 12, 13, and 14. Interestingly, we found an alternatively spliced transcript of the mRNA coding for the human mt-AspRS, which misses exon 13. Further characterization of this spliced transcript revealed that both full-length and exon 13-deleted mRNAs coexist in all tested tissues, are processed and handled by the polysomes, suggesting their active translations. Although we were unable to detect a protein corresponding to the spliced form, the large sequence diversity of the BID in almost all eukaryotes, together with the fact that we found alternatively spliced forms of mt-AspRS mRNA that lack other BID exons in other mammals, led us to speculate that there has been a relaxation of the selective pressure on the BID in the eukaryotic branch. It is possible that the appearance of mitochondrial AspRSs splice variants, defective in the BID, reflects the functional relaxation of the nuclear-encoded mt-aaRSs.

2. Material and methods

2.1. Generation of the multiple sequence alignment (MSA)

Sequences of the mt-AspRS proteins were examined in 81 eukaryotic organisms with: 27 Metazoa, 40 Fungi, nine Archaeplastida (*Viridiplantae*), and five Protists. To obtain an objective evaluation of the sequence divergence, the bacterial homologs were retrieved from 104 organisms encompassing all bacterial subgroups and removing redundancy by counting only non-identical sequences. Finally, the phylogenetic analysis (see below) was performed using nine sequences from archaeal and eukaryotic cytosolic AspRSs (*Cyanidioschyzon merolae*, *Pyrobaculum aerophilum*, *Aeropyrum pernix*, *Thermococcus kodakarensis*, *Methanothermobacter thermautotrophicus*, *Arabidopsis thaliana*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Homo sapiens*) as outgroup. The complete list of species is provided in the Supplementary Table 1.

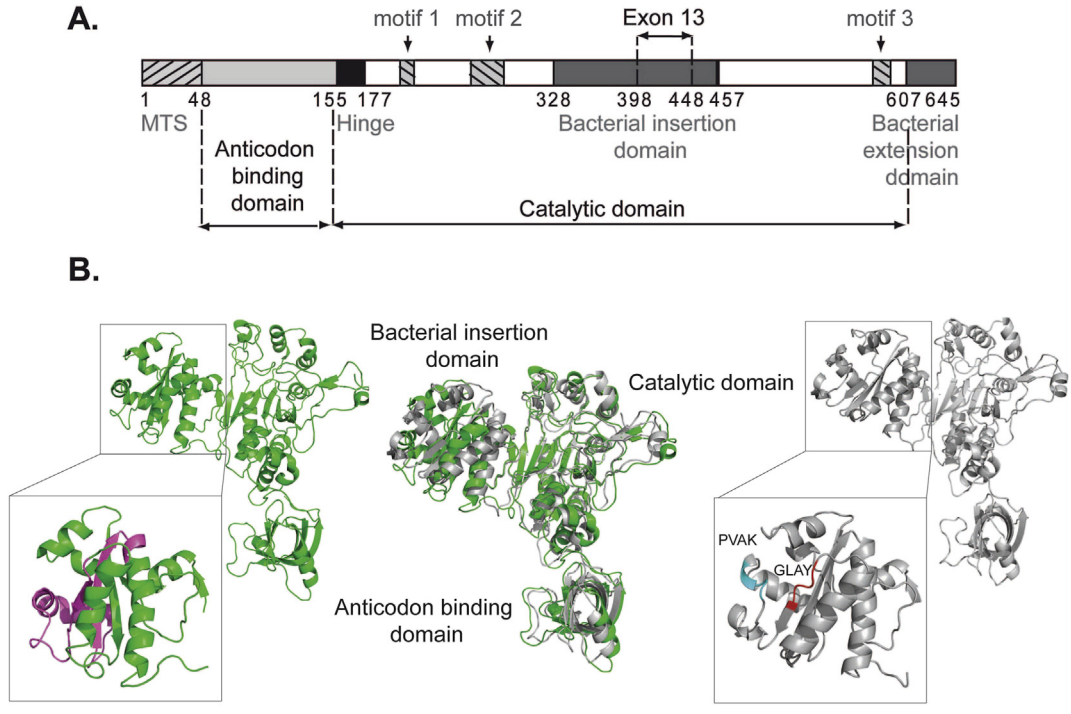

Fig. 1. Exon 13-encoded peptide in mt-AspRS. (A) Modular organization of full-length human mt-AspRS [7]. MTS stands for mitochondrial targeting sequence. Motifs 1, 2, and 3 constitute the catalytic core of the synthetase. (B) Crystallographic structure of the human mt-AspRS (green, on the left) (PDB: 4ah6) [10]. The bowed part shows the BID, where the location of the exon 13-encoded peptide is highlighted in magenta. Gray structure (right): the crystal structure of the *E. coli* AspRS (1eqr) [39], including a more detailed view of the BID where the highly conserved "G(L,I)Φ(Y,W,Φ)" and "PVAK" motifs in bacteria and viridiplantae, are colored in red and cyan, respectively. In the middle: superposition of both structures, emphasizing the overall fold conservation. Major structural domains are indicated. For sake of simplicity, only one monomer of AspRS (naturally found in a dimeric state) is shown in this figure. Structures have been drawn using PyMol (http://www.pymol.org).