Parallel Loss of Nuclear-Encoded Mitochondrial Aminoacyl-tRNA Synthetases and mtDNA-Encoded tRNAs in Cnidaria

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Abstract

Unlike most animal mitochondrial (mt) genomes, which encode a set of 22 transfer RNAs (tRNAs) sufficient for mt protein synthesis, those of cnidarians have only retained one or two tRNA genes. Whether the missing cnidarian mt-tRNA genes relocated outside the main mt chromosome or were lost remains unclear. It is also unknown what impact the loss of tRNA genes had on other components of the mt translational machinery. Here, we explored the nuclear genome of the cnidian Nematostella vectensis for the presence of mt-tRNA genes and their corresponding mt aminoacyl-tRNA synthetases (mt-aaRS). We detected no candidates for mt-tRNA genes and only two mt-aaRS orthologs. At the same time, we found that all but one cytosolic aaRS appear to be targeted to mitochondria. These results indicate that the loss of mt-tRNAs in Cnidaria is genuine and occurred in parallel with the loss of nuclear-encoded mt-aaRS. Our phylogenetic analyses of individual aaRS revealed that although the nearly total loss of mt-aaRS is rare, aaRS gene deletion and replacement have occurred throughout the evolution of Metazoa.

Key words: aminoacyl-tRNA synthetases, Cnidaria, mitochondrial evolution, subcellular targeting.

In contrast to other eukaryotic groups where mitochondrial (mt) genomes vary substantially in size and gene content, animal mtDNA is remarkably conserved. The typical mt genome of bilaterian animals contains 37 genes, which encode 13 proteins, 2 ribosomal RNAs, and 22 transfer RNAs (tRNAs); whereas the mtDNA of non–bilaterian animals often contains a few extra genes (Lavrov 2007). Exceptions to these generalities do occur and often involve the loss of tRNA genes. Extreme examples of mt-tRNA gene loss may be found in the mt genomes of the Phylum Cnidaria (Peery et al. 1995; Shao et al. 2006; Kayal and Lavrov 2008), the Keratosa (G1) group of demosponges (Wang and Lavrov 2008), and in Chaetognatha (arrow worms) (Helfenbein et al. 2004; Papillon et al. 2004), where no more than two mt-tRNAs appear to be retained.

Although the loss of mt genes is uncommon within animals, it has been a general trend throughout most of mt genome evolution prior to the origin of Metazoa. In particular, nearly all genes necessary for mt protein synthesis have either been transferred to the nucleus or supplanted by preexisting nuclear genes of similar function, resulting in a metazoan mt protein translation machinery that is co-encoded by the nucleus and the mitochondrion (Adams and Palmer 2003). Although, in principle, the functional transfer of mt-tRNAs to the nucleus could explain their absence from mt genomes, there has been no evidence so far of such import (Lithgow and Schneider 2010). Instead, imported cytosolic counterparts presumably have replaced lost mt-tRNAs (Duchêne et al. 2009).

If the absence of mt-tRNA genes from mt genomes represents a true loss, it should have an effect on the other components of the mt translational machinery with which mt-tRNAs coevolved. Aminoacyl-tRNA synthetases (aaRS), the enzymes that catalyze the specific attachment of amino acids to the 3′-ends of cognate tRNAs, should be especially affected by tRNA loss. In opisthokonts (animals, fungi, and their unicellular relatives), aaRS may be subdivided into three categories based on their functional localization: cytosol specific (cy-aaRS), involved in cytosolic protein synthesis; mitochondrion specific (mt-aaRS), involved in mt protein synthesis; and bifunctional, operating in both compartments (Antonellis and Green 2008). Throughout metazoan evolution, the members of these categories have undergone structural modifications in parallel with changes in tRNA identity determinants. In particular, there is clear evidence for coevolution between mt-aaRS and mt-tRNA structures (Chimnaronk et al. 2005; Sissler et al. 2005). Because of this coevolution, as well as their potentially distinct origins (e.g., Brindel et al. 2007), aaRS operating in one compartment may not be able to recognize tRNAs specific for another. Hence, we hypothesized that the actual loss of mt-tRNA genes would render mt-aaRS disposable by removing the evolutionary pressure to maintain them, while mt-tRNA transfer to the nucleus or to another mt chromosome would not affect them. In order to test this hypothesis, we surveyed the nuclear genome of the anthozoan cnidian Nematostella vectensis (Putnam et al. 2007) for evidence of mt-tRNA gene transfer as well as mt-aaRS gene loss.

We extracted tRNA-like sequences from the N. vectensis genome using tRNAscan-SE (Lowe and Eddy 1997) and used neighbor-joining (NJ) analysis (Saitou and Nei 1987;
Loss of mt-tRNAs and mt-aaRS in Cnidaria

...Studier and Keppler 1988) based on uncorrected (p) distances to reconstruct their phylogenetic relationships with mt-tRNA genes from four species of demosponges. Both cytosolic and mt-tRNAs can be detected and annotated using tRNAscan-SE in Cnidaria because of their conserved secondary structures. In total, 12,781 nuclear tRNA-like sequences were found in the N. vectensis genome after removing 20 bacterial contaminants and one mt-tRNAMet (present on a mt contig). We clustered all tRNA genes into 415 groups with \( \geq 90\% \) similarity and used one representative from each of these groups along with two tRNA genes from the previously published Nematostella sp. JVK-2006 mtDNA (Medina et al. 2006) and 101 mt-tRNA sequences from four species of demosponges for the phylogenetic analysis. The results of the NJ analysis revealed no close affinity of any N. vectensis nuclear tRNA genes to mt-tRNAs from demosponges. By contrast, both mt-tRNAs of Nematostella sp. were closely related to their demosponge counterparts (supplementary fig. S8, Supplementary Material online). Thus, there is no genomic evidence for a functional transfer of mt-tRNA genes to the nucleus in N. vectensis, consistent with previous studies on other organisms (Lithgow and Schneider 2010).

To assess whether the loss of mt-tRNA genes cooccurred with the loss of mt-aaRS genes, we applied a reciprocal best hits (RBH) approach (Rivera et al. 1998) to search the complete genome of N. vectensis for the presence of aaRS orthologs using the functionally verified aaRS sequences from Saccharomyces cerevisiae (fig. 1). As a control, we performed the same search in Homo sapiens. We identified sequences orthologous to all yeast cy-aaRS and H. sapiens except for GluRS, which is known to be fused with ProRS in Metazoa forming a bifunctional Glu-ProRS enzyme (Berthonneau and Mirande 2000). Furthermore, of the 14 distinctly mt (not bifunctional, see fig. 1) genes from yeast, our RBH search could identify 11 corresponding orthologs in human. Reversing the RBH search (using human sequences to query yeast) revealed that two of the remaining mt genes (for mt-LysRS and mt-ArgRS) are orthologous between yeast and human but underwent duplication and sublocalization in yeast. By contrast, although we found all 19 expected cy-aaRS orthologs in the N. vectensis genome, we were able to identify only two putative mt-aaRS orthologs (mt-PheRS and mt-TrpRS), suggesting that most mt-aaRS genes have been lost. This result contradicts the hypothesis that mt-tRNA loss would be precipitated in situations where mt-aaRS were able to aminoacylate imported cytosolic tRNAs (Schneider 2001; Lithgow and Schneider 2010).

The retention of two mt-aaRS in the nuclear genome of N. vectensis appears to be nonrandom. The realignment of the UGA termination codon to tryptophan in animal mtDNA likely explains the retention of the nuclear-encoded mt-TrpRS as well as the mtDNA-encoded mt-tRNA\(_{\text{Trp}}\). In particular, the cy-TrpRS specifically recognizes the anticodon of tRNATrp\(_{\text{CCA}}\) as a tRNA identity element, and thus, mt-tRNATrp\(_{\text{UCA}}\) cannot be aminoacylated by the cytosolic enzyme, necessitating the distinct mt enzyme (Charrière et al. 2006). The retention of mt-PheRS may be explained by its structural differences with the cytosolic counterpart. Unlike the majority of mt-aaRS that are monomers or homooligomers, both prokaryotic and cy-PheRS have \( \alpha_2\beta_2 \) heterotetrameric structures, with the active site located in the \( \alpha \)-subunit and tRNA-binding sites in both subunits. Conversely, the gene encoding metazoan mt-PheRS exists as a chimera of the \( \alpha \)-subunit and the C-terminal tRNA anticodon–binding domain of the \( \beta \)-subunit (Sanni et al. 1991). We suspect a hydrophobic domain of the N-terminal \( \beta \)-subunit may complicate its import into the mitochondrion, promoting the use of the chimeric protein (Haen KM, Lavrov DV, unpublished).

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**Fig. 1.** Evolutionary relationships among cytosolic and mt-aaRS in H. sapiens (HS), N. vectensis (NV), and Saccharomyces cerevisiae (SC). Row labels indicate aaRS specificity, and circles indicate presence/absence of aaRS. Filled, empty, and half-filled circles denote cytosolic, mt-, and bifunctional aaRS, respectively. Dashed circles indicate inferred losses of aaRS. Small circles indicate secondary duplication events. (A) aaRS for which distinct ancestral cy-aaRS (Cy) and mt-aaRS (Mt) can be inferred in Metazoa. (B) aaRS for which only a single ancestral bifunctional aaRS (Cy/Mt) was inferred.
The loss of mt-aaRS should necessitate the import of cy-aaRS into mitochondria, which would require the acquisition of mt-targeting signals (Wang et al. 2003). Dual targeting of the same aaRS to different cellular compartments is not an unusual phenomenon, and the acquisition of a mt-targeting signal by a cy-aaRS can serve as an indicator of mt-aaRS gene loss (Duchêne et al. 2005). Thus, to substantiate our inference about mt-aaRS loss in N. vectensis, we surveyed the genomic and expressed sequence tag (EST) sequences of cy-aaRS for the presence of upstream alternative start sites that could produce N-terminal targeting presequences. Indeed, we located putative mt-targeting signals for all the cy-aaRS expected to function in both the cytosol and mitochondria with scores within the range observed for experimentally verified targeting signals (supplementary table S1, Supplementary Material online). As expected, we did not identify a putative mt-targeting sequence in the N. vectensis cy-PheRS, but interestingly, we were able to identify such a sequence in cy-TrpRS, despite the maintenance of mt-TrpRS. Furthermore, MitoProt scores for both TrpRS were highly significant and virtually identical (~0.98). This result suggests that both tryptophanyl enzymes can be directed to the mitochondrion. The reason for this is unknown, but in dicotyledonous plants where two GlyRS are also directed to mitochondria, the second aaRS without mt-tRNA aminoacylation activity was hypothesized to be involved in the import of tRNA isoaacceptrs (Duchêne et al. 2001). Furthermore, we detected targeting signals for 10 out of 17 cy-aaRS in H. sapiens. Although the latter observations weaken the inference that can be made regarding the fate of mt-aaRS from the presence of mt-targeting signals in cy-aaRS, they potentially illustrate the first necessary step for the loss of mt-tRNAs and mt-aaRS. Further research detailing protein and tRNA targeting and import mechanisms will help clarify their function and subcellular localization.

Although the loss of nearly all mt-aaRS from cnidarian nuclear genomes represents an unusual case in the evolution of animal mt translation systems, the loss of either a mt or a cytosolic gene, accompanied by the duplication and neolocalization of the remaining aaRS gene to a different cellular compartment, has occurred several times throughout eukaryotic evolution. As an example, a previous study deduced the presence of eight bifunctional synthetases in the common ancestor of animals and fungi, suggesting that either the original mt or cytosolic gene was lost (Brindefalk et al. 2007; fig. 18). Our phylogenetic analyses of individual aaRS genes revealed that there was at least one additional round of duplication and differential sublocalization for six of these aaRS genes within the fungi–Metazoa group (fig. 18; supplementary figs. S1–S7, Supplementary Material online). This pattern of gene deletion and replacement explains why we were unable to find yeast orthologs in H. sapiens for mt-ThrRS using the RBH approach (supplementary fig. S5, Supplementary Material online).

To conclude, our study suggests the reported absence of mt-tRNAs in Cnidaria represents their genuine loss and demonstrates that mt-tRNA gene loss was accompanied by the loss of nuclear-encoded mt-aaRS and the acquisition of mt-targeting signals by cy-aaRS. Although it is hypothetically possible that all the remaining mt-aaRS and/or mt-tRNA genes are contained within the estimated 5% of unassembled protein-coding content of the N. vectensis genome (Putnam et al. 2007), we find it highly unlikely. We propose the following sequence of events in the loss of mt-tRNA and mt-aaRS genes: 1) Both cytosolic and mt-aaRS are targeted to mitochondria; 2) cytosolic tRNAs are imported into mitochondria, potentially together with cy-aaRS via the protein import pathway (e.g., Chacinska et al. 2009); 3) because cytosolic tRNAs are essential for cytosolic translation while mt-tRNAs may be replaceable by their nuclear analogs, a ratchet-like process leads to the disuse and eventual loss of mt-aaRS. This model is in agreement with cross aminoacylation studies in humans and yeast that showed cy-aaRS are generally inefficient for the aminoacylation of mt-tRNAs (Sissler et al. 2005). Our model also shows that neolocalization of cy-aaRS is a necessary, but not sufficient, condition for the deletion of mt-tRNA because even in the simplest scenario, an import signal should also evolve in cytosolic tRNA genes (Salinas et al. 2008). Furthermore, we speculate that the more frequent loss of mt-tRNAs observed in non–bilaterian animals (Wang and Lavrov 2008) and non-metazoan eukaryotes (reviewed in Duchêne et al. 2009) than in bilaterian animals is due to the more conserved structures of tRNAs encoded by their mtDNA, which are more similar to cytosolic tRNAs (Wolstenholme 1992), and thus can be more easily replaced by them. It is unclear whether the loss of nearly all mt-tRNAs and mt-aaRS observed in Cnidaria is simply an extreme example of the process that occurs in other metazoans or if it is a distinct phenomenon facilitated by additional factors. To this end, it would be very interesting to investigate whether a parallel loss of mt-aaRS occurred in Keratosa and Chaetognatha, two groups of animals that experienced a loss of mt-tRNA genes very similar to that in Cnidaria.

Supplementary Material

Supplementary figures S1–S8 and tables S1–10 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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References


Helfenbein KG, Fourcade HM, Vanjani RG, Boone JL. 2004. The mitochondrial genome of Paraspadella gotoi is highly reduced and reveals that chaetognaths are a sister group to protostomes. Proc Natl Acad Sci U S A. 101:10639–10643.


