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Visions & Reflections

A view into the origin of life: aminoacyl-tRNA synthetases

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The genetic code converts nucleic acid sequences into protein sequences through the action of aminoacyl-transfer RNA (tRNA) synthetases. These enzymes are responsible for the specific attachment of each amino acid to its cognate tRNAs. The aminoacylated tRNAs are then transported to the ribosomal complex by elongation factors, where the amino acid is incorporated into the translated polypeptide chain. The RNA world hypothesis states that modern aminoacyl-tRNA synthetases replaced aminoacylating ribozymes. From this perspective, the establishment of modern aminoacyl-tRNA synthetases would span the transition from the RNA world to the world of DNA and proteins. Thus, the study of the evolution of aminoacyl-tRNA synthetases could offer insights into that process. Here we review our current understanding of the origin and evolution of aminoacyl-tRNA synthetases, and discuss the implications of these studies on the origin of life.

The RNA background

The theory of an RNA world postulates that life evolved through a stage where all functions currently performed by proteins and DNA depended chiefly on RNA [1]. From physical and chemical considerations, this RNA-based metabolism has been proposed to be preceded by a 'chemical world', perhaps based on surface-based catalytic processes [2–6]. The products of these reactions laid the foundation for the eventual appearance of RNA-based protogenomes and membranes, essential requirements for the establishment of populations subjected to natural selection.

The categorical demonstration of the existence of an RNA world is impossible without the discovery of an extant form of RNA-based organisms. The probability of such discovery being made is small because one would expect such organisms to have been eliminated by competing species bearing protein-based physiologies. Thus, the theory of an RNA world springs out of theoretical considerations based on three observations: the central role of RNA and ribonucleoproteins in decoding genetic information [9]; the use of catalytic RNA molecules in limited aspects of modern cell metabolism [10, 11]; the potential of in vitro selected RNA molecules to catalyze important biochemical reactions [12–14].

These considerations all point to RNA as a plausible transition molecule, a jack-of-all-trades that was capable of undertaking the necessary functions required for establishment of a primitive cell [7, 15]. Eventually, the superior stability of DNA, and the greater catalytic plasticity and capacity of proteins, relegated RNA to limited metabolic chores, as evolution and selection pressed populations into the extant world of DNA and proteins.

The modern genetic code may have started as an operational RNA code or a primitive second genetic code. The codon–amino acid relationships were formed through interactions between residues and RNA molecules, perhaps out of aminoacylation reactions, that linked specific amino acids with specific RNAs. These RNAs may have been minihelix-like structures,

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resembling the acceptor arm of modern tRNA. These interactions layed the foundation for a primitive protein-coding mechanism, based on successive 'codons' along an RNA molecule [6–21].

The capacity of RNA molecules to carry out the aminoacylation of RNA molecules, including tRNA, has recently been demonstrated [21–24]. Possibly, a full RNA-based translation machinery emerged while the genetic code was being established. Part of this ribozyme machinery would include the functional ancestors of the extant aminoacyl-tRNA synthetases (ARSs), which emerged later, during the transition to the world of DNA and proteins.

The assembly of aminoacyl-tRNA synthetases

The three-dimensional structures of most synthetases have now been solved [25–47]. From this large body of data it is apparent that these enzymes assembled in a modular fashion, incorporating new domains around a conserved active site that represents the ancestral ARS fold [48–51]. On the basis of the architecture of this domain, ARSs are divided into two families of 10 enzymes each [52–55]. Class I enzymes have a catalytic core based on a Rossmann fold, whereas the active site of the class II enzymes is built around an antiparallel β sheet flanked by α helices. The active site domain of each enzyme has idiosyncratic insertions that facilitate specific interactions with the acceptor stem of its cognate tRNAs.

In extant enzymes the ancestral active site also incorporates new domains that carry out essential roles in tRNA recognition, aminoacylation and editing of tRNA molecules. Interestingly, these domains are not universally distributed among the ARSs of each class. In particular, different insertions were added to different enzymes at different stages (fig. 1) [48]. The extant functions of these domains suggest the nature of the selective pressures that drove their addition to the pre-existing enzymes. For example, the anticodon recognition domains that are present in most ARSs appeared later, when accurate molecular recognition of the tRNA anticodon became advantageous [45, 51, 56, 57].

Recently it has become apparent that open reading frames that code for isolated forms of these modular domains are widespread in nature [58–61]. The evolutionary relationships of these new genes with ARS genes are complex (fig. 1). With a few exceptions, the functions of the polypeptides that they encode are still unknown [60]. However, these new protein families have the potential to become the missing links in our understanding of the evolution of the two classes of ARSs.

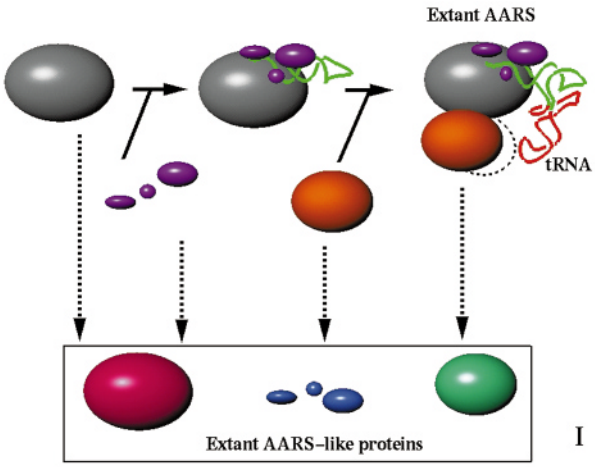

Figure 1. Model for the emergence of aaRS-like proteins from extant aminoacyl-tRNA synthetase structures, or their modular precursors. The class-defining domain of extant aminoacyl-tRNA synthetases (in grey) is likely to be the oldest module, and interacted with a minihelix-like domain (exemplified by a minihelix structure) through RNA binding elements (in purple) that were added in [15, 51]. Additional domains and insertions in extant aaRSs (in orange) were added later, perhaps to increase the specificity of each enzyme for its cognate tRNAs through new molecular interactions [15, 51]. The analysis of sequence and structure similarities between aaRS and aaRS-like proteins shows that aaRS-like proteins emerged at different stages of aaRS evolution [60, 61].

Evolution prior to the last universal common ancestor

Because each class of ARSs contains 10 enzymes [53], several gene duplication events were necessary to generate (from a common ancestor) all the enzymes of a specific class. These duplications, for the most part, had been accomplished by the time of appearance of the last universal common ancestor (LUCA) of all extant species [62–64]. This conclusion follows from the observation that, in the vast majority of ARSs, the phylogenetic relationships amongst the enzymes are clustered around substrate specificity, and not around species groups.

In contrast to phylogenies of whole species, which by definition tell us nothing about what happened before LUCA, information on the evolutionary events prior to LUCA might be obtained by analyzing the internal relationships of the enzymes in each class. It would be important, for instance, to establish the order of the duplications that gave rise to the different members of each family. This order should be compared with the theories of evolution of the genetic code. Correlations between these events could help us understand potential connections between the appearance of the code and the establishment of the extant aminoacyl-tRNA synthetases.

A good example of this type of analysis was provided by studies of sequences of lysyl-tRNA synthetase (LysRS) and $tRNA^{Lys}$. When the complete genomic sequence of *Methanococcus jannaschii* was obtained, it became apparent that this organism does not contain a gene coding for a canonical class II LysRS [65]. Because all ARSs are essential enzymes, a search was initiated to find the missing activity [66]. The enzyme catalyzing the aminoacylation of $tRNA^{Lys}$ with lysine in *Methanococcus maripaludis* turned out to be a member of the class I family of ARSs. So far, this represents the only instance of an aminoacylation activity that is catalyzed by a class I or class II ARS, depending on the species.

Class I LysRSs are mostly limited to archaebacterial species and a small number of eubacteria. On the other hand, class II LysRSs are present in all kingdoms of life, including some archaeal species [66–68]. Explanations for this gene distribution based on a hypothetical late lateral gene transfer of the class I *lysS* genes from bacteria to archaea (or vice versa) are not consistent with the phylogenetic analysis of LysRS sequences [66, 68]. More likely, the extant distributions of class I and class II LysRSs arose from a situation where an ancestral organism possessed both genes. This redundancy was resolved through the elimination of one of the two genes, either through genetic drift, or by the appearance of selective pressures in favor of one of the two molecules [68].

The realization that LUCA might have possessed two genes coding for two distinct LysRSs offered for the first time the possibility of determining the time of establishment of a tRNA identity relative to the appearance of its cognate ARS. A phylogenetic analysis of the relationships among sequences of $tRNA^{Lys}$ from species bearing class I or class II LysRSs supports the idea that at least one of the extant forms of these enzymes was established in the context of a preexisting $tRNA^{Lys}$, which remained universally distributed throughout the phylogenetic tree [68]. This prediction subsequently received support from the biochemical analysis of the aminoacylation properties of class I lysyl-tRNA synthetase [57, 67].

Details of the types of metabolism that preceded LUCA can also be obtained through the biochemical and phylogenetic analysis of ARSs. A case in point is the mechanism for aminoacylation of $tRNA^{Gln}$, which in some organisms requires an initial aminoacylation by glutamyl-tRNA synthetase, and a subsequent transamidation catalyzed by the enzyme Glu-$tRNA^{Gln}$ amidotransferase [69, 70]. Current phylogenetic analyses of this system point to the existence of an ancient transamidation pathway in the physiology of pre-LUCA organisms [58, 71–74]. The same studies indicate that the emergence of glutaminyl-tRNA synthetase is a relatively late event that probably took place within the emerging euykaryotic line of descent, via duplication and mutation of the glutamyl-tRNA synthetase gene. This new ARS was then laterally transferred to some proteobacteria, where it is still functional today [58, 71–74]. Thus, the analysis of the biochemical and phylogenetic characteristics of GlnRS offers us a window to the early metabolism of LUCA, as well as an indication of the cellular interactions that took place at the onset of the eukaryotic evolutionary branch.

Evolution after the last universal common ancestor

The aminoacyl-tRNA synthetases follow the evolutionary lines of all extant and extinct species in earth. By defining these evolutionary pathways, information can be gathered about events preceding crucial points in the origin of species. A case in point is the origin of the eukaryotic cell, and the processes of endosymbiosis that gave rise to the nucleus and other cellular organelles.

Many observations support an endo-symbiotic origin for the modern eukaryotic cell, and in particular of mitochondria and plastids [75, 76]. Although certain basal eukaryotes such as the diplomonad *Giardia lamblia* lack mitochondria, whether mitochondria were initially part of all basal eukaryotes is still debated [77–84]. Some of the current theories on the origin of eukaryotes propose that the incorporation of mitochondria preceded the development of the nucleus. These theories explain the increase in complexity in the ancestral eukaryotic cell (increase in genome size, incorporation of nucleus and endoplasmic reticulum and so on) [80, 85] as a consequence of the establishment of functional mitochondria. These proposals imply that all ancestral eukaryotic cells were mitochondriates, and that those species that presently do not contain mitochondria (such as *G. lamblia*) must have lost them a posteriori.

Regardless of the timing of this endosymbiotic event, it is reasonable to assume that the loss of genes from a mitochondrial ancestor (or the transfer of genes to the emerging nucleus) was gradual and extensive. Even the largest known mitochondrial genome (*Reclinomonas americana*) contains only a fraction of the genes found in the smallest prokaryote chromosome [86]. ARSs were among the gene families involved in this process of loss of genes from the ancestral mitochondrial genome. All *aarS* genes from the ancestor of mitochondria were either transferred to the nucleus or, in most cases, lost and functionally replaced by their nuclear-equivalent loci, of archaeal origin.

Less common is the case of valyl-tRNA synthetase (ValRS). All eukaryotic ValRSs (including that of the amitochondriate *G. lamblia*) are of bacterial origin, most likely due to a substitution of the archaeal *valS*

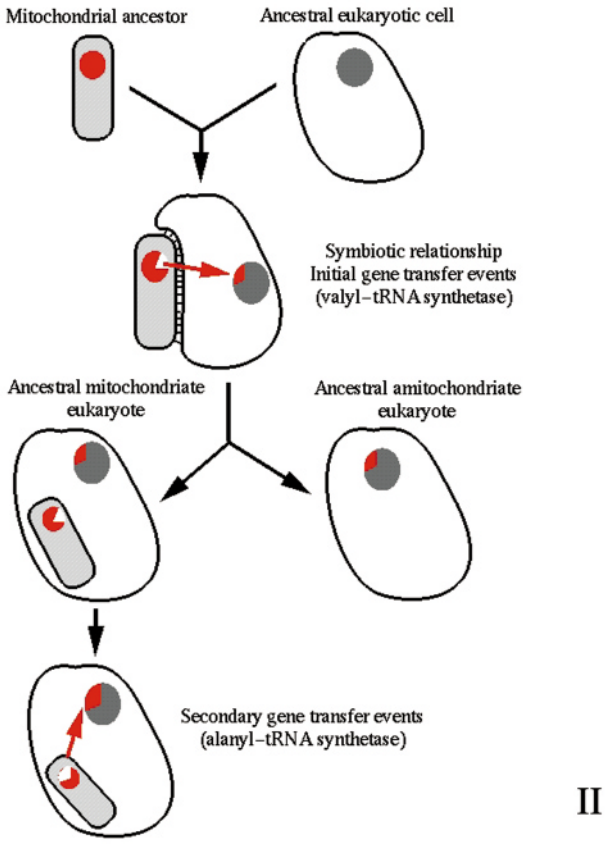

Figure 2. Model of the intermediate stages of mitochondria genesis as suggested by the phylogenies of valyl- and alanyl-tRNA synthetases. An initial phase of gene transfers suggests a close level of interaction between the mitochondrial ancestor and the primordial eukaryotic cell. The final integration of the translation apparatuses of both cells (including the replacement of the nuclear *alaS* gene by its bacterial counterpart) does not take place in amitochondriate diplomonads (such as *G. lamblia*) [88]. This observation suggests that these cells did not undergo the full mitochondrial integration experienced by the rest of eukaryotic branches leading to extant mitochondriate organisms [88].

gene by the mitochondrial *valS* [87]. Given that *G. lamblia* does not contain mitochondria, this finding was taken as an indication that species that lack mitochondria must have lost these organelles at some point in their evolutionary history [87].

This view is challenged by the phylogenetic analysis of alanyl-tRNA synthetase (AlaRS), which results in a radically different evolutionary pattern to that of ValRS [88]. The phylogenetic relationships of cytoplasmic and mitochondrial AlaRSs show that extant cytoplasmic AlaRSs from mitochondriate eukaryotes originated from mitochondrial AlaRS genes. Mitochondrial and cytoplasmic AlaRSs (both nuclear-encoded) form a sister group with bacterial AlaRS, indicating that all cytoplasmic AlaRSs of extant mitochondriate eukaryotes are of mitochondrial origin [88]. Most likely this situation is due to the replacement of the original archaeal *alaS* gene by the mitochondrial *alaS*, in a similar fashion to that observed for valyl-tRNA synthetase [87].

However, in the case of AlaRS it is very apparent that *G. lamblia* AlaRS is more closely related to archaeal AlaRS than to either bacterial or other eukaryotic AlaRSs. Thus, the gene encoding for AlaRS shows different patterns of acquisition and retention between an amitochondriate and mitochondriate eukaryotes.

The simplest explanation for these findings is that diplomonads like *G. lamblia* diverged early from the main eukaryotic branch, retaining their original *alaS* gene (of archaeal origin) (unpublished observations). This contrasting pattern of gene substitution offered by the *Giardia* AlaRS and ValRS sequences is consistent with the course of endosymbiosis and integration of mitochondrial genes being gradual, and the existence of a primitive eukaryotic cell that lacked mitochondria. The evolution of AlaRS indicates that *Giardia* represents an intermediate stage in the development of the eukaryotic cell, because the full integration of mitochondria in other eukaryotes resulted in the nuclear substitution of the archaeal *alaS* by a bacterial *alaS* imported from the mitochondrial genome (fig. 2).

Thus, the analysis of the evolutionary events that gave rise to all extant ARSs has the potential to unravel hidden aspects of the early evolution of life. This exploration has just begun, and we can expect more insights to come out of the analysis of the ARS families that remain to be characterized in detail.

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