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Letter to the Editor

Two Classes of tRNA Synthetases Suggested by Sterically Compatible Dockings on tRNA Acceptor Stem

The 20 aminoacyl-tRNA synthetases establish the genetic code through aminoacylation reactions that link specific amino acids to tRNAs that bear triplet anticodons. Their universal distribution across the phylogenetic tree suggests they are among the oldest polypeptide families. All of these proteins fall into one of two classes comprised of 10 enzymes each. No explanation for the two classes is known. By examination of synthetase-tRNA cocrystals, we noted the possibility for pairwise docking of a specific synthetase from each class on the tRNA acceptor stem. Remarkably, the pairs were not arbitrary, but fit closely with the subdivisions in each of the two classes.

The active sites of class I enzymes are formed from a Rossmann nucleotide binding fold that binds the tRNA acceptor stem from the minor groove side, using two highly conserved sequences (Webster et al., 1984; Hountondji et al., 1986; Ludmerer and Schimmel, 1987; Moras, 1992; Cusack, 1997). In contrast, the active site of class II enzymes is a core of antiparallel β strands that binds to the major groove side of the acceptor stem, using three degenerate sequence motifs (Eriani et al., 1990; Cusack, 1993). Consistent with their opposite approaches to the tRNA acceptor stem, class I and class II enzymes catalyze the aminoacylation of different hydroxyl groups of the terminal tRNA adenosine (Fraser and Rich, 1975; Sprinzl and Cramer, 1975). No evidence for a common ancestor to these two classes has been uncovered.

Based on their mode of binding to the tRNA acceptor stem, both classes of tRNA synthetases have been subdivided into three subclasses. These subclasses are designated as Ia,b,c and IIa,b,c (Figure 1). Given that the enzymes from the two classes bind to opposite sides of the acceptor stem, the possibility of virtual docking of a synthetase from each class to a single tRNA was investigated. This was done by superimposing the molecular coordinates of each tRNA in the available structures of tRNA synthetase-tRNA complexes of class I enzymes to the equivalent tRNA coordinates in complexes of class II enzymes. To avoid bias caused by small conformational differences in the tRNA structures of the published synthetase-tRNA complexes, all superimpositions were done to optimize fitting of the acceptor-stem and elbow region of the L-shaped tRNA structures.

Once the tRNAs were superimposed, the relative orientations of the protein coordinates from the complexes being studied were analyzed. Only the active site–containing domain, including its idiosyncratic loops and insertions, was considered. This domain is thought to be the historical enzyme and it includes the region that docks to the acceptor stem, using in part idiosyncratic RNA binding insertions (Brown and Doolittle, 1995; Nagel and Doolittle, 1995; Schimmel and Ribas de Pouplana, 1995). It was soon evident that, from all possible pairing combinations, specific pairs could be obtained where the coordinates of the class I and II active sites could codock without major steric clashes. This specificity arises because, within each class, the orientation of the active sites with respect to the tRNA acceptor stem varies significantly for the members of each subclass.

Remarkably, these pairings linked two members of class Ia (isoleucyl- and valyl-tRNA synthetase, IleRS and ValRS) with two members of class IIa (threonyl- and seryl-tRNA synthetase, ThrRS and SerRS), a member of class Ib (glutaminyl-tRNA synthetase, GlnRS) with one of class IIb (aspartyl-tRNA synthetase, AspRS), and one from class Ic (tyrosyl-tRNA synthetase, TyrRS) with a member of class IIc (phenylalanyl tRNA synthetase, PheRS) (Figure 2) (Rould et al., 1989; Cusack et al., 1990; Ruff et al., 1991; Bedouelle et al., 1993; Goldgur et al., 1997; Nureki et al., 1998; Sankaranarayanan et al., 1999; Fukai et al., 2000). Because the structures of the active site domains of synthetases in any one subclass are closely related (Cavarelli and Moras, 1993; Doublié et al., 1995; Nureki et al., 1995), the pairings presented in Figure 2 most likely would also link each of the other members of class Ia, whose structures are not yet

Class I	Class II
1a	2a
Leucine Isoleucine Valine Arginine Cysteine Methionine	Serine Threonine Alanine Glycine Proline Histidine
1b	2b
Glutamate Glutamine Lysine-I	Aspartate Asparagine Lysine-II
1c	2c
Tyrosine Tryptophan	Phenylalanine

Figure 1. Classification of Aminoacyl-tRNA Synthetases by Class and Subclass

Modified from Cusack (1995), on the basis of structural data on glycyl-tRNA synthetase (Logan et al., 1995) and sequence analyses of alanyl-tRNA synthetase (Ribas de Pouplana et al., 1993; Shiba et al., 1995). While most lysine-tRNA synthetases (LysRS) are found in class II, examples of class I LysRS are known (Ibba et al., 1997). The dotted box around class I LysRS represents its tentative assignment to class Ib.

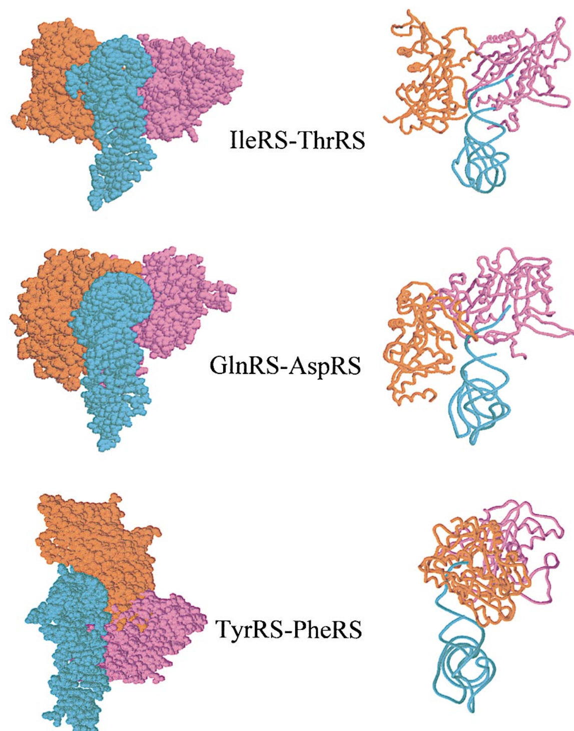

Figure 2. Graphical Representations of the Sterically Compatible Pairs of Synthetases Discussed in This Study

(See: Bedouelle et al., 1993; Cusack et al., 1990; Fukai et al., 2000; Goldgur et al., 1997; Nureki et al., 1998; Rould et al., 1989; Ruff et al., 1991; and Sankaranarayanan et al., 1999.) For the tyrosyl-tRNA synthetase-tRNA complex, we used a previously published model of this structure (Bedouelle et al., 1993), which is practically identical to the recently determined cocrystal structure (S. Cusack, personal communication). The IleRS-ThrRS pairing is equivalent to the pairings of IleRS-SerRS, ValRS-SerRS, and ValRS-ThrRS (not shown). The representations on the left are space-filling models viewed along the longitudinal axis of the tRNA acceptor stem that is perpendicular to the plane of the paper. The representations on the right show the backbone displays of the same structures after a 90° rotation toward the reader. Class I active sites are colored in red. Class II active sites are colored in purple. The tRNA molecule is colored in blue.

known, with those of class IIa, and so on for members of class Ib and IIb, and of class Ic and IIc.

A suggestion that these compatible combinations correspond to ancestral pairings of tRNA-synthetase domains comes from the large differences in the relative positions of the borders of different pairs around the axis of the acceptor stem (Figure 3). These differences are both rotational and translational and explain why a class Ia synthetase (e.g., IleRS or ValRS) can be better paired with a class IIa enzyme (ThrRS or SerRS) or a class Ib synthetase with one from IIb (Figure 2). The exceptional docking of PheRS and TyrRS, which each bind to the acceptor stem with an orientation different than that of other enzymes of their respective classes, is especially noteworthy. In particular, while the unusual docking of PheRS can accommodate a synthetase from more than one class I subclass, TyrRS can only be co-docked with PheRS and not with any other class II (or class I) enzyme.

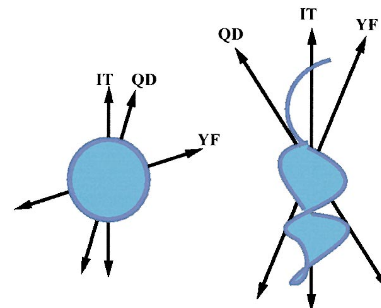

Figure 3. Simplified Representation of the Comigration (around the Acceptor Stem) Found among the Compatible Pairs of Active Sites

The arrows represent the longest line of the interface between each of the aaRS pairs displayed in Figure 2. The left figure corresponds to the view along the longitudinal axis of the tRNA acceptor stem (represented as a blue circle). The right figure corresponds to the view perpendicular to the one on the left (after a 90° rotation toward the reader) showing an acceptor-TΨC minihelix structure. IT = IleRS-ThrRS pair; QD = GlnRS-AspRS pair; YF = TyrRS-PheRS pair.

Strikingly, the subclasses paired according to this criterion recognize amino acid sets that are sterically related (Figure 1). The active site domains are ancient ATP binding sites that might have been adapted for tRNA binding because of the role of ATP in amino acid activation. The later reaction and aminoacylation itself may have been executed by ribozymes of limited amino acid specificity (Lee et al., 2000). In this context, it is also easy to imagine how the two primordial synthetases in a given pair may also have developed the same amino acid specificity. This functional duplication would eventually give rise to two enzymes, with different class architectures, but using the same substrates. This situation closely mirrors that found with class I and II lysyl-tRNA synthetases, which are the only synthetases known to date that can be either of class I or class II architecture (Ibba et al., 1997). From this perspective, class I lysyl-tRNA synthetases (whose structures are unknown) are predicted to belong to class Ib.

These observations raise the possibility that early tRNA synthetases acted as "chaperones" to cover and protect the acceptor end of tRNA (perhaps in a high-temperature environment), possibly to cover the CCA end and preserve aminoacylation. Thus, a specific primordial chaperone/synthetase pair may have been involved in the discrimination between tyrosine and phenylalanine (class Ic and IIc, respectively). Likewise, glutamate (and glutamine) (Ib) had to be separated from aspartate (and asparagine) (IIb), and valine (and isoleucine) (Ia) from threonine (IIa) (isosteric with valine). Eventually, the individual members of each pair may have acquired exact amino acid specificity. In this way, the primordial chaperone/synthetase pairs would have contributed to the final development of the genetic code. As the members of each pair gained exact amino acid specificity, and the pairs split apart, new codons would have to be assigned to differentiate between similar amino acids.

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Lluís Ribas de Pouplana* and Paul Schimmel
Departments of Molecular Biology, and Chemistry
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, California 92037

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* To whom correspondence should be addressed (lluis@scripps.edu).