The renaissance of aminoacyl-tRNA synthesis

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The role of tRNA as the adaptor in protein synthesis has held an enduring fascination for molecular biologists. Over four decades of study, taking in numerous milestones in molecular biology, led to what was widely held to be a fairly complete picture of how tRNAs and amino acids are paired prior to protein synthesis. However, recent developments in genomics and structural biology have revealed an unexpected array of new enzymes, pathways and mechanisms involved in aminoacyl-tRNA synthesis. As a more complete picture of aminoacyl-tRNA synthesis now begins to emerge, the high degree of evolutionary diversity in this universal and essential process is becoming clearer.

Introduction

Aminoacyl-tRNA synthesis is the ubiquitous cellular process that provides substrates for ribosomal translation of messenger RNA during protein synthesis. Much of the long-lived interest in aminoacyl-tRNA and how it is made, derives from its pivotal role in providing the interface between nucleic acid and amino acid sequences in translation. Consequently, the specificity of aminoacyl-tRNA synthesis in pairing the appropriate tRNAs and amino acids is a key determinant of the faithful transmission of genetic information (Figure 1). The synthesis of any given protein may require the co-translational insertion of up to 20 canonical amino acids plus selenocysteine, whose unusual formation was elucidated more recently (see below). In turn, this necessitates the use of at least 21 distinct pathways of aminoacyl-tRNA formation. Recent years have seen dramatic advances in our knowledge of all aspects of aminoacyl-tRNA synthesis, which have emphasized not only the conserved features, but also the mechanistic complexity and diversity of this essential process.

Aminoacyl-tRNA synthesis: back to diversity

The direct attachment of the correct amino acid to a specific tRNA is carried out by an aminoacyl-tRNA synthetase (AARS) in a two-step process involving ATP-dependent amino acid activation followed by transfer of the amino acid onto tRNA (reviewed in Ibba and Söll, 2000). The discovery of 20 distinct AARSs was a milestone in the study of these enzymes, as it confirmed one of the central tenets of Crick's adaptor hypothesis (Crick, 1958). The subsequent finding that these 20 enzymes could be partitioned into two highly conserved structural classes of 10 members, each with unrelated active site topologies, provided a satisfying symmetry which seemed to support the notion that aminoacyl-tRNA synthesis varies little among contemporary organisms (see for example Arnez and Moras, 1997, for discussion of the AARS classes). Numerous studies endorsed the notion that aminoacyl-tRNA synthesis had been highly conserved during evolution, and as recently as 1997 a detailed and fairly complete picture of the various processes was seemingly in place. Answers to some long-standing questions were also starting to emerge. For example, the means by which tRNA^{Ile} induces the editing (i.e. removal) of misactivated valine by isoleucyl-tRNA synthetase [IleRS; first observed in 1966 (Baldwin and Berg, 1966)] and the alternative route to Asn-tRNA synthesis [suspected since 1972 (White and Bayley, 1972)] were both becoming more transparent (Curnow *et al.*, 1996; Hale *et al.*, 1997). As has subsequently become clear, however, rather than tidying up loose ends, these and many other studies have marked the starting point for the remarkable recent progress in our understanding of many diverse aspects of aminoacyl-tRNA synthesis. Various mechanisms of this process have proven more complex and dissimilar than anticipated, prompting re-assessments of entrenched views on their origin and evolution. Similarly, AARSs and AARS-like proteins

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Fig. 1. The cellular synthesis of an aminoacyl-tRNA and its role in protein synthesis. An uncharged tRNA and the corresponding amino acid are first selected from the cellular pools of similar molecules by the appropriate AARS (see text for details). After synthesis and release from the AARS the aminoacyl-tRNA is delivered to the ribosome, where its anticodon can then interact with the corresponding codon in mRNA (reviewed in Al-Karadaghi *et al.*, 2000). The example shown illustrates how this leads to the translation of the codon UGG as tryptophan during the elongation phase of protein synthesis.

have been found to play a variety of unexpected roles outside of translation. These range from group I intron splicing (e.g. Chen *et al.*, 2000; Rho and Martinis, 2000) to apoptosis (reviewed with other examples in Schimmel and Ribas de Pouplana, 2000). In all these respects, the title of a keynote address given at a 1997 Keystone Symposium by Dino Moras, 'Aminoacyl-tRNA synthetases: back to diversity', has proven to be remarkably prophetic.

Quality control during translation

Correctly aminoacylated tRNAs are essential for faithful translation of the genetic code: any mistakes in the matching of tRNAs and amino acids may be perpetuated as errors in protein synthesis. The range of unique tRNA structures available from the different combinations of nucleotides, both unmodified and modified, ensures that the correct nucleic acid substrate can be specifically selected from the cellular pool by the appropriate AARS. By comparison, the structural simplicity of the amino acid substrates presents a more challenging problem. AARSs achieve the amino acid substrate specificity necessary to keep errors to an acceptable level in essentially two ways, by preferential binding of the correct (cognate) amino acid and by selective editing of incorrect (non-cognate) amino acid(s). While many structural and biochemical principles underlying amino acid selection and rejection have long been understood (e.g. Jakubowski and Goldman, 1992; Fersht, 1999), recent advances have provided data at the molecular level and revealed unanticipated editing reactions. For example, the crystal structures of IleRS complexed with the non-cognate amino acid valine (Nureki *et al.*, 1998) and with tRNA^{Ile} (Silvian *et al.*, 1999) confirmed the existence of distinct catalytic and editing sites in the enzyme, as had long been suspected based on biochemical studies. Subsequent investigations have now revealed the mechanism by which tRNA^{Ile} translocates misactivated non-cognate amino acids such as valine, threonine, α -aminobutyrate and cysteine between these catalytic and editing sites, which are over 25 Å apart (Nomanbhoy and Schimmel, 2000). Interestingly, many of the principles of editing described for IleRS have recently been observed in other AARSs. For example valyl-tRNA synthetase (ValRS), which is required to discriminate valine from isoleucine and threonine, displays a high degree of structural homology to IleRS in terms both of its catalytic and editing sites

and the role of its cognate tRNA in facilitating hydrolysis of non-cognate amino acids (Fukai *et al.*, 2000). The parallels between IleRS and ValRS are not entirely unexpected, given that the two enzymes are closely related members of the class I AARSs. Indeed leucyl-tRNA synthetase, which is also closely related to IleRS and ValRS, appears to contain a homologous editing domain (Cusack *et al.*, 2000) possibly required for discrimination against the metabolite homocysteine (Jakubowski, 1995). More surprisingly, structural studies have also shown that editing of misactivated serine by threonyl-tRNA synthetase (ThrRS), a class II AARS, is also achieved by tRNA-dependent translocation of the non-cognate amino acid between catalytic and editing sites (Sankaranarayanan *et al.*, 2000). Structure-based comparisons between ThrRS and the class I enzymes IleRS and ValRS indicate that tRNA-dependent editing in both AARS classes requires conformational switching of the tRNA's 3'-terminal CCA end between a hairpin and a helical conformation; the two classes' mechanisms are effectively mirror images of each other (Dock-Bregeon *et al.*, 2000). This surprising finding reinforces previous observations that class I and II AARSs appear to be mirror images of each other with respect to tRNA and ATP binding (Ruff *et al.*, 1991), and it substantiates proposals that this property reflects the origin of the two different AARS classes. Significant insight into this last point has come from the finding that certain class I and II AARSs form sterically compatible pairs, whose respective amino acid substrates are themselves sterically related (Ribas de Pouplana and Schimmel, 2001; see also below).

In addition to the editing mechanisms which target mischarged tRNAs as a means of quality control, the aminoacylation of tRNAs may also provide an important check-point for translational fidelity. In much the same way that tRNA maturation and transport are dependent on the recognition of specific conformations (e.g. McClain and Seidman, 1975; Lipowsky *et al.*, 1999) a number of tRNAs are only recognized and exported from eukaryotic nuclei after aminoacylation (Lund and Dahlberg, 1998; Sarkar *et al.*, 1999). Recent studies have shown that many of the AARSs present in mammalian nuclei are part of a large multi-enzyme complex (Nathanson and Deutscher, 2000), which might itself also facilitate tRNA export through association with elongation factor 1 (Grosshans *et al.*, 2000). The existence of a nuclear multi-synthetase complex is not in itself surprising given that comparable cytoplasmic complexes have been well documented (see for example Quevillon *et al.*, 1999 and refer-

Fig. 2. Examples of phylogenetically diverse bacteria and archaea with non-canonical aminoacyl-tRNA synthesis pathways as inferred by genome sequence analysis [see text for details; data derived from DDBJ/EMBL/GenBank and IGwit, *Bifidus* data (F. Arigoni, Nestle Research Center, personal communication)]. Organisms within the blue oval do not encode CysRS; within the red oval encode a class I LysRS; within the green oval lack GlnRS; enclosed within the yellow oval lack AsnRS. Thus, for example, *Rhodobacter* lacks AsnRS and GlnRS and contains a class I LysRS, whereas *Chlamydia* also lacks AsnRS and GlnRS but does not contain a class I LysRS. The position of *Rhodopseudomonas* indicates that it contains GlnRS and a class I LysRS but lacks AsnRS, while *Pseudomonas* only lacks AsnRS.

ences therein). In addition to AARSs, the cytoplasmic complexes also contain accessory proteins (e.g. Quevillon *et al.*, 1999), elongation factors (e.g. Kim *et al.*, 2000) and molecular chaperones (Kang *et al.*, 2000). Accessory proteins functional in tRNA-binding have also been described in bacteria, suggesting that higher order aminoacylation complexes may also exist outside the eukaryal kingdom (Swairjo *et al.*, 2000). While the exact functional significance of such higher order complexes is not known, it is possible that they may provide a means of optimizing aminoacyl-tRNA synthesis and its subsequent channeling to the ribosome (discussed in Grosshans *et al.*, 2000; Nathanson and Deutscher, 2000).

The biochemical scope of aminoacyl-tRNA synthesis

Recent years have seen dramatic advances in our understanding of canonical aminoacyl-tRNA synthesis, i.e. the direct attachment of cognate tRNAs to amino acids by the 20 canonical AARSs. At the same time it has become clear that the formation of a minority of aminoacyl-tRNAs does not follow the canonical pattern, either in terms of the enzymes and/or the pathways employed for synthesis (Ibba *et al.*, 2000). Pathways whereby aminoacylated tRNAs are further modified prior to their use in translation are known for Asn-tRNA, Gln-tRNA, selenocysteinyl-tRNA and formylmethionyl-tRNA formation (reviewed in Blanquet *et al.*, 2000; Ibba *et al.*, 2000), and AARSs with novel enzymatic activities have been identified (for Cys-tRNA and Lys-tRNA formation). Non-canonical aminoacyl-tRNA synthesis is most prevalent in microbes; for example, some archaea have been shown to use such enzymes to synthesize up to five of the 21 aminoacyl-tRNAs they require for protein synthesis (Figure 2).

Recent advances in our knowledge of non-canonical aminoacyl-tRNA synthesis are summarized below.

The transamidation pathways for Gln-tRNA and Asn-tRNA synthesis. Many bacteria and archaea lack genes encoding the asparaginyl- (AsnRS) or glutaminyl-tRNA synthetases (GlnRS) or both, as shown in Figure 2. In these organisms, Asn-tRNA and Gln-tRNA are instead synthesized by aminoacylation-transamidation (equations 1–4).

tRNA^{Gln} is first misaminoacylated with glutamate (equation 1) by a non-discriminating glutamyl-tRNA synthetase (GluRS), which is able to synthesize both Glu-tRNA^{Glu} and Glu-tRNA^{Gln} (Sekine *et al.*, 2001). The resulting mischarged tRNA is then converted by glutamyl-tRNA^{Gln} amidotransferase (GluAdT) into Gln-tRNA^{Gln} (equation 2). Similarly, Asn-tRNA^{Asn} is formed via a non-discriminating aspartyl-tRNA synthetase (AspRS, equation 3) and an aspartyl-tRNA^{Asn} amidotransferase (AspAdT, equation 4). In bacteria and archaea, transamidation (equations 2 and 4) is catalyzed by two related multimeric enzymes, GatCAB and GatDE (Tumbula *et al.*, 2000). To date GatDE has only been found in archaea, where it functions solely as a GluAdT. In contrast GatCAB can function as both a GluAdT and as AspAdT, and has been shown to perform one or both reactions in a genome-context specific fashion (Table I; see also Curnow *et al.*, 1998).

Selenocysteinyl-tRNA synthesis. The mechanism by which selenocysteine (Sec) is formed from serine and then co-translationally

Table I. Known tRNA-dependent amidotransferase activities

Gene	Function ^a		
	GluAdt	AspAdt	AspAdt and GluAdt
<i>gatCAB</i>	many bacteria, chloroplasts	few bacteria and archaea	some bacteria
<i>gatDE</i>	archaea	none known	none known

^aFunction is defined as the proven ability of the given gene products to catalyze the specified reaction *in vivo* and/or *in vitro*, and is dependent upon the presence of the corresponding non-discriminating aminoacyl-tRNA synthetases. The absence of known mitochondrial GlnRS encoding genes suggests that GluAdt might also exist in some examples of this organelle.

inserted into nascent polypeptides has been extensively described in the bacterium *Escherichia coli* (Commans and Böck, 1999). tRNA^{Sec} is first charged with serine by seryl-tRNA synthetase, and the seryl-moiety subsequently converted to Sec by selenocysteine synthase. The resulting Sec-tRNA^{Sec} is then used to insert Sec in response to certain in-frame UGA stop codons. The means by which the UGA codon is recognized in archaea and eukaryotes has recently been found to differ from that used in bacteria. In bacteria, the elongation factor SelB recognizes an mRNA stem-loop structure immediately following UGA, whereas archaea and eukaryotes recognize a structure (SECIS) in the mRNA 3'-untranslated region. The archaeal (Rother *et al.*, 2000) and eukaryal (Tujebajeva *et al.*, 2000; Fagegaltier *et al.*, 2000) counterparts of SelB, eEFsec, preferentially bind Sec-tRNA^{Sec} but not the appropriate mRNA secondary structures. This function is performed by SECIS-binding protein 2 (Copeland *et al.*, 2000), which associates with the Sec-tRNA^{Sec}-eEFsec complex to facilitate UGA decoding as Sec by an unknown process.

Another tRNA-dependent amino acid transformation, albeit not involved in protein synthesis, is the formation of glutamate 1-semialdehyde, the first precursor in porphyrin biosynthesis in many organisms (Moser *et al.*, 1999 and references therein). This glutamate derivative is formed by glutamyl-tRNA reductase, a unique oxidoreductase with sequence-specific aminoacyl-tRNA recognition, in an NADPH-dependent reduction of standard Glu-tRNA^{Glu}. This shows the close relationship between aminoacyl-tRNA synthesis and intermediary metabolism.

Class I and class II lysyl-tRNA synthetases. As mentioned above, the AARSs are divided into two mutually exclusive structural groups, classes I and II. The assignment of an AARS of particular substrate specificity to one or the other of these classes is almost completely conserved throughout evolution—that is to say, regardless of its origin, a particular AARS will always belong to the same class. To date, the only known exception is found among the lysyl-tRNA synthetases (LysRSs) which are class I enzymes in most archaea and a few bacteria (Ibba *et al.*, 1997a, b), but are otherwise members of class II. Functionally, the class I and class II forms of LysRS behave as archetypal members of their respective classes (see for example Söll *et al.*, 2000), providing no obvious clues as to why two unrelated LysRS families exist.

The dual function prolyl/cysteinyI-tRNA synthetase. Perhaps the most unexpected of all the non-canonical routes of aminoacyl-tRNA synthesis is an alternative pathway for Cys-tRNA formation.

The possibility that Cys-tRNA can be formed independently of cysteinyl-tRNA synthetase (CysRS) was first raised when it became clear that some archaeal genomes do not encode CysRS (reviewed in Doolittle, 1998). Functional studies with one such archaeon, *Methanococcus jannaschii*, revealed that both Cys-tRNA^{Cys} and Pro-tRNA^{Pro} can be synthesized by prolyl-tRNA synthetase (ProRS; Stathopoulos *et al.*, 2000). Subsequent investigations have shown that, while the ability to synthesize Cys-tRNA^{Cys} is not a feature of all ProRS proteins, it is nevertheless observed in some organisms (e.g. *Giardia*, *Methanococcus maripaludis* and *Thermus thermophilus*) that also encode a conventional CysRS (Bunjun *et al.*, 2000; Stathopoulos *et al.*, 2000; C. Stathopoulos and I. Ahel, unpublished results). The mechanism that allows certain ProRS enzymes to specifically synthesize both Cys-tRNA^{Cys} and Pro-tRNA^{Pro} is not apparent from the high-resolution structure of the protein (Yaremchuk *et al.*, 2000). Despite some initial dispute (Lipman *et al.*, 2000), recent biochemical studies indicate that discrimination between the two cognate amino acid substrates is achieved by a specific requirement for binding tRNA^{Cys} prior to cysteine activation, but not for tRNA^{Pro} prior to proline activation (Stathopoulos *et al.*, 2001).

The origins of aminoacyl-tRNA synthesis

The intimate connection between aminoacyl-tRNA synthesis and the genetic code has made this a fertile area for speculation on the origins of coded protein synthesis. While a consensus has arisen that aminoacyl-tRNA synthesis *per se* may have originated as an RNA-catalyzed process (e.g. Illangasekare and Yarus, 1999; Lee *et al.*, 2000), the evolution of extant AARSs remains more contentious. Phylogenetic comparisons (Nagel and Doolittle, 1995) and structural sub-groupings (Ribas de Pouplana and Schimmel, 2001) indicate clear relationships between the AARSs but, with few exceptions (see below), do not reveal how they may have evolved from a presumably smaller number of ancestral proteins. The only AARSs for which clear evolutionary histories have been established are GlnRS and AsnRS. Phylogenetic and structural considerations leave little doubt that GlnRS and AsnRS were the last AARSs to emerge, resulting from duplication and diversification of ancestral GluRS and AspRS, respectively (reviewed in Woese *et al.*, 2000). Whether the comparatively recent emergence of GlnRS and AsnRS also reflects a late recruitment of glutamine and asparagine to coded protein synthesis is more open to question, as it seems likely that Gln-tRNA^{Gln} and Asn-tRNA^{Asn} were originally synthesized by tRNA-dependent transamidation reactions. Nevertheless, given the widespread use of the AspAdt-catalyzed reaction as the sole

means of asparagine synthesis in many bacteria (e.g. Becker and Kern, 1998; Curnow *et al.*, 1998; B. Min and D. Söll, unpublished results), it is tempting to speculate that asparagine may have been one of the last amino acids recruited to protein synthesis.

Outlook

Advances in structural biology and functional genomics have already provided an unprecedented new impetus to the study of aminoacyl-tRNA synthesis, and will undoubtedly continue to do so. For example, genome-wide array analyses offer a means to see how aminoacyl-tRNA synthesis fits into the broader process of translation, and together with gene knockouts and expression arrays may also suggest functions for some of the numerous AARS-like proteins (Schimmel and Ribas de Pouplana, 2000). While AARS interactions with synthetases or other proteins are well known (see above) in higher eukaryotes, little is known about the existence or potential relevance of such interactions in other organisms. Genomic tools provide an obvious means to address this issue. For example, the protein–protein interaction map of *Helicobacter pylori* (Rain *et al.*, 2001) indicates a variety of potential interactions of interest that include not only AARSs and a subunit of the GluAdT, but also other proteins of unknown function. The correlation of function with new protein domains (obtained by structural genomic and computational analyses) may lead to *in silico* approaches to tease out of the amino acid sequences of unknown open reading frames their putative enzymatic activities. This may be a way of searching for ‘lost’ progenitors of components of the current aminoacyl-tRNA synthesis machinery. Despite the obvious advantages such new methodologies offer, the full realization of their potential still requires careful checks by more classical approaches. For example, transposon insertion studies of the minimal genome of *Mycoplasma genitalium* initially had suggested, quite surprisingly, that both the isoleucyl- and tyrosyl-tRNA synthetases might be dispensable for cellular viability (Hutchison *et al.*, 1999). However, subsequent genetic and biochemical experiments indicated that transposon mutagenesis had removed only non-essential portions of these AARS, and thus not disrupted aminoacyl-tRNA synthesis (B. Ruan and D. Söll, unpublished results). Finally, it is worth noting that current technologies offer no obvious means to rapidly investigate the true nature of the tRNAs themselves in terms of the large number of modified nucleotides they are known to contain (see for example Björk *et al.*, 2001 and references therein). Given that key roles in the recognition of AARSs and regulation of translation have already been ascribed to modified RNAs, such modifications may contribute to a further unanticipated level of complexity in the process of aminoacyl-tRNA synthesis itself.

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