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EMBO WORKSHOP REPORT
Aminoacyl-tRNA synthetases: a family of expanding functions
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Introduction

The aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes well known for their role in protein synthesis. More recent investigations have discovered that this classic family of enzymes is actually capable of a broad repertoire of functions that not only impact protein synthesis, but also extend to a number of other critical cellular activities (Figure 1; Martinis *et al*., 1999). Specific aaRSs play roles in cellular fidelity, tRNA processing, RNA splicing, RNA trafficking, apoptosis and transcriptional and translational regulation. A recent EMBO workshop entitled *Structure and Function of Aminoacyl-tRNA Synthetases* (Mittelwihr, France, October 10–15, 1998) highlighted the diversity of the aaRSs' role within the cell. These novel activities, as well as significant advances in delineating mechanisms of substrate specificity and the aminoacylation reaction, affirm the family of aaRSs as pharmaceutical targets. Herein we report major advances in the field during the short period since the last aaRS meeting (Francklyn *et al*., 1997).

aaRSs perform dual and essential roles in the cell

Eukaryotic pre-tRNAs are synthesized in the nucleus and require extensive processing to yield functional tRNAs. An elegant series of experiments clearly show that mature tRNAs are charged in the nucleus prior to export to the cytoplasm (Lund and Dahlberg, 1998). Blockage of the tRNA export machinery results in accumulation of nuclear charged tRNAs. Introduction of specific aaRS inhibitors decreases export of the relevant uncharged tRNA. This nuclear aaRS-dependent aminoacylation reaction was proposed to serve as a stringent quality control mechanism prior to export of mature tRNA to the cytoplasm where it is required for protein synthesis. Nuclear aminoacylation of tRNAs (Arts *et al*., 1998; Lund and Dahlberg, 1998) presumably requires import of aaRSs from the cytoplasm. Analysis of *Saccharomyces cerevisiae* sequences determined that 15 cytoplasmic aaRSs contain potential nuclear localization signal sequences that are lacking in the corresponding *Escherichia coli* enzyme (Schimmel and Wang, 1999). While aaRS nuclear import remains to be observed experimentally, these comparisons suggest discrete structure–function differences that emerged during the evolution of bacterial and eukaryotic aaRSs.

Under apoptotic conditions, TyrRS was shown to be specifically secreted and proteolytically cleaved, generating two cytokines from its N- and C-terminal halves (Wakasugi and Schimmel, 1999). The full-length enzyme is inactive in cytokine activity assays, yet the endothelial monocyte-activating polypeptide II (EMAP II)-like C-terminal domain of bovine (Kornelyuk *et al*., 1999) and human (Wakasugi and Schimmel, 1999) TyrRSs exhibits monocyte chemotaxis activity as well as stimulating production of TNFα and tissue factor. The human TyrRS C-terminal domain was also tested and shown to have leukocyte chemotaxis activity and to induce production of myeloperoxidase. Moreover, human mini-TyrRS, which retained the N-terminal aminoacylation domain but lacked the C-terminal EMAP II-like domain, acts as an IL-8-like chemokine (Wakasugi and Schimmel, 1999). Mutation of a conserved 'ELR' amino acid sequence motif in the human mini-TyrRS (also found in certain chemokines) abolished leukocyte chemotaxis.

Interestingly, EMAP II-like domains are also fused to *E.coli* and *Caenorhabditis elegans* MetRSs as well as human and bovine TyrRS (Simos *et al*., 1996; Kleeman *et al*., 1997). In addition, the domain is appended to polypeptides, such as p43 from humans and Arc1p from yeast, which interact with aaRSs (Simos *et al*., 1996; Quevillon *et al*., 1997). The N-terminal domain of Arc1p interacts with both yeast GluRS and MetRS, while the C-terminal EMAP II-like domain binds the tRNA, enhancing affinity and stimulating catalytic activity (Simos *et al*., 1998). Arc1p has been proposed to serve as a mobile *trans*-acting tRNA binding domain involved in tRNA channeling by facilitating transport of tRNA out of the nucleus via the nuclear pores.

Some aaRSs are involved in their own transcriptional or translational regulation (Putzer *et al*., 1995). Transcriptional regulation is mainly based on anti-termination mechanisms (Henkin, 1994; Condon *et al*., 1996). However in one case, *E.coli*, AlaRS binds to the promoter of its own gene and represses transcription (Putney and Schimmel, 1981). Now, *Thermus thermophilus* PheRS has also been found to bind specifically to DNA, although the functional implications remain undefined (Lechler and Kreutzer, 1998). In *E.coli*, ThrRS regulates its own expres-

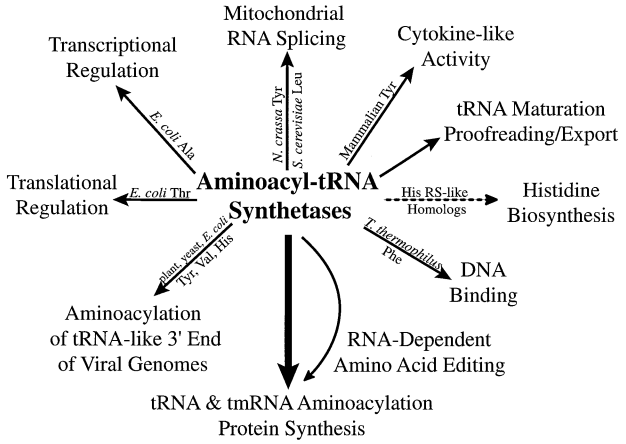

Fig. 1. Cellular roles and functions of aaRS. Particular aaRSs involved in each of the represented activities are indicated by the three letter code of the amino acid that they activate.

sion at the translational level by binding to two hairpin domains of the mRNA operator that separately mimic the anticodon stem–loop of $tRNA^{Thr}$ (Romby *et al*., 1996) and repress their own translation by occluding ribosome binding (Sacerdot *et al*., 1998).

Mitochondrial *Neurospora crassa* TyrRSs as well as *S.cerevisiae* LeuRS are essential protein factors in certain group I intron splicing activities (reviewed in Dujardin and Herbert, 1997; Lambowitz *et al*., 1999). It was reported that human mitochondrial and *Mycobacterium tuberculosis* LeuRSs complement a yeast null strain lacking the endogenous mitochondrial LeuRS (S.Martinis *et al*., unpublished data). A short C-terminal deletion and single-site substitutions within a conserved CP1 region were previously shown to affect yeast mitochondrial LeuRS-induced splicing activity (Li *et al*., 1996). Similar mutations in *M.tuberculosis* LeuRS also decreased or abolished complementation of the yeast null strain. Thus, unlike the TyrRS-promoted splicing model, prokaryotic and eukaryotic sources of LeuRS may contain universal determinants to facilitate essential mitochondrial splicing activities.

Some bacterial genomes contain an open reading frame named *hisZ* (Delorme *et al*., 1992). The translated product resembles the catalytic domain of HisRS, yet it lacks essential catalytic residues, rendering the protein inactive (Sissler *et al*., 1999). Upon disruption of *hisZ,* bacteria will only grow on minimal medium supplemented with histidine or histidinol, the last intermediate in the biosynthesis of histidine. Interestingly, the *hisZ* gene correlates with the *hisG* gene length encoding ATP phosphoribosyltransferase (the first enzyme in the histidine biosynthesis pathway). When *hisZ* is present, the *hisG* gene product is ~100 amino acids shorter than that encoded by the *hisG* in bacterial species lacking *hisZ*. Thus, it was suggested that the HisRS-like HisZ protein can complement an incomplete ATP phosphoribosyltransferase.

aaRS crystal structures address mechanisms of aminoacylation

Structural analysis of class I [i.e. IleRS (Nureki *et al.*, 1998; T.Steitz, unpublished data), ArgRS (Cavarelli *et al*., 1998), GlnRS (Rath *et al*., 1998), TyrRS (Brick *et al*., 1988) and TrpRS (Doublié *et al*., 1995)] and class II aaRSs (reviewed in Arnez and Moras, 1997; Cusack, 1997) in complexes with various substrates has provided unique snapshots of the aminoacylation reaction. Enzymes from both classes appear to use a lock and key mechanism for the side chain of the amino acid and an induced fit mechanism for other substrates including ATP, tRNA and the peptidic moiety of the amino acid (Arnez and Moras, 1997; Cusack, 1997; Carter *et al*., 1999). One exception is HisRS where the histidine side chain binding site is created by induced fit of a crucial active site loop (S.Cusack *et al.*, unpublished data). Also, comparison of the heterologous complex between yeast $tRNA^{Asp}$ and *E.coli* AspRS revealed a tRNA-induced mechanism that dictates specificity with key control played by the acceptor stem (D.Moras *et al.*, unpublished data). Catalytic and structural roles for three bound magnesium ions are also now well established for several class II tRNA synthetases including SerRS, AsnRS and AspRS (Belrhali *et al*., 1994; Berthet-Colominas *et al*., 1998; Schmitt *et al*., 1998a).

Additional strategies for specific substrate selection have been highlighted. For example, ArgRS (Cavarelli *et al*., 1998) is characterized by two nucleic acid binding modules: an anticodon binding domain similar to that of MetRS and IleRS, and an RNA binding module with topology overlapping the S4 ribosomal protein. The class II ThrRS contains four domains (Moras *et al.*, unpublished data) including a novel N-terminal bi-modal domain that interacts with the minor groove of the tRNA acceptor stem. Structures of ProRS– and ThrRS–tRNA complexes have re-emphasized anticodon binding as a key mechanism for tRNA recognition and discrimination. Although RNA binding and recognition deform the anticodon loop in each case, a common pattern for base-specific recognition has not yet been identified.

Aminoacylation fidelity is ensured by two aaRS catalytic sites

Two valine binding sites were identified within *T.thermophilus* IleRS (Nureki *et al*., 1998, 1999). One was in the Rossmann fold-based activation site and the other was located near a cleft formed by two completely conserved peptides in the CP1 editing domain (Lin *et al*., 1996). Mutational analysis established conserved residues that are important for editing. The aaRS editing mechanism is also tRNA-dependent and at least for IleRS relies specifically on the tRNA D-loop (Hale *et al*., 1997). A small RNA minihelix mimicking the $tRNA^{Ile}$ D-loop failed to stimulate editing activity, demonstrating that the D-loop-based editing determinants require presentation in the context of the full-length tRNA (Nordin and Schimmel, 1999). Although the role of the D-loop has been clearly established, it remains unclear how this outside corner of the tRNA interacts, either directly or indirectly, with the protein to stimulate editing of misactivated or mischarged amino acids.

A co-crystal structure of *Staphylococcus aureus* IleRS complexed to *E.coli* tRNA illuminated a potential RNA–protein 'editing complex' (T.Steitz *et al.*, unpublished data). Although electron density only extended through the C74 base of $tRNA^{Ile}$, modelling of a helical CCA

3′ terminus placed A76 near the critical amino acids of the hydrolytic editing cleft. One clear question that persists is how the misactivated amino acid or the 3′-end of the mischarged tRNA is translocated by >25 Å from the synthetic active site to the CP1-based editing cleft. Steitz proposed that the tRNA shuttles its misacylated 3′-end between the editing cleft and the Rossman fold analogous to the DNA polymerase mechanism of editing. Thus, the hydrolytic site could sample the RNA-linked amino acid to determine if it is correct or mischarged. It remains unclear though, how the misactivated valyl-adenylate could be translocated to the editing site.

Misacylated tRNAs are essential for novel translation pathways

Certain methanogenic Archaea appear to lack CysRS. Potential alternative routes to charging cysteine tRNA may include thiolation of serine mischarged to $tRNA^{Cys}$, similar to the selenocysteine transformation (Ibba *et al*., 1997a; Kim *et al*., 1998). While *Methanobacterium thermoautotrophicum* SerRS could aminoacylate its cognate $tRNA^{Ser}$, it did not misacylate $tRNA^{Cys}$ (Kim *et al*., 1998). However, recombinant *Methanococcus jannaschii* SerRS mischarges serine to *M.jannaschii* $tRNA^{Cys}$ (M.Saks, unpublished data) indicating that this aaRS has relaxed tRNA specificity. Although neither the transformation pathway, nor the enzyme(s) required for converting Ser-$tRNA^{Cys}$ to Cys-$tRNA^{Cys}$ have been identified, it is possible that SerRS may play a pivotal role in the incorporation of serine, selenocysteine and cysteine into proteins in some organisms.

Asparagine- and glutamine-charged tRNAs require mischarged Asp-$tRNA^{Asn}$ (Curnow *et al*., 1996; Becker and Kern, 1998) and Glu-$tRNA^{Gln}$ (Curnow *et al*., 1996; Schön *et al*., 1988) intermediates, respectively in some organisms. These mischarged tRNAs undergo a tRNA-dependent transamidation reaction to convert the linked amino acid prior to incorporation into proteins. The genes encoding the heterotrimeric tRNA-dependent amidotransferase (AdT) were cloned from both *Bacillus subtilis* (Curnow *et al*., 1997) and *Deinococcus radiodurans* (Curnow *et al.*, 1998). Interestingly, both proteins catalyze both Asn-$tRNA^{Asp}$ and Gln-$tRNA^{Glu}$ transamidase activities. Notably, *T.thermophilus* EF-tu discriminates the mischarged tRNA unlike its *E.coli* counterpart (Becker and Kern, 1998). *Deinococcus radiodurans* (Curnow *et al*., 1998) and *T.thermophilus* (Becker *et al*., 1997; Becker and Kern, 1998) have genes for both the tRNA-dependent AdT and the corresponding aaRS, implicating functional duplication. Both organisms also lack an apparent asparagine synthesis pathway. The auxotroph has been suggested to balance its redundant pathways by using the more efficient direct AsnRS aminoacylation route when asparagine is present, yet in its absence the cell resorts to the two-step transamidase pathway to produce Asn-$tRNA^{Asn}$ (Becker and Kern, 1998). Alternatively, the tRNA-dependent transamidase pathway has been proposed to serve as a sole route to asparagine synthesis, perhaps as a remnant from an RNA world (Curnow *et al*., 1998).

Because of the lack of GlnRS in Archaea and the transamidase pathway which relies on a non-discriminatory GluRS to misacylate $tRNA^{Gln}$, evolution of the GlxRS family is particularly intriguing. GlnRS was proposed to arise via duplication of the GluRS gene deep in the evolutionary branches of the eukarya and then to be horizontally transferred to bacteria (Lamour *et al*., 1994; Brown and Doolittle, 1999). Phylogenetic analyses demonstrate that Archaea GluRSs are more closely related to both GlnRS and GluRS from eukarya than to bacterial GluRS (Siatecka *et al*., 1998; Brown and Doolittle, 1999), and suggest that the core GluRSs existed in the last common ancestor that relied on the transamidation pathway to produce Gln-$tRNA^{Gln}$. Moreover, since the anticodon-binding modules of GlnRS and GluRS are completely distinct, the C-terminal domain would have been appended subsequent to the bacteria and Archaea/eukarya split (Siatecka *et al*., 1998).

Specific aaRS–tRNA interactions

aaRSs rely on identity sets to aminoacylate their cognate tRNAs with high specificity (reviewed in Giegé *et al*., 1998). Several aaRSs even charge non-canonical tRNA-like substrates such as the 3′-ends of viral genomes (Florentz and Giegé, 1995; Goodwin and Dreher, 1998) and tmRNA (Himeno *et al*., 1997; Felden *et al*., 1998) which also contain discrete identity elements. Recent developments have highlighted the importance of cryptic (Saks and Sampson, 1996) and permissive elements (Frugier *et al.*, 1998; Sissler *et al.*, 1998), as well as alternative identity sets for the same specificity (Sissler *et al.*, 1996). Identity elements may partially vary along the evolutionary scale as shown by species-specific differences for proline (Stehlin *et al*., 1998) or cysteine identity (Lipman and Hou, 1998). A dramatic evolutionary change has been observed for the well established tRNA G3:U70 base pair linked to alanine identity. Although nearly universally conserved, G3:U70 is virtually lacking in $tRNA^{Ala}$ sequences from animal mitochondria, presenting a potential novel scenario for $tRNA^{Ala}$ recognition (Chihade *et al*., 1998).

Identity set evolution has been directly linked to the co-evolution of cognate aaRSs. ProRS sequences can be divided into two groups based in part on the motif 2 loop sequence (Stehlin *et al*., 1998). Cysteine-scanning mutagenesis as well as cross-linking experiments suggest that an arginine of this loop interacts with the G72 base, a major determinant for the *E.coli* enzyme in one group, but not in human aaRS representing the other group (K.Musier-Forsyth *et al*., unpublished data). Interestingly, conserved lysine-specific tRNAs are recognized by either a class II or I LysRS (Ibba *et al*., 1997b, 1999). Specifically, two different class I LysRSs rescue an *E.coli* strain deficient in class II LysRS. Both class I and II LysRSs recognize the discriminator base and anticodon nucleotides, yet apparently approach and interact with the acceptor stem helix from the major and minor groove sides, respectively. Phylogenetic analysis suggested that at least one type of LysRS appeared after $tRNA^{Lys}$ was established (Ribas de Pouplana *et al*., 1998), and recent studies by Ibba *et al*. (1999) are consistent with this prediction (Schimmel and Ribas de Pouplana, 1999).

tRNA aminoacylation results in a conformational change of its 3′-terminal adenosine based on NMR and crystal structures of tRNA terminus model compounds

(Nawrot *et al*., 1997), the crystal structure of formyltransferase bound to fMet-tRNA^{fMet} (Schmitt *et al*., 1998b) as well as fluorescence studies of a tRNA in which the 3′-adenosine residue was substituted by formycin (M.Sprinzl *et al.*, unpublished data). Specifically, the ribose of the 3′-terminal adenosine of an aminoacylated tRNA adopts the 2′-*endo* conformation. The terminal adenosine is destacked and could be recognized as a structural determinant to distinguish aminoacylated tRNAs from the pool of uncharged tRNAs (Nawrot *et al*., 1997). Furthermore, M.Sprinzl proposed that it could act as a switch to trigger proofreading by the CP1-based hydrolytic editing domain of aaRS. The 5′-terminal phosphate of tRNA can also be an identity element. For example, peptidyl-tRNA hydrolase, which recycles premature translational termination products, requires a 5′-phosphate to select N-blocked aminoacylated elongator tRNAs and reject fMet-tRNA^{fMet} (Fromant *et al*., 1999).

Unnatural aaRSs and novel aminoacylation procedures

In vitro protein synthesis incorporating unnatural amino acids into specific predetermined positions has been successful using chemically protected amino acids covalently linked to tRNAs. Subsequent deblocking of the incorporated atypical amino acid yields the catalytically competent enzyme (S.Hecht *et al.*, unpublished data). An *in vivo* approach is currently being developed based on an 'orthogonal' suppressor tRNA and a mutant GlnRS evolved to charge it functionally *in vitro* and *in vivo* (Liu *et al*., 1997a,b). A ribozyme-based aminoacylation activity was also isolated by *in vitro* selection (Suga *et al*., 1998a,b). Specifically, the RNA contains a tRNA-like structure and can transfer amino acids from a short ribonucleotide to tRNA (H.Suga, unpublished data).

Inhibitors targeting aaRSs may lead to novel antibiotics

Pharmaceutical efforts to identify new inhibitors of aaRSs have led to analogs of aminoacyl-adenylate and pseudomonic acid (Schimmel *et al*., 1998). Spectrofluorimetric techniques detect differences in the modes of binding of each class to IleRS (Pope *et al*., 1998a,b). Novel chimeric compounds based on the two inhibitors led to a compound with exceptional affinity for IleRS (K_D <10 fM), comparable to the most potent reversible enzyme inhibitors (A.Pope, unpublished data). Other pharmaceutical efforts have selected peptides from combinatorial phage display libraries that bind specifically to aaRSs. One inhibitory peptide binds to *E.coli* ProRS (L.A.Paige, R.Hyde-DeRuyscher, N.Hyde-DeRuyscher, P.T.Hamilton, D.M.Fowlkes, P.Wendler, P.Gallant, J.Kranz, A.Lim, M.Namchuk, J.Zhang and S.Rocklage, unpublished data) with K_I values of 0.5 and 0.4 μM for proline and ATP binding, respectively (J.Tao, T.Li, G.Connelly, X.Shen, J.A.Silverman, F.Houman, P.Wendler and F.P.Tally, unpublished data). Induction of this peptide also provided protection against *E.coli* in a lethal infection model. Peptides were also isolated from phage display libraries that bind with nanomolar affinity to *Haemophilus influenzae* TyrRS (L.A.Paige, unpublished data). A fluorescence polarization assay incorporated TyrRS bound to these peptides and was used in competition assays to detect small molecule binders with high sensitivity.

Summary

A wealth of information from diverse disciplines has yielded exquisite insight into the aaRSs' interactions with substrates and catalytic mechanisms. The discovery of fascinating new roles also reflects a vast network of functional activities that extends far beyond the aminoacylation reaction. Full realization of the aaRSs' diverse and essential functional roles will not only present evolutionary models for the development of the cell, but also provide enormous opportunities for pharmaceutical advances.

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