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DOMAIN 4 SYNTHESIS AND PROCESSING OF MACROMOLECULES

Aminoacyl-tRNA Synthetases in the Bacterial World

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ABSTRACT Aminoacyl-tRNA synthetases (aaRSs) are modular enzymes globally conserved in the three kingdoms of life. All catalyze the same two-step reaction, i.e., the attachment of a proteinogenic amino acid on their cognate tRNAs, thereby mediating the correct expression of the genetic code. In addition, some aaRSs acquired other functions beyond this key role in translation. Genomics and X-ray crystallography have revealed great structural diversity in aaRSs (e.g., in oligomery and modularity, in ranking into two distinct groups each subdivided in 3 subgroups, by additional domains appended on the catalytic modules). AaRSs show huge structural plasticity related to function and limited idiosyncrasies that are kingdom or even species specific (e.g., the presence in many *Bacteria* of non discriminating aaRSs compensating for the absence of one or two specific aaRSs, notably AsnRS and/or GlnRS). Diversity, as well, occurs in the mechanisms of aaRS gene regulation that are not conserved in evolution, notably between distant groups such as Gram-positive and Gram-negative *Bacteria*. The review focuses on bacterial aaRSs (and their paralogs) and covers their structure, function, regulation, and evolution. Structure/function relationships are emphasized, notably the enzymology of tRNA aminoacylation and the editing mechanisms for correction of activation and charging errors. The huge amount of genomic and structural data that accumulated in last two decades is reviewed, showing how the field moved from essentially reductionist biology towards more global and integrated approaches. Likewise, the alternative functions of aaRSs and those of aaRS paralogs (e.g., during cell wall biogenesis and other metabolic processes in or outside protein synthesis) are reviewed. Since aaRS phylogenies present promiscuous bacterial, archaeal, and eukaryal features, similarities and differences in the properties of aaRSs from the three kingdoms of life are pinpointed throughout the review and distinctive characteristics of bacterium-like synthetases from organelles are outlined.

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INTRODUCTION

Key Role of Aminoacyl-tRNA Synthetases in Biology and Focus of the Review

Aminoacyl-tRNA synthetases are ancient proteins present in all living organisms whose origin intermingles with that of life. They are responsible for correct expression of the genetic code at the translational level, where they catalyze the attachment of amino acids on tRNA. For biological necessity, this seminal function has to be specific (although errors often occur) and is governed by rules mainly conserved in evolution that act as an

operational second genetic code (1, 2). Because of their pivotal role, synthetases were the subject of intensive research covered in various reviews (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). Extant synthetases were shaped by evolution and show structural diversity in the tree of life, but amazingly they also show new functions beyond their key role in translation (15, 16, 17).

This essay focuses on bacterial synthetases and their paralogs, emphasizes how structures account for functional properties, outlines the consequences of their evolutionary history, and highlights enzymologic aspects, including the editing function that ensures correction of aminoacylation errors. The regulation of synthetase expression in bacteria is also covered, and approaches to inhibit their enzymatic activities and to engineer their structure are outlined. Since the phylogeny of synthetases presents complex patterns with promiscuous bacterial, archaeal, and eukaryal features (3, 14, 18), similarities and differences in properties of synthetases from the three kingdoms of life will be pinpointed all along the review and distinctive characteristics of bacterium-like synthetases from organelles will be outlined. Finally, perspectives toward future discoveries and deeper understanding are proposed.

Abbreviations and Visualization of Three-Dimensional Structures

Main abbreviations

aaRS, aminoacyl-tRNA synthetase, with the three-letter code for amino acids, e.g., AlaRS for alanyl-tRNA synthetase; ND-aaRSs, Non-Discriminating aaRSs, namely ND-AspRS or ND-GluRS that mischarge $tRNA^{Asn}$ or $tRNA^{Gln}$ in organisms lacking AsnRS or GlnRS—a contrario, D-AspRS or D-GluRS for the Discriminating enzymes, i.e., that are specific for their cognate transfer RNAs and mt-aaRS for mitochondrial-aaRS (if not otherwise indicated, aaRSs are discriminating); tRNA, transfer RNA (e.g., $tRNA^{Ala}$ for a molecule specific for alanine and alanyl-$tRNA^{Ala}$ when this tRNA is charged with alanine) and ASL for Anticodon Stem Loop; $mRNA^{aaRS}$, messenger RNA of aaRS. Most cited aaRS domains are abbreviated as follows: ABD, Acceptor-stem-Binding Domain; ACB, AntiCodon Binding; CP, Connective Peptide (in CP1 and CP2 versions); EMAP, Endothelial Monocyte Activating Polypeptide; OB-fold, Oligonucleotides/oligosaccharides Binding fold and SC-fold for Stem-Contact fold. Trbp stands for TrNA-binding protein. The nomenclature of tRNA is as given in the tRNA database (19), with, e.g., A_{76} for amino acid accepting 3′-terminal adenosine and $N_{34}N_{35}N_{36}$ for anticodon triplet; abbreviations of modified nucleosides cited in the text are as follows: m^2A, 2-methyl-adenosine; t^6A, N^6-threonyl-carbamoyl-adenosine; k^2C, lysidine (2-lysyl-cytidine); Q, queuosine (a 7-deazaguanosine derivative with a dihydroxy-cyclopentene ring bound at its *C7* carbon); s^2U, 2-thiouridine; and mnm^5s^2U, 5-methyl-aminomethyl-2-thiouridine.

Visualization of structures

To facilitate understanding of aaRS architectures, readers can visualize and manipulate their three-dimensional (3D) structures on the websites of the RCSB PDB (Protein Data Bank of the Research Collaboratory for Structural Bioinformatics consortium) (http://www.pdb.org) (20) or the user-friendly *Proteopedia* free encyclopedia (http://www.proteopedia.org) (21), using the four-digit accession codes given in the text (in italics and bold); as an example, visualize the structure of *Escherichia coli* MetRS (***3h99***). Readers may also use the tools of PyMol (Delano Scientific, http://www.pymol.org).

Historical Background

The first aaRS was discovered in 1958 as an amino acid activation enzyme (22), and it was rapidly suggested that the aaRS enzymes are partners of protein synthesis, where they have a dual role—first to activate amino acids and second to transfer the activated amino acids on tRNA (23, 24):

ATP + amino acid	⇋	aminoacyl-adenylate + PPi	(activation step)
aminoacyl-adenylate + tRNA	⇌	aminoacyl-tRNA + AMP	(transfer step)
ATP + amino acid + tRNA	⇌	aminoacyl-tRNA + AMP + PPi	(overall reaction)

For a long time, aaRSs were considered as a family of 20 functionally homogeneous proteins, although they were characterized by a huge diversity in sequence, subunit size, and oligomeric structure. The surprise was great when it was realized in 1990, after analysis of the accessible aaRS sequences and early crystallographic work, that these enzymes fall into two groups of 10 members each that would aminoacylate tRNAs by different mechanisms (25, 26). This dichotomy is a result of evolution; it is not completely understood and is still under debate (27, 28, 29, 30, 31, 32, 33), although recent data favor the view of a common origin of both aaRS classes as suggested by the synthesis of functional class I and class II aaRS-mimics (in fact amino acid activating enzymes) that could have been coded by opposite strands of the same gene (34).

Understanding how aaRSs recognize tRNA was and remains an important issue approached by all available theoretical and experimental tools, often applied to bacterial systems. Early data pinpointed the importance of the tRNA-accepting end and of the anticodon for recognition by aaRSs (reviewed in reference 5) and contributed to the progress of genetic methods for the *in vivo* search of tRNA identity determinants (35, 36). On the other hand, it was realized that specificity of aaRSs for both amino acid activation and tRNA aminoacylation is rather low *in vitro* (reviewed in references 37 and 38). For biological necessity, it was then conjectured that editing mechanisms should exist to correct activation and aminoacylation errors. Today, these precursory seminal suggestions find support from structural biology and genomics as reported below.

Overview of the aaRS World

General features of aaRSs and peculiarities in *Bacteria*

The world of aaRSs shows a large diversity with enzymes of modular structure ranking in two evolutionarily distinct classes (Figure 1) and featuring significant variations in sequence, length, and oligomery (Table 1). In addition to the two types of class-specific catalytic domains and the ACB domains needed to ensure the seminal tRNA aminoacylation function, aaRSs contain additional modules of diverse structure (N- or C-terminally located or inserted into the protein core formed by the catalytic and ACB domains) that participate in varied functions (e.g., editing in the three kingdoms of life and kingdom-specific ancillary activities). Structural and functional complexity of aaRSs increases in *Eukarya* with the systematic addition of new domains and motifs (see below). Distinctive features in bacterial aaRSs are diverse, such as the large insertion in the catalytic domain of AspRSs (18, 39) and many discrete elements revealed by computational methods in other aaRSs (40). Furthermore, bacterial aaRSs differ from other aaRSs by physicochemical characteristics, such as isoelectric points (pI) in the mild acidic range (pI ~5 to 6, as compiled for *E. coli* and *Thermus thermophilus* aaRSs), in contrast to human cytosolic and mitochondrial aaRSs, where pI are shifted toward alkaline values (pI ~5.5 to 8.5) (41).

The first known aaRS sequence was that of dimeric TrpRS from *Bacillus stearothermophilus* (or *Geobacillus stearothermophilus*), the smallest aaRS, with a subunit of 327 amino acids (52). The first amino acid sequence deduced from DNA sequencing was that of tetrameric AlaRS from *E. coli*, with a subunit of 875 amino acids (53). Note that genome sequencing revealed strain-dependent differences in *E. coli* proteins, notably in ProRS, with 572 amino acids in an enterohemorrhagic strain (54) and 590 amino acids in an uropathogenic strain (55). Altogether, the *E. coli* aaRSs reveal a complex evolutionary history with unexpected connections with other protein families and intricate horizontal gene transfer events (39). Surprisingly, several aaRSs are even encoded in viral genomes (56, 57).

Partition of aaRSs in two families (Figure 1) is based on different structures of their active sites. It was revealed by sequence peculiarities in *E. coli* ProRS (25, 26) and unprecedented crystallographic features in *E. coli apo*-SerRS (25) (not in PDB; see [***1ses***] for a structure with a seryl-adenylate analog) and the yeast AspRS:tRNA^{Asp} complex (***1asy***) (58) (also commented upon in references 18, 59, 60, and 61). While in class I aaRSs the ATP-binding domain is a Rossmann fold similar to the dinucleotide-binding fold discovered in glyceraldehyde-3-phosphate dehydrogenases (62), it is a seven-stranded antiparallel β-sheet in class II.

In class I aaRSs, the signature motifs HIGH and KMSKS (in fact, HiGh and kmSKs, with amino acids in lowercase letters being less conserved) are responsible for the interaction with the universal substrate ATP. These signatures are always located close to the α-phosphate of ATP and assist catalysis. In class II aaRSs, residues of the so-called motifs 2 (fRxe) and 3 (gxgxgfd/eR) play this role. A third signature motif, called motif 1 (gΦxxΦxxP, with Φ for hydrophobic residues), is responsible for dimerization. Class I aaRSs are mostly monomers, and class II aaRSs are mostly dimers (Table 1). It is noteworthy that class I TrpRS (63) and TyrRS (64) are dimers and recognize tRNA as do class II aaRSs, thereby making a link with dimeric class II enzymes. From the side of function, aaRS partition explains the regiospecific tRNA acylation on either the 2′-OH group (class I and class II PheRS) or the 3′-OH group (class II, except PheRS) of the ribose at terminal A_{76} (65, 66, 67) and the differential ATP- and tRNA-binding modes (see "Aminoacylation of tRNA," below).

The high degeneracy of the three motifs in class II aaRSs has to be noticed. With only one proline and two arginine residues strictly conserved, these signatures could not be discovered on a sole sequence inspection but required

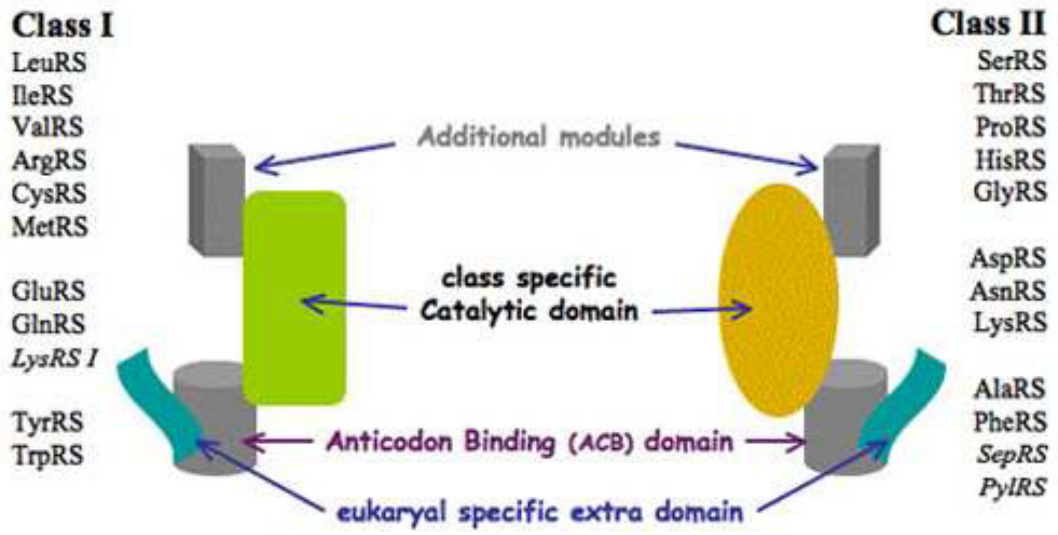

Figure 1 Modular architecture of aminoacyl-tRNA synthetases and their partition in two classes with subclasses. Modularity in aaRS structure was first revealed after analysis of the *E. coli* AlaRS sequence (50) and was confirmed by crystallography of many other aaRSs (51). *SepRS* (*O*-phosphoseryl-tRNA synthetase) and *PylRS* (pyrrolysyl-tRNA synthetase) are noncanonical aaRSs found in *Archaea*, except a few bacterial PylRSs (see "The ambiguous status of archaeal aaRSs—a short synopsis," below, for details).

knowledge of 3D structures in which the single-signature amino acids are similarly located in specific architectural frameworks. This means that conservation of 3D structure is not necessarily accompanied by strict conservation of sequence, since 15% sequence identity can be sufficient to account for conservation of the core structure of aaRSs (68). This has implications for exploring the evolution and phylogeny of aaRSs that should be structure based (40, 68). In this view, the discovery by advanced bioinformatic tools of hidden protein motifs that connect structurally and functionally unrelated aaRSs should be stressed. Thus, Rossmannoid motifs (i.e., structural motifs derived from the classical dinucleotide-binding fold or Rossmann fold that are present in a variety of proteins) could be identified in the ACB domain of class II aaRSs, notably in ProRS and GlyRS (69).

For a long time it was believed that each amino acid has its own aaRS and, consequently, that all organisms should contain 20 aaRSs. However, it was observed early on that some *Bacteria* require a misacylating GluRS and an amidotransferase for formation of glutaminyl-tRNA^{Gln} (70, 71). This pathway remained mysterious for a long time, although it was readily suggested that it might serve to compensate for a defective GlnRS (71). Today we know that GlnRS, and also AsnRS, are missing in some *Bacteria* and in most *Archaea*, where their absence is compensated for by the presence of ND-GluRS or ND-AspRS. These nondiscriminating aaRSs are actors in an indirect pathway of aminoacyl-tRNA synthesis via transamidation of mischarged tRNA^{Gln} or tRNA^{Asp} (7, 72). Notice, by the way, that some ND-aaRSs have lost the capability to efficiently charge their cognate tRNA and specialized to be only misacylating enzymes. This is the case of GluRS-2 from *Helicobacter pylori* that specialized to misacylate tRNA^{Gln} with glutamate (73). (See "Alternative functions of bacterial aminoacyl-tRNA synthetases," below, for details on the biology of ND-aaRSs.)

It was also found that two distinct genes coding for distinct aaRSs of the same specificity could coexist in a same bacterium. Such duplicated aaRSs are known for various specificities (e.g., Arg, Asp, Cys, Glu, Ile, Lys, Ser, Thr, Trp, and Tyr). Thus, two forms of ArgRS occur in *Oenococcus oeni* (74), two of AspRS occur in *T. thermophilus* (75), two of CysRS occur in *Mycobacterium smegmatis* (76), two of GluRS occur in *H. pylori* and *Acidithiobacillus ferrooxidans* (77, 78, 79), two of IleRS occur in *Staphylococcus aureus* (80), two of LysRS occur in *E. coli* (81), two of SerRS occur in *Streptomyces* taxa (82), two of ThrRS and TyrRS occur in *Bacillus subtilis* (83), and two of TrpRS occur in various bacterial genera, including the *Salmonella* taxa (84). Duplications also occur in *Archaea* and *Eukarya*. Different reasons can account for this fact. In the case of the two *E. coli* LysRS forms, their differential regulation could provide a metabolic strategy to adapt the microorganism to different physiological conditions (85). For duplicated AspRS,

Table 1 Overall structural characteristics of aminoacyl-tRNA synthetases, with emphasis on *E. coli* members

Class I aaRSs			Class II aaRSs		
Subclasses	**Oligomeric structure**	**No. aa/subunit (*E. coli* aaRSs)**	**Subclasses**	**Oligomeric structure**	**No. aa/subunit (*E. coli* aaRSs)**
Class Ia			**Class IIa**		
LeuRS	α or αβ[a]	860	SerRS	α_2	430
IleRS[l]	α	937[b]	ThrRS	α_2	642
ValRS	α	951	ProRS	α_2	572 or 590
ArgRS	α	577	HisRS	α_2	424
CysRS	α	461	GlyRS	$\alpha_2\beta_2^c$	303 & 689
MetRS	α_2 or α	642			
Class Ib			**Class IIb**		
GluRS	α[d]	471	AspRS	α_2	590
GlnRS	α	551	AsnRS	α_2	467
LysRS I[e]	α	–[f]	LysRS	α_2	505
Class Ic			**Class IIc**		
TyrRS	α_2	424	AlaRS	α_4	875
TrpRS	α_2	334	PheRS[g]	$\alpha_2\beta_2^h$	327 & 795
			PylRS[i]	α_2	–[j]
			SepRS[h]	α_4	–[k]

[a]Can be αβ heterodimeric in a few *Bacteria*, such as *Aquifex aeolicus* from the phylum *Aquificae* (see "Structures of aminoacyl-tRNA synthetases," below).

[b]Revised amino acid sequence by means of mass spectrometry peptide mapping (42).

[c]Tetrameric $\alpha_2\beta_2$ GlyRSs are exclusively found in *Bacteria*; however, a few *Bacteria* contain dimeric α_2 GlyRSs (of archaeal/eukaryal-type). A dimeric α_2-type GlyRS in *Eukarya*, was as first found in baker's yeast (43).

[d]A catalytically active homodimeric form of *Mycobacterium tuberculosis* GluRS has been characterized that is in equilibrium with the monomer (44).

[e]Present in a few *Bacteria*, but mainly in *Archaea*.

[f]523 amino acids in archaeal *Pyrococcus horikoshii* (39).

[g]Small catalytic α-subunit is the class II characteristic domain in PheRSs.

[h]Monomers in mitochondria.

[i]Noncanonical archaeal aaRSs (PylRS and SepRS); note the presence of PylRS in some *Bacteria*, such as in *Desulfitobacteria hafniense* (46) from the phylum *Firmicutes*.

[j]454 amino acids in the subunit of the *Methanococcus mazei* PylRS (47).

[k]534 amino acids in the subunits of the *Archaeoglobus fulgidus* SepRS (48, 49).

[l]Underlined aaRSs: protein sequence available.

For additional references on aaRS sequences, see reference 10.

regulation of the D and ND forms keeps protein synthesis efficient when the direct pathway of aspartic acid biosynthesis is repressed. On the other hand, duplicated aaRS genes were found to code for paralogous proteins that have acquired new functionalities. Thus, the second CysRS in *M. smegmatis* was identified as the product of the *mshC* gene encoding an ATP-dependent Cys:GlncN-Ins ligase (76). Likewise, a putative LysRS gene in *Salmonella* codes for the PoxA protein (86) that lysylates a protein mimic of tRNA (see "Paralogs of bacterial aminoacyl-tRNA synthetases," below, for comments).

As a last point, notice that a single aaRS can aminoacylate isoaccepting tRNA species. The degeneracy of the genetic code often accounts for this property, a prominent example being the SerRSs that serylate the five $tRNA^{Ser}$ isoacceptors needed to read the six serine codons (87).

Peculiarities of eukaryal aaRSs and differences with the bacterial orthologs—a short synopsis

Eukaryal cytosolic aaRSs have an enhanced structural complexity with the presence of additional domains and motifs in comparison with the bacterial orthologs (18, 88). These extrastructures exhibit a variety of functions, some indirectly related to tRNA aminoacylation but most without apparent connection, and they were progressively incorporated during evolution into virtually

all aaRSs following a scenario correlating well with the progressive evolution and complexity of eukaryal organisms (15, 88). Thus, N-terminal appended domains are found in both class I (e.g., CysRS, GlnRS, and MetRS) and class II (e.g., GlyRS, AspRS, AsnRS, and LysRS) aaRSs. Likewise, C-terminal extensions are present in several class I aaRSs (e.g., CysRS, MetRS, and TyrRS) and in class II SerRS. As an example, the 70-residue-long N-terminal extension in *Saccharomyces cerevisiae* AspRS (see "Structure of aminoacyl-tRNA synthetases," below) contains a lysine-rich helical domain that recognizes the anticodon stem-helix of $tRNA^{Asp}$ and thereby enhances significantly its binding (89). Such N-terminal appendices encompassing helices with RNA-binding motifs are present in other eukaryal AspRSs (89) and in mammalian LysRSs (90). Four other extradomains (EMAP II and the associated ELR tripeptide, leucine zipper, glutathione S-transferase, and WHEP domains) are shared by several aaRSs in higher *Eukarya* (GluRS, GlyRS, HisRS, MetRS, ProRS, and TrpRS), and, among them, the WHEP domain is of special interest. This WHEP domain (so named because it was discovered in WRS [TrpRS], HRS [HisRS], and EPRS [GluProRS, a fused aaRS build by GluRS and ProRS]), besides having regulatory functions in TrpRS, serves also as the linker that joins GluRS to ProRS in EPRS (15). The origin of EPRS in *Eukarya* is ancient and dates back to the most primitive *Metazoa* (91). There are also motifs specific to only one eukaryal aaRS and not found in other proteins. To date, eight such motifs have been characterized in AspRS, CysRS, GluRS, IleRS, LeuRS, PheRS, SerRS, and ThrRS (15). As noticed by the authors who discovered them in databases, once added to a given aaRS in the course of evolution, they were irreversibly retained until the appearance of humans. Mutations in these domains that are not concerned with the tRNA aminoacylation function can cause severe pathologies in humans (92, 93, 94). Furthermore, eukaryal aaRSs often exist under various isoforms that can be produced *in vivo* either through alternative mRNA splicing or by natural proteolytic fragmentation, as occurs for human TrpRS (95, 96) and TyrRS (97), as well as by posttranslational modifications (98). This structural diversity reflects functional diversity. Thus, human mini-TrpRS has antiproliferative and antiangiogenic activity (95). The mini-TyrRS is a cytokine that activates angiogenic signal transduction pathways (97), and the EPRS duplex regulates inflammatory gene expression via phosphorylation of two serine residues in the WHEP domain connecting GluRS and ProRS (91). Another fascinating property of the eukaryal aaRSs, found in higher *Eukarya*, is their assemblage in a large supramolecular architecture, named the MARS complex. This complex comprises nine aaRSs (ArgRS, AspRS, GlnRS, GluRS, IleRS, LeuRS, LysRS, MetRS, and ProRS) and three auxiliary proteins (p18, p38, and p43) (99, 100). Although its structure and functions are not fully elucidated, the newest evidence indicates a dynamic organization, a role in cellular channeling, and participation in regulatory pathways (99, 101, 102).

Most aaRSs from eukaryal organelles show bacterium-like features (for a short survey, see "Bacterium-like aminoacyl-tRNA synthetases," below). However, some of them deviate from this scheme and present unprecedented idiosyncratic properties. This is the case of mt-PheRSs that are small monomers, in contrast to the large $(\alpha\beta)_2$ heterotetramers in *Bacteria*, *Archaea*, and the cytosol of *Eukarya* (103, 104). It is noteworthy that the human mt-PheRS is one of the smallest aaRSs with a chimerical architecture (***3cmq***) combining the catalytic domain of the α-subunit with the ACB domain of the β-subunit of canonical large PheRSs (103) (see also "Structure of aminoacyl-tRNA synthetases," below).

The recent interest in aaRSs from unicellular *Protozoa* (e.g., amoeboids, flagellates, apicoplasts, and kinetoplastids) that are often pathogens for humans deserves a comment. One reason lies in the presence of distinctive structural features in these aaRSs, absent in the orthologs from other eukaryal genera, particularly in human aaRSs, making these proteins potential new targets in the search for new antipathogen drugs. Three examples illustrate the point. First, a specific insertion was found in the ACB domain of the AspRS from apicomplexan pathogens that are the vectors of malaria (105). Another insertion is present between the catalytic and ACB domains in the AspRS from the parasite *Entamoeba histolytica*. Second, analysis of its 3D structure (***3i7f***) (106) suggests that the insertion enhances $tRNA^{Asp}$ binding and thereby plays a role similar to the RNA-binding motif from the N-terminal extensions in the AspRSs from lower *Eukarya* that are absent in *Entamoeba*. The third example comes from the knowledge of the HisRS structures from *Trypanosoma brucei* (***3hri***) and *Trypanosoma cruzi* (***3hrk***), the vectors of leishmaniases. In this aaRS, the binding pocket for the adenine moiety of ATP differs substantially both from the binding site in bacterial structures and from the homologous pocket in human HisRS (107).

The ambiguous status of archaeal aaRSs—a short synopsis

In contrast to all *Eukarya* and most *Bacteria* that are equipped with a full set of the 20 canonical aaRSs, *Archaea* are lacking one or several aaRSs (i.e., AsnRS, CysRS, GlnRS and/or LysRS) whose absence is compensated for by the presence of noncanonical aaRSs, namely ND-aaRSs, SepRS (*O*-phosphoseryl-tRNA synthetase) and/or class I LysRS-1 (108). In addition, some methanogenic *Archaea* encode a SepRS and/or a PylRS (pyrrolysyl-tRNA synthetase), two atypical and strictly archaeal aaRSs. PylRS charges the unusual 22nd proteinogenic amino acid pyrrolysine on cognate $tRNA^{Pyl}$ (109), and, in those *Archaea* lacking CysRS, SepRS charges *O*-phosphoserine to $tRNA^{Cys}$ (108). PylRSs and SepRSs belong to class II aaRSs, where they are ranked into class IIc because of structural similarities with PheRSs (Table 1). The dimeric α_2 PylRSs (110) show close relationship with the β-subunit of PheRSs, as seen by crystallography (e.g., ***2zcd*** [46, 47]) and the tetrameric α_4 SepRSs (111) resemble the catalytic α-subunit of PheRSs, as seen in structures of *apo*- (***2odr***) (48) and tRNA-bound ***2du3*** (112) versions of these proteins. The fact that the monomer of α_4 SepRS shares strong resemblance to the catalytic domain of $\alpha_2\beta_2$ PheRS supports the idea of a common origin of these two class II aaRSs (113). The structural similarity of the SepRS and PheRS catalytic domains accounts for the idiosyncratic aminoacylation of $tRNA^{Sep}$ and $tRNA^{Phe}$ at the 2′-OH position instead the 3′-OH position normally recognized by class II aaRSs including PylRS (114).

GlnRS is absent from all *Archaea*, and AsnRS is absent from most *Archaea*, where they are substituted by ND-GluRS and ND-AspRS that mischarge noncognate $tRNA^{Gln}$ and $tRNA^{Asn}$. Then, synthesis of asparaginyl-$tRNA^{Asn}$ and glutaminyl-$tRNA^{Gln}$ occurs by an indirect route similar to that elucidated for *Bacteria* (see "Alternative functions of aminoacyl-tRNA synthetases," below). Cysteinyl-$tRNA^{Cys}$, as well, is produced indirectly when CysRS is lacking in a pathway utilizing a Sep-tRNA:Cys-tRNA synthase that converts phosphoseryl-$tRNA^{Cys}$ to cysteinyl-$tRNA^{Cys}$ (48). As to noncanonical class Ib LysRS-1, also found in a few *Bacteria* (Table 1 and "Structure of aminoacyl-tRNA synthetases," below), it is the lysine-specific aaRS in most *Archaea*, and, in a few cases, it is present together with a canonical class II LysRS.

For reasons not completely understood, archaeal aaRSs of a given amino acid specificity can be of strictly archaeal type (e.g., GlyRSs, MetRSs, and most ThrRSs) and/or can encompass structural features of bacterial and/or eukaryal aaRSs (18). The first known example of an archaeal aaRS with bacterial features was AspRS from a *Pyrococcus* strain (115). At opposite, archaeal aaRSs can lack domains present in bacterial enzymes, as found in the crenarchaeal ThrRSs from *Sulfolobus solfataricus* (116) and *Aeropyrum pernix* (***3a32***) (117) that are deprived of a *cis*-editing domain. In the majority of other archaeal ThrRSs, as in *Pyrococcus abyssi*, the bacterial editing domain (also found in eukaryal ThrRSs) is replaced by a D-aminoacyl-tRNA deacylase-like domain (***2hl1***) (118) (see "Error correction" under "Aminoacylation of tRNA," below, for details). Remarkably, the crenarchaeal ThrRSs are of the strictly archaeal-type, as well as the freestanding editing domain found in this group of *Archaea* (115). On the other hand, the editing domain of archaeal PheRSs resembles that of eukaryal PheRSs (119). Altogether, these intricate properties confer an ambiguous status to archaeal aaRSs, reflecting their ancient origin and their subsequent intricate evolutionary history.

Conserved aaRS properties and the challenge to understand their global biology

The preceding short survey on the aaRS world has highlighted their universal presence in all living organisms where they have the pivotal role in protein synthesis in mediating the correct expression of the genetic code. For each proteinogenic amino acid, evolution shaped a specific aaRS that specifically charges that amino acid on a cognate tRNA molecule. Amazingly, this universal function is accompanied by great structural diversity, as illustrated in Figure 1 and Table 1. Most intriguing is the partition of the aaRSs into two classes of 10 members each with class-specific catalytic domains. Likewise, the architectural modularity of aaRSs with a huge variety of modules appended to or inserted within their catalytic domains and a trend of larger structures in *Eukarya* is puzzling. This survey has also sketched other fascinating facts, notably the ubiquitous presence of aaRS paralogs in living organisms and the participation of aaRSs in various biological processes unrelated to protein synthesis. Furthermore, it was shown that aaRSs are of interest for human medicine (e.g., as targets to inhibit pathogens or when mutated as causative agents of various diseases). A general belief argues that all of these features reflect the evolutionary history of aaRSs and, more generally, that of the genetic code and even of life. Altogether, this calls

for a global interdisciplinary approach where information gathered on aaRSs from the three kingdoms of life and even from viruses must be compared. In this context, the bacterial aaRSs are of interest because of the ancient origin of *Bacteria* and the relative ease of experimental studies. Thus, the search for primordial features accounting for aaRS function is facilitated in *Bacteria*. Likewise, the relative ease of access to pure bacterial aaRSs and the availability of sophisticated tools for their study turned out to be essential to gather a huge and diversified corpus of structural and functional characteristics, some idiosyncratic to bacterial aaRSs, others shared by archaeal and eukaryal aaRSs. How do we rationalize data that are seemingly unrelated? How does genomics meet structure-function problems and evolution in aaRS research? How should we assemble the pieces of the puzzle? It is the aim of this essay to bring answers to these questions with focus on bacterial aaRSs.

STRUCTURE OF AMINOACYL-tRNA SYNTHETASES

Toward a Structural Understanding of Bacterial aaRSs

Crystallogenesis of aaRSs and aaRS:tRNA complexes

In 1971, a monomeric active fragment of *E. coli* MetRS obtained by limited trypsin digestion of the native enzyme was the first crystallized aaRS (120). It took 30 years to get satisfying structures of a free monomeric MetRS, in *apo*-form or with bound methionyl-adenylate analogs, either from *E. coli* (121, 122, 123) (***1p7p***) or *T. thermophilus* (124) (***1a8h***). The first crystals of a complex between aaRS and tRNA (the yeast aspartate complex) were grown in 1980 (125, 126) when it was realized that tRNA interaction with aaRS and aminoacylation capacity were maintained above 1.0 M ammonium sulfate (125, 126, 127, 128). This finding, restricted to ammonium sulfate, was a surprise because salts at high concentration were known to inhibit tRNA aminoacylation and to disrupt protein/nucleic acid interactions, particularly in the aaRS field (129, 130). At present, ammonium sulfate is widely used as a major crystallant of aaRS:tRNA complexes (51). As to *Bacteria*, the first crystallization successes concerned the *E. coli* GlnRS:$tRNA^{Gln}$ complex (131) followed by the *T. thermophilus* SerRS:$tRNA^{Ser}$ (132) and PheRS:$tRNA^{Phe}$ (133) complexes (both with native tRNAs from *T. thermophilus*). Note that many crystals were grown with aaRSs (sometimes truncated but still active) from thermophilic *Bacteria* or *Archaea* that crystallize more readily than the orthologs from mesophiles. Today, representatives of all aaRS families, either free or with ligands, have been crystallized, leading to ~630 crystal structures (including aaRS fragments and paralogs) deposited in the RCSB Protein Data Bank.

A panel of bacterial crystal structures

Although bacterial aaRSs represent the majority of solved structures (48% versus 20%, 31%, and <1% for *Archaea*, *Eukarya* [cytosol], and mitochondria), their distribution in *Bacteria* is uneven (Table 2). Structures from *E. coli* and *T. thermophilus* aaRSs are the most represented with ~100 and ~70 structures, respectively, corresponding to 12 amino acid specificities for *E. coli* and 15 for *T. thermophilus*. In the case of *E. coli* aaRSs, ~60 structures correspond to *apo*-proteins or proteins in interaction with small ligands and 33 to aaRS:tRNA complexes (including 18 GlnRS:$tRNA^{Gln}$ complexes) in different functional states. Figure 2 is a gallery of bacterial aaRS structures representative of the 20 amino acid identities, displaying when available structures of aaRS:tRNA complexes. It is noteworthy that the structure of *Aquifex aeolicus* AlaRS (***1yfr***) corresponds to a truncated molecule restricted to the catalytic and tRNA-recognition domains (with 453 instead of 866 amino acids for the complete protein) (134). This truncated molecule alanylates $tRNA^{Ala}$ and is monomeric and homologous to a fragment from *E. coli* AlaRS so far not crystallized. A full-size tetrameric α_4-structure of an AlaRS is still missing, but a model based on crystal structures of the two halves of archaeal *Archaeoglobus fulgidus* AlaRS, namely $AlaRS_{\Delta C}$ (***2ztg***, comprising catalytic, tRNA recognition, and editing domains) and $AlaRS_C$ (***2zvf***, dimerization domain) allowed to propose a dimeric butterfly-like organization (135).

Besides the large panel of aaRS structures from *E. coli* (closely related to *Salmonella,* for which no crystallographic structure is yet known, except a putative LysRS structure [***3g1z***] from *Salmonella enterica* serovar Typhimurium), 3D structures are also available for 10 other *Proteobacteria* (*Acinetobacter baumannii*, *Brucella abortus*, *Brucella melitensis*, *Campylobacter jejuni*, *Coxiella burnetii*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa*, *Rhodopseudomonas palustris*, *Vibrio cholerae*, *Yersinia pestis*). Additional structures come from 14 other *Bacteria*, notably from four species closely rooted with *Archaea* (*A. aeolicus, Deinococcus radiodurans, Thermotoga maritima*, *T. thermophilus*), five *Bacilli* from the *Firmicutes* phylum (*B. stearothermophilus*, *Bacillus subtilis*, *Enterococcus faecalis*, *S. aureus*, *Staphylococcus*

Table 2 Crystallographic structures of free or ligand-bound native bacterial aminoacyl-tRNA synthetases, with emphasis on *E. coli* members[a]

	E. coli structure[a]		Other bacterial[c] structures	
aaRSs	*apo*-aaRSs or in complex with small ligands[b]	aaRS:tRNA[b]	*apo*-aaRSs or in complex with small ligands	aaRS:tRNA
Class I aminoacyl-tRNA synthetases				
Class Ia				
LeuRS	–	3zit, 4aq7, 4ari, 4as1, 4cqn, 3zgz, 4arc	*Mmo, Tth*	*Tth*
IleRS	–	–	*Tth*	*Sau*
ValRS	–	–	*Tth*	*Tth*
ArgRS	4oby	–	*Tth, Kpn, Cje*	–
CysRS	*1li5*, 1li7	1u0b	–	–
MetRS	*1qqt*, *3h97*, *3h9c*, 1f4l, 1p7p, 1pfu, 1pg2, 1pg0, 1pfy, 1pfw, 1pfv, 3h99, 3h9b	–	*Aae, Bab, Bme, Msm, Sta, Tth*	*Aae*
Class Ib				
GluRS	–	–	*Bbu, Bth, Mtu, Sel, Tma, Tth*	*Pae, Tma, Tth*
GlnRS	*1nyl*, 2rd2, 2re8	1gsg, 1gts, 1qrs, 1qru, 1qrt, 1qtq, 1euq, 1euy, 1exd, 1o0b, 1o0c, 1zjw, 1gtr, 4jyz, 4jxx, 4jyz	*Dra, Pae*	–
Class Ic				
TyrRS	1x8x, 1wq4, 1vbm, 1vbn, 1wq3, 2yxn, 4oud	–	*Bst, Mtu, Sau, Tth*	*Tth*
TrpRS	–	–	*Bst, Bsu, Cje, Dra, Mpn, Tma, Tth, Vch, Ype*	–
Class II aminoacyl-tRNA synthetases				
Class IIa				
SerRS	not in PDB	–	*Aae, Tth*	*Tth*
ThrRS	*1evk*, *1tje*, *1tke*, 1tkg, 1tky, 1evl, 1fyf, 4hwo, 4hwp, 4hwr, 4hws, 4p3o, 4p3p	1qf6, 1kog[d]	*Sau*	–
ProRS	–	–	*Efa, Rpa, Tth*	*Tth*
HisRS	1kmn, 1kmm, 2el9, 1htt	–	*Aba, Bth, Nostoc, Sau,Tth*	*Tth*
GlyRS	–	–	*Cje, Tma, Tth*	–
Class IIb				
AspRS	*1eqr*	1c0a, 1il2	*Msm, Mtu, Tth*	*Pae, Tth*
AsnRS	–		*Tth* (not in PDB)	–
LysRS	1lyl, 1bbu, *1bbw*, 1e1o, 1e1t, 1e22, 1e24	–	*Bst, Bth*	–
Class IIc				
AlaRS	—[e]	–	*Aae*	–
PheRS	*3pco*	–	*Pae, Sha, Tth*	*Tth*

[a] *E. coli* aaRSs (~200 structures) are designated by their PDB accession code (identifiers of *apo*-forms without bound ligands are in italics).

[b] Small ligands bound to aaRSs (or aaRS:tRNA complexes) can be inhibitors.

[c] *Bacteria* abbreviated as follows: *Aba*, *Acinetobacter baumannii*; *Aae*, *Aquifex aeolicus*; *Bab*, *Brucella abortus*; *Bme*, *Brucella melitensis*; *Bst*, *Bacillus* (*Geobacillus*) *stearothermophilus*; *Bsu*, *Bacillus subtilis*; *Bth*, *Burkholderia thailandensis*; *Bbu*, *Borrelia burgdorferi*; *Cje*, *Campylobacter jejuni*; *Cbu*, *Coxiella burnetii*; *Dra*, *Deinococcus radiodurans*; *Efa*, *Enterococcus faecalis*; *Kpn*, *Klebsiella pneumoniae*; *Mmo*, *Mycoplasma mobile*; *Msm*, *Mycobacterium smegmatis*; *Mtu*, *Mycobacterium tuberculosis*; *Nostoc*, *Nostoc sp.* PPC 7120; *Pae*, *Pseudomonas aeruginosa*; *Rpa*, *Rhodopseudomonas palustris*; *Sau*, *Staphylococcus aureus*; *Sha*, *Staphylococcus haemolyticus*; *Sel*, *Synechococcus elongatus*; *Tma*, *Thermotoga maritima*; *Tth*, *Thermus thermophilus*; *Vch*, *Vibrio cholerae*; *Ype*, *Yersinia pestis*. For more details and PDB identifiers see reference (51). Note the presence in *Borrelia burgdorferi* and other *Spirochetes*, and in a few α-*Proteobacteria*, of an atypical class I LysRS (136), which structure has been solved for the archaeal *P. horikoshii* enzyme (*1irx*) (45). For the remaining ~200 known bacterial structures, the Table gives only their phylogenetic origin.

[d] ThrRS with bound tRNA-like domain of $mRNA^{ThrRS}$.

[e] Only the structure of the catalytic fragment of *E. coli* AlaRS is known under 8 versions (wild-type or mutant with small/without ligands) (*3hy1*, *3hxv*, *3hxw*, *3hxx*, *3hxy*, *3hxz*).

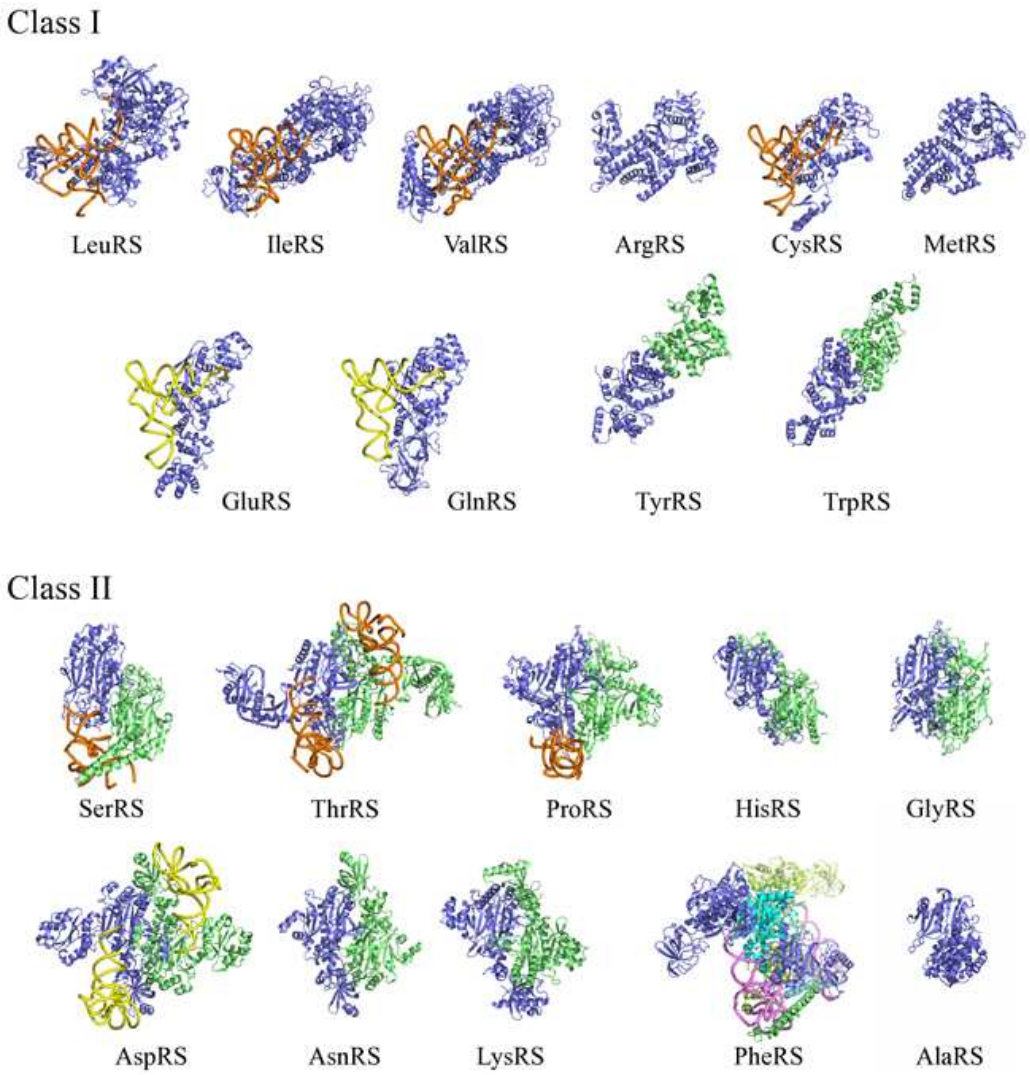

Figure 2 A gallery of canonical bacterial aminoacyl-tRNA synthetase structures. AaRSs in each class are displayed with their catalytic domain in the same orientation. Subclass distribution is indicated by tRNA colors, with orange, yellow, and pink backbones for subclasses a, b, and c, respectively (see Table 2 for subclass distribution). For dimeric aaRSs of classes Ic, IIa, and IIb, the second monomer is green. For tetrameric class IIc PheRS, one αβ-heterodimer is shown in blue and the other is shown in green; for AlaRS, the structure corresponds to a monomeric active fragment (see text). Identification of the displayed structures: class I *T. thermophilus* LeuRS with $tRNA^{Leu}$ in posttransfer editing conformation (***2bte***) (137), *S. aureus* IleRS with $tRNA^{Ile}$ (***1qu2***) (138), *T. thermophilus* ValRS with $tRNA^{Val}$ (***1gax***) (139), *T. thermophilus* ArgRS (***1iq0***) (140); *E. coli* CysRS with $tRNA^{Cys}$ (***1u0b***) (141), *E. coli* MetRS (***1qqt***) (122), *T. thermophilus* GluRS with $tRNA^{Glu}$ (***2dxi***) (142), *E. coli* GlnRS with $tRNA^{Gln}$ (***1o0b***) (143), *E. coli* TyrRS (***1x8x***) (144), and *T. thermophilus* TrpRS (***2el7***) (unpublished from RIKEN Structural Genomics Initiative); class II *E. coli* SerRS with $tRNA^{Ser}$ (not in PDB) (25), *E. coli* ThrRS with $tRNA^{Thr}$ (***1qf6***) (145), *T. thermophilus* ProRS with $tRNA^{Pro}$ (***1h4s***) (146), *E. coli* HisRS (***1kmn***) (147), *T. thermophilus* GlyRS (***1ati***) (148), *E. coli* AspRS with $tRNA^{Asp}$ (***1c0a***) (149), *T. thermophilus* AsnRS (not in PDB) (150), *E. coli* LysRS (***1e1o***) (151), *T. thermophilus* PheRS with $tRNA^{Phe}$ (***2iy5***) (152), and *A. aeolicus* AlaRS (***1yfr***) (134).

haemolyticus), two Gram-positive *Actinobacteria* (*M. mobile*, *Mycobacterium tuberculosis*), two *Cyanobacteria* (*Synechococcus elongatus*, *Nostoc*), and one spirochete (*Borrelia burgdorferi*) (Table 2).

Many structures correspond to snapshots of conformational states occurring during the tRNA aminoacylation step, including the editing step, and thus inform about aaRS functioning. This is the case of *E. coli* LeuRS, MetRS, GlnRS, TyrRS, ThrRS, HisRS, AspRS, and LysRS and *B. stearothermophilus* TrpRS (see "Aminoacylation of tRNA," below, for mechanistic details). For other aaRSs, the motivation was pharmacological with the aim of drug/antibiotics development. This concerns *E. coli* (153, 154, 155) and *T. thermophilus* (156) LeuRS, *S. aureus* (138) and *T. thermophilus* (157) IleRS,

M. smegmatis MetRS (158), *E. coli* ThrRS (159), and *S. aureus* TyrRS (160) in complex with inhibitors acting as potential antipathogens (see "Inhibition and engineering of bacterial aminoacyl-tRNA synthetases," below, for details). Note the increasing number of aaRS structures in complex with inhibitors and the structures solved recently by structural genomics consortia (e.g., by the Seattle Center for Structural Genomics of Infectious Disease).

The structure of the ND-AspRS from *T. thermophilus* (***1n9w***) that aspartylates both $tRNA^{Asp}$ and $tRNA^{Asn}$, is of interest (161). This atypical AspRS is an archaeal-type with strong similarity to *Pyrococcus kodakaraensis* AspRS (***1b8a***) (162) and misses the bacteria-specific insertion found in the catalytic core of *T. thermophilus* (***1l0w***, ***1g51***) and *E. coli* (***1eqr***) AspRSs (163, 164, 165). Interestingly, this AspRS has strong structural resemblance with yeast AspRS (166), although it is missing the eukaryal N-terminal extension (Figure 3). Generalizing this observation, most bacterial aaRSs show overall conserved structures in evolution, but differ from their archaeal and eukaryal orthologs by kingdom-specific appendices and/or inserted or deleted domains (18).

The resolution of structures is comprised between 1.4 and 3.3 Å with a tendency of the highest resolutions for aaRSs interacting with ligands, e.g., 1.4 Å for a mutant MetRS from *E. coli* complexed with methionine (***3h99***) (167). All structures systematically contain regions not well resolved and characterized by high crystallographic *B*-factors (these factors, also known as temperature factors, are correlated with mobility/disorder of atoms in crystals). In early reports, this fact was completely overlooked. At present, the structural plasticity of aaRSs is partly understood and reflects important functional features. Indeed, disordered domains not seen in the *apo*-proteins, or in complexes with small ligands, mostly consist of loops that make contacts with ligands. This intrinsic plasticity of aaRSs, often reflected by induced-fit/allosteric conformational changes, is required for their

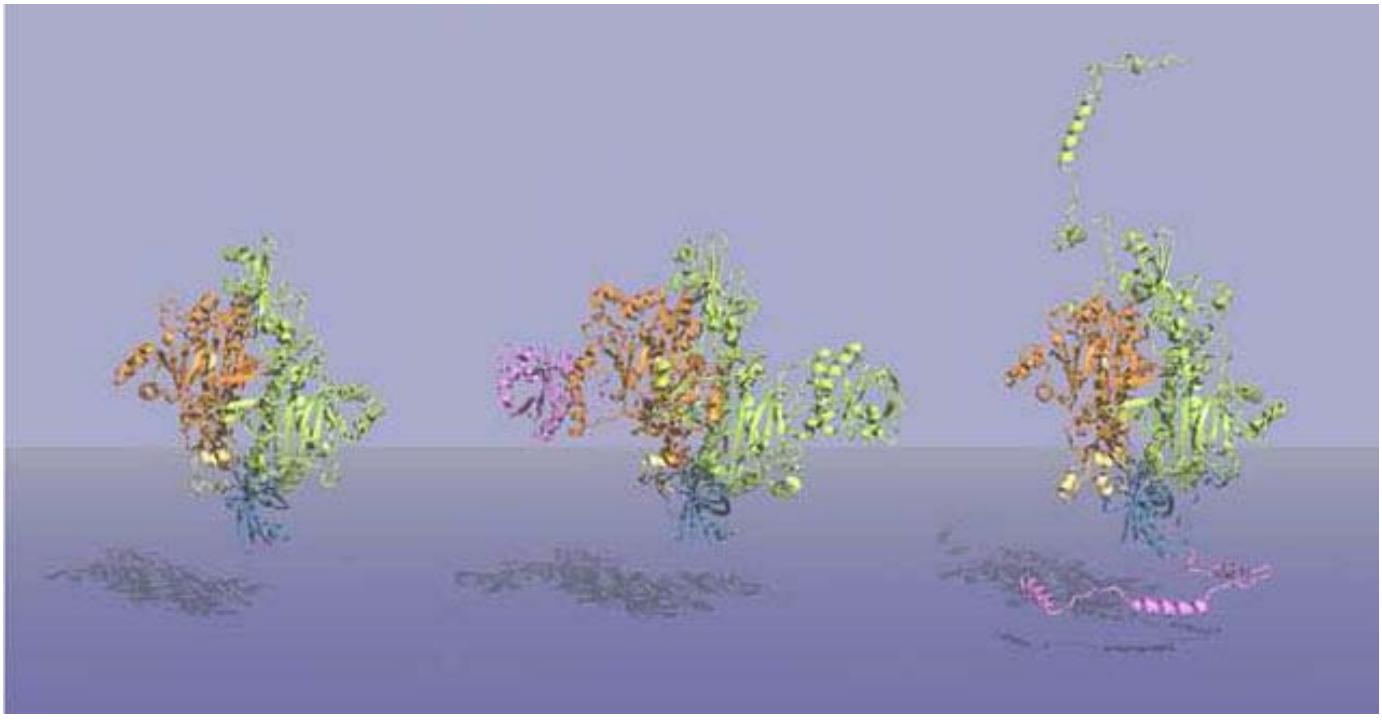

Figure 3 Variability of aminoacyl-tRNA synthetase structures during evolution: the case of AspRS. The figure shows the organization of dimeric AspRS in the three kingdoms of life with bacterial-type (*E. coli*) (***1eqr***) (149) AspRS in the middle surrounded by archaeal-type (*P. horikoshii*) (***1b8a***) (162) and eukaryal-type (*S. cerevisiae*) (***1eov***) (166) AspRS at left and right, respectively. AspRSs are displayed with the anticodon-binding domain in blue, the hinge region in yellow, and the catalytic domain in orange. The second monomer is in light green, and the kingdom-specific domains or extensions are in pink. The exact fold of the N-terminal extension of yeast AspRS is unknown, and the present model is based on structure predictions (89).

biological functioning either during the tRNA aminoacylation process or the interplay with other cellular partners (see "Aminoacylation of tRNA," below, for details on aaRS plasticity).

Subclass Characteristics and Other Remarkable Anatomies of Bacterial aaRSs

All extant aaRSs have a class-defining catalytic domain and an ACB domain of variable architecture, both being decorated by idiosyncratic additional domains (Figure 1), often defining the subclasses. ACB domains are mainly conserved in evolution and correspond either to α-helical bundles, sometimes called DALR motifs in the seven class Ia aaRSs (because of conservation of D, A, L, and R amino acids; note that in the ArgRS nomenclature the domain is called Add2); α-helical folds (α-ACB in GluRSs, TyrRSs, TrpRSs, and LysRSs); β-sheeted folds (β-ACB in GlnRSs); α/β-mixed architecture (α/β-ACB in ThrRSs, HisRSs, and ProRSs); OB-folds (OB-ACB in AspRSs, AsnRSs, and LysRSs); or ferredoxin folds (FDX-ACB in PheRS β-subunits) (39). Additional details on the structure of the 20 aaRS families can be found in reference 18; for functional aspects see the next sections and reference 18.

Class Ia ArgRS, CysRS, IleRS, LeuRS, MetRS, and ValRS

These are monomeric proteins, with the exception of MetRSs that can be dimeric, as is native *E. coli* MetRS. This enzyme, however, remains fully active as a monomer after proteolytic C-terminal truncation (168). Class Ia aaRSs contain three subclass characteristic domains, namely, a flexibly linked CP1 domain of variable length (~150 to 200 amino acids) inserted into the Rossmann fold, except in CysRS and ArgRS (called Ins-2 in ArgRS), where it is half this size, a smaller CP2 also inserted in the Rossmann fold, and, in the C-terminal location, an α-helical bundle domain for recognition of tRNA anticodon. ACB domains are connected to the Rossmann domain by an SC-fold of β-α-α-β-α topology that contains the class I-specific KMSKS motif (***1a8h***) (124). Four class Ia members (IleRS, LeuRS, CysRS, and MetRS) contain one or two Zn-binding motifs.

MetRS: Bacterial MetRSs have a C-terminal extension that can occur as an autonomous paralog in some organisms, notably the Trbp111 protein (comprising 111 amino acids) in *A. aeolicus*, the bacterial CsaA proteins (acronym derived from the name of the *B. subtilis csaA* gene), and mammalian EMAP II cytokines (see "Paralogs of bacterial aminoacyl-tRNA synthetases," below, for details). In *E. coli* MetRS, the C-terminal extension is a Trbp111-like domain, while in Gram-negative *Borrelia burgdorferi* it is closely related to the EMAP II domain (18). Remarkably, MetRSs can be classified in four groups on the basis of their Zn-binding features (the presence of one or two identical Zn-binding knuckles either containing or void of zinc) (122). These knuckles, located far away from the catalytic site, are inserted into the short CP1 domain connecting the two halves of the Rossmann fold. Likely, they have an intrinsic fold similar to that of the isolated *E. coli* MetRS-derived knuckle solved by NMR (169). Interestingly, monomeric MetRS from *M. smegmatis* revealed a new conformation of the KMSKS domain triggered by the binding of adenosine (***2x1l***, ***2x1m***) (158).

IleRS, LeuRS, and ValRS: These aaRSs are large proteins of elongated shape (Figure 2) that are related to evolution. They have a large and flexible CP1 globular insertion that protrudes from the catalytic domain and a smaller CP2 insertion that binds Zn^{2+} ions in certain IleRSs. The CP1 domain participates in editing activity (this is not the case in MetRSs, where the CP1 insertion is of smaller size and without editing function). A similar domain named ABD is present in class Ib enzymes, where it serves for recognition of the tRNA acceptor end.

The CP1 insertion reveals a conserved core and conserved residues at the editing site, suggesting a common editing mechanism in class Ia enzymes (see "Error correction," below, for details). The location of CP1 in the sequence of the catalytic domain, subsequent to the first Zn-binding insert, is exclusive to bacterial and mitochondrial LeuRSs. Another peculiarity of bacterial LeuRSs is an additional insertion of ~50 amino acids in the catalytic core prior to the conserved KMSKS signature. This insertion was first identified in *T. thermophilus* LeuRS (***1h3n***) (170). This well-ordered and flexibly linked extradomain protrudes out of the protein core and participates in recognition of the $tRNA^{Leu}$ acceptor stem (137, 170). Activity of deletion mutants and variants with chimerical swaps within the LeuRS-specific domain of *E. coli* confirms the critical role of this insertion in aminoacylation and excludes a direct participation in editing (171). Finally, bacterial LeuRSs have an exclusive C-terminal RNA-binding extension (~60 amino acids), only visible in the LeuRS:$tRNA^{Leu}$ complex, where it is close to the tertiary G_{19}•C_{56} pair that links together the

D- and T-loops of tRNA (***2byt***) (137). A flexible peptide tether (of length more important than sequence) controls accessibility of this extension and facilitates its rigid-body movements (172). Note the peculiar αβ-heterodimeric LeuRS from the deep-rooted bacterium *A. aeolicus* whose structure is split in two parts in the catalytic domain, with part of the catalytic domain and the CP1 module constituting the large α-subunit and the remaining part of the catalytic domain with the KMSKS signature and the tRNA-binding domain constituting the small β-subunit (173). Note also the peculiar structure of *Mycoplasma* LeuRSs that have highly degenerate CP1 modules and in the case of *M. mobile* LeuRS is lacking the CP1 domain (***3ziu***) (155).

IleRS and ValRS structures mainly stem from studies of the *T. thermophilus* enzymes, either in *apo*-versions or in complexes with small ligands and/or tRNA. Structures of *S. aureus* IleRS:$tRNA^{Ile}$ complexes are also available (18, 51). These structures were essential for deciphering the mechanism of tRNA isoleucylation and valylation, as well as the editing mechanism of mischarged $tRNA^{Ile}$ or $tRNA^{Val}$ (174), notably for understanding the role of conformational changes (see "Aminoacylation of tRNA," below, for details on catalysis and editing). Note that the ACB domain of IleRSs contains additional Zn-binding and α/β modules and that of ValRSs includes a α/β module.

CysRS and ArgRS: These two aaRSs deserve special attention because of unusual structural and functional properties (18). CysRSs exhibit a small size resulting either from the absence of an insertion in the catalytic domain or from the addition of small insertions within the Rossmann fold as is apparent in the *E. coli* crystal structure (***1li5***) (175). This enzyme does not need editing to reject serine and alanine (176), in agreement with the small size (75 amino acids) of its CP1 insertion (175).

ArgRSs are quite large proteins and show a remarkable structural modularity mainly conserved in evolution with several domains appended around their catalytic core (140, 177). Add1 and Add2 (Additional domains) are the two nucleic acid-binding modules attached at the N- and C-terminal sides of the catalytic domain. Ins-1 and Ins-2 (Insertion domains) are inserted modules in the first and second half of the Rossmann fold (RF1 and RF2). Add1 has the topology of a motif from the ribosome-recycling factor (RRF). Add2 corresponds to the class Ia characteristic ACB domain and is located in ArgRSs, as are the CP1 domains in the class Ia editing aaRSs. This structural organization is explicitly seen in *E. coli* ArgRS (***4oby***) (178). The crystal structure of *T. thermophilus* ArgRS reveals an additional insertion within RF2 (Ins-3) that is bacterial specific (***1iq0***) (140). Finally, and of great functional significance, ArgRSs belong to the few aaRSs that recognize the D-stem and loop of tRNA (via idiosyncratic Add1) and require cognate tRNA for amino acid activation.

Class Ib GlnRS, GluRS, and LysRS-1

These aaRSs need their cognate tRNA for amino acid activation in the first step of the aminoacylation reaction (in fact for the pyrophosphate exchange reaction). This functional feature is shared with ArgRS, that in addition shows resemblance with structural modules from GluRS and GlnRS. This confers an ambiguous status to ArgRSs that are sometimes ranked in class Ib aaRSs (see e.g., reference 3).

GlnRS and GluRS: These two aaRSs are evolutionarily linked and form the GlxRS superfamily of intricate evolutionary history with gene duplications and lateral gene transfers between *Archaea*, *Eukarya*, and *Bacteria* (18). GlxRS enzymes contain five structural domains, namely the class I-specific Rossmann fold (domain 1), the ABD insertion (domain 2), the SC-fold (domain 3), and two distal domains (4 and 5) interacting with the tRNA anticodon arm, as was explicitly visualized in the crystal structures of the *E. coli* GlnRS:$tRNA^{Gln}$ complex (***1gsg***) (179) and that of native *T. thermophilus* GluRS (***1gln***) (180). While the N-terminal halves (domains 1 to 3) in bacterial GlnRSs and GluRSs present a high degree of similarity, the C-terminal halves (domains 4 and 5) are fundamentally different, being two α-helix bundles in GluRSs and antiparallel β-sheets forming a β-barrel in GlnRSs (***1gtr***). As in class Ia aaRSs, the catalytic domain of GlxRS enzymes is split in two parts, namely, the Rossmann fold (domain 1) and the ABD insertion designed to recognize the acceptor end of tRNA (domain 2). In *E. coli* GluRS, this ABD insertion contains a Zn^{2+} ion sequestered by a Cys-x-Cys-x_n-Cys-x-His (n = 6 to 25) signature typical of the SWIM domain (a Zn-chelating domain designated after SWI2/SNF2 and MuDR proteins that are predicted to interact with DNA and/or proteins [181]), which finds its homolog in *E. coli* GlnRS where a leucine residue stabilizes the $tRNA^{Gln}$ acceptor end (182). On the other hand, *T. thermophilus* GluRS does not contain zinc (180), and several other

bacterial GluRSs do not contain in their ABD insertion the residues needed to bind zinc (182). Note that ABD insertions in class Ib aaRSs have structural features in common with the CP1 domains present in class Ia aaRSs. Note also a structural similarity of *E. coli* GlnRS with MetRS (183).

Extant GlxRSs likely originate from an ancestral ND-GluRS (184). In modern ND-GluRSs, discrete amino acid substitutions in domain 4 likely abolish the ability to discriminate between glutamine ($_{34}CUG_{36}$ and $_{34}UUG_{36}$) and glutamate ($_{34}CUC_{36}$ and $_{34}UUC_{36}$) anticodons, respectively. This assumption is supported by data on *T. thermophilus* GluRS (***2dxi***) that become nondiscriminating after replacement of Arg_{358}, the residue recognizing C_{36} in $tRNA^{Glu}$, by smaller glutamine (185). As could be anticipated, the overall architecture of the complex with tRNA is conserved in the cocrystal structure of ND-GluRS from *T. maritima* with noncognate $tRNA^{Gln}$ and a stable analog of glutamyl-adenylate (***3akz***) (186). It is noteworthy that comparison with the binary complex of *T. thermophilus* D-GluRS with cognate $tRNA^{Glu}$ reveals a quite different structure in the outer corner of the L-shaped $tRNA^{Glu}$ and $tRNA^{Gln}$, especially in their D-loops (Figure 4). This feature is important for the recognition of $tRNA^{Gln}$ by GatCAB in the glutamine transamidosome (186) (see "Alternative functions of bacterial aminoacyl-tRNA synthetases," below, for details).

LysRS-1: Bacterial class Ib LysRS (i.e., LysRS-1) has a special status. This aaRS is of archaeal origin and is present in only a few *Bacteria*, notably in α-*Proteobacteria* and in spirochetes such as *B. burgdorferi*. Like the other class Ib members, it requires cognate $tRNA^{Lys}$ for adenylate formation (190). The 3D structures of bacterial class Ib LysRSs resemble that of LysRS-1 from archaeal *P. horikoshii* (***1irx***) (45). Ranking of LysRS-1 proteins in class Ib is justified because of their striking architectural similarity to GluRSs. Remarkably, comparison of class I and class II LysRSs suggests similar strategies for substrate recognition within their unrelated catalytic site topologies (45).

Class Ic TyrRS and TrpRS

TyrRSs and TrpRSs are small dimeric proteins of elongated shape and atypical properties that share strong structural resemblance despite low sequence identity (191). Bacterial TrpRSs and TyrRSs, however, differ from their eukaryal and archaeal orthologs in that they are missing extensions whose functions are unrelated with aminoacylation but play a role, e.g., in signal transduction pathways, notably for stimulating or inhibiting angiogenesis, as is the case, respectively, for fragments of human TyrRS and TrpRS (192) (see "An overview of the aaRS world," below). Both TyrRSs and TrpRSs bind the tRNA-accepting stem from the major groove side in a way reminiscent of class II aaRSs and with the tRNA molecule spanning across the dimer, as was first proposed for *B. stearothermophilus* TyrRS on the basis of protein engineering data (193) and later confirmed by crystallography for both enzymes. The structural biology of bacterial TyrRSs and TrpRSs is well documented and covers 12 organisms (see Table 2). About 20 crystal structures were solved for each (http://www.pdb.org), but only one structure of a complex with tRNA, that of TyrRS from *T. thermophilus* (***1h3e***) (64), is available. Note the absence of amino acid editing by the two aaRSs, despite the presence of a CP1-like insertion in the catalytic domain (note that typical CP1 domains are inserted in the catalytic domain of those class Ia aaRSs with editing activity, namely, IleRSs, LeuRSs, and ValRSs). This insertion is of small size (~50 amino acids) in comparison with that from class I editing aaRSs and, instead of editing, is involved in dimer interface interactions and binding of the tRNA-accepting ends (64, 194, 195).

TyrRS: These aaRSs present an overall conserved organization in the three kingdoms of life, with a N-terminal catalytic domain and a C-terminal region of variable architecture that contains the ACB domain (18, 194). In *Bacteria*, this C-terminal region has a bipartite architecture with a α-helical domain (α-ACB) followed by an S4-like domain (as present in the superfamily of RNA-binding proteins homologous to the ribosomal protein S4) specific to *Bacteria* and mitochondria. The crystal structure of *B. stearothermophilus* TyrRS shows well-ordered catalytic and α-ACB domains, but the C-terminal domain is not seen in the electron density map (***3ts1***) (196). This domain is mobile and as determined by NMR has a well-defined S4-fold (***1jh3***). It consists of a five-stranded β-sheet packed against two α-helices on one side and one α-helix on the other side (197). Mobility concerns also the C-terminal EMAP II-like domain in eukaryal TyrRSs. In crystallized TyrRSs, the C terminus is often resected or when present not seen in the crystal structures. This structural organization and the functional implications are fully confirmed by the crystal structure of the *T. thermophilus* TyrRS:$tRNA^{Tyr}$

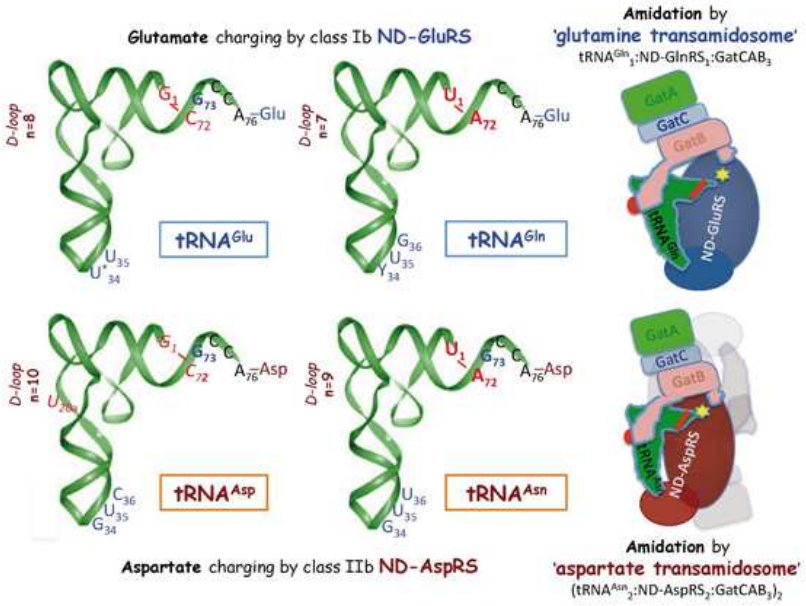

Figure 4 Dual tRNA aminoacylation by ND-GluRS and ND-AspRS and tRNA-dependent amino acid amidation, the two steps of the indirect pathway of glutaminyl-tRNA^{Gln} and asparaginyl-tRNA^{Asn} formation. (Left) The two tRNA couples (tRNA^{Glu} / tRNA^{Gln} and tRNA^{Asp} / tRNA^{Asn}) aminoacylated by ND-GluRS and ND-AspRS, respectively, and (Right) schematized representations of the *T. maritima* glutamine (***3al0***) (186) and *T. thermophilus* aspartate (***3kfu***) (187) transamidosomes. The main structural and functional features important for tRNA aminoacylation by ND-aaRSs and for tRNA-dependent conversion of the glutamyl and aspartyl residues into glutamine and asparagine are shown, as well as the amidation site (yellow star) in the transamidosomes. The major identity determinants for aminoacylation of the four tRNAs by their cognate aaRSs are shown in blue (6, 188). In *Bacteria*, position 34 is a modified U in tRNA^{Glu} and a pyrimidine (Y) in tRNA^{Gln}. Notice the quite similar identity sets in the tRNA^{Glu} / tRNA^{Gln} and tRNA^{Asp} / tRNA^{Asn} couples, in agreement with their dual aminoacylation by the ND-aaRSs. In bold red: U_1–A_{72}, the major identity determinants for amidation in tRNA^{Gln} and tRNA^{Asn}; in red italics: notably the antideterminant G_1–C_{72} pair that prevents glutamate and aspartate amidation in charged tRNA^{Glu} and tRNA^{Asp} (186, 189). The longer length of the D-loop in tRNA^{Glu} and tRNA^{Asp} (as compared to tRNA^{Gln} and tRNA^{Asn}, a feature conserved in *Bacteria* [19]) is a further antideterminant that prevents amidation. Transamidosomes show an overall conserved organization based on the association of ND-aaRS, tRNA, and heterotrimeric GatCAB. Notice the Yqey domain of GatB that contacts the D-loop of tRNA and thereby plays a key role in transamidation. Notice further the different sizes of the two transamidosomes. While the glutamine transamidosome is formed by five entities (as seen in the figure), the much larger aspartate transamidosome is formed by 14 macromolecular entities (for clarity, only half of the structure is shown, with the second subunit of AspRS and its tRNA ligand shown in light grey). This architectural variation is due to structural differences in ND-GluRSs (class Ib monomers) and ND-AspRSs (class IIb dimers) and the correlated mechanistic differences in the aminoacylation and transamidation steps occurring within the two types of transamidosome (see Fig. 6 in "Aminoacylation of tRNA" and "Indirect pathways of specific tRNA aminoacylation for ribosome-mediated translation," below, for details).

complex (64). Thus, the anticodon of tRNA^{Tyr} contacts the α-ACB domain of one subunit while its accepting arm interacts with the catalytic domain of the other subunit. The mobile C-terminal S4 domain is required for contacting and anchoring the *Bacteria*-specific long variable arm of the tRNA on the protein and thus is visible in the complex. In *Eukarya* and *Archaea*, tRNA^{Tyr} has a small variable region, and TyrRS has a different type of ACB domain that is homologous to that of TrpRS, implying protein-RNA contacts in the TyrRS:tRNA^{Tyr} complexes from eukaryal and archaeal organisms different from those occurring in *Bacteria* (194).

TrpRS: Bacterial TrpRSs have the smallest subunit in the aaRS world (e.g., 328 amino acids for *B. stearothermophilus* TrpRS) and represent good approximations of a minimalist aaRS (see "Mimicry of catalytic domains," below, for details on the engineering of an artificial aaRS named the TrpRS urzyme [a neologism, with the prefix *ur-* emphasizing the possible ancestral nature of the enzyme] [198]). They are excellent model systems for exploring conformational changes during the functional cycle of aaRSs (199). A large set of crystal structures is known for *B. stearothermophilus* TrpRS (191, 195, 199, 200) showing the bipartite organization of the protein

with the catalytic domain followed by a small C-terminal ACB domain (called SD, for Small Domain, in the TrpRS literature) as well as different functional states of the enzyme. It is noteworthy that, when ATP and tryptophan bind to TrpRS, relative rigid-body movements of the ACB/SD domain occur (***1d2r***, ***1maw***) (195, 199). Note that the complex of TrpRS with an ATP analog (***2ov4***) reveals an untwisting of the ACB/SD domain relative to the Rossmann-fold domain during aminoacylation, in contrast to TrpRS under ATP/PP_i exchange conditions in which no conformational change takes place (200). The topology of the last C-terminal ~60 amino acids (forming a long discontinuous α-helix running from one extremity of a monomer to the dimer axis) is idiosyncratic to TrpRS (195). Remarkably and unforeseen, the structure of TrpRS from the thermophilic bacterium *T. maritima* (***2g36***) differs from other TrpRSs by the presence of an iron-sulfur cluster [4Fe–4S] coordinated in its C-terminal ACB domain by a 4-cysteine motif (201). The discovery of such a cluster in the aaRS world is unprecedented and its biological role remains elusive. However, since the tRNA-modifying enzyme MiaB from *T. maritima* is involved in thiolation and methylation of A_{37} in tRNA anticodon loops (including that of $tRNA^{Trp}$) and contains, as well, an iron-sulfur cluster sequestered in a 4-cysteine motif (202), it is conceivable that the cluster in the thermophilic TrpRS participates in the specific recognition of the anticodon loop from $tRNA^{Trp}$ (201).

Class IIa SerRS, ThrRS, ProRS, HisRS, and GlyRS

Class IIa aaRSs constitute a rather heterogeneous family of homodimeric α_2-proteins (except GlyRS that is either of α_2- or of $\alpha_2\beta_2$-type) showing important subunit size variations (see Table 1). These aaRSs charge small and polar amino acids (glycine, serine, threonine, proline, and histidine) on tRNA. Overall, the core of the monomers comprises two main modules that are the catalytic domain with the canonical class II signature sequences and a C-terminal ACB domain of mixed α/β-architecture. This ACB domain is the distinctive feature of class IIa aaRSs, except in SerRS that lacks this domain (either lost or not appended during evolution). Despite this absence, ranking of SerRS in class IIa is justified since it is structurally and phylogenetically related to ThrRS and ProRS (14). Idiosyncratic insertions and/or appended domains distinguish the different enzymes of this subclass, which otherwise show large functional diversity (e.g., only ThrRS and ProRS are editing enzymes encompassing distinct editing domains within class IIa aaRSs). Remarkably, GlyRS and ProRS have bipartite and evolutionarily distinct distributions in *Bacteria*, that is, of bacterial- or archaeal/eukaryal-type for GlyRSs and of bacterial- or eukaryal-type for ProRSs.

SerRS, ThrRS, and ProRS: These three aaRSs are structurally and phylogenetically related (14), despite unique structural and functional features characterizing each of them. Interestingly, the structural relatedness concerns also the three amino acids, with serine and threonine capable of forming an internal H-bonded five-membered ring structure that mimics the ring structure of proline (14).

The crystal structure of *E. coli* SerRS reveals an unprecedented N-terminal domain forming an antiparallel α-helical coiled-coil conserved among SerRSs that is stretching 60 Å out into the solvent and is stabilized by interhelical hydrophobic interactions (25). The antiparallel coiled-coil domain is well seen on the structure of the SerRS:$tRNA^{Ser}$ complex from *T. thermophilus* (***1set***) (203). It is also visible on Figure 2 in the structure of the *E. coli* complex (not in PDB) but becomes more obvious under a different orientation. Note that $tRNA^{Ser}$ spans over the two subunits of SerRS and that the extended coiled-coil domain of one subunit makes contacts with the long variable arm and the T-loop of $tRNA^{Ser}$ and thereby directs the acceptor stem into the active site of the other subunit (203). Interestingly, the tRNA anticodon is not recognized by SerRS; this is also the case for AlaRS (class IIc) and LeuRS (class Ia). SerRSs lack an editing domain, but, as shown with the yeast enzyme, possess a hydrolytic activity toward noncognate aminoacyl-adenylates (204).

The ThrRS enzymes, and particularly *E. coli* ThrRS, are interesting for other reasons. They are editing aaRSs and, in the case of *E. coli* and in *Bacteria* closely related to *E. coli*, were shown to recognize both tRNA and a tRNA-like domain encrypted in the operator region of its own mRNA. This recognition is governed by the identity rules (as described below), with threonine identity elements in $tRNA^{Thr}$ and $mRNA^{ThrRS}$ recognized in an equivalent manner by the ThrRS (145, 205, 206) (see "Regulation strategies," below, for other details).

ThrRSs belong to the TGS superfamily (after ThrRS, GTPase, and SpoT guanosine polyphosphate hydrolase)

because of the presence of the small TGS subdomain (~50 amino acids) in their N-terminal part (39). TGS features are common to many enzymes, including AlaRSs. ThrRSs have a conserved overall architecture with a N-terminal editing domain located ~39 Å away from the threonylation site and the protein core formed by a central catalytic domain and a C-terminal ACB domain similar to that of the GlyRS, HisRS, and ProRS families. The N-terminal domain, linked to the core by a long α-helix, is divided into two subdomains, named N1 and N2. This editing domain has strong sequence and folding analogy with the editing domain of AlaRSs (145, 207, 208). Its small N1 subdomain has the topology of proteins from the ubiquitin family, while the N2 subdomain that hydrolyzes mischarged seryl-tRNA^{Thr} has a new fold consisting of a α-helix surrounded by antiparallel β-sheets. Several crystal structures of the isolated editing domain (residues 1 to 224) show how serine is recognized and threonine rejected (209). As seen in the structure of the ThrRS:tRNA^{Thr} complex (***1qf6***), subdomain N2 contacts the acceptor arm of tRNA on its minor groove side, so that the tRNA is clamped between the catalytic and N2 domains (145). Structures with small ligands from *S. aureus* ThrRS (***1nyq***) confirm the structural scheme found for the *E. coli* protein and reveal conformational changes important for activity (210).

As for ProRSs, phylogeny and crystallography indicate that they belong to two evolutionary groups of bacterial- and eukaryal/archaeal-types and show structures that fold in at least five distinct architectures (211). Thus, *E. coli* ProRS (no crystal structure available), *E. faecalis* (***2j3l***), and *R. palustris* (***2i4l***) ProRSs (211) are bacterium-like and *T. thermophilus* (***1hc7***) ProRS is eukarya-/archaea-like (212). The main differences between the two groups are the presence in the eukarya/archaea-like enzymes of a C-terminal Zn-binding module appended to the class IIa-specific ACB domain and in bacterium-like ProRSs of a large INS insertion domain (~180 amino acids) in the catalytic domain. Although ProRSs are editing enzymes, the editing INS domain can be missing, as in *R. palustris* ProRS, but, in that case, its absence is compensated for by freestanding homologs of the INS domain acting in *trans* (see "Error correction" under "Aminoacylation of tRNA" below).

HisRS: A series of crystallographic structures at 2.4- to 2.8-Å resolution of bacterial HisRSs either free or with small ligands bound, namely from *E. coli* (e.g., ***1kmn*** [147, 213] and a structure with a histidyl-adenylate analog [***2el9***, unpublished from RIKEN Structural Genomics Initiative]), *T. thermophilus* (***1h4v***) (146, 214) and *S. aureus* (***1qe0***) (215), give a clear picture of the HisRS architecture and its conformational plasticity that is essential for activity. The homodimeric *E. coli* enzyme has a globular shape, with monomers (424 amino acids) consisting of three domains: first, a N-terminal catalytic core displaying the three class II signatures within a six-stranded antiparallel β-sheet sitting on two α-helices and superposes well with the homologous domain of other class II aaRSs; second, a α-helical insertion (~60 amino acids) interrupting the catalytic domain between motif 2 and motif 3; and third, a C-terminal α/β-domain (100 amino acids) resembling half of a β-barrel and oriented so as to contact the anticodon stem and part of the anticodon loop of tRNA^{His}. Comparison of the different structures reveals slight conformational changes in subdomains that correlate with different functional states, notably, a significant mobility of the insertion domain, as suggested by poor electron density in the *T. thermophilus* HisRS structure (***1adj***, ***1ady***) (214). Mobility of the insertion domain likely favors contacts with the acceptor stem of tRNA^{His} and is associated with adenylate formation (216).

GlyRS: These are enzymes of two mutually exclusive α_2- and $\alpha_2\beta_2$-types without sequence similarity and a phylogeny that does not correspond to the taxonomic classification of organisms. While *Archaea* and *Eukarya* use dimeric α_2 GlyRSs, *Bacteria* use both α_2 and $\alpha_2\beta_2$ GlyRSs (14, 68). This reflects an intricate evolutionary history. Crystal structures of bacterial $\alpha_2\beta_2$ GlyRSs, as present in *E. coli*, are not yet available; however, several structures of archaeal/eukaryal α_2-type GlyRSs are known (Table 2). Their architecture is illustrated by the GlyRS from *T. thermophilus* (***1ati***) (148). It contains the three class II motifs, but motif 1 does not contain the proline believed to be a class II invariant. Each monomer consists of an active site resembling that of AspRS and SerRS, a C-terminal ACB domain of 100 residues and a third domain unusually inserted between motif 1 and 2, almost certainly interacting with the acceptor arm of tRNA^{Gly}. This insertion domain is a rubredoxin-like zinc ribbon (217). The C-terminal domain has a novel five-stranded parallel/antiparallel β-sheet structure with three surrounding helices. The residues responsible for substrate recognition, particularly in the glycine-binding pocket, were readily identified because of the conserved nature of the class II active sites (148).

Class IIb AspRS, AsnRS, and LysRS

These homodimeric aaRSs are closely related in structure and ligand recognition. In *Bacteria* and *Archaea*, their monomers have a bipartite structure, with the ACB module joined to the catalytic core by short hinges. N-terminal extensions with different biological functions (e.g., enhancing tRNA binding, important for aaRS regulation) are appended on the ACB modules and characterize the eukaryal enzymes (89). The ACB domains are made of OB-folds (40), a common motif with β-barrel architecture found in many proteins (218). These OB-folds recognize tRNA anticodon loops, notably the related anticodon identity determinants (aspartate GUC, asparagine GUU, and lysine UUU triplets) and thus play a key role in tRNA discrimination for specific aminoacylation. Discrimination relies on the positioning of the so-called L_{45} loop in the OB-folds of AspRS, AsnRS, and LysRS in the complexes with their cognate tRNA and on peculiar sequence conservation within the L_{45} loops of these aaRSs (219). Distinction between D- and ND-AspRSs relies also on structural differences in their OB-folds (161, 220, 221). Furthermore, discrete amino acid changes in the aspartate-, asparagine-, or lysine-binding pockets prevent amino acid misrecognition by the class IIb aaRSs (222).

AspRS: The distinctive feature of bacterial AspRSs is the large extradomain (~120 amino acids) inserted in the catalytic domain between class II signature motifs 2 and 3. This domain, discovered in *T. thermophilus* AspRS (***1l0w***) (163, 164), has the architecture of a five-stranded antiparallel β-sheet flanked by three α-helices and resembles the so-called ferredoxin fold (163). This distinctive feature of bacterial AspRSs is well seen in the crystal structure of the *E. coli* enzyme displayed in pink in Figure 3. It also has strong homology with a domain found in archaeal GatB proteins and thus was designated the GAD domain (after GatB/AaRS/Domain) (39). This homology reflects an evolutionary link between bacterial AspRSs and GatB transamidation enzymes and suggests a role of the GAD domain in the indirect pathway of $tRNA^{Asn}$ charging (see also "Alternative functions of bacterial aminoacyl-tRNA synthetases," below, for details). As in the case of $tRNA^{Glu}$ and $tRNA^{Gln}$ charging by ND-GluRS (see above), the structure of tRNA plays a critical role in the mechanism of tRNA aspartylation by ND-AspRS (Figure 4).

The *E. coli* AspRS:$tRNA^{Asp}$:aspartyl-adenylate complex (***1c0a***) shows different binding modes of the two branches of the L-shaped $tRNA^{Asp}$ with AspRS (149). While the anticodon branch, including the three anticodon bases (but not the bacterial Q-modification at position 34 of $tRNA^{Asp}$), binds the β-barrel of the N-terminal ACB domain exclusively through direct interactions, the water-solvated acceptor arm establishes both direct and water-mediated hydrogen bonds with AspRS. For the recognition of aspartyl-adenylate, *Bacteria*-specific Gln_{231} (replaced in *Eukarya* and *Archaea* by serine), together with class II-conserved arginine in motif 2, plays the key role in stabilizing the transition state of the aspartylation reaction. Note the closed conformation of the so-called flipping loop within the catalytic domain that anchors aspartic acid or aspartyl-adenylate in its binding pocket and helps correct positioning of the tRNA terminal A_{76} to facilitate the transfer of aspartic acid to its ribose 3′-OH group. In contrast, when tRNA is absent, this flipping loop has an open conformation (149, 165).

AsnRS: The crystallography of AsnRSs is poorly documented, with only one bacterial structure reported—that of *T. thermophilus apo*-AsnRS (not in PDB) (150). This structure is remarkably similar to that of eukaryal and archaeal AspRSs and to class II LysRSs but is lacking the large insertion within the catalytic domain characterizing bacterial AspRSs. Its catalytic site is similar to that of AspRSs, but with a notable difference for discrimination of the related aspartic acid and asparagine substrates. The three structures of free and adenylate-bound archaeal *P. horikoshii* AsnRS at high resolution (1.45 to 1.98 Å) (***1x54***, ***1x55***, ***1x56***) shed light on a peculiar water-assisted asparagine recognition, in contrast to the situation in AspRSs, in which aspartic acid recognition is achieved exclusively through extensive interactions with amino acid residues (223).

LysRS: Crystal structures of *E. coli* LysRS (the constitutive LysS form), in complex with small ligands (***1e22***, ***1lyl***) (151, 224), show as anticipated, similarities with AspRS and AsnRS structures. The LysU form (the product of the *lysU* gene expressed under extreme physiological conditions, such as heat shock) differs slightly (88% sequence identity) from the major LysS form that is synthesized under normal growth conditions (85). It is interesting to note that the LysU enzyme synthesizes Ap_4A and other adenyl-dinucleotide compounds known as alarmones and is involved in the heat shock response (225, 226). Two structures of *T. thermophilus* LysRS in complex with either homologous unmodified $tRNA^{Lys}$ transcript

or modified *E. coli* $tRNA^{Lys}$ are also available (not in PDB) (227). These bacterial LysRS structures show three well-resolved metal ions (Mn^{2+} mimicking the biological Mg^{2+} ions) coordinating ATP and lysine with conserved residues of the catalytic site and thereby stabilizing a pentavalent transition state (151). Two structures of *B. stearothermophilus* LysRS in complex with lysyl-adenylate analogs (***3e9h***, ***3e9i***) highlight the functional role of a conserved glutamate residue (Glu_{411} in *B. stearothermophilus*) for the nucleophilic attack of PP_i in the ATP/PP_i exchange reaction (228). As to the LysRS:$tRNA^{Lys}$ complex, its structure shows an ordered tRNA anticodon branch in contact with the ACB-OB-fold of the protein in a way reminiscent of what is seen in AspRS, with best electron density for the anticodon identity determinants interacting with the aaRS (227). This observation supports a common class IIb interaction mode of tRNA, where interaction of anticodon with the aaRS is the first trigger for productive tRNA aminoacylation (166). Finally it is worth mentioning the structure of PoxA from *S. enterica* (229) (***3g1z***), a paralog of LysRS, and to remember the existence in a few *Bacteria* of a class Ib LysRS that shares similarities with GluRS (see above).

Class IIc AlaRS and PheRS

AlaRS and PheRS are the largest aaRSs and are made of four subunits arranged in intricate topologies. Tetrameric α_4-AlaRS and especially $\alpha_2\beta_2$-PheRS have complex multidomain arrangements. Both demonstrate editing activity. Their ranking in class IIc, however, is based not on robust evidence (AlaRS was first ranked in class IIa) but rather on idiosyncrasies that differentiate them from the other class II members. The oligomeric organization of AlaRS and PheRS is conserved during evolution, except in mitochondria, where PheRS is monomeric (see "Bacterium-like aminoacyl-tRNA synthetases," below). Note that the true topology of cytosolic PheRSs is dimeric of $(\alpha\beta)_2$-type with two heterodimers. Interestingly, among aaRSs, AlaRSs have the highest degree of sequence conservation and have limited similarity with other aaRSs (18).

AlaRS: The AlaRS monomers (~800 amino acids) are organized into four functional domains that are, starting from the N terminus, the class-defining catalytic domain, a tRNA recognition domain (that is split into two structural modules), an editing domain, and a C-terminal oligomerization domain. Although a complete crystallographic structure of a native AlaRS is still missing, data on AlaRS fragments give a rather comprehensive view on structure/function relationships. Thus, the catalytic fragments of *A. aeolicus* AlaRS (453 amino acids) (***1yfr***) show class II characteristics and a bipartite organization of the tRNA recognition domain made of a helical module with a hairpin motif critical for acceptor-stem recognition and a C-terminal module of mixed α/β-fold. Docking of $tRNA^{Ala}$ suggests critical contacts with three AlaRS domains (230). For archaeal *A. fulgidus* AlaRS, two halves of the enzyme were solved (135), namely, the large active truncation comprising the catalytic/tRNA-recognition domain and the editing domain ($AlaRS_{\Delta C}$) and the smaller C-terminal truncation restricted to the dimerization domain ($AlaRS_C$). These structures inform about the insertion of the editing domain in the enzyme core via tight hydrophobic interactions to the catalytic/tRNA-recognition domains, on the side opposite from that in ThrRS. Thus, tripartite $AlaRS_{\Delta C}$ (***2ztg***) forms a groove containing the amino acids required for recognition of the alanine $G_3{\bullet}U_{70}$ identity pair in tRNA. Therefore, this groove appears to be an alternative tRNA-binding site that specifically recognizes the $G_3{\bullet}U_{70}$ pair in the acceptor stem of $tRNA^{Ala}$. This implies that both tRNA recognition and editing are triggered by the same determinant. The dimerization domain (***2zvf***) consists of helical and globular modules. The helical module mediates dimerization by forming a helix-loop-helix zipper, while the globular module with its positively charged face suitable for tRNA binding is important for the aminoacylation and editing activities.

PheRS: Extensive sequence analysis and solution studies on various PheRS proteins, together with detailed crystallographic investigations on the PheRS from *T. thermophilus* (152, 231, 232, 233, 234) and more recently from *E. coli* (235), provide a good understanding of these enzymes that have both aminoacylation and editing activity. The heterotetrameric $(\alpha\beta)_2$ PheRSs have an unprecedented architecture shaped as a leatherback turtle with large flippers (Figure 5) formed by the N-terminal parts of the β-subunits as first revealed by the crystal structure of the *T. thermophilus* enzyme (***1pys***) (234). This structure, and refined versions with ligands (231, 233, 235), including tRNA (152, 232) (***2iy5***), highlights 11 domains (3 in the catalytic α-subunit, namely, A0 to A2—with N-terminal A0 disordered in the *T. thermophilus* structure—and 8 domains in the β-subunit, namely, B1 to B8). The small catalytic α-subunit (327 to

Figure 5 Shape convergence in biomacromolecular structures and morphology of living organisms, or when macromolecular structures meet zoology. The two panels represent the mimicry of the morphology of a leatherback turtle (*Dermochelys coriacea*) (left) with the crystal structure of a representative PheRS (e.g., from *T. thermophilus* [***2iy5***]) (right). Notice the bilateral symmetry in the shape of both turtle and pseudodimeric $(\alpha\beta)_2$ PheRS (with catalytic short α-subunit in green CPK amino acid models displayed on a yellow background and a large β-subunit in cyan). The large front flippers of the turtle show astonishing mimicry with the B_1–B_5 domains from the β-subunit of the PheRS. Other shape convergences can be found when comparing the structures of dimeric class IIb aaRSs or the dimerization domain of $AlaRS_C$ with the symmetric morphology of butterflies (try to find these mimicries, and others, after clicking on the PDB accession codes of aaRS structures given in the text). Whether such shape convergences have biological meaning remains an open question.

350 amino acids) comprises the active site (A1 and A2) and domain A0 with a compact triple-helix structure that contains a GxxG RNA-binding sequence for a specific contact with $tRNA^{Phe}$ (232, 235). The large β-subunit (775 to 797 amino acids) contains a heterodimeric domain (B1 and B5) similar to the DNA-binding module of CAP (Catabolite gene Activator Protein); a domain inserted in B1 (B2), similar to the EMAP II and OB-folds found, respectively, in MetRS and class IIb aaRSs, where they contact the tRNA anticodon region; an editing domain of intricate architecture (B3 and B4) distant from the aminoacylation domain; a cryptic "catalytic-like" domain without class II signatures (B6 and B7); and a recognition domain of $tRNA^{Phe}$ anticodon (B8). This domain is missing in eukaryal PheRSs so that recognition of $tRNA^{Phe}$ differs in *Bacteria* and *Eukarya* in agreement with differences in the catalytic efficiency of $tRNA^{Phe}$ charging. It is noteworthy, and most intriguing, that B8 shares structural similarity with the U1A spliceosomal protein and B3/B4 shares structural similarity with the biotin synthetase/repressor protein (BirA) from *E. coli*, with a DNA-binding α/β-motif (B3), and a SH3-like (for SRC Homology) motif found in proteins of signaling pathways (B4) (236). Remarkably, comparison of the *T. thermophilus* PheRS structure with the *E. coli* PheRS structure uncovers significant rearrangements of the structural domains involved in $tRNA^{Phe}$ binding/translocation (234). The high-resolution PheRS structure from pathogenic *S. haemolyticus* (***2rhq***) (237) and *P. aeruginosa* (***4p71***) (238) overall agrees with the architecture of the *T. thermophilus* and *E. coli* orthologs but reveals idiosyncratic pockets for drug discovery.

General Conclusions on the Similarity and Diversity in aaRS Structures

AaRSs are modular enzymes globally conserved in the three kingdoms of life. All of them catalyze the same two-step reaction, namely, the attachment of a proteinogenic amino acid on their cognate tRNAs. Based on the tertiary structure of their catalytic domains, they are ranked in two distinct groups of 10 enzymes each. On the basis of other structural features, each of the two groups is subdivided into three subgroups. Structural di-

versity comes from the nature, the number, and the size of various additional domains appended on the catalytic cores—in particular, different types of anticodon-binding domains and, in the case of certain amino acid specificities, of editing domains where noncognate amino acids mischarged on tRNA are hydrolyzed. The oligomery of α-, α_2-, α_4-, or $a_2\beta_2$-type contributes further to the structural diversity of aaRSs. Genomic and X-ray crystallography methods were the main tools that led to these general conclusions but allowed also the discovery a plasticity of the aaRS architectures related to different functional states, as well as the characterization of faint structural idiosyncrasies within a given group of aaRSs specific for the same amino acid that could be correlated with kingdom and even species functional differences, such as for cognate tRNA recognition or for new functions. In this regard, the recent identification of novel protein domains in cyanobacterial aaRSs deserves attention (239). In addition to these general trends, one should notice the following: (i) the absence of GlnRS and AsnRS in many bacterial and most archaeal organisms, compensated for by the presence of non-discriminating GluRS and AspRS that aminoacylate, respectively, noncognate $tRNA^{Gln}$ and $tRNA^{Asn}$ in addition to their cognate $tRNA^{Glu}$ and $tRNA^{Asp}$; (ii) the presence in some *Bacteria* of a class I LysRS (LysRS-1) instead of the otherwise conserved class II LysRS, thus breaking the unicity of the aaRS ranking rule; and (iii) the presence of atypical aaRSs in *Archaea* (ND-aaRSs, LysRS-1, and the two strictly archaeal PylRS and SepRS).

AMINOACYLATION OF tRNA

aaRS-Class-Specific Features for Substrate Binding

Difference in size and in the architectural organization of the active sites accounts for different binding modes of substrates in the two aaRS classes. Thus, in class I aaRSs, the active-site subdomain is smaller than in class II enzymes as a result of differences in the number of amino acids forming the two class-specific catalytic domains (~150 residues in the class I Rossmann fold and ~250 residues in class II antiparallel β-sheet domain). Moreover, topological differences in the protein fold and in the overall shape of the two types of active sites favor discrimination of amino acids with similar chemical groups, but with a different size (i.e., glutamate and aspartate or arginine and lysine on class I and class II enzymes, respectively). These differences lead to two binding modes for ATP (Figure 6). In class I aaRSs, ATP adopts an extended conformation reminiscent of that found in other proteins containing a Rossmann fold, while in class II enzymes it exhibits a bent conformation with the γ-phosphate folded back over the adenine base. In both cases, Mg^{2+} ions and strongly conserved amino acids are involved in ATP binding as well as in stabilization of the reaction transition state (i.e., residues from the flexible KMSKS signature loop in class I and the two invariant arginines in motif 2 and 3 in class II aaRSs [240]).

In the two classes, Mg^{2+} ions play a dual role in amino acid activation by stabilizing the conformation of ATP and participating in adenylate formation (18). They assist catalysis and facilitate adoption of the productive conformation of the PP_i moiety in ATP. However, and in contrast with the class-dependent conserved ATP-binding modes, those of Mg^{2+} ions are diverse and depend more on the aminoacylation system than on the aaRS class (241). For instance, class I *E. coli* GlnRS has one Mg^{2+}-binding site controlling interaction with the β- and γ-phosphates of ATP (242). In *B. stearothermophilus* TrpRS, a Mg^{2+} ion controls tryptophan activation by subtle allosteric effects (243). Several class II enzymes have three Mg^{2+} sites, notably in *E. coli* AsnRS (151) and *P. kodakaraensis* AspRS (162), where the catalytic Mg^{2+} is located between the α- and β-phosphates and the two others are seated on each side of the γ-phosphate bond. In class II *E. coli* HisRS, only two Mg^{2+} ions are observed, with the catalytic ion replaced by an arginine residue, absent in other class II aaRSs (147). This arginine residue, Arg_{259}, is common to all HisRSs and, as a Mg^{2+} ion, has a catalytic role in maintaining an interaction with the α-phosphate of ATP (***1kmn***) (147) or histidyl-adenylate (***1htt***) (213) (see also "Mechanistic of tRNA aminoacylation" for details).

Furthermore, the crystal structures of the glutamine and aspartate aaRS:tRNA complexes have revealed two evolutionary conserved aaRS class-dependent tRNA recognition schemes. In bacterial systems, this is best seen when comparing the structures of *E. coli* class I GlnRS (179, 244) and class II AspRS (149, 245) in complex with their cognate tRNAs (Figure 6). Thus, class I and class II aaRSs recognize opposite sides of tRNA as a consequence of the different entry mode of their helical acceptor stems in the catalytic sites. Class I aaRSs bind this stem on the minor groove side and class II enzymes on the major groove side. This different tRNA positioning im-

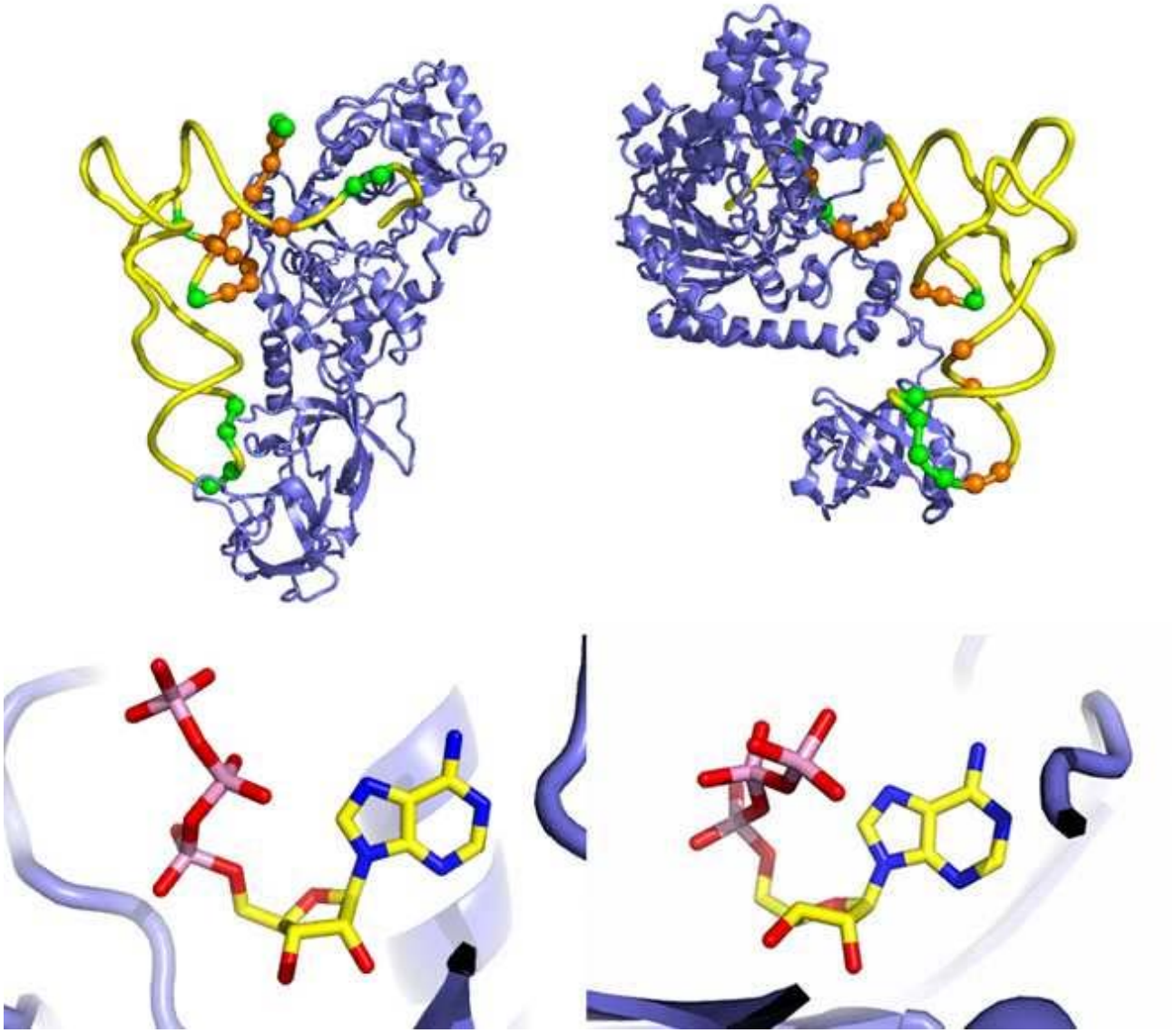

Figure 6 Different substrate recognition modes by class I and class II aminoacyl-tRNA synthetases. The differences are illustrated by the structures of *E. coli* glutamine (left) and aspartate (right) complexes ([***1gsg***] [179, 244] and [***1c0a***] [149], respectively): tRNA recognition (top); ATP recognition (bottom). For clarity, only one subunit of the AspRS:tRNA^{Asp} complex is displayed. The tRNAs are shown as yellow ribophosphate backbones with contact residues represented as colored spheres (contacts with CCA are not shown): identity determinants are in green (a few do not contact the aaRS; see text) and other contact residues in orange. The class II adenylate conformation is from the archaeal AspRS from *P. kodakaraensis* (***1b8a***) (162). In the *E. coli* GlnRS:tRNA^{Gln} complex, 13 tRNA^{Gln} nucleotides (nt) are both determinants and make contact with GlnRS (nt 1, 2, 3, 10, 34, 35, 36, 37, 38, 70, 71, 72, 73) and 10 other nucleotides make additional contact with GlnRS (nt 4, 5, 6, 7, 8, 11, 12, 13, 14, 15). In the *E. coli* AspRS:tRNA^{Asp} complex, 8 tRNA^{Asp} nucleotides are both determinants and contact residues (nt 2, 10, 34, 35, 36, 38, 71, 73) and 11 other residues make additional contacts with AspRS (nt 11, 12, 25, 28, 32, 33, 67, 68, 69, 70, 72).

plies conformational adaptation of the acceptor end of tRNA. In class II AspRS, the backbone of the terminal $-N_{73}CCA_{76}$ sequence keeps a helical conformation, whereas in class I GlnRS it makes a sharp hairpin turn toward the inside of the L, with a disruption of the first U_1–A_{72} pair. In both cases, binding of tRNA maintains the terminal ribose in a position that facilitates attack of the α-phosphorus of the adenylate intermediate. As a final result, the class-dependent entry of the tRNA into the active site accounts for the correct positioning of the 2′-OH (class I) or 3′-OH (class II) of the terminal ribose for receiving the amino acid.

Fidelity of Amino Acid Recognition and Functional Implications

A first prerequisite of faithful tRNA aminoacylation is fidelity of the amino acid activation step. For that, the extant aaRS family evolved to preferentially recognize the L-enantiomers of amino acids and to become selective for the 20 canonical proteinogenic members from the present genetic code. However, the too great relatedness of amino acid structure and shape should probably be fatal for recognition fidelity by the aaRSs with the consequence that these enzymes would make errors, as anticipated in the fifties by Pauling (246) and since then amply documented. To overcome this intrinsic weakness and exclude nondesired amino acids from protein synthesis, evolution developed strategies, both to avoid incorrect amino acid (and tRNA) recognitions and to clear errors due to amino acid misactivation and correlated tRNA mischarging (see, e.g., references 247 and 248 and below for details on tRNA recognition and editing).

The basis of selectivity for the L-enantiomers is intriguing and was only investigated in a few instances. For *E. coli* AspRS and TyrRS, molecular dynamics simulations and energy calculations support this preference (249). A similar approach shows the unfavorable electrostatic surrounding in the active site of *E. coli* HisRS for D-amino acid binding (250).

Amino acid recognition by aaRSs was studied in depth for *E. coli* AspRS by structure-based computer simulations and site-directed mutations (222, 251). They revealed the existence of a network of electrostatic interactions and a charge distribution in the active site accounting for optimal binding of charged aspartate. Binding of aspartate is stronger than for related compounds, particularly for neutral asparagine. Importantly, the intricate interplay between the amino acids constituting the aspartate-binding pocket protects AspRS against most binding errors and renders its engineering, e.g., in view of specificity switches, difficult (251).

For class Ia ArgRS and class Ib aaRSs, binding of amino acids in the catalytic site requires the presence of tRNA (reviewed in reference 252). This unique property is explained by crystallography. As seen in the structure of the *T. thermophilus* GluRS:$tRNA^{Glu}$:Glu ternary complex (***2cv0***), tRNA and aaRS collaborate to form a specific binding pocket for L-glutamate. This cooperation occurs by subtle conformational changes allowing specific amino acid binding and activation. In contrast, in the GluRS:Glu binary complex (***2cuz***), when $tRNA^{Glu}$ is absent, this conformational change does not occur. As a consequence, the amino acid binding site is defective, thus accounting for binding of incorrect amino acids and lack of glutamate activation (142). Further evidence comes from a mutational study with *E. coli* GluRS. Thus, when $tRNA^{Glu}$ is present, a $Cys_{100}Tyr$ variant (with an altered Zn-binding motif in the SWIM-fold involved in recognition of the acceptor-end of tRNA, see above) has a lower affinity for L-glutamate than the wild-type enzyme, while in the absence of $tRNA^{Glu}$, glutamate binds with the same affinity to both variant and to wild-type GluRS (253). Such effects likely apply as well for amino acid activation by GlnRSs (254, 255). It is noteworthy that site-directed mutagenesis on the wild-type *E. coli* GlnRS explicitly demonstrated that amino acid selectivity relies on the collective remodeling of the active site and not on direct amino acid contacts with the enzyme, since switching the activation specificity from glutamine to glutamate necessitates 22 amino acid substitutions and one deletion in the Rossmann fold of the wild-type GlnRS (256). It is anticipated that tRNA-dependent activation of arginine by ArgRSs (257, 258) and lysine by LysRS-1 (45) follows similar schemes. Note that aaRSs requiring cognate tRNA for activation can tightly bind their amino acid substrates in the absence of tRNA, thereby leading to inactive conformers as seen, e.g., in *E. coli* ArgRS (***4oby***) (178) and *B. burgdorferi* GluRS (***4gri***) (unpublished from the Seattle Structure Genomics Center for Infectious Disease). In these enzymes, the functional catalytic site is shaped by subtle conformational changes triggered by cognate tRNA. In summary, RNA is the specificity effector for amino acid recognition and activation.

Fidelity of tRNA Recognition and the Identity Problem

General concepts and definitions

Specific recognition of tRNA by aaRSs depends on thermodynamic and kinetic parameters, with a first discrimination by K_D for binding (259) and additional discrimination by V_{max} brought by the aminoacylation reaction (260). Identity rules account for these effects and are presently phenomenologically understood (6, 188, 261, 262, 263), although many mechanistic aspects remain elusive (264). These rules are referred to as the second genetic code (1) and rely in each aaRS–tRNA system on a limited number of tRNA determinants that contact the aaRS or act by indirect effects, but also on less known antideterminants that prevent false tRNA

interactions with noncognate aaRSs. Determinants and antideterminants are defined as nucleosides (more precisely, as chemical groups on these nucleosides) and form the so-called recognition/identity sets. They are located mainly at the two distal ends of the tRNA molecule and, in most cases, contact identity amino acids on aaRSs. Identity sets can be completed by permissive elements. Such elements, only characterized in a few tRNAs, depend on the nucleotidic context (265). In a more structural perspective, crystallographic data show a limited number of contact points between aaRS and tRNA with some of them involving identity elements (see, e.g., reference 18 for specific references). Note that tRNA backbone interactions, or the related concept of indirect readout of tRNA (recognition by aaRSs of sequence-dependent conformations of tRNA via the sugar-phosphate backbone or nonspecific portions of the bases) (266), can have a functional role as first explicitly seen in the recognition of the acceptor stem of $tRNA^{Asp}$ by *E. coli* AspRS (149, 267) and documented for a few other systems (266). Table 3 gives an outlook of the structural features in tRNA that are important for recognition and specificity toward aaRSs.

Because anticodon bases specify correct reading of the genetic message on mRNAs and form the relationship between the amino acids forming proteins and the trinucleotides of the genetic code, they were soon considered to be prime candidates for specifying tRNA recognition by aaRSs. This assumption turned out to be correct in many systems but does not apply to the alanine, leucine, and serine identities (6, 188, 261). Based on other considerations, participation of tRNA residues near the amino acid CCA-acceptor end was assumed to be crucial. A wealth of experimental data and thoughts on the origin of tRNA aminoacylation confirmed the importance of the anticodon and accepting branch in tRNA identity, and at present it is accepted that tRNA identity rules are universal, with nevertheless idiosyncratic distinctions in the three kingdoms of life and even species-dependent subtleties (262, 264, 277). In a few cases, experimental evidence shows an initial role of the accepting branch when contacting the catalytic site of aaRSs (e.g., in class Ib, see above) or of the tRNA anticodon domain as for AspRS (166), as well as of other structural features (e.g. the relationship between D-loop, variable region, and T-loop [278]). The importance of the tRNA shape, as well, should not be overlooked because it provides the structural framework that dictates the correct positioning of the identity elements for optimized interaction with the aaRSs (see "Role of tRNA and identity determinants in tRNA aminoacylation," below).

Three main strategies were used to find identity determinants. In its simplest version, the first strategy consists in searching consensus sequences of all tRNAs charged by a same aaRS. In a more elaborate version, the search is computer-assisted as first applied for *E. coli* tRNAs (279). This strategy was recently ameliorated thanks to novel computational tools (280, 281, 282). The second strategy, widely used, is the *in vitro* transcription of artificial tRNA genes that can readily produce any type of tRNA transcript for aminoacylation assays (283). This method, however, does not evaluate the role of post-transcriptional modifications in identity. Atomic mutagenesis (removal or replacement of chemical groups in tRNA) would be the method of choice to discover the chemical signals in tRNA nucleosides important in identity (284). But, because of methodological difficulties, the approach was restricted to those systems where aaRSs can aminoacylate small RNA substrates (285). The third strategy, also widely used, especially for *E. coli* tRNAs, allows the *in vivo* study of mutated suppressor tRNAs with a reporter system based, for instance, on the reading by engineered suppressors of an amber mutation at position 10 of a dihydrofolate reductase gene (286, 287).

The strength of an identity determinant is given by the functional effect produced by its mutation (k_{cat}/K_m of aminoacylation for *in vitro* methods or suppression strength for *in vivo* methods). The strongest determinants are located mainly at both extremities of the L-shaped tertiary structure of tRNA and are essentially conserved in evolution. Completion of an identity set is verified by transplantation of the putative identity set into the background of a tRNA with another identity. This conceptual framework allowed characterization of most of the strongest determinants and also a gross understanding of the role of tRNA architecture but not to unravel subtleties underlying expression of identities (6, 188, 261, 264).

Determinants and antideterminants

The distribution of identity determinants in *E. coli* tRNAs is listed in Table 4. It is reminiscent of that observed for *S. cerevisiae* and a few other eukaryal and archaeal organisms (6, 188, 262, 288). The most striking

Table 3 Structural features within tRNA involved in interactions with aminoacyl-tRNA synthetases and/or important for the specificity of tRNA aminoacylation

Structural features	Effect[a] on Interaction	Effect[a] on Specificity	Comments and references
Shape of tRNA recognized by an aaRS			
Canonical L-shape	+++	±	*Under special conditions, all tRNAs can be recognized by certain aaRSs, but tRNA mischarging efficiency is low (37)
Atypical shapes tRNAs with large variable region, tRNA-like domains, many mitochondrial tRNAs, etc	+++	++	*For tRNAs with large variable region (e.g., $tRNA^{Leu}$) or atypical tertiary interaction networks (e.g., $tRNA^{Cys}$) (268) *For tRNA mimics in mRNA, e.g., in *E. coli* $mRNA^{Thr}$ (206) *Aminoacylation of tRNA mimics can be efficient, e.g., in viral tRNA-like structures (264) *For atypical mitochondrial tRNAs, e.g., reference (269)
Determinants in tRNA for specific tRNA aminoacylation *directly* read by an aaRS			
Bases Restricted number (2–11) in identity sets; mainly in single-stranded regions; sometimes in WC pairs	+++	+/+++++	*System-specific contacts, e.g., in *E. coli* aspartate and glutamine system (149, 244) and identity sets (6, 188) *Atomic determinants in *E. coli* alanine system (270)
Sugar (from identity bases)	+	+	*Few examples of direct contacts of ribose O2′ with aaRSs, e.g., in the *E. coli* aspartate system (149) *Only a few examples of ribose as identity determinant (271, 272)
Modified residues	+	++	*Few documented system-specific examples (273)
Determinants in tRNA for specific tRNA aminoacylation *indirectly* read by an aaRS			
Sequence-dependent Individual or collective	+/++	+++	*Recognition of idiosyncratic conformations in cognate tRNAs (266) *Water mediated recognition of individual identity determinants, e.g., in *E. coli* aspartate system (149)
Antideterminants in tRNA *for noncognate tRNA rejections* by an aaRS			
Individual nucleosides WC or modified nucleosides; possibly idiosyncratic tRNA domains	–	++++	*Not systematically searched; only a few cases supported by experimental validation (188, 274, 275)
Other nucleotidic constituents *for stability of a given aaRS:tRNA complex and for tuning its specificity*			
Bases	+/+++	±/++	*System-specific contacts contributing to overall binding affinity, e.g., in the *E. coli* glutamine system (149, 244)
Ribophosphate backbones	+/+++	±/++	*System-specific contacts (as above), e.g., reference 266

[a]Effects: +++++, strongest;++++, very important; +++, important; ++, medium; +, weak; ±, low. WC, Watson-Crick; n.d., not determined. See the text for more detail. Note that the structural features in tRNA are mirrored by proteic elements in the aaRSs (less well defined and characterized than their counterparts on tRNA), notably amino acids that contact identity nucleotides (e.g., in the *E. coli* AspRS Arg_{76}, Glu_{93}, and Gln_{46} make hydrogen-bond interactions with identity determinants Q_{34} and U_{35} from the anticodon of cognate $tRNA^{Asp}$ [149]). Two of these amino acids (Gln_{46} and Glu_{93}) are conserved through evolution in AspRSs (276) and therefore can be considered as aspartate identity amino acids.

difference between bacterial systems and other systems concerns tyrosine identity given by G_1–C_{72} in *Bacteria* (and organelles) and C_1–G_{72} in *Eukarya* and *Archaea* (194). This differential expression of tyrosine identity in evolution correlates with kingdom-specific features in $tRNA^{Tyr}$, namely, large variable regions in *Bacteria* and small ones in *Eukarya* and *Archaea* (19, 194). The crystal structure of both *T. thermophilus* and *Methanococcus jannaschii* TyrRS:$tRNA^{Tyr}$ complexes explains the functional idiosyncrasies that are mainly due to different acceptor stem recognitions and involvement of the S4-like domain of bacterial TyrRSs in recognition of the large variable region of bacterial $tRNA^{Tyr}$ (64, 289). Note that the human mitochondrial TyrRS, closely related with bacterial TyrRSs, disobeys the universal identity rules since the G_1–C_{72} pair is not needed (290). In contrast, however, *E. coli* tRNAs make ample use of residues in the tRNA acceptor helix. For instance, in the aspartate system, the two first base pairs in this helix are determinants (291, 292). Whether idiosyncratic identity determinants within the acceptor stem of mitochondrial tRNAs contribute to aminoacylation

Table 4 Distribution in tRNA of identity determinants for aminoacylation by bacterial aminoacyl-tRNA synthetases and aminoacylation capacity of tRNA minihelices[a]

	Aminoacyl-tRNA synthetases	
tRNA domains	**Class I**	**Class II**
Amino acid accepting branch		
nt$_{-1}$ (1 identity)	–	H
Discriminator base 73 (18 identities)	C, E, I, L, M, Q, R, V, W, Y	A, D, F, G, H, K, N, P, S
Acceptor stem[b] (12 identities)	C, E, I, M, Q, V, W	A, D, G, H, T, S
Core region[c] (13 identities)	C, E, L, M, Q, R, W	A, D, F, G, P, S
Anticodon stem-loop branch		
Anticodon stem[d] (2 identities)	Q	K
Anticodon loop[e] (3 identities)	E, M, Q	–
Anticodon triplet[f] (17 identities)	C, E, I, M, Q, R, V, W, Y	D, F, G, H, K, N, P, T
tRNA structure (collective participation)[g]	C, E, I	F, K
Amino acid accepting minihelices		
Identity of accepting RNA	I, L[h], M, Q, V	A, D, G, H, P, S

[a]AaRSs are abbreviated by the one-letter code; underscored letters mean participation in identity of modified nucleotides (273).

[b]Base pair (bp) 1–72, 2–71, 3–70, 4–69.

[c]Concerns mainly conserved or semiconserved residues important for tRNA architecture, such as the atypical Levitt G_{15}•G_{48} pair for cysteine identity (293).

[d]bp 29–41, 30–40, 31–39.

[e]Residues 37 and 38.

[f]Triplet 34, 35, 36 (numbering of tRNA residues is according to reference 19).

[g]As a result of decreased catalytic aminoacylation efficiency of unmodified tRNA transcripts in comparison with the efficiency of fully modified native tRNAs (data only available for six *E. coli* systems, with AlaRS not sensitive to the presence of tRNA modifications [273]). Most data on identities were retrieved from references 6 and 188; see also newer literature for the importance of the tRNA core region in cysteine identity (294), of N_{73} and other elements in leucine and proline identities (295, 296, 297), and of anticodon stem elements for tRNA recognition by *E. coli* LysRS and GlnRS (298). For data on minihelices, see references 270, 285, 299, and 300.

[h]Only *A. aeolicus* LeuRS.

specificity, e.g., in the human tyrosine system, remains to be tested.

Antideterminants preventing false recognition of tRNA are not well known, and so far only a few have been discovered. The hypermodified lysidine residue (k^2C or 2-lysyl-cytidine) at the first anticodon position of minor *E. coli* $tRNA_2^{Ile}$ is responsible for the rejection of this tRNA by MetRS and was the first bacterial antideterminant discovered (274). Likewise, A_{36} in *E. coli* $tRNA^{Arg}$ is an antideterminant against TrpRS (301), and the G_2•U_{71} pair in *B. burgdorferi* $tRNA^{Lys}$ (cognate for class Ib LysRS) prevents misrecognition by class IIb LysRS (190).

Specificity determinants and antideterminants exist at the protein level but were not systematically searched and identified by mutagenesis analyses. Several examples are worth mentioning. Thus, Arg_{83} in AsnRS is involved in the recognition of U_{36} at the third identity position of $tRNA^{Asn}$ anticodon (223). Likewise, identity amino acids have been described in bacterial TyrRSs. They consist of conserved or semiconserved residues recognizing anticodon (Asp_{259}, Asp_{423}) and acceptor arm (Glu_{154}, Arg_{198}) identity bases (194, 290). On the other hand, amino acids acting as antideterminants were found in *E. coli* MetRS, *B. stearothermophilus* TyrRS, and *H. pylori* GluRS. Thus, Asp_{449} and Asp_{456} from the C-terminal ACB-domain of MetRS are negative signals that reject noncognate tRNA anticodons (302). Likewise, Glu_{152} of TyrRS rejects noncognate tRNAs by electrostatic and steric repulsion (303), and Arg_{350} in the ACB region of GluRS rejects $tRNA^{Gln}$ (77).

Role of modified nucleosides

Five modified nucleosides were explicitly characterized as identity determinants in *E. coli* tRNAs, namely, k^2C_{34} (in $tRNA_2^{Ile}$), s^2U_{34} (in $tRNA_1^{Gln}$ and $tRNA^{Glu}$), $mnm^5s^2U_{34}$ (in $tRNA^{Lys}$), Q_{34} (in $tRNA^{Tyr}$), and t^6A_{37} (in $tRNA^{Ile}$), all located in anticodon loops (273) (for the meaning of modification symbols, see the Introduction, above). The role of k^2C_{34} in minor *E. coli* $tRNA_2^{Ile}$ is remarkable since this residue is both an antideterminant against MetRS (see above) and a determinant for IleRS (274). However, the determinant role could be indirect, since the major $tRNA_1^{Ile}$ has a G at position 34 that is structurally different from k^2C, which could mean that *E. coli* IleRS does not make a direct functional contact at position 34. On the other hand, the drastic reduction of the k_{cat}-dependent isoleucylation capacity of *E. coli* $tRNA_1^{Ile}$ after replacement of t^6A_{37} by A_{37} shows that t^6A_{37} is an isoleucine identity determinant and probably is involved in a direct interaction with *E. coli* IleRS (304). The case of the *E. coli* glutamate system is interesting since isolated hypomodified $tRNA^{Glu}$ species (modivariants) have variable aminoacylation capacities (305). The strongest impairment concerns the modivariants lacking $mnm^5s^2U_{34}$ and m^2A_{37}. The fact that another modivariant solely containing s^2U_{34} is efficiently aminoacylated conclusively indicates that the mnm^5 group (5-methyl-aminomethyl

group) is not important for aminoacylation, which in turn indicates that the s^2 group acts as a determinant for $tRNA^{Glu}$ aminoacylation (305).

Minimalist tRNAs as means for understanding aaRS functions and evolution

The aminoacylation capacity of minimalist tRNAs restricted to an accepting branch, or part of it, is so far documented in 11 systems (Table 4). This property allowed discovery of novel identity elements in tRNA, yielded information on the mechanism of identity expression, and provided insights into how evolution established the identity rules (2, 270, 285). Note that the ability to prepare charged or mischarged minihelices turned out to be useful for studying editing (299, 306, 307). Below is a short survey of significant outcomes from these studies.

The first data came from the *E. coli* alanine system and indicated that native L-shaped tRNA is not a prerequisite for the aaRS aminoacylation function (308, 309). By atomic mutagenesis it was conclusively shown that the unpaired exocyclic 2-amino group of G_3 positioned in the minor groove of the wobble $G_3 \bullet U_{70}$ alanine identity pair is required for aminoacylation by AlaRS (309). Moreover, it was shown that the A_{73} discriminator base of $minihelix^{Ala}$ is a determinant of the transfer step of aminoacylation and that substitution of the exocyclic amino group of A_{73} with a keto-oxygen results in negative discrimination (310).

Likewise, RNA hairpin helices based on the acceptor stem of $tRNA^{Met}$ and $tRNA^{His}$ were shown to be specifically aminoacylated by MetRS and HisRS (311). Other studies with a $tRNA^{Tyr}$-derived microhelix gave the clue of the tyrosylation species specificity that is solely determined by the N_1–N_{72} base pair (312). Furthermore, and most interesting, it was shown that a single atomic group in an RNA helix based on *E. coli* $tRNA^{Pro}$ (the 6-keto group of C_{72}) is needed for positive discrimination by cognate ProRS and is an antideterminant for negative discrimination by noncognate AlaRS (313). As for serine identity, serylation rates for $minihelix^{Ser}$ variants revealed that *E. coli* SerRS recognizes five base pairs (1–72 to 5–68) of the $tRNA^{Ser}$ acceptor stem with major recognition elements clustered between base pair positions 2–71 and 4–69 (314). Serylation efficiency of these minihelices revealed the role of functional groups from the major groove, in agreement with the structure of the *T. thermophilus* SerRS:$tRNA^{Ser}$ complex (132). This allows SerRS to recognize the set of $tRNA^{Ser}$ isoacceptors that present sequence variations within the five last base pairs in the accepting arms. Other studies on the charging ability of $tRNA^{Asp}$- and $tRNA^{Val}$-derived minihelices shed new light on tRNA recognition by ValRS and AspRS (315, 316). Furthermore, in the *E. coli* histidine system, atomic mutagenesis probed the exact role of the additional G_{-1}–C_{73} base pair in $tRNA^{His}$ (a characteristic of all $tRNA^{His}$ species) in histidylation by *E. coli* HisRS (317). Results indicated that G_{-1} serves to position the 5′-monophosphate, which is critical for aminoacylation, and additionally that C_{73} and G_{-1} contain exocyclic atomic groups located in the major groove of the accepting RNA helix that contribute to HisRS recognition.

As a last point, note that tRNA fragments restricted to an anticodon stem loop (ASL) can interact with aaRSs. Thus, an ASL^{Val} containing the anticodon $_{34}CAC_{36}$ valine identity determinants stimulates somewhat valylation of a free-standing $tRNA^{Val}$-derived minihelix (315). Likewise, an ASL molecule derived from *E. coli* $tRNA^{Glu}$ containing the s^2U_{34} identity determinant (see above) binds to GluRS and inhibits aminoacylation of native $tRNA^{Glu}$ (318). Most interesting in this context is the glutamylation of bacterial $tRNA^{Asp}$ on the Q-base at position 34 of its anticodon by a mimic of the catalytic domain of bacterial GluRS (comments in [319] and [320], and see "Mimicry of Catalytic Domains", below for details and implications).

In the perspective of evolution, it is important to know that the functionality of minimalist tRNAs (mini- or microhelices, ASLs) depends on the presence of identity determinants, since their absence impairs or completely abolishes their aminoacylation or inhibition capacities. This strongly suggests that primordial aminoacylation systems consisted of pairs of minimalist aaRS and tRNA (2, 198, 321, 322), whose specificity was governed by recognition rules excluding evolutionarily more recent signals from ACB domains in aaRSs and ASLs in tRNAs. Also related to evolution is the question of the coding properties of tRNA identity bases in the acceptor and ASL domains. An answer comes from a recent physicochemical-based study showing that the anticodon encodes the hydrophobicity of each amino acid side chain and the acceptor stem codes preferentially for the surface area or size of each side chain (323). These orthogonal properties suggest that genetic coding of protein 3D structures evolved in distinct stages, based initially on the

size of the amino acid and later on its compatibility with globular folding in water (323).

Mechanism of tRNA Aminoacylation

Catalytic mechanisms of tRNA aminoacylation have been discussed in depth in several reviews and, altogether, an overall common mechanism emerged (6, 7, 240, 324, 325). In this section, emphasis is given to landmark features of the reactions catalyzed by emblematic representatives of the two classes of bacterial aaRSs (remenber that the partition of aaRSs in two classes is based on different structures of their catalytic sites). Other aspects, either atypical or idiosyncratic (e.g., editing and activity of aaRS fragments) are covered in "Error correction" and "Truncated aaRSs," below.

Amino acid activation

After entry of amino acids and ATP to their respective binding pockets, with ATP in the conformation characteristic of each aaRS class, the α-carboxylate group of the amino acid substrates attacks the α-phosphate of ATP, leading to intermediates stabilized by the aaRS class-conserved residues and Mg^{2+}-assisted hydrolysis of the phosphate bond followed by PP_i release. Binding of the adenylate on the protein (that can be enhanced by tRNA binding) facilitates the amino acid transfer on tRNA and prevents the activated amino acid from reacting with other nucleophiles present in the solvent (e.g., water) or on the surface of the aaRS (e.g., the side chain of lysine residues). In all systems so far investigated, conformational changes occur in the aaRSs upon amino acid binding, e.g., in *E. coli* LysRS (***1e1o***) (326), bacterial-type ProRSs (***2j3m***) (221), and *B. stearothermophilus* TrpRS (***1m83***, ***1man***) (327).

Kinetic investigations and isotope-exchange methods combined with crystal structure analysis suggest an overall similar in-line mechanism by nucleophilic attack for four class I aaRSs (IleRS, MetRS, TrpRS, and TyrRS) (240). Refined views emerged when function was interpreted in the light of crystallographic and molecular dynamics results. In the case of *E. coli* class II HisRS, energy variation during the mutual approach of histidine and ATP to form adenylate shows that the surrounding nanospace of the protein confines the reactants in geometry suitable for the in-line nucleophilic attack. Electrostatic potential data indicate a role of Mg^{2+} and Arg_{259} in the active site facilitating the process by reducing the negative charge distributed over the oxygen atoms of the ATP α-phosphate in a way mimicking that of the two Mg^{2+} cations from the catalytic site of other class II aaRSs (250). For *B. stearothermophilus* TrpRS, a conformational transition state accompanies tryptophan activation (327). Moreover, linking new TrpRS crystal structures (with bound AMP, PP_i, and/or tryptophan) and molecular simulations techniques (i) gave support to the existence of a transiently covalently linked adenosine for stabilizing closed TrpRS conformations, (ii) showed high affinity of the KMSKS loop for the β-phosphate of ATP, (iii) revealed a conformational free-energy barrier early to the induced-fit phase of the catalytic process (328), and (iv) showed the importance of Mg^{2+} in the catalytic mechanism (329). For *E. coli* MetRS, recent structural and functional studies (high-resolution structures at 1.6-Å resolution combined with an advanced mutagenesis analysis) elucidated the mechanism of amino acid selectivity by this aaRS. Interestingly, this selectivity switches from an induced fit in the native protein to a lock-and-key mechanism in a MetRS with altered amino acid specificity (167).

Amino acid attachment on tRNA

Once tRNA is bound to its cognate aaRS and A_{76} is properly located, the second step of the aminoacylation reaction can proceed. The 2′- or 3′-hydroxyl of the terminal tRNA ribose donates its proton to the phosphate of the adenylate, thereby forming a second transition state. Then, one of the two free-oxygen atoms of the α-phosphate attracts the proton from the attacking OH of the terminal ribose, forming a cyclic intermediate that converts into the ester linkage between the amino acid and the tRNA ribose. This general mechanism was proposed for *E. coli* GlnRS and *S. cerevisiae* AspRS, the two archetypes of class I and class II enzymes (18, 149, 242). As usual for catalysts, the main function of the aaRS is to correctly orient the substrates and to stabilize the intermediate transition states. Juxtaposition of the reactive groups of the substrates triggers the reaction, and the structure of the intermediates promotes by itself its forward progression. The site of amino acid charging on the tRNA seems to be the only difference between the two classes (65, 66, 67, 330). This difference is explained in part by the mode of tRNA entry into the catalytic site that dictates proper positioning of the acceptor OH. The significance of the conservation of this 2′ or 3′ specificity through evolution remains obscure, since a rapid isomerization of the amino acid occurs between the 2′- and 3′-hydroxyls after release from the

enzyme (331). For acylations on 2′-OH, an isomerization is required to produce the 3′ species used for protein synthesis. This step could be assisted *in vivo* by elongation factor EF-Tu, since this protein was shown to stabilize the orthoester isomerization intermediate (332).

Asymmetric functioning of oligomeric aaRSs

Half-of-sites reactivity and asymmetric functioning were proposed for dimeric class Ic aaRSs. A large number of mechanistic studies on *B. stearothermophilus* TyrRS demonstrated that this protein acts as an asymmetric dimer (only one site in action) in charging tRNA (333). Likewise, structural studies on *B. stearothermophilus* TrpRS under different conformational states (i.e., after ligand binding) led to similar conclusions and showed open complexes of the enzyme interacting with tryptophan and ATP that are asymmetric in the manner observed in *apo*-TrpRS (199).

Functional and structural asymmetry is also documented for class II aaRSs, notably dimeric bacterial AspRS, HisRS, LysRS, and ThrRS, and tetrameric GlyRS. For *E. coli* AspRS, the tRNA-dependent structural asymmetry of the dimer is enhanced when heterologous yeast $tRNA^{Asp}$ is bound (291) and correlates with the functional asymmetry found in the yeast AspRS, revealed by the existence of two different affinity constants and K_m-values for ATP (334). For HisRS and ThrRS from *E. coli*, amino acid activation (as well as misactivation of serine by ThrRS) occurs at different rates in the two active sites when tRNA is absent (335, 336), but half-of-sites reactivity is not observed in HisRS (334). To clarify the basis of this asymmetry, fluorescence resonance energy transfer experiments using differential labeling of the two HisRS monomers were undertaken. They allowed measurement of similar adenylate formation rates, but they were asymmetric with respect to the two active sites of the dimer. Furthermore, these experiments revealed rigid-body rotation of the HisRS α-helical insertion domain, suggesting that conformational changes are rate limiting for product formation. This "alternating site" model for HisRS catalysis may be common to other class II aaRSs (216). Likewise, asymmetry for nucleotide binding was predicted in the LysU isoform of *E. coli* LysRS on the basis of molecular dynamics simulations (338). Enzymology of GlyRSs dates back to the 1960s and suggested soon an asymmetric functioning of these heterotetrameric aaRSs. However, the exact nature of the functional asymmetry (by half-of-sites or flip-flop mechanisms) remains elusive (339).

Role of tRNA and identity determinants in tRNA aminoacylation

Optimal aminoacylation efficiency depends both on tRNA shape and full sets of identity determinants in tRNA, the relative contribution of each being system dependent. Thus, the specificity of serylation depends principally on recognition of the shape of $tRNA^{Ser}$. This shape is peculiar because of the presence of a large variable hairpin region protruding from the canonical L-shape that is contacted by SerRS. Large variable regions occur in all $tRNA^{Ser}$ species (except the mitochondrial ones), as well as in $tRNA^{Leu}$ and bacterial $tRNA^{Tyr}$ species (19), conferring a peculiar tripod-like shape to these tRNAs that can be considered as an identity determinant in serine, leucine, and tyrosine systems. Likewise, most $tRNA^{Cys}$ species present a structural idiosyncrasy because of the noncanonical nature of their N_{15}•N_{48} tertiary pair, and, interestingly, recognition of $tRNA^{Cys}$ by *E. coli* CysRS was shown to be shape dependent (141). This conclusion finds robust support in the crystal structure of the SerRS:$tRNA^{Ser}$ complex from *T. thermophilus* that explicitly shows that recognition of tRNA principally relies on backbone contacts with SerRS and secondarily on sequence-specific interactions (203).

In the other systems where tRNAs have a small variable region, efficient aminoacylation predominantly relies on full identity sets (6, 188). As an example, in the *E. coli* histidine system, anticodon and core regions in $tRNA^{His}$ play critical roles in the initial binding/discrimination between cognate and noncognate tRNAs, whereas acceptor stem residues, particularly at identity position 73, influence the reaction after tRNA binding (340). Rapid kinetics using $tRNA^{His}$ and HisRS mutants more precisely defined the functional role of the identity elements. Thus, mutations at identity positions in histidine anticodon preferentially affect the thermodynamics of initial complex formation; in contrast, mutations in the acceptor stem of $tRNA^{His}$ or the conserved signature motif 2 in HisRS impose a specific kinetic block on aminoacyl transfer and decrease tRNA-mediated kinetic control of amino acid activation (334). As a second example, in the *E. coli* tryptophan system, stopped-flow fluorimetric investigations indicate that the identity determinant A_{73} of $tRNA^{Trp}$ contributes to stabilization of the transition state during tryptophanyl-$tRNA^{Trp}$ synthesis (341). In general, however, the functional relationship between identity determinants is poorly understood. For instance, the structural basis underlying the cooperative, additive, or anticooperative relationships of identity determinants

in the aspartate system remains to be explicitly elucidated (342). Thus, pairs of determinants located far apart in the 3D structure of $tRNA^{Asp}$ behave cooperatively, while those clustered in the GUC anticodon act additively or anticooperatively. It is noteworthy that the strong anticooperative effect of the triple anticodon mutant CAU (with a global effect that is less severe than the sum of individual effects) can be explained by the loss of all tRNA contacts with the ACB domain of AspRS, making this mutant a mimic of an aspartate minihelix marked by the sole G_{73} determinant (316). A structural interpretation of additive or cooperative effects is less straightforward and implies transfer of chemical information from the anticodon region to the catalytic site. Only few such studies have been undertaken (see, e.g., another example in the glutamine system [343]). It can be anticipated that such long-range effects, such as those occurring between distant regions in the aaRS structure, are widespread (see below).

A last point of practical importance concerns incomplete charging (e.g., tRNA aminoacylation plateaus less than 100% that can be as low as ~1%), frequently observed with noncognate tRNAs (occurs also with cognate and mutant tRNAs, as well as in reactions catalyzed by mutant aaRSs). Incomplete tRNA charging is aaRS dependent, and this phenomenon remains puzzling for many researchers. In fact, it reflects the equilibrium between forward acylation and reverse deacylation and is linked to the fragility of the ester bond the amino acids make with the terminal ribose of tRNA (344). Thus, incomplete charging does not necessarily mean the presence of inactive tRNA molecules but mainly reflects the functioning of charging reactions with low acylation and high aminoacyl-tRNA deacylation rates.

Exit of charged tRNA from aaRSs

Early steady-state kinetic investigations that determined the rate-determining step for tRNA aminoacylation gave a phenomenological view on the exit of charged tRNA from aaRSs. No common picture emerged, and dissociation of charged tRNA from the enzyme is not always rate limiting as could be expected (345). The problem is still under debate, and it is not excluded that rate-limiting steps are modulated by experimental conditions. Nevertheless, more recent data on GluRS and GlnRS resulting from global approaches combining crystallographic, physicochemical, and functional analyses rejuvenated the question and brought structural understanding. Thus, for *E. coli* GlnRS, exit of charged tRNA was actually found to be rate limiting according to pre-steady-state kinetics that revealed a rapid burst of product formation followed by a slower linear increase of glutaminyl-$tRNA^{Gln}$ formation. In addition, the data conclusively demonstrated the existence of long-distance pathways of communication through the GlnRS:$tRNA^{Gln}$ complex (i.e., by allosteric phenomena; see below). In support of this assumption, mutation of U_{35} in the $tRNA^{Gln}$ anticodon loop decreases the aminoacylation rate and weakens glutamine-binding affinity, indicating that the active-site configuration depends on enzyme-tRNA contacts ~40 Å apart (343). For *T. thermophilus* GluRS, a computational study examined factors affecting release of charged $tRNA^{Glu}$ (protonation states of amino acids and substrates present in the active site and the presence and absence of AMP and EF-Tu) that gave a more intricate picture of the exit mechanism of charged tRNA. Thus, distinct nonequilibrium posttransfer states were identified, and the undocking of AMP or charged tRNA was shown to proceed along thermodynamically competitive pathways. Release of the tRNA acceptor stem appeared to be further accelerated by the deprotonation of the α-ammonium group on the charging amino acid. It is noteworthy that the addition of EF-Tu to the aaRS:tRNA complex stimulated the dissociation of the tRNA core and the tRNA acceptor stem (346).

Structural and functional plasticity in aaRS:ligand systems—motions and allosteric phenomena

Disorders in early crystallographic structures of aaRSs (196) and mechanistic studies on the amino acid activation step (reviewed e.g., in references 4, 12, and 13) indicated enzyme flexibility and induced-fit mechanisms during catalysis. On the other hand, one can conjecture that aaRSs are allosteric enzymes since they are contacted outside their catalytic site by identity determinants of tRNA during the aminoacylation process (342, 347). This fact implies conformational rearrangements that would occur upon tRNA binding, as documented for classical allosteric enzymes when interacting with their allosteric effectors (see, e.g., reference 348 for a review). In the early phenomenological picture of the functioning of aaRS:tRNA systems, it was suggested that tRNA binding takes place through discrete kinetic-dependent steps involving scattered recognition sites, with best mutual adaptation of the two interactants in the cognate complexes (5). From the standpoint of allostery (and induced-fit phenomena), tRNA-triggered conformational changes in

active aaRSs, as well, were soon suggested (5). However, despite intensive work, the allosteric effector role of aaRS ligands during the aminoacylation process remained elusive for years and it is only in the last decade that advanced physico-chemical, mutagenesis, and computational approaches brought new insight into the fine mechanistic understanding of these enzymes.

Structural plasticity in aaRSs and in their macromolecular tRNA ligands, and long-range domain-domain communication in their structures (263, 264, 349), are functional necessities to ensure the specificity of tRNA aminoacylation and other aaRS functions. Indeed, functional aaRS complexes must process the chemical information brought by the interaction of the small (amino acid, ATP, aminoacyl-adenylate, PP_i, and Mg^{2+}) and macromolecular (tRNA) substrates. Contacts with substrates necessarily occur in the catalytic site and also in remote domains, mainly in ACB domains located ~50 to 70 Å apart from the catalytic site and in editing domains (see "Error correction," below). These contacts are accompanied by induced fit/allosteric phenomena. On the other hand, the fact that aaRSs are multidomain proteins implies the existence of communication between domains and of coupled domain motions. In other words, functional aaRS:tRNA complexes can be considered as "signal transduction" systems in which specific conformational changes occur, which can be subtle or dramatic (264, 350, 351). A few examples taken from bacterial systems illustrate the point.

Subtle conformational changes in aaRSs essential for tRNA aminoacylation are well documented in the aspartate system. Thus, the mutual adaptability of *E. coli* AspRS with its substrates is accompanied by flexibility in the N-terminal ACB domain and great mobility of the so-called flipping loop (that controls the proper positioning of aspartate) not seen in the crystal structure when tRNA is absent (165). Interestingly, all regions of AspRS that contact $tRNA^{Asp}$ show local flexibility in the *apo*-enzyme, which suggests that analyzing the flexibility of *apo*-aaRSs may be informative about tRNA binding (165). Other examples, based on functional and molecular dynamics methods, come from *E. coli* and *M. smegmatis* MetRSs, *E. coli* ThrRS, and *B. stearothermophilus* TrpRS. For the MetRSs, flexibility of the amino acid-binding pocket is required to accommodate methionine (121) and adenosine (158). Moreover, domain-domain communication and motion (352) as well as rigid-body rotation of the catalytic and ACB domains occur upon tRNA binding (347) in the *E coli* MetRS. For *E. coli* ThrRS, a study of the active-site dynamics during the aminoacyl transfer step, when threonyl-adenylate and $tRNA^{Thr}$ are bound, explains the catalytic mechanism in which a histidine residue plays a key role (353). For *B. stearothermophilus* TrpRS, domain motion closes and twists the ACB and catalytic domains of the aaRS, with the ACB domain moving as a rigid-body with both catalytic HIGH and KMSKS class I signatures (with HIGH replaced by TIGN in *B. stearothermophilus* TrpRS) to deliver ATP to the tryptophan carboxyl group in the active site within the Rossmann fold (328) (see "Overview of the aaRS world," above, for the meaning of the features defining class I aaRSs). In this process, ATP acts as an allosteric effector for TrpRS (199). Selection of the amino acid requires also domain motion in the catalytic domain triggered by a remote structural motif (354). Other allosteric effects are triggered by Mg^{2+} when the metal ion assists catalysis of tryptophan activation (329).

The crucial question, still unsolved, is how chemical signals in aaRS and tRNA (i.e., from identity elements) trigger specific aminoacylation. Phenomenological evidence clearly demonstrates that mutations in anticodon identity elements differentially affect catalytic efficiency of tRNA aminoacylation (342). On the other hand, crystallographic data indicate conformational changes in aaRS:tRNA complexes associated with different functional states of the aaRSs or generated by mutations in the aaRSs. Attempts to understand how signals are transduced from the aaRS anticodon domain to the catalytic site were approached by molecular dynamics simulations on *E. coli* MetRS (352, 355, 356). Four communication pathways between the active site and the ACB domain ~50 Å away were identified in the MetRS body (355). When comparing the intramolecular mobility of native MetRS with that of a variant deficient in aminoacylation after mutating Trp_{461} (a conserved residue in contact with the anticodon of cognate $tRNA^{Met}$), significant differences were found. While native MetRS shows mobility in all motifs important for catalysis and correlated motions between distant residues (e.g., residues from the active site or the Zn-binding motif and residues from the ACB domain), mobility and correlated motions decrease significantly but not uniformly in the aminoacylation-deficient variant (352). Other simulations on the bacterial GluRS:$tRNA^{Glu}$ complex from *T. thermophilus* found intricate dynamical communication networks between identity elements of $tRNA^{Glu}$ and residues within the GluRS catalytic sites, with differences between pretransfer and

posttransfer networks (357). The general conclusion is that residues within both GluRS and $tRNA^{Glu}$ are essential for information transduction. Unexpectedly, the allosteric networks in GluRS display considerable similarities with those in an archaeal LeuRS:$tRNA^{Leu}$ complex (357) despite the remarkably different interactions GluRS (***1n78***) and LeuRS (***2v0c***) make with their cognate tRNAs (358, 359). In the case of the *E. coli* GlnRS:$tRNA^{Gln}$ complex, pre-steady-state kinetics on GlnRS variants (with contact points with tRNA mutated) were used to discover allosteric signaling pathways in the aaRS body that would regulate glutamine binding and glutaminyl-tRNA formation. Interestingly, data suggest long-range signal propagation from the tRNA anticodon to the catalytic site and reveal protein contacts that weaken glutamine-binding affinity across distances up to 40 Å (360).

Coupled motions also occur between catalytic and editing domains of bacterial aaRSs. For instance, normal mode analyses in *T. thermophilus* LeuRS suggest that sparsely distributed amino acid clusters are critical for long-range mechanochemical motions in this enzyme (361). In bacterial-like *E. coli* ProRS, coupled dynamics occur between the INS region in the editing domain and protein segments containing the catalytically important proline-binding loop. Interestingly, it was suggested that multiple pathways are possible between the editing and catalytic domains and that the amino acids engaged in the motions are evolutionarily conserved and/or have co-evolved (362).

On the other hand, in the context of the multidomain architecture of aaRSs, it is worth mentioning the extreme case of structural mobility occurring in the GlnRS from *D. radiodurans* (***2hz7***). Indeed, this large aaRS (852 amino acids) contains a well-structured C-terminal extension of 215 residues (a paralog of Yqey proteins, the freestanding proteins of elusive function consisting of two different α-helical bundles) appending the ACB domain not seen at all in the 2.3-Å crystal structure of this GlnRS because of mobility in the crystal lattice (363, 364). The function of the Yqey appendix likely is to enhance the stability of the complex with tRNA by contacts in *cis* (363, 364). It is noteworthy that Yqey constitutes also the C-terminal domain of GatB, a subunit of the trimeric aminoacyl-tRNA amidotransferases, where it contacts the D-loop of misacylated tRNA. Because of the structural similarity with GatB (see "Alternative functions of bacterial aminoacyl-tRNA synthetases," below), it can be suggested that Yqey proteins participate in amidation pathways.

In summary, the allosteric phenomena in the aaRS field that for a long time were based on speculation have received robust experimental support in the past decade. The existence of molecular communication pathways in aaRSs and aaRS:tRNA complexes is presently demonstrated for ~15 aaRS specificities (reviewed in reference 351). Today the large body of results demonstrating the plasticity of aaRS structures and the structural mobility of their constitutive domains, together with thermodynamics informing about cooperativity and anticooperativity in aaRS reactions, provide the experimental background for further efforts to understand the physical basis of signal communication, in particular, between identity determinants in tRNA or aaRS and residues from the aaRS catalytic sites. This needs accurate analyses of both the local and extended networks of amino acids participating in allostery, as investigated e.g., for TrpRSs (365, 366). However, despite the recent breakthroughs, many open questions remain. For instance, can the conclusions claiming alternative communication networks, as seen e.g., in *E. coli* ProRS (362), be generalized to the ensemble of aaRSs or are system-specific idiosyncrasies possible? Likewise, the idea of evolutionary conserved amino acids in allosteric communication pathways needs clarification. Also better correlations between thermal fluctuations, as reflected by the crystallographic *B*-factors, and functional dynamics are needed (367). For that, more high resolution structures of aaRSs under different functional states are awaited, as well as more enzymologic investigations combined with enhanced computational methods for monitoring the mechanochemistry of these enzymes.

Error Correction

Amino acid misactivation and tRNA mischarging

Fidelity of translation results from the accuracy of three processes involving tRNA, namely, (i) tRNA aminoacylation (7), (ii) selection of aminoacylated tRNAs by initiation (368) and elongation (369) factors, and (iii) decoding of the genetic message on mRNA by tRNA anticodons (370). It has been estimated that the summed error frequency of these processes does not exceed 1/3,000 *in vivo* (371). Faithful tRNA aminoacylation depends primarily on the successful discrimination between cognate and noncognate amino acids and tRNA substrates. Inaccuracy in amino acid selection (10^{-4} to 10^{-5}) is more frequent than tRNA selection (10^{-6}) because of the larger surface area of the tRNA molecules and the resulting greater structural diversity of the contact

regions. Half of the bacterial aaRSs (class Ia LeuRS, IleRS, ValRS, and MetRS and class II SerRS, ThrRS, ProRS, LysRS, AlaRS, and PheRS) misactivate noncognate amino acids that are similar in shape to their cognate substrates (Table 5), and many aaRSs catalyze tRNA mischarging (as a result of noncognate tRNA recognition followed by the attachment of the cognate amino acid) (37). Also worth emphasizing (and often overlooked) is the nonabsolute discrimination between L- and D-amino acids by aaRSs as first observed ~40 years ago in the case of *E. coli* and *B. subtilis* TyrRSs (372). Today, significant D-aminoacylation of tRNA has been characterized for *E. coli* AspRS, HisRS, LysRS, TrpRS, and TyrRS and was suggested for a few others (373, 374, 375, 376).

Loose specificity was certainly advantageous in primitive aaRSs when chemical evolution established life on Earth because it generated diversity in an emerging protein world. It still remains a biological necessity in modern ND-aaRSs that catalyze tRNA mischarging in organisms lacking GlnRS or AsnRS (72). Unspecific tRNA aminoacylation became toxic when the genetic code and the translation machinery were fixed because high levels of mischarged tRNA would lead to coding ambiguity with codons translated by statistical distributions of amino acids rather than by the specific amino acid. Thus, aaRSs became more accurate and some, stepwise, acquired proofreading/editing capability to correct activation and aminoacylation errors. The implication in modern life is that defective corrections would produce cellular disorders. This conjecture was verified with *E. coli* cells harboring editing-defective aaRSs that show perturbed viability (381, 382) and, in the case of mammalian cells, editing-defective aaRSs (AlaRS, GlyRS, ValRS) that produce diverse cellular disorders, including human neurodegenerative pathologies (383, 384, 385).

A recurrent question concerns the functional specificity and distinction of editing-defective aaRSs, since some of them catalyze amino acid misactivation (e.g., in plant ArgRSs [386]) and/or tRNA mischarging (e.g., in ArgRS, GluRS, GlnRS of different origins [37]), while others achieve high amino acid specificity (e.g., in *E. coli* CysRS [387]). The point is of interest for CysRS and ArgRS that both belong to the same structure-based group of aaRSs (class Ia) as the editing IleRS, ValRS, LeuRS, and MetRS. It concerns also class Ib and class IIb aaRSs that recognize structurally related amino acids (note that ND-GluRSs and ND-GlnRSs are mischarging enzymes). For these aaRSs, three mechanisms were shaped by

Table 5 Error correction by bacterial editing aminoacyl-tRNA synthetases[a]

aaRS	Amino acids misactivated[b]	Editing domain[c]	Pretransfer editing	Posttransfer editing	*trans* editing
Class I					
IleRS	V, *T*, *C*	CP1	+[d]	+	No
ValRS	I, T, *C*, *S*, *A*	CP1	+[d]	+	No
LeuRS	I, M, V, D, N	CP1	+	+	No
MetRS	Only nonstandard[e]	Aminoacylation site	+[d]	?	No
Class II					
SerRS	T, C	No separate editing domain	+	–	No
ThrRS	S, V	N2 subdomain or paralog	–[f]	+	+
ProRS	A, C	INS insertion or paralog	+	+	+
LysRS	*M*, *L*, *C*, *A*, *T*	Aminoacylation site	+	–	No
AlaRS	G, S, *C*	C-terminal domain or paralog	+[d,g]	+	+
PheRS	Y, I, L, M	B3/B4 subdomain	+	+	No

[a]Data are retrieved from references 38, 248, 325, and 377.

[b]Only standard amino acids are given; for amino acids in italics, the relative rate of activation is extremely low (38, 377).

[c]Only mischarging with misactivated amino acids is given – note that most of the editing aaRSs also can misrecognize tRNA and catalyze mischarging with their cognate amino acid (mostly under particular conditions, either *in vitro* and *in vivo*, e.g., reference 37).

[d]On the basis of increased rates of the ATP–PP_i exchange reaction.

[e]Homocysteine, norleucine, ethionine, selenomethionine (activated selenomethionine is transferred to $tRNA^{Met}$ and not edited, a useful property for crystallography since this allows production of selenomethionine-labeled proteins used for phasing by the Multiwavelength Anomalous Dispersion, or MAD, method [see references 378 and 379]).

[f]A Zn^{2+} ion prevents misactivation of valine (isosteric with threonine) but does not prevent serine activation (see reference 380).

[g]No definitive and direct structural proof.

evolution to achieve the specificity level compatible with fidelity of protein synthesis: (i) kinetic driven specificity with high catalytic discrimination between the correct and the incorrect reactions (this concerns essentially tRNA mischarging [37] but also misactivation followed by tRNA charging of the false amino acid, as observed with plant ArgRSs [386]), (ii) tRNA-induced remodeling of the catalytic site for enhanced amino acid binding (see below), and (iii) tuning a proper balance of tRNA and aaRS concentrations (388). The direct implication is cellular toxicity in the case of perturbed homeostasis due to elevated aaRS concentrations or, in other words, high levels of mischarged tRNAs that produce errors in protein synthesis (often observed when overproducing aaRS genes).

Editing by deacylases

Since certain aaRSs can mischarge their cognate tRNA with D-amino acids (see above), it was anticipated that cells should be equipped with enzymes capable to clear the D-aminoacyl-tRNAs. D-Tyrosyl-tRNA^{Tyr} deacylase is the enzyme capable of fulfilling this task (372). It has wide amino acid specificity, and the deletion of its gene is toxic for *E. coli* as shown by an accumulation of intracellular D-tyrosyl-tRNA (373, 375). Distribution of this dimeric tRNA-dependent deacylase is universal in *Bacteria* and *Eukarya* (376). (*B. subtilis* is an exception and lacks a gene encoding a functional D-aminoacyl-tRNA deacylase; see "Regulation in aminoacyl-tRNA synthetase gene expression in *E. coli*," below, for details). The crystal structure of the *E. coli* D-tyrosyl-tRNA^{Tyr} deacylase (***1jke***) reveals a β-barrel architecture, assembled together with its two subunits, that is closed on one side by a β-sheet lid (373). The ortholog from *Haemophilus influenzae* (***1j7g***) shows the same structural organization (389). The active site, at the interface of the two subunits, accounts for the broad editing specificity that likely occurs by a conserved mechanism in D-tyrosyl-tRNA^{Tyr} deacylases (390). It is noteworthy that a D-aminoacyl-tRNA-like domain of a structure similar to the *E. coli* deacylase was added in the course of evolution to most archaeal ThrRSs for posttransfer editing of mischarged seryl-tRNA^{Thr} (118, 373, 391, 392). This domain differs from the freestanding editing domain in *Crenarchaea*, such as *S. solfataricus*, that hydrolyzes seryl-tRNA^{Thr} (116).

Editing by aaRSs

General concepts: To ensure the viability of organisms, cellular aaRS expression is generally regulated by growth rates to keep levels of tRNA misrecognition and hence mischarging low (393) (see "Regulation of aminoacyl-tRNA synthetase gene expression in *E. coli*," below for details). Correction mechanisms that clear mistakes before mischarged L-amino acids would be misincorporated into proteins therefore enhance accuracy of translation. First indications on corrections mediated by IleRS, ValRS, or PheRS came from observations on aaRS-dependent deacylation of correctly or incorrectly charged tRNAs (344, 394, 395, 396) and led to the proposal of "kinetic proofreading" (174) and the generalization of "chemical proofreading" in the prevention of tRNA mischarging (397). Altogether, 10 editing aaRSs act in *Bacteria* (Table 5).

A realistic "double-sieve" model rationalized the phenomenology of editing (398). This is a "steric-exclusion" mechanism that received strong structural support with the discovery of a separate editing site in many aaRSs distinct from the catalytic site ensuring tRNA aminoacylation. Thus, a "coarse sieve" corresponds to the catalytic/synthetic site and would reject the amino acids that are larger than the cognate one but would bind smaller and isosteric amino acids. A second "fine sieve" corresponds to a distinct editing site that would hydrolyze the noncognate amino acids that were misactivated (pretransfer editing) or mischarged on tRNA (posttransfer editing). This scheme was explicitly visualized by crystal structures of class I *T. thermophilus* IleRS (***1ile***) in complex with L-isoleucine or L-valine (399) and of *S. aureus* IleRS in complex with tRNA^{Ile} and mupirocin (an analog of isoleucyl-adenylate; see "Inhibition and engineering of bacterial aminoacyl-tRNA synthetases," below, for details) (***1qu2***) (138) in conjunction with functional data on *E. coli* IleRS (400, 401). As a result, the misactivated adenylate or the mischarged acceptor strand of tRNA bound to editing IleRS via its anticodon stem-loop have to shuttle between the catalytic/synthetic and editing sites ~30 Å apart. This scheme is also valid for ValRS and LeuRS. The situation is more intricate for MetRS and class II editing aaRSs in which the "double-sieve" model does not apply *stricto sensu* because amino acids larger than or dissimilar to the correct ones can be misactivated, transferred to tRNA, and cleaved. Although posttransfer editing domains distinct from the synthetic domains have been characterized in most class II editing aaRSs, editing by these enzymes proceeds by different mechanisms and can even be catalyzed in *trans* by freestanding editing proteins. This was first described for YbaK, a homolog to the editing domain of *H. influenzae* ProRS (299) followed by AlaX homologous to that of archaeal

AlaRSs (402) and led to the proposal of a "triple-sieve" mechanism with the third sieve being the separate protein (403). The present understanding of the diverse aaRS-mediated editing types (Table 5) is based on extensive biochemical, genetic, and structural data that are covered in several reviews (248, 325, 404, 405, 406). The prominent and newest facts are summarized below.

Pretransfer editing: This editing mode is difficult to demonstrate because of the connected mechanistic features not easy to separate by experiment, such as the inherent lability of aminoacyl-adenylates, tRNA dependence, and occurrence of this editing possibility in posttransfer editing background (e.g., with GlnRS [407], IleRS [401, 408, 409, 410], LeuRS [297, 411], and ValRS [410, 412]). For *E. coli* LeuRS with posttransfer editing capability, activity of variants with either point mutations in CP1 or entire CP1 resected suggests a pretransfer translocation step that moves misactivated adenylates from the activation site to that for editing (413) and supports the conclusion that a latent pretransfer editing mechanism is activated upon deletion of CP1 (the large connective peptide inserted in the catalytic domain of LeuRS) (414). In *A. aeolicus* LeuRS pretransfer editing is favored (411), while in an editing-defective enzyme mutated in CP1 a tRNA-independent editing activity likely occurs in the synthetic site (415). This mechanism is exclusively used in *Mycoplasma* pathogens whose LeuRSs are missing or contain a degenerate CP1 domain (416). For *E. coli* ValRS, explicit evidence of pretransfer editing is lacking, although mutational analysis revealed a direct relationship between the ability of a tRNA to be valylated and its ability to stimulate editing activity (412). In support to pretransfer editing, molecular dynamics simulations indicate that noncognate threonyl-AMP and threonyl-A_{76} substrates bind more strongly than cognate valyl-AMP and valyl-A_{76} in both pre- and posttransfer editing sites of *T. thermophilus* ValRS and that mutations in the CP1 domain decrease the binding ability of the pretransfer threonyl-AMP substrate (417). For *E. coli* IleRS, novel kinetic approaches show a tRNA-dependent hydrolysis of valyl-adenylate that is largely insensitive to mutations in the CP1 editing domain and a pretransfer editing in IleRS likely residing in its catalytic/synthetic site, suggesting a kinetically controlled partitioning of the noncognate aminoacyl-adenylate between the editing and synthetic sites (418). This result indicates that both pre- and posttransfer editing are important in IleRS, in contrast to ValRS and LeuRS, where editing occurs nearly exclusively by posttransfer hydrolysis in the editing domain (418, 419). Finally, computational methods applied to *A. aeolicus* MetRS, strongly suggest existence of substrate-assisted pretransfer editing of homocysteine and homoserine in which the carboxylate from an aspartate residue (Asp^{259}) in the active site acts as a base in the hydrolytic mechanism (420). This mechanism may occur in IleRS, LeuRS, and ValRS since similarly located aspartate or glutamate residues are found in the synthetic site of these aaRSs (420).

Pretransfer editing in CP1-containing aaRSs (IleRS, LeuRS, MetRS, ValRS) probably is ancient and would have been present in primitive aaRSs before addition of the CP1 domain. In modern aaRSs, however, it is not the major route and, as seen in *A. aeolicus* and *E. coli* LeuRS, represents only 5%, the remaining 95% being CP1-dependent posttransfer editing (415, 419). Importantly, this conclusion concerns also norvaline, a nonstandard amino acid naturally found in *Bacteria* that is efficiently misactivated by *A. aeolicus* LeuRS and edited in the posttransfer pathway (415).

The case of *E. coli* GlnRS, a nonediting class I aaRS, deserves a special comment since this enzyme hydrolyzes glutaminyl-adenylate by a tRNA-dependent mechanism. This hydrolysis is analogous to pretransfer editing of noncognate aminoacyl-adenylates by editing aaRSs such as IleRS. Because GlnRS does not possess a spatially separate editing domain, this absence shows that a pretransfer editing-like reaction can occur within a class I catalytic site (407).

Pretransfer editing is also found in class II aaRSs. Thus *E. coli* ProRS misactivates alanine and hydrolyzes noncognate alanyl-adenylate before alanine transfer to $tRNA^{Pro}$ (421), likely within the prolylation active site (422). LysRS as well uses pretransfer editing with methionine, leucine, cysteine, alanine, threonine, and a few nonstandard amino acids (377). The situation in ThrRS is peculiar since valine, isosteric with threonine, is expected to be misactivated but is not. This is explained by the presence of a Zn^{2+} ion in the active site that participates in threonine recognition and prevents binding of the methyl group of valine (380). Thus, the Zn^{2+} ion acts as the "first sieve" against valine. ThrRS, however, activates serine, but there is no evidence of pretransfer editing of seryl-adenylate (423).

The editing abilities of MetRS and class II LysRS are unique for different reasons. MetRS misactivates only nonstandard amino acids, preferentially homocysteine,

the cellular precursor of methionine, but also unnatural norleucine, selenomethionine, and even telluromethionine that can be incorporated in proteins under nonphysiological conditions (38, 424). Editing of homocysteinyl-adenylate involves a cyclized homocysteine thiolactone intermediate and occurs within the MetRS synthetic site that appears partitioned for aminoacylation and editing activities. The case of LysRS is a priori intriguing since, besides efficiently misactivating ornithine and a series of standard amino acids (although less efficiently), it mischarges $tRNA^{Lys}$ with these noncognate amino acids (377). Ornithine is readily edited via a cyclization pathway, so that the charging extent of $tRNA^{Lys}$ is less than 1%. Mischarging with standard amino acids can reach plateau levels of 90% (e.g., for arginyl-$tRNA^{Lys}$) and is quasi-abolished when aminoacylations are conducted in the presence of lysine (377) and thus is tolerated for cellular life.

Finally, the pretransfer editing status of PheRS remains puzzling since it has to select between phenylalanine and larger tyrosine by a mechanism differing from the "double-sieve" model. Explicit evidence for tyrosine discrimination came from fast kinetic data obtained with yeast PheRS (425) and was extended to *E. coli* PheRS (426). The observation of PheRS-dependent tyrosyl-adenylate pyrophosphorolysis (425) suggests a kinetically driven process that could occur within the catalytic site, an interpretation consistent with the plasticity of the catalytic site seen in crystal structures of *T. thermophilus* PheRS in complex with substrates larger than phenylalanine (***2akw***, ***2aly***) (233). As for LeuRS (see below), this editing activity likely is a remnant of the early evolution of PheRS that acquired later posttransfer editing capacity.

Posttransfer editing: Occurrence of posttransfer editing is documented for seven aaRSs (class I IleRS, LeuRS, ValRS and class II AlaRS, PheRS, ProRS, ThrRS) and relies on specialized editing sites different from the synthetic aminoacylation sites. This necessitates motion of tRNA on the aaRSs. In class I enzymes, the flexible 3′-end of the misacylated tRNA is translocated from the aminoacylation site to the hydrolytic site on their appended CP1 domains, implying that tRNA interactions are partially distinct in the two catalytic steps, as illustrated by a tRNA-dependent translocation of ~25 Å of misactivated valine on *E. coli* IleRS (409). This translocation of the tRNA amino acid accepting end is accompanied by a clear segregation of nucleotide determinants for the editing and aminoacylation functions of tRNA. In class II aaRSs, the situation is more intricate since different types of editing domains, and hence different editing mechanisms, have been identified and that editing can be achieved in *trans* by helper proteins.

In closely related IleRS and LeuRS, posttransfer editing likely occurs by a similar mechanism. This conclusion is supported by the presence in their homologous CP1 editing domains of a conserved threonine-rich peptide and of a second conserved region separated by ~100 amino acids that make up each part of the editing site in both *E. coli* enzymes (427). However, some idiosyncratic positions in LeuRS and/or IleRS account for specific substrate recognition (427, 428). Crystallography and computational analysis suggest that during the editing pathway the CP1 domain rotates via its flexible β-strand linker relative to the main aaRS body with the end of the N-terminal β-strand acting as a hinge. From the viewpoint of physiology, the vital importance of editing is illustrated by the toxicity of many amino acids in *E. coli* when LeuRS editing is inactivated (429). From the viewpoint of structure, two complexes of *T. thermophilus* LeuRS with analogs of pre- or posttransfer editing substrate show binding of the pretransfer editing analog in both the catalytic/synthetic and editing active sites (***1obh***), while the binding of the mischarged tRNA posttransfer editing analog occurs solely in the editing site (***1obc***) (430). Moreover, LeuRS structure of an isolated CP1 editing domain of *A. aeolicus* (***3pz6***) (431) and mutagenesis of the C-terminal domain of *M. tuberculosis* LeuRS indicate the importance of these two domains in regulating quality control of leucyl-$tRNA^{Leu}$ synthesis (432). Most interesting, crystal structures of *E. coli* LeuRS in the aminoacylation (***4arc***) and editing conformation (***4aq7***) show correlated rotations of four flexibly linked LeuRS domains and the unexpected role of the editing domain that stabilizes tRNA during aminoacylation (433). Besides the conserved CP1 editing domain, the related IleRS, LeuRS, and ValRS contain, in addition, a bipartite CP2 domain that is spatially close to the acceptor stem of tRNA (137, 138, 139) and thus could play a role in editing. Studies with engineered LeuRS molecules bring support to this expectation and show that CP2 indeed is needed for posttransfer editing and leucine activation (434). In addition, CP2 helps correct orientation of the mischarged tRNAs into the CP1 editing site since deletion mutants missing CP2 are editing defective (434).

Note the unusual editing properties of deep-rooted *A. aeolicus* LeuRS that is capable of editing the complete set of mischarged tRNAs that can be generated by LeuRS,

IleRS, and ValRS (307). Interestingly, this conclusion comes from studies on the editing of charged or mischarged RNA minihelices with triple leucine, isoleucine, and valine identity, suggesting that the editing domain of *A. aeolicus* LeuRS preserved properties of an ancestral editing domain (307).

Posttransfer editing used by class II aaRSs follows different routes and appears fundamentally different from the editing of class I enzymes. In *E. coli* ProRS, it is the 180-amino-acid-long INS (INSertion) domain located between class II signature motif 2 and 3 that catalyzes deacylation of mischarged alanyl- or cysteinyl-tRNA^{Pro} (435), with the single Cys_{443} residue within motif 3 (dispensable for ProRS aminoacylation activity) critical for the hydrolytic editing of alanyl-tRNA^{Pro} (421). It is noteworthy that the INS domain can act as an independent protein (299) and finds paralogs in nature (403). Posttransfer editing by ThrRS and AlaRS deserves particular attention because of intriguing similarities, but also peculiarities, that have their origin in the different evolutionary histories of the two aaRSs. Thus, both bacterial enzymes have structurally related editing sites on which the overall catalytic domain is fused C-terminally in ThrRS and N-terminally in AlaRS (401). More precisely, in *E. coli* ThrRS, posttransfer editing is associated with a N-terminal domain and is independent from aminoacylation since a resected variant missing this domain can misacylate tRNA^{Thr} with serine, but is unable to hydrolyze seryl-tRNA^{Thr}. The editing site is located in a cleft of subdomain N2 and is triggered by conserved amino acids (Asp_{180}, His_{73}, His_{77}) in the bacterial, yeast, and human ThrRSs (423). This editing domain found in *Bacteria* and *Eukarya* is absent in *Archaea*, in contrast to the similar AlaRS editing domain present in the three branches of the tree of life (207).

The case of AlaRS is paradoxical since glycine and serine, sterically smaller and larger than alanine, are both misactivated. Editing of glycyl-tRNA^{Ala} is in line with the idea that the aaRS sieves out amino acids that are smaller than alanine, but the editing of seryl-tRNA^{Ala} does not fit this scheme. The crystal structures of an active fragment of *A. aeolicus* AlaRS in complex, separately, with Mg^{2+}/ATP, alanine, glycine, or serine shed light on the paradox: while alanine and glycine are bound in similar orientations in a pocket where they are stabilized by salt bridges and H-bonding with class II conserved Asn_{194}, serine in contrast forces pocket expansion and has its binding reinforced by that of Mg^{2+}/ATP in the active site and by coordination with Asn_{194}, thereby breaking the sieve for serine exclusion (134). On the other hand, serine exclusion could involve participation of the AlaXp protein (a *trans*-editing protein homologous to the editing domain of AlaRS) widely represented in *Bacteria* (402). Importantly, the small C-terminal C-Ala domain universally tethered to the editing domain promotes cooperative binding of both AlaRS aminoacylation and editing domains to tRNA^{Ala} (436).

Biological necessity of posttransfer editing of tyrosyl-tRNA^{Phe} is well illustrated by tyrosine incorporation into proteins at phenylalanine positions when an editing-defective PheRS is overproduced *in vivo* (437). Its mechanism in *Bacteria* is partly deciphered based on structural and functional results (233, 438, 439, 440). Thus, the posttransfer editing site is localized within the large β-subunit of PheRS at the B3/B4 interface (B3 and B4 are two of the eight subdomains constituting the β-subunit) (439) ~35 Å apart from the catalytic site on the small α-subunit. Importantly, binding of tyrosine in the editing site is seen by crystallography (***2amc***) (233). Moreover, the activity of deletion mutants indicates that the adjacent B2 subdomain on the β-subunit, similar to an EMAP-fold with tRNA-binding capacity in other aaRSs, acts as a secondary tRNA-binding site that could contribute to editing by promoting the translocation of mischarged tRNA to the editing site (440). Note that the EMAP-fold, also present in MetRSs and a few eukaryal aaRSs, is a distinct version of the OB-fold. Its acronym derived from "Endothelial Monocyte Activating Polypeptide" reminds that the fold was discovered in a cytokine bearing no structural resemblance with other cytokines but with aaRSs, thus accounting for the cytokine activity of fragments of eukaryal aaRSs or of proteins associated with aaRSs such as p43 or Arc1p (10). Finally, a new crystal structure of *T. thermophilus* PheRS complexed with puromycin (***4tva***) and molecular mechanics calculations revealed the architecture of the enzyme liganded with a mimic of the tyrosyl-A_{76} moiety of misacylated tRNA^{Phe} and allowed to propose a universal hydrolytic editing mechanism (441).

Relative contributions of pre- and posttransfer editing: The relative contribution of both editing modes in bacterial aaRSs has been evaluated for class I LeuRS, IleRS, and ValRS and class II ThrRS. It is noteworthy that, in *T. thermophilus* LeuRS, the editing CP1 domain binds the pre- and posttransfer substrates in largely overlapping sites, suggesting a similar mechanism of hydrolysis for

both editing substrates (430). As a result, the contributions of the two editing modes of *E. coli* LeuRS are similar, but, for reasons not yet understood, the posttransfer editing mode is disfavored (29%) by the *A. aeolicus* LeuRS (411). Furthermore, in either *E. coli* or *A. aeolicus* LeuRS, preediting is low and does not exceed 10% of the total editing activity (411). In contrast, tRNA-dependent pretransfer editing reaches 35% in *E. coli* LeuRS and up to 65% in *A. aeolicus* LeuRS and appears to depend on the interaction of the tRNA acceptor end with the CP1 editing domain (411). For *E. coli* IleRS and ValRS, kinetic effects and the terminal A_{76} of tRNA control partitioning of pre- and posttransfer editing (418, 442). For *E. coli* ThrRS, it is the rate of aminoacyl transfer that modulates the relative contribution of pre- and posttransfer editing, and pretransfer hydrolysis of seryl-$tRNA^{Thr}$ contributes to editing only when the rate of transfer is low (332).

Motion and plasticity of editing domains: Dramatic structural changes occur in editing systems and imply movement of ~30 Å of the accepting end of tRNA during translocation of mischarged tRNAs from the aminoacylation site to the hydrolytic editing sites. This translocation is transient and its molecular understanding remains elusive, although it is likely accompanied by conformational changes in the whole body of both RNA and protein. In *E. coli* IleRS it utilizes two amino acids from the hinge region between the core of the enzyme and the separate editing domain (401). In *E. coli* LeuRS it is a peptide within the editing domain (443) and a tRNA-dependent communication during pretransfer editing that adjust the conformational rearrangements at the disordered interface between the synthetic and editing domains (444). As for ThrRS, the bipartite architecture of its subunits (with the C-terminal core comprising the catalytic domain connected by a linker helix of 18 residues to the N-terminal domain with the N2 editing site) makes the whole protein flexible as revealed by different crystal structures of *S. aureus* and *E. coli* ThrRSs. In particular, variations of the relative orientation of the two ThrRS parts could be seen as well as four mobile regions participating in tRNA binding (210). This overall flexibility of ThrRS most likely facilitates the needed shuttling of the mischarged CCA end of $tRNA^{Thr}$ from the catalytic site to the N2 editing site.

Editing polypeptides resected from bacterial aaRSs

The CP1 editing domain (210 to 275 amino acids) from IleRS, LeuRS, and ValRS, and its smaller version in the other class Ia aaRSs, can be resected from the host proteins. Its structural core with a conserved hydrolysis site shows distinct archaeal, bacterial, or eukaryal insertions (***2wfe***) (445). Active CP1 domains were derived from IleRS, LeuRS, and ValRS. The crystal structure of CP1 from *E. coli* LeuRS was solved in three versions, either in *apo*-form (***2ajg***) or in complexes with methionine (***2ajh***) and isoleucine (***2aji***). The structures show a rigid binding pocket formed by conserved amino acids that is best adapted to recognize isoleucine and methionine, but not leucine (446). Likewise, the structure of the fragment from *T. thermophilus* IleRS was solved in *apo*-form (***1udz***) or in complex with valine (***1ue0***) or adenylate analogs mimicking the substrates in pre- and postediting states (***1wnz***, ***1wk8***) (447, 448). Resolution in the 1.6- to 2.0-Å range visualizes the editing site large enough to accommodate valine but not isoleucine. Isolated CP1 domains from *E. coli* IleRS and *B. stearothermophilus* ValRS hydrolyze valyl-$tRNA^{Ile}$ and threonyl-$tRNA^{Val}$, respectively, but not correctly charged tRNAs. In contrast, the smaller CP1 insertions of MetRS and CysRS (100 and 50 amino acids, respectively) have no deacylation activity (310).

For *E. coli* LeuRS, early data suggested an inability of the isolated CP1 domain to catalyze editing (449). However, more recent data obtained with rationally designed *E. coli* CP1 fragments show that the β-strands, which link the domain to the aminoacylation catalytic core, are required for editing activity (450), thus explaining the apparently contradictory results obtained with domains missing these linkers (449). Activity of the freestanding CP1 domain from *A. aeolicus* αβ-LeuRS was straightforwardly found using mischarged isoleucyl-$tRNA^{Leu}$ and isoleucyl-$minihelix^{Leu}$. Interestingly, the short β-stranded peptide (20 amino acids) from this freestanding CP1 domain was shown to confer editing capacity after transplantation into the inactive CP1 domain from *E. coli* LeuRS. Likewise, fusion of the β-subunit (with the tRNA-binding domain) of the αβ-LeuRS to the *E. coli* editing domain activates its editing function (300). Noticeably, a $minihelix^{Leu}$ with triple leucine, isoleucine, and valine identity, mischarged with isoleucine, valine, or threonine, is edited by the separate CP1 domain from *A. aeolicus* LeuRS. This contrasts with the editing capacity of the freestanding CP1 domains from IleRS and ValRS that cannot clear mischarged minihelices (307). Therefore, it was proposed that the editing domain from *A. aeolicus* LeuRS has preserved ambiguous properties from an ancestral editing domain (307).

Editing domains resected from bacterial class II aaRSs (ProRS and AlaRS) have been produced as independent proteins with hydrolytic activity. Thus, an isolated INS domain (a large insertion of ~180 amino acids in the catalytic domain) from *E. coli* ProRS, when expressed as an independent protein, deacylates mischarged alanyl-microhelix^{Pro} (299). In the alanine case, editing of seryl-tRNA^{Ala} by resected *E. coli* AlaRS (438 to 875 fragment) depends on the C-terminal domain (731 to 875) linked to the inactive editing core (438 to 730) (451).

Autonomous bacterial *trans*-editing proteins

trans-editing proteins homologous to the editing domains inserted into ProRS, AlaRS, and ThrRS have been discovered in many genomes from the three kingdoms of life. When expressed as freestanding proteins, they are able to hydrolyze mischarged alanyl-tRNA^{Pro} (299, 402, 403), seryl-tRNA^{Ala} (402), or seryl-tRNA^{Thr} (115). In *Bacteria*, autonomous proteins complete the editing action of ProRS and AlaRS and in some organisms compensate for the lack of or poor editing activity of their ProRS or AlaRS that have lost or not acquired the appropriate editing domain. To date, three bacterial *trans*-editing proteins have been structurally and functionally characterized, namely, YbaK, ProX, and AlaX.

YbaK and ProX proteins have sequence similarity with ProRSs and are widespread in the three kingdoms of life. The crystal structure of *H. influenzae* YbaK (158 amino acids) solved at 1.8-Å resolution (***1dbu***, ***1dbx***) shows a unique globular fold made of a seven-stranded β-sheet surrounded by six short helices (452). A similar fold exists in *E. coli* ProX (***2dxa***) (unpublished, from RIKEN Structural Genomics Initiative). YbaK/ProX proteins are aminoacyl-tRNA deacylases with preferential deacylation activity of cysteinyl-tRNA^{Pro} (453, 454). They are paralogs of the editing domain inserted into the catalytic core of most ProRSs and catalyze *trans*-editing in *α-Proteobacteria* and the higher *Eukarya* coding for ProRSs missing the internal editing domain (211, 402). A bioinformatics analysis revealed that, in addition to INS and YbaK, there are four other INS-like domains throughout the bacterial kingdom (ProXp-ala, ProXp-x, ProXp-y, and ProXp-z, with the last three of unknown function), as e.g., in *Caulobacter crescentus* where YbaK and ProXp-ala edit cysteinyl- and alanyl-tRNA^{Pro} (455), but without tRNA specificity for YbaK (456). Deletion screens, together with *in vitro* deacylation assays, showed that the ProXp-x/y/z *trans*-editing factors recognize multiple tRNAs and prevent mistranslation errors caused by a number of aaRSs, including AlaRS, LysRS, SerRS, and ThrRS (457).

AlaX proteins from *Archaea* have autonomous editing activity. They are structural homologs of AlaRS editing domains with an architectural organization comprising a N-terminal domain with a glycine-rich motif and a C-terminal domain with two Zn-binding motifs (***1wnu***, ***1v4p***, ***2e1b***) (208, 458, 459). A related AlaXp protein is widely distributed in *Bacteria* and *Eukarya* (402). Its serine-editing activity could be demonstrated *in vivo* by using *E. coli* cells harboring an editing-defective AlaRS unable to clear seryl-tRNA^{Ala} and thus showing serine toxicity that could be rescued by an AlaXp-encoding transgene (460). Likewise, artificial recombinant fragments resected from *E. coli* AlaRS show hydrolytic editing of seryl-tRNA^{Ala} or glycyl-tRNA^{Ala}. Furthermore, a fragment of ~110 amino acids from *A. aeolicus* AlaRS corresponding to the C-Ala domain (the C-terminal domain universally tethered to the editing domain) located N-terminally in AlaRSs and forming a stable crystallizable structure, acts in *trans* in seryl-tRNA^{Ala} editing when tethered to the AlaXp-like editing domain of AlaRSs by a linker of α-helical coiled-coil architecture (***3g98***) (436). Interestingly, isolated C-Ala binds to the elbow formed by the D- and T-loops of tRNA^{Ala}, indicating that the L-shape of tRNA is required for editing and thus explaining why mischarged truncated tRNA^{Ala} molecules missing the elbow are not edited (306). Taken together, the accepting branch of tRNA interacts cooperatively with the flexibly tethered editing/C-Ala structure. Binding capacity of C-Ala to tRNA is explained by the resemblance of the distal portion of C-Ala with the DNA binding motif of RecJ DNA repair exonucleases (well conserved in *Bacteria*). Thus, C-Ala appears to be an ancient single-stranded nucleic acid binding motif that may have played a role in the evolution of AlaRS by coupling aminoacylation to editing (436). On the other hand, a mutational analysis examined the activity of the *P. horikoshii* AlaX editing domain with seryl-tRNA^{Ala} and alanyl-tRNA^{Ala} as substrates. Results show that wild-type AlaX preferentially deacylates seryl-tRNA^{Ala} but only ~12-fold faster than alanyl-tRNA^{Ala}. The impact of mutations was rather limited on seryl-tRNA^{Ala} deacylation that was decreased at most ~10-fold and even slightly reversed for two mutants that became more active on alanyl-tRNA^{Ala} than on seryl-tRNA^{Ala}. This indicates a relatively modest specificity of the AlaRS editing domain and may account for the widespread phyloge-

netic distribution of AlaX freestanding editing domains, thereby contributing a further mechanism to lower concentrations of misacylated $tRNA^{Ala}$ (461). However, it was shown that a single residue in AlaX-S, the smallest of extant AlaX enzymes, is important to determine the deacylation of seryl-$tRNA^{Thr}$ and seryl-$tRNA^{Ala}$, suggesting that the AlaX domain was used to maintain translational fidelity in the earlier stages of genetic code evolution when mis-serylation of tRNAs was frequent (462).

Editing determinants

Editing determinants in tRNA have a diverse nature and are less known than aminoacylation determinants. In the alanine system, the tRNA itself is a major editing determinant (306). In the other systems, determinants are discrete nucleosides and they could even be atomic groups from these nucleosides. Thus, in *E. coli* $tRNA^{Ile}$, major determinants are located in the corner of its L-shape (463), in contrast to aminoacylation where they are found in the anticodon triplet (304), demonstrating a segregation of the signals for editing and aminoacylation. In *A. aeolicus* $tRNA^{Leu}$, editing determinants are located in the anticodon arm (464), while in the *E. coli* valine system, atomic mutagenesis suggests that the unprotonated N1 atom in $tRNA^{Val}$ 3′-terminal A_{76} is a determinant for both editing and aminoacylation (465). However, most of these data remain incomplete and more work is required to define complete sets of editing determinants in the tRNAs concerned. Importantly, for *E. coli* AlaRS it could be conclusively shown that editing of seryl-$tRNA^{Ala}$ is sensitive to the G_3•U_{70} pair that is also the identity determinant for tRNA alanylation (451). This result indicates that two distinct domains in AlaRS can recognize the same base pair in tRNA. A significant breakthrough is the recent characterization of editing determinants in mischarged alanyl- and cysteinyl-$tRNA^{Pro}$ that are recognized by the freestanding bacterial *trans*-editing INS and ProXp-ala proteins: these determinants are the same (G_{72}/A_{73} and G_{35}/C_{36}) as those recognized by ProRS for aminoacylation (456).

On the protein side, editing signature motifs and determinants have been identified in a few aaRSs. Thus, two conserved HxxxH and CxxxH Zn-binding motifs in the editing domains of *E. coli* ThrRS and AlaRS dictate amino acid binding and rejection (207, 423). In several *E. coli* aaRSs, conserved amino acids act as editing determinants, such as (i) Thr_{252} in the CP1 editing site of LeuRS (466), (ii) Lys_{279} in the INS domain of ProRS that acts indirectly in alanyl-$tRNA^{Pro}$ hydrolysis (467), and (iii) highly conserved Tyr_{59} in the synthetic site of IleRS that modulates both posttransfer editing and aminoacylation (468).

Additional corrections by elongation factor

Some mischarged tRNAs can escape editing and are released by aaRSs. Consequently they can associate with elongation factor and be conveyed to the ribosome for protein synthesis. Importantly, in view of accuracy, the association with elongation factor is dynamic so that the mischarged species can rebind with aaRSs, allowing resampling by the product-editing pathway. As a result, accuracy of translation mediated by elongation factor was found increased over 10-fold *in vitro*, supporting the existence of an additional quality control step before translation (469).

Considerations on evolution

Mutations create diversity in macromolecules and are a major driving force in evolution. In such a view, the aaRSs are important actors in producing diversity in proteins because of their rather loose specificity. During chemical evolution toward life, proto-aaRSs likely were in limited number and had minimalist structures restricted to catalytic domains with poor specificity. When the protein synthesis machinery was fixed, the need for diversity declined and the catalytic site of aaRSs became more selective for amino acid recognition. But errorless recognition of amino acids was not possible for amino acids too closely related because of chemical constraints as prophesied by Pauling (246). Therefore, evolution had to invent editing strategies. Although not explicitly proven, this scenario finds support from the phylogenetic analysis of aaRS structures and by the characterization in aaRSs from contemporary organisms of relics of ancestral features. This is the case of redundant editing pathways in LeuRS or ProRS and of different types of posttransfer mechanisms that have evolved independently. This is reflected by the presence in extant aaRSs of different and unrelated internal editing domains and even of freestanding editing domains.

In another perspective, the addition of new amino acids into the early genetic repertoire, such as asparagine, glutamine, and pyrrolysine, was certainly related with mutational tinkering in the aaRS world. A consequence of this tinkering is the structural resemblance of the aaRSs specifying the new amino acids with older aaRSs.

This is well seen when comparing the structure of AsnRS or GlnRS with, respectively, AspRS or GluRS. Alternatively, the recent understanding of the mechanisms ensuring specificity and specificity switches in the aaRSs has found unprecedented applications with the design of orthogonal aaRS/tRNA pairs for reprogramming the genetic code and for incorporating unnatural amino acids in proteins (470) (see "Inhibition and engineering of bacterial aminoacyl-tRNA synthetases," below, for details).

To conclude, it is tempting to propose that the structural complexity of extant aaRSs, together with their rather loose specificity but with error correction potential, are biological necessities that protect these macromolecules against concerted mutational effects that would generate variant aaRSs perturbing the accuracy of protein synthesis and, in the worst case, the universality of the genetic code. Thus, structure and function of aaRSs reflect evolution in action in maintaining an exquisite balance between error and specificity.

Truncated aaRSs

The activity of truncated aaRSs provide strong evidence supporting the modular architecture of these enzymes and informs about the evolutionary history of aaRSs and about their mechanism of action. The first known example of a functional fragment was a truncated *E. coli* MetRS obtained by mild proteolysis of the native homodimeric (2x676 amino acids) enzyme (168). This truncated enzyme, deprived of its C-terminal domain involved in dimerization, therefore is monomeric (551 amino acids) but fully active. On the other hand, the crystal structure of the isolated C-terminal domain of dimeric MetRS from *P. abyssi* (***1mkh***) (471) has an α-helix bundle architecture and resembles that of Trbp111, a dimeric tRNA-binding protein found in many *Bacteria* and *Archaea*. This domain displays nonspecific tRNA-binding properties, and functional assays on *E. coli* MetRS variants show that the presence of the appended C-domain improves binding affinity of $tRNA^{Met}$. Because the oligomeric state of MetRSs is species dependent, and the monomeric forms (present in the three kingdoms of life) display the shortest sequences, it can be concluded that ancient MetRSs were short monomers.

The other historically important example of active versions of a truncated aaRS came from studies on tetrameric α_4-AlaRS and brought the first explicit demonstration of aaRS modularity. Based on structural and functional results, alanine activation, tRNA charging and editing activities were found to depend on the size of the truncature. While the smallest fragment by itself activates alanine (53), aminoacylation, editing, and oligomerization require additional domains (50, 135, 451) (***2zvf***). Interestingly, these active fragments are monomeric, having lost the oligomerization domain, suggesting, in addition, that oligomerization (as for MetRS) is not essential for enzymatic activity. These observations suggest that the ancestral enzyme was restricted to the active site domain and that extant AlaRSs emerged after simple peptide fusions. This view finds robust support by the alanylation capacity of artificial AlaRS fusions (472) making up the *E. coli* alanyl-adenylate domain fused to "artificial" peptide sequences (28 amino acids) shown previously to bind to the acceptor arm of $tRNA^{Ala}$ (473). Certain fusions were predicted to be functional and indeed could alanylate hairpin microhelices designed after the acceptor stem of $tRNA^{Ala}$ (provided they contain the alanine identity $G_3 \bullet U_{70}$ base pair), thus demonstrating the chemical logic underlying tRNA aminoacylation (472).

Functional studies on fragments derived from monomeric *E. coli* GluRS shed light on the evolution of this enzyme and showed its need of $tRNA^{Glu}$ for glutamate activation. Thus, two C-truncated enzymes ($GluRS_{1–313}$ with domains 1 to 3 and $GluRS_{1–362}$ with domains 1 to 4) glutamylate specifically $tRNA^{Glu}$ and require $tRNA^{Glu}$ for glutamate activation as does full-length $GluRS_{1–471}$. The k_{cat} of tRNA glutamylation by the $GluRS_{1–362}$ variant is ~2,000-fold lower than that determined with the native enzyme but is strongly stimulated by the addition of free domain 5 ($GluRS_{363–471}$). These functional data indicate that covalent connectivity with domain 5 is not required for activity. Furthermore, since truncated GluRSs form productive complexes with $tRNA^{Glu}$, these imply that the tRNA acceptor branch interacts first with domains 1 to 3 and that the anticodon branch interacts with domain 5 in a second step. The fact that the decreased catalytic activity of the shorter truncated $GluRS_{1–313}$ lacking the small domain 4 (49 residues) is not stimulated by its C-complement $GluRS_{314–471}$ reveals the importance of the covalent connectivity between domain 3 and 4 for the aminoacylation reaction. Altogether, the activity of the truncated versions of GluRS confirms the structural modularity of this aaRS and indicates that proto-GluRS was a minimalist protein restricted to domains 1 to 3 that acquired domains 4 and 5 later in evolution (322).

In summary, studies on truncated aaRSs turned out to be extremely useful in uncoupling the different activities that were encrypted by evolution in extant aaRSs. In this respect, the studies on AlaRS and GluRS were enlightening and informed about the domains essential or dispensable for tRNA aminoacylation. Truncated aaRSs were also essential in many structural biology projects. This was the case of *E. coli* MetRS and of many other aaRSs that crystallized more readily when rendered structurally more stable after resection of floppy extensions (51) (see "Structure of aminoacyl-tRNA synthetases," above).

AaRS-Dependent Quality Control of tRNA Aminoacylation

The need of faithful protein synthesis and the apparently contradictory facts showing nonabsolute fidelity of many molecular processes during translation lead to the idea of "quality control" that would account for mistranslation levels compatible with life. This is achieved by two types of mechanisms, namely during aaRS-dependent production of cognate aminoacyl-tRNAs and decoding of the charged tRNAs on the ribosome, so that globally mistranslation levels are minimized and synthesized proteomes functional (see, e.g., references 474, 475, 476, and 477, for reviews).

Two examples on aaRS-dependent production of cognate aminoacyl-tRNAs, namely prolyl-tRNA^{Pro} and leucyl-tRNA^{Leu}, illustrate the significance of the quality control concept in *Bacteria*. (i) In a mechanistic perspective, it is known that proofreading of mischarged alanyl- and cysteinyl-tRNA^{Pro} is achieved by two types of mechanisms involving the INS domain of ProRS (*cis*-editing) and the freestanding YbaK protein (*trans*-editing) (see "Error correction," above). The poorly understood Ybak-dependent *trans*-editing pathway of cysteinyl-tRNA^{Pro} was elucidated recently, and, remarkably, revealed cyclization of tRNA-bound cysteine prior to hydrolysis and the crucial role of the YbaK backbone atoms in stabilizing the transition state, while the product is stabilized by the 2′-OH of tRNA (478). (ii) In a more physiological perspective, it was shown that the prime biological function of editing by *E. coli* LeuRS is not, as anticipated, to prevent incorrect isoleucine incorporation into proteins but to prevent misincorporation of the nonstandard amino acid norvaline (479). This quality control mechanism is a key feature for the bacterial adaptive response to oxygen deprivation, and the nonessential role for editing under normal bacterial growth has important implications for the development of resistance to antimicrobial agents targeting the editing site of LeuRS (479).

INHIBITION AND ENGINEERING OF BACTERIAL AMINOACYL-tRNA SYNTHETASES

Adaptation of pathogens to antibiotics calls for new target macromolecules and new strategies to find new antipathogenic agents. AaRSs that acquired subtle kingdom- and sometimes even species-specific idiosyncrasies in their structure, function, or regulation during evolution make them attractive for such strategies. In addition, discovery and design of small molecules that inhibit aaRS function has the advantage of being useful for aaRS crystallography and enzymology (480, 481, 482, 483, 484, 485, 486). On the other hand, engineering of aaRSs is one of the powerful tools to approach their structure/function relationships. Also, the emerging biotechnologies and biology-inspired chemistry that aim to produce proteins having incorporated unnatural amino acids need advanced methods of aaRS engineering. Engineering of orthogonal aaRS/tRNA pairs is the prerequisite to reach this last goal. The present understanding of tRNA identity and knowledge of aaRS structures provides the conceptual background allowing this engineering.

Natural-Product Inhibitors and Derivatives

A number of natural products of bacterial origin inhibit aaRSs, notably pseudomonic acid A (known as mupirocin) and furanomycin (IleRS), granaticin and Agrocin 84 (LeuRS), indolmycin and chuangxinmycin (TrpRS), SB-219383, an atypical dipeptide with the second amino acid bearing a bicyclic scaffold (TyrRS), albomycin derivatives and 4-hydroxyderricin (SerRS), borrelidin (ThrRS), microcin C (AspRS), ascamycin (AlaRS), and ochratoxin A and other natural products (PheRS) (480, 481, 483, 487, 488, 489). These compounds are substrate mimics containing amino acid moieties and compete more or less efficiently for cognate amino acid or aminoacyl-adenylate binding in the catalytic site of the aaRSs. Thus, isoleucyl-adenylate and mupirocin, although of rather hidden mimicry (a methyl terminus of monic acid mimicking the side chain of isoleucine and a pyran ring mimicking adenosine), have a common recognition in the adenylate-binding pocket of IleRS as seen in crystal structures of *S. aureus* (***1qu3***, ***1ffy***) and *T. thermophilus* (***1jzs***,***1jzq***) IleRS. This similar recognition explains why

muporicin blocks binding of ATP and isoleucine on IleRS (138, 157). It is noteworthy that a mutagenesis analysis revealed that the selectivity of mupirocin binding in *T. thermophilus* IleRS over human IleRS comes from differences in only two residues in the aaRS active site (157). Similarly, the active moiety of antibacterial microcin C that inhibits AspRS is an aspartyl-AMP analog with an *N*-acyl phosphoroamidate linkage generated *in vivo* by processing its heptapeptide-nucleotidic precursor (490). The panel of natural products inhibiting bacterial aaRSs was recently enlarged to compounds of plant origin. Thus, the bioactive chalcone 4-hydroxyderricin isolated from *Angelica keiskei* covalently modifies *S. aureus* SerRS and thereby inhibits serylation of tRNAs (491).

So far, only mupirocin is on the market as a bacteriostatic compound used in dermatology as a topical antibiotic and, more generally, in eradication of methicillin-resistant *S. aureus*, which is a cause of death in hospitalized patients who have received antibiotic therapy (492, 493). Based on a better understanding of aaRS and inhibitor structures, several of these natural products were rationally modified to improve their inhibition capacities. This is notably the case of dipeptidic SB-219383, a bacterial fermentation product that inhibits TyrRS from staphylococci and streptococci (494) and of AspRS-specific microcin C (495). The crystal structure of *S. aureus* TyrRS with the dipeptidic inhibitor bound (***1jii***) (160) shows its interaction in the active site and provides the framework for designing novel antimicrobial agents. Finally, engineering of the mupirocin structure led to molecules with improved potency (496, 497).

On the other hand, resistance to antibiotics is becoming a worldwide public health problem and touches specifically bacterial pathogens that are becoming resistant to aaRS inhibitors (498, 499, 500). For example, sequencing the *ileS* gene from several strains of *S. aureus* with various degrees of susceptibility to mupirocin revealed a variety of point mutations clustering all on the surface of the IleRS (501). Interestingly, mutations do not solely lie in the Rossmann fold at positions critical for the antibiotic binding but are also found at the N and C termini of IleRS at positions distal to the tRNA-binding face and, more puzzling, within the CP1-editing domain or even outside the tRNA-binding site (501). Resistance can also occur by antibiotic-mediated aaRS gene induction or amplification. Thus, the second TrpRS gene (*trpRS1*) from *Streptomyces coelicolor* is inducible by indolmycin (502), and amplification of the IleRS gene as a result of secondary mutations provides adaptation to mupirocin resistance in *S. enterica* (503). Altogether, these phenomena explain the recent interest to better understand the biology and pharmacology of natural compounds interacting with aaRSs (e.g., the mechanism of mupirocin resistance [504]) and to search for antibiotic analogs, as is the case for mupirocin (499).

Synthetic Inhibitors

A huge number of synthetic inhibitors targeting the complete set of aaRSs were discovered by high-throughput screening of chemical libraries or were rationally designed as substrate analogs, primarily for pharmacological reasons, but also for crystallization/crystallographic purposes (481, 482, 484, 486). The refined understanding of aaRS structures supports efforts for renewed rational approaches to discover new inhibitors of pathogenic aaRSs. Thus, in view of new antituberculosis drugs, the use of the adenosine-binding site for inhibitor binding to *M. smegmatis* MetRS was evaluated and a potential binding site for a specific allosteric inhibitor was identified (158).

Stable nonhydrolyzable analogs of aminoacyl-adenylates (aa-AMP), such as aminoalkyl-adenylates (aa-ol-AMP) or aminoacylsulfamoyl-adenosines (aa-AMS), have been widely used in structural and mechanistic studies of aaRSs (481, 505, 506, 507) and for solving most of the crystallographic structures discussed in this review. The β-ketophosphonates are other synthetic adenylate analogs that were used, e.g., to inhibit bacterial GlnRSs and GluRSs (508). Potential inhibitors docking the aminoacyl-adenylate binding sites in aaRSs were discovered by virtual or library high-throughput screening. Among them, several molecules with only partial or apparently unrelated structures with aaRS substrates were found to selectively target, e.g., *S. aureus* MetRS (509, 510, 511), *Staphylococcus epidermidis* TrpRS (512), *E. coli*, *S. aureus* and *P. aeruginosa* TyrRSs (513, 514, 515), *S. aureus* ProRS (516), mycobacterial AspRSs (517), *S. aureus* LysRS (516), and bacterial PheRSs (518).

The boron-containing antifungal agent AN2690 is of particular interest because it inhibits posttransfer editing of LeuRS. A crystal structure of *T. thermophilus* LeuRS shows binding of an AMP-AN2690 adduct in the editing site (***2v0c***) and reveals the inhibition mechanism (156). This mechanism requires boron and the oxaborole ring of AN2690 and implies binding of the inhibitor to the

cis-diol of 3′-terminal ribose of tRNA via its boron atom with the consequence of tRNA being trapped in the editing site (156). Interestingly, a derivative of AN2690 binding to the freestanding editing domain of *S. pneumoniae* LeuRS (***4k47***, ***4k48***) inhibits LeuRS activity, thus being a potent antipneumococcal agent (519). Another organoboron LeuRS inhibitor targets clinical anaerobic *Bacteria*, such as bacteroidetes and clostridia (520). These results open up opportunities for the development of new aaRS inhibitors targeting posttransfer editing sites of aaRSs via boron chemistry. Likewise, bacterial supramolecular particles containing aaRSs, such as transamidosomes (the particles where glutamyl-tRNA^{Gln} or asparaginyl-tRNA^{Asn} is formed in organisms lacking GlnRS or AsnRS; see "Alternative functions of bacterial aminoacyl-tRNA synthetases," below, for details), are other attractive targets for specific inhibition of pathogens. Thus, among a series of new chloramphenicol analogs, one compound was identified as being a strong competitive inhibitor of the amidation of aspartyl-tRNA^{Asn} by the GatCAB amidotransferase in the asparagine transamidosome of *H. pylori* (521).

Engineering of aaRSs, Toward Orthogonality, and Design of Chimeras

In the early 1980s, the TyrRS from *B. stearothermophilus* was one of the first proteins to be engineered by site-directed mutagenesis (522, 523, 524). Today, engineering of aaRSs is routine practice, both for structural and functional studies (see, e.g., reference 256 and the many other examples given in this review). Remarkably, in the past decade, this engineering has moved in important new directions with the design of orthogonal aaRSs, more precisely of aaRS/tRNA pairs that are orthogonal to all endogenous pairs in a given organism, notably in *Bacteria*. (An orthogonal aaRS:tRNA pair consists of variant aaRS and tRNA molecules engineered in such a way that, first, the orthogonal aaRS becomes specific to an unnatural amino acid and would solely charge it on the orthogonal tRNA partner, and, second, the orthogonal tRNA would not be charged by a natural amino acid by any other aaRSs present in the protein synthesis system for which the orthogonal aaRS:tRNA pair was designed.) In addition, emerging trends aim to design aaRS chimeras.

Availability of orthogonal pairs enabling specific tRNA aminoacylation by unnatural amino acids is the prerequisite for ribosome-dependent synthesis of alloproteins, i.e., proteins containing chemically or spectroscopically active or structurally diverse amino acid analogs. To date, orthogonal aaRS/tRNA pairs have been used both *in vitro* and *in vivo* to incorporate ~200 unnatural amino acids into proteins in either *E. coli* (notably ~55 unnatural amino acids incorporated *in vivo*), yeast or mammalian cells (470, 525, 526). The method relies on orthogonal aaRSs able to charge stop codon (amber, ochre, or opal) reading suppressor tRNAs or frameshift suppressor tRNAs. Knowledge of identity rules, aaRS structure, and natural orthogonal aaRS/tRNA pairs (see above and, e.g., references 46, 527, and 528) was essential for the rational choice of the aaRSs to be engineered. Because of kingdom-specific distinctions in tRNA aminoacylation systems, expression of alloproteins in *E. coli* cells essentially relies on engineered aaRS/tRNA pairs of heterologous origin (e.g., *M. jannaschii* TyrRS/tRNA^{Tyr} or *S. cerevisiae* AspRS/tRNA^{Asp}, GlnRS/tRNA^{Gln}, TyrRS/tRNA^{Tyr}, or PheRS/tRNA^{Phe} pairs). Interestingly, based on a GlnRS/tRNA^{Gln} pair, it was possible to generate a complete set of orthogonal pairs active for reading amber, ochre, and opal codons (529). Orthogonality is reached by adapting the active site of the aaRSs to the desired new amino acid analog either by structure-based mutagenesis or by selection of the desired variants from structure-based libraries. Thus, 22 unnatural amino acids could be incorporated into proteins by evolving the orthogonal *E. coli* TyrRS/tRNA^{Tyr} and LeuRS/tRNA^{Leu} pairs (470). Noticeably, mutagenesis in editing domains of aaRSs could become useful to improve orthogonality (248). Altogether, the methods based on aaRS orthogonality have great potential, either in biotechnology and molecular medicine, besides being a powerful source of basic information on genetic code and aaRS evolution and the mechanism of tRNA recognition and aminoacylation.

In another perspective, and given the modular structure of aaRSs, a logical follow-up would be to fabricate aaRS chimeras encompassing structural elements from aaRSs of different specificities and/or of different phylogenetic origins. This idea has been explored based on conformational similarities in the catalytic domain (i.e., Rossmann fold) and the nearby SC-fold (i.e., a β-α-α-β-α fold that properly orients tRNA on the ACB domain) of *E. coli* MetRS (***1qqt***) and GlnRS (***1exd***). Thus, MetRS chimeras were designed with portions of the structurally similar SC-fold, but with highly degenerated sequence, of GlnRS motif or with alanine residues. The chimerical variants retained significant tRNA methionylation

activity and indicated that the structural integrity of the SC-fold contributes more to tRNA aminoacylation than does amino acid identity (530). It can be anticipated that such studies will help to reveal new structure/function relationships between aaRSs and to get deeper insight into the evolutionary processes that shaped the modularity of these enzymes.

REGULATION OF AMINOACYL-tRNA SYNTHETASE GENE EXPRESSION IN *E. COLI*

General Organization and Genes

The 20 aaRSs from *E. coli* are encoded by 23 genes. Most (17 of 20) of the aaRSs are encoded by a single gene; the three exceptions are PheRS, GlyRS, and LysRS. The heteromultimeric PheRS and GlyRS are encoded by two different genes corresponding to their two different subunits. LysRS is encoded by two genes for two homologous but different proteins, one being constitutively synthesized (the housekeeping enzyme) and the other being under multiple regulations (see below). The *E. coli* genome also carries a number of genes paralogous to aaRS genes, such as *genX* (*yjeA*) that encodes a protein homologous to the catalytic core of LysRS (85, 531, 532) and *yadB* that encodes a protein homologous to the catalytic domain of GluRS (533, 534). The properties of these shorter aaRS versions are described in "Paralogs of bacterial aminoacyl-tRNA synthetases," below.

The aaRS genes of *E. coli* are generally scattered throughout the whole chromosome, with the exception of the genes for the heteromultimeric enzymes mentioned above, which are grouped. Also, the genes for ThrRS (*thrS*) and PheRS (*pheST*) are grouped at 38 min (Table 6) and only separated by three genes, and those for AspRS (*aspS*) and ArgRS (*argS*) are located at 42 min and separated by nine genes. All the available bacterial genome sequences show that aaRS genes are generally scattered, even if, as for *E. coli*, some of these genes may be clustered. The clustering is higher for *B. subtilis*, where about half of the aaRS genes are situated between 323° and 268° (0/360° is the replication origin).

Inspection of bacterial genomes, other than *E. coli*, also tells us that the two genes corresponding to the two subunits of PheRS (*pheS* and *pheT*) are mostly adjacent in *Proteobacteria*, the phylum to which *E. coli* belongs, but may be found separated by a single open reading frame, as for *Pasteurella multocida* (a *γ-Proteobacteria* as *E. coli*), or by several open reading frames, as for *Neisseria gonorrhoeae* and *Rhodospirillum centenum* (both *β-Proteobacteria*). In more distant phyla, such as *Cyanobacteria*, *pheS* can be found separately from *pheT*. For even more distant phyla, such as *Aquifica*, the two PheRS genes can be found grouped (*T. maritima*) or separately (*D. radiodurans* and *A. aeolicus*). The situation is similar for GlyRS, although its two genes were already found separately in the phylogenetic tree close to *E. coli*, as, e.g., in some *γ-Proteobacteria* (*Francisella tularensis*). Concerning the genes of different aaRSs that are grouped such as *thrS* and *pheST* (separated by three genes in *E. coli*), genomic sequences indicate that they remain grouped in *γ-Proteobacteria*, but also sometimes in much more distant phyla, such as some *Firmicutes* (*Clostridium acetobutylicum*), in a way not obviously related to the phylogenetic tree. The looser association of *aspS* and *argS* that are separated by nine genes in *E. coli* is generally conserved in *Enterobacteria* but not further in *γ-Proteobacteria*.

In *E. coli*, unlike the rRNA operons that are divergently transcribed from *oriC*, there is no correlation between the orientation of the transcription of the different aaRS genes and the direction of replication. Moreover, no correlation is found between the position of the aaRS genes and the tRNA genes that are also scattered over the chromosome, with the exception of the gene encoding GluRS (*gltX*), which is adjacent to three genes encoding $tRNA^{Val}$ that are transcribed divergently from the aaRS gene (576). Although aaRSs have a key role linking amino acid biosynthesis to translation, cotranscription of aaRS genes with biosynthetic genes is not found in *E. coli*, whereas such examples have been described in *B. subtilis*, for instance, with the *gltX-cysE-cysS* operon (577).

All aaRS genes from *E. coli* have been experimentally found to be essential (578, 579) (https://www.genome.wisc.edu/resources/essential.htm), with the exception of *lysS* and *lysU* (see below), which may replace each other. The deletion of *lysU* shows no clearly identifiable phenotype (85), whereas that of *lysS* causes cold sensitivity mainly because *lysU* is hardly expressed at temperatures below 37°C (559) (see below).

Putative σ^{70}-promoters have been assigned to almost all *E. coli* aaRSs (Table 6). Although these enzymes are quite abundant and their codon content is that of relatively highly expressed genes, their promoters have sequences

Table 6 Properties of the *E. coli* aminoacyl-tRNA synthetase genes

aaRS gene and map position[a]	Coordinates[b]	Promoter[c]	Regulation[d]
alaS **60.72** (–)	2,817,403 ← 2,820,033 UG: *recX* (–) DG: *csrA* (–) p	alaSp TIS: 2820112; 79 nt from TIC (535)	Transcriptional autoregulation (536)
argS **42.2** (+)	1,958,086 → 1,959,819 UG: *yecM* (–) DG: *yecT* (+)	argSp TIS: 195828; 58 nt from TIC ρ-independent terminator (537)	
asnS **21.27** (–)	986,808 ← 988,208 UG: *pcnB* (–) t DG: *ompF* (–) p	asnSp TIS: 988267; 59 nt from TIC (538)	
aspS **41.96** (–)	1,946,774 ← 1,948,546 UG: *yecD* (+) DG: *nudB* (–) p	aspSp TIS: 1948641; 95 nt from TIC aspSp1: 1948725; 179 nt from TIC (539)	
cysS **11.94** (+)	553,834 → 555,219 UG: *ppiB* (–) DG: *ybcI* (–)	cysSp TIS: 553800 (+); 34 nt from TIC Putative ρ-independent terminator (540)	
glnS **15.2** (–)	705,316 → 706,980 UG: *nagE* (–) t DG: *ybfM* (–)	glnSp2 TIS: 705222; 94 from TIC (541) glnSp1 TIS: 705283; 33 from TIC ρ-independent terminator (542)	Possible regulation by incompletely characterized loci: *glnU* and *glnR* (543); see also 544 for comments. glnSp1: −10 carries a Dam methylation site. Expression of *glnS* is decreased by methylation (545). glnSp2: σ^{70} (D) and σ^{32} (H) activated (541). Possibly under director indirect control of OmpR (546)
gltX **54.26** (–)	2,517,279 ← 2,518,694 UG: *valU* (+) DG: *yfeD* (+)	gltXp1 TIS: 2518806: 112 nt from TIC gltXp2: 2518798; 104 nt from TIC gltXp3 TIS: 2518752; 58 nt from TIC (547)	Repressed by Fis (483)
glyQ **80.23** (–) *glyS* **80.19** (–)	3,722,430 ← 3,723,341 3,720,351 ← 3,722,420 UG: *ysaB* (–) DG: *sokA* (+)	*glyQS* operon Putative glyQp TIS: 3723377; 36 nt from TIC ρ-independent terminator (549)	
hisS **56.84** (–)	2,637,323 ← 2,638,597 UG: *sroE* (–) DG: *yfgM* (–) p	hisSp TIS: 2638664; 67 nt from TIC (550)	
ileS **0.48** (+)	22,391 → 25,207 UG: *ribF* (+) p DG: *lspA* (+) p	ribFp: 21383; 1008 nt from TIC ileSp1: 21833; 558 nt from TIC ileSp2: 22034; 357 nt from TIC (551) ileSp3: 22229; 162 nt from TIC (541)	ileSp3: σ^{32} (H) activated (541)
leuS **14.47** (–)	671,424 ← 674,006 UG: *ybeL* (+) p DG: *lptE* (–)	No experimental evidence, but putative promoter and terminator proposed (552)	Possible regulation by incompletely characterized mutants *leuX* and *leuY* (553, 554) and/or *leuR* (555); see also reference 544 for comments

lysS **65.34 (–)**	3,031,679 ← 3,033,196 UG: *prfB* (–) p DG: *idi* (+)	*xerD-dsbC-recJ-prfB-lysS* operon xerDp: 3037873; 108 nt from TIC (*xerD*) (541) dsbCp2: 3037655; 811 nt from TIC (dsbC) (556) (dsbCp): 3036856; 12 nt from TIC (*dsbC*) (557) prfBp: 3034342–47; 38–43 nt from TIC (*prfB*) (558)	xerDp: σ^{32} (H) (556) and σ^{24} (E)-activated promoter (541, 557) dsbCp2: σ^{24} (E)-dependent promoter (556) dsbCp: CpxR and σ^{24} (E) activated promoter (557)
l*ysU* **93.78 (–)**	4,351,223 ← 4,352,740 UG: *yjdL* (–) DG: *yjdO* (+)	lysUp1: 4352828; 88 nt from TIC lysUp2: 4352820; 80 nt from TIC (559)	lysUp1: repressed by Lrp (560). Under control of *rlu* and *lrp*; see text
metG **47.25 (+)**	2,192,322 → 2,194,355 UG: *mrp* (–) DG: *molR* (+) p	metGp0+ 2192081; 241 nt from TIC metGp+: 2192290;32 nt from TIC (561)	Possible autoregulation by a tRNA-like structure in the *mrp* gene (561)
pheM **(leader peptide)** **38.74 (–)** *pheS* **(α subunit)** **38.71 (–)** *pheT* **(β subunit)** **38.66 (–)**	1,797,250 ← 1,797,294 1,795,983 ← 1,796,966 1,793,581 ← 1,795,968 UG: *rplT* (–) t DG: *ihfA* (–) p	*pheST* operon pheMp: 1797325 (–); 31 nt from *pheM* (562, 563)	Regulated by phenylalanyl-tRNA^{Phe} levels (leader peptide-dependent attenuation) (562, 564). Possibly regulated by oxygen availability (Fnr and ArcA) via readthrough from *rplT* (565, 566). Regulated by IHF and SOS (567); see text
proS **4.68 (–)**	217,057 ← 218,775 UG: *yaeB* (–) DG: *yaeF* (–)	proSp: 218837; 62 nt from TIC putative ρ-independent terminator. Gene called DrpA (568)	
serS **20.23 (+)**	938,651 → 939,943 UG: *rarA* (+) DG: *dmsA* (+) p	serSp: 938589; 62 nt from TIC (569)	Possible regulation by incompletely characterized locus serR (555); see also reference 544 for comments
thrS **38.77 (–)**	1,798,666 ← 1,800,594 UG: *arpB* (+) DG: *infC* (–) p	*thrS-infC* operon thrSp: 1800757 (–); 163 nt from TIC (570)	Translational autoregulation (205, 563), see text. NsrR binding site in regulatory regions (571)
trpS **75.67 (–)**	3,510,656 ← 3,511,660 UG: *rpe, gph* (–) p DG: *yhfZ* (–)	*rpe-gph- trpS* operon rpep: 3513175; 94 from TIC of *rpe* (572) trpSp: 3511719; 59 from TIC of *trpS* (573)	Not under autogenous *miaA*or *trpR* control (573)
tyrS **36.94 (–)**	1,713,972 ← 1,715,246 UG: *pdxH* (–) p DG: *pdxY* (–) p	*pdxH-tyrS-pdxY* operon tyrSp2: 1715307 (–); 61 from TIC (538) tyrSp1: 1715293 (–); 47 from TIC (574)	
valS **96.54 (–)**	4,479,005 ← 4,481,860 UG: *holC* (–) p DG: *yjgN* (+)	valSp1: 4481950; 90 nt from TIC valSp2: 4481942; 82 nt from TIC; ρ-independent terminator (575)	

[a]Listed are the names of the aaRS genes and the positions in centisomes. The direction of transcription is with increasing (+) or decreasing (–) centisomes.

[b]Listed are the coordinates of the different genes of the *E. coli* K12 MG1655 strain (http://ecocyc.org/) and the name of the upstream gene (UG) and downstream gene (DG), with the direction of transcription followed by either p, indicating that the gene has his own promoter, or by t, indicating that the gene is followed by a (putative) ρ-independent terminator. The information comes from EcoCyc (http://ecocyc.org/) or regulon database (http://regulondb.ccg.unam.mx/index.jsp) (538).

[c]Listed are the coordinates of the transcription initiation site (TIS) and the distance in nucleotides between the TIS and the A of the AUG of the translation initiation codon (TIC). The gene is followed by a sequence resembling a ρ-independent terminator when indicated.

[d]Given is the type of regulation, if known.

that are often quite far from the classical σ^{70}-consensus. In some cases several promoters are being used to express a single aaRS gene with one of the promoters activated by an alternative σ-factor (Table 6).

Heterodimeric Enzymes and Multifunctional Operons

PheRS and GlyRS are the only aaRSs in *E. coli* composed of two different subunits. In both cases, the genes for the two different subunits are not only grouped on the *E. coli* chromosome, but are also expressed as operons where the promoter proximal gene codes for the smaller subunit. In the case of GlyRS, the genes for the two subunits, *glyQ* and *glyS*, are located at 80 min, and the evidence that they are cotranscribed is based on the polar effects of Tn*5* insertions in *glyQ*, the promoter proximal gene, on the expression of *glyS*, the promoter distal gene (580). The intergenic distance between the promoter proximal *glyQ* gene and the distal *glyS* gene is only nine nucleotides. In the case of PheRS, the genes of the two subunits, *pheS* and *pheT*, are located at 38 min and are also cotranscribed (562, 581). The distance between the two genes of the operon is 14 base pairs, similar to the intercistronic distance in the *glyQS* operon. The *pheST* operon is controlled by attenuation and is part of a region, which includes the structural gene for ThrRS (see below).

Some aaRS genes are expressed from larger transcription units with apparently unrelated genes. For example, the gene for IleRS (*ileS*) is located at 0.48 min and is part of an operon composed of five cistrons: the first, *ribF*, encodes the flavokinase and FAD synthetase; the second, *ileS*, encodes IleRS; the third, *lspA*, encodes the prolipoprotein signal peptidase; and the two last cistrons, *fkpB* and *ispH*, encode a peptidylprolyl isomerase and a 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase (http://biocyc.org/ECOLI/). The whole operon is expressed from a promoter upstream of *ribF* (ribFp). Three other promoters are located within *ribF* (ileSp1, ileSp2, ileSp3), and the last one is located within *ileS* upstream of *lspA* (lspAp) (551) (http://biocyc.org/ECOLI/). The characterized terminator of this operon is located after the *ispH* gene.

Another example is the constitutively synthesized gene for LysRS (the housekeeping enzyme), *lysS*, that is expressed from an operon located at 65 min of which the first cistron is the gene *pfrB* encoding the peptide-chain-release factor 2 (RF2), and the second cistron is the *lysS* gene originally called *herC* (558). The gene *lysS* might also be expressed from longer transcripts carrying *recJ* (involved in recombination) and *dsbC* (a subunit of the protein disulfide isomerase) sequences upstream of *pfrB* (http://biocyc.org/ECOLI/). The expression of *prfB* is autoregulated by a posttranscriptional mechanism involving a +1 frameshifting at a naturally occurring UGA codon early in the gene (582). The expression of *lysS*, located immediately downstream, might thus be influenced by the translation of *prfB*. Still another example is *trpS* encoding TrpRS, which is expressed as a distal gene of a complex operon (572) made of seven genes expressed from seven promoters (http://biocyc.org/ECOLI/).

A striking example of a larger transcription unit is that carrying the gene for ThrRS (*thrS*), which is located at 39 min and belongs to a region of six genes transcribed in the same direction and all involved in translation. The *thrS* gene is followed by *infC* (translation initiation factor 3), *rpmI* (ribosomal protein L35), *rplT* (ribosomal protein L20), and the genes *pheS* and *pheT* (the two subunits of PheRS). The gene *thrS* is expressed from the thrSp promoter and makes two detectable mRNAs: the shorter covers *thrS* and *infC* and stops at terminator t1, and the longer traverses t1 and also covers the next two genes, *rpmI* and *rplT*, and stops at terminator t2 (583). These results indicate that *thrS* is always expressed with *infC* and sometimes with *infC*, *rpmI*, and *rplT*. The expression of *thrS* is negatively autoregulated at the translational level (205). However, neither the expression of *infC* nor that of the other next genes is noticeably affected by this regulation, since these genes are mainly expressed from their own promoters. Transcripts that carry *thrS* and the very distal genes *pheS* or *pheT* were not detected. The latter genes are expressed from their own promoter, pheMp, but may also be expressed from upstream promoters since the terminator t2, located after *rplT* and upstream of *pheM*, the leader peptide of the *pheST* operon, is very leaky *in vivo* (584).

With reference to the association between aaRS genes with unrelated genes that exist in *E. coli*, the gene for ThrRS, *thrS*, is found associated with *infC* and *rpmI-rplT*, in almost all *γ-Proteobacteria* and *β-Proteobacteria* and more rarely so in *α-Proteobacteria*. This association persists in some species belonging to quite distant phyla such as *Bacteroidetes* or *Chlorobium* and even *Firmicutes* (*C. acetobutylicum*). Other associations, such as *ribF* and *lspA* with *ileS*, are found in *E. coli* and most

γ-Proteobacteria and *β-Proteobacteria* and less so with *α-Proteobacteria* and seldom with more distant phyla. The *prfB-lysS* association is even less conserved.

In conclusion, it appears that in *E. coli* some aaRS genes are expressed as multicistronic operons carrying apparently unrelated genes. However, the significance of cotranscription of other genes with aaRS genes is unknown. In the case of the highly regulated *thrS-pheS-pheT* region, the existence of long transcripts cannot, at the present time, be associated with any cross-regulation (e.g., ThrRS regulating the expression of the downstream gene *infC* or ribosomal protein L20, the regulator of *rpmI* and *rplT*, regulating the expression of the downstream *pheST* genes).

Regulation Strategies

Specific regulations

Specific regulations deal with the modulation of the expression of individual aaRSs in response to growth medium and/or genetic changes. The study of several amino acid biosynthetic operons in *E. coli* and *Salmonella enterica* serovar Typhimurium showed that aaRSs could be involved in the regulation of these operons (585). Since the expression of the biosynthetic operons is generally induced under cognate amino acid starvation conditions, the question was raised of whether or not the expression of the genes for aaRSs themselves is controlled in a similar way in *E. coli*. To test this hypothesis, many experiments were performed to study the effect of amino acid shortage on the expression of cognate aaRSs (reviewed in reference 544). Although different studies gave contradictory results, it seems that many aaRSs are transiently derepressed under cognate amino acid shortage but much less than the corresponding biosynthetic operons under the same experimental conditions (586, 587). In addition to these physiological studies, some more detailed molecular analyses have been performed to identify the *cis*-acting signals and the *trans*-acting genes that are involved in aaRS expression regulation. Some of these results have been reviewed previously (544, 588) and are summarized in Table 6; a few are described below.

In vitro studies of the expression of the *alaS* gene encoding AlaRS indicated that this enzyme was able to inhibit its own transcription in the presence of alanine (536). In addition, the enzyme was shown to bind to the −10 region of its own promoter, protecting two palindromic sequences from DNase I digestion. Unfortunately, no experiments are available to confirm that this apparently unique regulatory mechanism among *E. coli* aaRSs occurs *in vivo*.

In *E. coli,* LysRS is encoded by two different genes, *lysS* and *lysU,* located at 65 and 94 min, respectively (Table 6). This example is the only one in which two different aaRSs are able to activate the same tRNA isoacceptor family in *E. coli*. The first gene, *lysS*, corresponds to the housekeeping aaRS and is constitutively expressed from one or several σ^{70}-dependent promoters but might also be expressed from σ^{24} and σ^{32}-dependent promoters (Table 6). The second gene, *lysU*, corresponds to a form that is not constitutively expressed but is induced by various stimuli such as high temperature shifts, external pH changes, anaerobiosis, or the presence of specific metabolites in the growth medium (559, 589, 590). The expression of *lysU* is controlled by several genes such as *lrp*, *rpoH*, and *rlu*, which code, respectively, for the leucine response protein, the heat shock σ-factor, and an uncharacterized regulatory locus, all described below.

The expression of the *lysU* gene is induced by heat shock but only in the presence of a wild-type copy of *htpR* (or *rpoH*), the structural gene for the heat shock σ-factor (591). The role of *htpR* in *lysU* expression is unclear since induction of *htpR* synthesis, without temperature shift, does not cause *lysU* derepression (592). A second regulatory locus for *lysU* was found by genetic means: second-site suppression analysis indicated that *lysU* expression is under the control of a locus named *rlu* (regulation of *lysU*), located at 49.5 min on the *E. coli* map (593). The third regulator of *lysU* expression is the *lrp* gene, which encodes the regulatory protein of the Leu-Lrp regulon and causes repression or activation of many genes in *E. coli* (594, 595). The direct involvement of *lrp* in *lysU* expression was shown by DNase footprinting of Lrp protein to DNA upstream of *lysU* (560, 596).

Although a great deal of information is available about the control of *lysU* expression, many points are still unclear. How are the effects of the different stimuli (temperature, pH, and metabolites) related? What is the role of *rlu*? What is the indirect mechanism by which *htpR* controls *lysU* expression? What is the effect of *prfB* (encoding RF2) autoregulation on *lysU* expression? Although sensible reasons have been proposed to explain the existence of a second gene for LysRS (85), the *raison d'être* of this second inducible gene remains hypothetical.

The two subunits of PheRS, a heterodimeric aaRS of the $\alpha_2\beta_2$-type, are expressed from an operon (see above) whose expression is mainly due to a promoter located far upstream (~400 base pairs) of *pheS*, the first structural gene of the operon (562). Nucleotide sequencing and transcription studies (562) showed the presence of a ρ-independent transcription terminator between this promoter and the structural genes of the operon. Elements similar to those found upstream of amino acid biosynthetic operons controlled by attenuation were found between the promoter and the terminator of the *pheST* operon. These elements are a small 14-amino-acid-long open reading frame with five phenylalanine codons called the leader peptide and sequences that allow the formation of alternative structures on the transcript. These control elements were shown to be responsible for the induction under conditions of phenylalanyl-tRNA^{Phe} starvation (564). The model for *pheST* expression regulation, similar to that described for the histidine and tryptophan biosynthetic genes (585), is as follows. Under conditions of phenylalanine starvation, the ribosome pauses in the leader peptide at the phenylalanine codons, which allows the downstream mRNA to fold into a structure, called the antiterminator, which is incompatible with the formation of the terminator located downstream. The absence of transcription termination allows RNA polymerase to reach the structural genes of the operon. On the contrary, if phenylalanine is synthesized at adequate levels, the leader peptide is translated normally and the antiterminator structure cannot form, allowing the terminator structure to form, which decreases transcription of the structural genes. A strong indication that the expression of the *pheST* operon is controlled by a leader peptide-dependent attenuation mechanism comes from the finding that the operon is induced in the presence of a mutation in the *miaA* gene, formerly called *trpX*, which codes for Δ2-isopentenyl-pyrophosphate transferase, a tRNA modification enzyme in *E. coli* (e.g., for tRNA^{Trp} and tRNA^{Phe} modification). A mutation in this gene also causes induction of the biosynthetic *trp* and *pheA* operons, which are controlled by the same kind of leader peptide-dependent attenuation mechanism. In the presence of the *miaA* mutation, translation of the leader peptide is slowed down, favoring antiterminator structure formation downstream on the mRNA and thus increased expression of the structural genes. The involvement of specific attenuator-like mRNA structures in control of *pheST* expression was shown by detailed mutational analysis of the leader region (584, 597). The *pheST* operon is also under *himA* and SOS control (567). The former control may be direct because sequences resembling the IHF binding site consensus are present at the *pheST* promoter. SOS control is probably indirect since no consensus LexA binding site was found next to this promoter. Global transcription studies (565, 566) indicate that the expression of *rplT*, the gene encoding ribosomal protein L20 located immediately upstream of the *pheST* operon, is decreased during anaerobic growth and increased in Fnr- and ArcA-deficient strains (both are global transcriptional regulators involved in the response to oxygen). Since the terminator between *rplT* and *pheST* is weak (see above), *pheST* expression could also be regulated by aerobiosis.

Many *Enterobacteria* show, after the *rplT* transcription terminator and upstream of *pheST* structural genes, features corresponding to a leader peptide-dependent attenuation described above. Inspection of the regions upstream of *pheS* in other *γ-Proteobacteria* belonging to orders different from *Enterobacteria*, such as *Alteromonadales* (*Shewanella putrefaciens*), *Pasteurellaceae* (*H. influenzae*), *Pseudomonadaceae* (*Pseudomonas aeruginosa*), and *Vibrionaceae* (*Vibrio cholerae*), do not show features corresponding to leader peptide-dependent attenuation. Despite the limited occurrence of this type of regulation for the PheRS genes, leader peptide-dependent attenuation has been found to regulate the transcription of at least one other aaRS in *Bacteria*. For instance, the auxiliary TrpRS encoded by the *trpS1* gene of *S. coelicolor* has been shown to be inducible by several antibiotics using the same type of mechanism (500).

The most complete work about regulation of aaRS gene expression in *E. coli* has been performed with ThrRS, which follows a mechanism completely different from PheRS. ThrRS negatively regulates the expression of its own gene (*thrS*) at the translational level (563). The protein binds to a site, the operator, positioned in the leader of its own mRNA upstream of the Shine-Dalgarno sequence and inhibits translational initiation by competing with the binding of the 30S ribosomal subunit (598). The RNA operator is composed of four structural domains with tRNA^{Thr} anticodon-like sequences present in the loops of two of the four domains. The role of these two anticodon-like sequences is somewhat different since mutations in the main anticodon-like domain (that closest to the translation initiation site) abolish control, whereas mutations in the other have only a limited effect. The fact that these sequences are recognized as anticodon-like sequences is highlighted by biochemical

and genetic data (205). In particular, the first base of the anticodon-like sequences can be changed without major effect on control, whereas any change in the second or third base abolishes control. This pattern of mRNA recognition is reminiscent of that of the $tRNA^{Thr}$ isoacceptors for which the first base can either be C, G, or U, but neither the second nor the third base can be changed without abolishing aminoacylation (599). Perhaps the most convincing data indicating that the RNA operator carries a tRNA-like structure comes from an experiment showing that, if its identity is switched from threonine to methionine, the gene *thrS* becomes regulated by MetRS instead of ThrRS levels in the cell. Indeed, the replacement of the anticodon-like CGU sequence in the first (and main) anticodon-like domain of operator by CAU, the $tRNA^{Met}$ anticodon, abolishes the negative feedback control by ThrRS and establishes control by MetRS both *in vitro* (600) and *in vivo* (601).

ThrRS is a homodimeric enzyme, with each subunit composed of three different domains. The N-terminal domain gives the enzyme a winged shape, while the catalytic and the C-terminal domain form the core of the protein (145) (see "Structure of aminoacyl-tRNA synthetases," above, for more details on structure). The crystal structures of ThrRS complexed with two $tRNA_2^{Thr}$ molecules (***1qf6***) (145) and to the main anticodon-like domain of $mRNA^{ThrRS}$ (***1kog***) (206) provide a detailed picture of how this aaRS performs two distinct functions, aminoacylation and control. In aminoacylation, each tRNA interacts mainly with one subunit of the enzyme with the anticodon loop of $tRNA^{Thr}$ recognized by the C-terminal domain of the protein and the acceptor arm sequestered between the catalytic and the N-terminal domains. In the regulatory complex, the two anticodon-like domains occupy positions equivalent to those of the anticodon arm of $tRNA^{Thr}$ with the two apical loops of the operator in interaction with the two C-terminal domains of the ThrRS dimer. Remarkably, the same amino acids interact with the CGU anticodon sequence of the tRNA and with the analogous residues in the main anticodon-like sequence of the operator. In agreement with the structure, changes of amino acids in the C-terminal domain of ThrRS affect both aminoacylation and regulation (602). In conclusion, the conjunction of genetic, biochemical, and structural data definitively proves the existence of mimicry in the way ThrRS recognizes the anticodon arm of tRNA and the two analogous regions of the operator on its own mRNA. Other tRNA mimics have been described (see above), and their role in gene regulation has been discussed elsewhere (603).

In addition to the enterobacterial order, the entire *thrS* operator with its four domains is found in several other γ-proteobacterial orders such as the *Alteromonadales* (*Shewanella putrefaciens*) and *Pseudomonadaceae* (*P. aeruginosa, putida*, and *syringae* groups). In more distant γ-proteobacterial orders, such as the *Pasteurellaceae*, either the entire operator (*H. influenzae, Pasteurella multocida*) or a partial operator carrying only the translation initiation proximal part (domains 1 and 2) of the operator (*Haemophilus ducreyi*) is found. In the case of *V. cholerae* belonging to the order of *Vibrionaceae*, only a partial (domains 1 and 2) operator is found (206).

It should be noted that the regulation of aaRS in *E. coli* and related *Bacteria* is very different from that of *Firmicutes*. In *B. subtilis*, the derepression levels after cognate amino acid starvation of several aaRS genes resemble those obtained with biosynthetic genes (83, 604, 605). Also, the control regions of most *B. subtilis* aaRS genes show common characteristics, something that is not found in *E. coli*. These common features are located downstream of the transcription initiation site and include a 14-base-pair-long conserved sequence (the T-box) located upstream of a transcription termination site (83, 605, 606). These regulatory regions are generally composed of three helical domains, a pseudoknot element preceding the T-box and mutually exclusive secondary structures, one of them being the terminator. A key element of this regulation is a triplet, called the "specifier codon," which is placed in the first helical domain (607). The "specifier codon" always corresponds to the amino acid specificity of the downstream aaRS gene. Under amino acid starvation conditions, the level of aminoacylation of the cognate tRNA decreases. This starvation allows the cognate uncharged tRNA to bind to the nascent mRNA in such a way that the tRNA anticodon recognizes the "specifier codon" and the 3′ CCA uncharged end of the tRNA attaches to a downstream sequence of the mRNA (608). This tRNA attachment favors the formation of an antiterminator structure excluding the formation of the terminator. This allows transcription beyond the terminator into the structural aaRS gene. Interestingly, these regulatory elements can also be found upstream of amino acid biosynthetic and uptake genes in bacilli (609). The presence of such regulatory elements is exactly what could be expected from the apparently interconnected role of aaRSs

and biosynthetic genes. At the present time, more than 1000 T-box elements have been annotated in all groups of Gram-positive *Bacteria*, as well as in some other phyla such as *Deinococcus* and *Thermus* and in a few Gram-negative phyla such as *Geobacter* and *Chloroflexus* (608, 610, 611). Several variations in this mechanism were recently described, notably the discovery in organisms of the phylum *Actinobacteria* of a novel group of T-box leader sequences of IleRS genes (ileS) lacking conserved elements essential for interaction with tRNA in other T-boxes. In these organisms, regulation requires $tRNA^{Ile}$-dependent structural rearrangements in the T-box riboswitches (612). Another peculiarity occurs in *Clostridium acetobutylicum*, where efficient antitermination of the AlaRS gene (alaS) is explained by correlated sequence variations in $tRNA^{Ala}$ and alaS T-box leader RNA (613). Lastly, a novel regulatory mechanism is found in *B. subtilis* strains lacking a D-aminoacyl-tRNA deacylase. This mechanism regulates expression of the two TyrRSs (TyrS and TyrZ) present in these strains via a T-box riboswitch and a MarR (Multiple antibiotic resistance Regulator) factor. This is accounted by differentially organized tyrS and tyrZ operons (with a T-box in both operons and a MarR sequence in the tyrZ operon) and their growth-dependent expression (606). Remarkably, tyrZ is mainly expressed when *B. subtilis* needs D-tyrosine under stationary phase growth and biofilm formation, and when TyrS is inactivated. Since TyrZ is less efficient than TyrS and more selective for L-tyrosine over D-tyrosine it is concluded that these features may provide the mechanism preventing misincorporation of D-tyrosine in proteins (606).

Global regulations

The bacterial cell becomes larger and contains more DNA, RNA, and protein when cellular growth rate increases. The relative increase in RNA is larger than that of proteins that, in turn, is larger than that of DNA (614). In addition, the proportion of stable RNA (ribosomal RNA and tRNA) to total RNA increases with growth rate. The number of ribosomes per cell increases from ~7,000 to ~70,000 when the doubling time varies from 100 to 24 min (614). Also, the number of tRNA molecules per cell increases from ~63,000 to ~700,000 when the cellular doubling time varies to the same extent (614).

Quantitative 2D gel analysis of cells grown at different growth rates shows that the cellular concentration of the majority of the aaRSs analyzed increases with growth rate (615). This increase is, if normalized to the total cellular proteins, between 1.5- and 3-fold. In the case of AlaRS and LysRS expressed from the regulated gene, *lysU*, the cellular concentration actually decreases with growth rate. The average increase with growth rate in the concentration of housekeeping aaRSs is 1.7-fold if normalized to total cellular proteins, which is a limited increase compared with that of ribosomes under the same growth conditions.

Although the molecular mechanisms of growth rate-dependent regulation are unknown for most aaRS genes (see discussion in reference 544), precise information is available for ThrRS. As explained above, the synthesis of this enzyme is negatively autoregulated at the level of translation because of its ability to bind to its RNA operator on its own mRNA causing translation inhibition. Interestingly, operator mutations that abolish autoregulation also abolish growth rate-dependent regulation (570) indicating a clear relationship between the specific and the more global regulation for this aaRS. A simple explanation for these results is that, at high growth rates when the $tRNA^{Thr}$ concentration is higher, the majority of ThrRS is involved in aminoacylation and less is available to bind to its mRNA, increasing its translation. The involvement of $tRNA^{Thr}$ is indicated by the fact that, if $tRNA^{Thr}$ concentration is increased by the presence of a multicopy plasmid carrying the gene for a $tRNA^{Thr}$ isoacceptor, the expression of *thrS* is induced independently of growth rate (570).

Several aaRS genes carry a recognition sequence (GATC) for the Dam methylase in their regulatory regions. This is the case for *trpS* (at the 5′ end of the −35 box), *glnS* (over the −10 region), and possibly for *cysS* (two sequences upstream of the −35 box). The expression of a few *E. coli* genes is influenced by Dam methylation (616). The expression of *glnS* was shown to be under Dam methylase control since *glnS-lacZ* translational or transcriptional fusions are derepressed in a strain mutated in the *dam* gene (545). The expression of *trpS* was also reported to be derepressed in a *dam* strain (617).

At high growth rate, there are ~7×10^5 tRNAs/cell and protein synthesis rates are of approximately 5×10^5 amino acids/s/cell (614). These numbers mean that, on average, an aminoacylated tRNA is discharged and recharged about every second. This, in turn, means that, as soon as the supply of an amino acid is restricted, the concentration of the corresponding aminoacylated tRNA

is immediately limiting (544). As a consequence, stable RNA synthesis is immediately stopped in *E. coli*. The effect of amino acid starvation on stable RNA synthesis is called the stringent response and depends on the *relA* and *spoT* genes that allow pppGpp and ppGpp synthesis. The synthesis of individual proteins is differentially affected by amino acid starvation. A study of the effect on aaRS synthesis of a decrease of aminoacylated tRNA indicates that most of the aaRSs are not under stringent control, i.e., less affected by amino acid starvation than, for instance, ribosomal proteins, which are known to be under stringent control. Among the nine aaRSs that were studied, ArgRS and maybe ValRS could be under weak stringent control (618).

ALTERNATIVE FUNCTIONS OF BACTERIAL AMINOACYL-tRNA SYNTHETASES

Indirect Pathways of Specific tRNA Aminoacylation for Ribosome-Mediated Translation

Besides direct charging of the amino acid onto its cognate tRNA(s) by an aaRS, there are also indirect pathways with tRNA-dependent modification of a noncognate amino acid mischarged to tRNA (619). Three such cases occur in *Bacteria* and concern formation of asparaginyl-tRNA^{Asn}, glutaminyl-tRNA^{Gln}, and selenocysteinyl-tRNA^{Sec} (with Sec for selenocysteine, the 21st amino acid in ribosome-mediated protein synthesis). A fourth case, tRNA-dependent biosynthesis of cysteine from *O*-phosphoserine in methanogens (107), is archaea-specific and will not be further discussed here.

ND-GluRSs and ND-AspRS for tRNA-dependent glutamine and asparagine biosynthesis

In those *Bacteria* (also in most *Archaea* and in some organelles) deprived of AsnRS or GlnRS, asparagine or glutamine can be synthesized by tRNA-dependent mechanisms after the mischarging of tRNA^{Asn} or tRNA^{Gln} by ND-AspRS or ND-GluRS (ND means Non-Discriminating for efficient tRNA charging by these ND-aaRSs of both their cognate tRNAs and a related noncognate tRNA) followed by amidation of mischarged aspartate or glutamate by a tRNA-dependent amidotransferase (AdT) (7, 72). AdT enzymes designated by the generic name Gat (for Glutamine amidotransferase) are of different oligomeric organizations (heterotrimeric GatCAB in *Bacteria* and heterodimeric GatDE in *Archaea*) with structurally different subunits that amidate both tRNA-bound aspartate and glutamate. Surprisingly, in the *H. pylori* system, AspRS was shown to increase the affinity of aspartyl-tRNA^{Asn} for the GatCAB amidotransferase (620), suggesting a coupling between aaRS and amidotransferase activities (see below, for structural validation). Note that the GatE subunit contains a subdomain that is included in the insertion domain of bacterial AspRSs making a structure-function link between aaRSs and tRNA-dependent amidotransferases. Also of interest is the presence in a few *Bacteria* of ND-AspRSs of archaeal-type coexisting with D-AspRSs (e.g., in *T. thermophilus* and *D. radiodurans*) (621, 622), making an evolutionary link between *Bacteria* and *Archaea*. In *Bacteria*, however, ND-AspRSs are mostly of bacterial-type (e.g., in *P. aeruginosa* and *H. pylori*) (221, 623). Simultaneous presence of the two GluRS forms (D and ND) in *Bacteria* is also documented in *H. pylori* and *Acidithiobacillus ferrooxidans* and, intriguingly, the ND-version does solely mischarge tRNA^{Gln} and has lost the ability to aminoacylate tRNA^{Glu} (77, 78, 79). Altogether, the phylogenetic distribution of ND-aaRSs is intricate and not well understood, but likely recapitulates part of the evolutionary history of the class Ib GluRS/GlnRS and class IIb AspRS/AsnRS couples. At the tRNA level, the identity sets of the tRNA^{Glu}/tRNA^{Gln} and tRNA^{Asp}/tRNA^{Asn} couples are related with participation of discriminator N_{73} and anticodon $N_{34}N_{35}N_{36}$ residues (188). Finally, it should also be emphasized that both direct and indiect pathways for the synthesis of asparaginyl-tRNA^{Asn} can be operational in the same organism, e.g., in *T. thermophilus* (624), in *Synechococcus elongatus*, and probably in other *Cyanobacteria* (239).

Based on these considerations, it is anticipated that the relaxed specificity of ND-AspRSs and ND-GluRSs relies on altered recognition of the tRNA identity determinants because of correlated architectural idiosyncrasies within the ND-aaRSs. In the AspRS case, the ND-enzyme has to efficiently recognize both tRNA^{Asp} and tRNA^{Asn}, two tRNAs sharing the same major identity determinants with the sole exception of the third anticodon determinant C_{36} in tRNA^{Asp} replaced by U_{36} in tRNA^{Asn} (Figure 4). Thus, the structural understanding appears straightforward and relies primarily on peculiarities in the OB-fold ACB domain of ND-AspRSs. In the particular case of archaeal-type *T. thermophilus* ND-AspRS (***1n9w***), this domain is lacking a helix inserted between two β-strands of the OB-fold and contains a peculiar L1 loop differing from the large loops interacting with tRNA^{Asp} identity determinant C_{36} in conventional D-AspRSs (161). More generally, these features are accompanied by additional

idiosyncrasies in archaeal-type ND-AspRSs (625), but remain more elusive in bacterial-type ND-AspRSs (220, 221). Although of the same nature, the recognition of $tRNA^{Gln}$ and $tRNA^{Glu}$ by ND-GluRSs appears more intricate, especially since ND-GluRS has lost the ability to aminoacylate cognate $tRNA^{Glu}$ (77, 78) and since the nonconservation of the glutamine G_{73} identity determinant that can be A_{73} in $tRNA^{Glu}_2$, as in *H. pylori* and in *Archaea* (19). A structural basis accounting for the relaxed specificity of ND-GluRSs is brought by the crystal structure of the enzyme from the thermophilic cyanobacterium *Thermosynechococcus elongatus* (***2cfo***) (626). As expected, it shares the overall architecture of a D-GluRS (that of *T. thermophilus* [142]) and shows a sievelike recognition of the third anticodon base N_{36} (C_{36} in $tRNA^{Glu}$ and G_{36} in $tRNA^{Gln}$). However, while in D-GluRS from *T. thermophilus*, Arg_{358} recognizes C_{36}, it is Gly_{366} that fulfills this role in ND-GluRSs. This amino acid change, conserved in most other ND-GluRSs, triggers relaxation, because the smaller glycine allows both small C_{36} and bulkier G_{36} to be tolerated by the protein (626). In agreement, homologous Arg_{350} from the ACB region in *H. pylori* D-GluRS rejects $tRNA^{Gln}$ for glutaminylation (77).

For reasons of functional efficiency, specificity, and evolution, one can conjecture that biosynthesis of asparaginyl-$tRNA^{Asn}$ or glutaminyl-$tRNA^{Gln}$ is coupled within supramolecular ternary aaRS:tRNA:AdT complexes in organisms utilizing the indirect tRNA-dependent pathway of asparagine or glutamine synthesis (Figure 4). Experimental support for this idea comes from the analysis of the crystal structure of archaeal *Methanothermobacter thermautotrophicus* GatDE complexed to $tRNA^{Gln}$ (***2d6f***) (627). This structure visualizes a 40-Å-long channel for ammonia transport connecting the active sites in GatD and GatE and reveals the interactions that the accepting branch and the D-loop of $tRNA^{Gln}$ make with GatE (627). In addition, the structure allows straightforward docking of GluRS for forming the aaRS:tRNA:AdT complex. Existence of such a ternary complex, called a transamidosome, is known for the asparagine (628) and glutamine (186) systems (Figure 4). Thus, the asparagine transamidosome assembles *T. thermophilus* ND-AspRS with cognate mischarged $tRNA^{Asp}$ and AdT (GatCAB) and remains stable during the aspartate to asparagine transformation process. By channeling the mischarged aspartyl-$tRNA^{Asn}$ intermediate between the ND-AspRS and AdT active sites, it prevents toxic interactions of the mischarged tRNA with elongation factor. Specificity of aspartyl-$tRNA^{Asn}$ amidation is conferred by the U_1–A_{72} pair of $tRNA^{Asn}$ and prevented on aspartyl-$tRNA^{Asp}$ by its G_1–C_{72} pair and U_{20a} in the D-loop that act as antideterminants (189). This scheme finds robust experimental support from crystallographic and solution data on the archael-type *T. thermophilus* asparagine transamidosome that is a particle formed by 14 macromolecular units, namely, two GatCAB trimers, two ND-AspRS dimers, and four asparaginyl-$tRNA^{Asp}$ molecules (***3kfu***) (187).

The distinct structure of the asparagine transamidosome from *Pseudomonas aeruginosa* (***4wj3***) (629) is representative of *Bacteria*, while that from *T. thermophilus* is representative of *Archaea*. In the *Thermus* archaeal-type structure, the Yqey domain of GatB contacts specifically the D-loop of $tRNA^{Asn}$ (Yqey refers to the name of the *Yqey* genes that code for a family of bacterial and yeast proteins of unknown function containing a multihelical domain consisting of two different α-helical bundles; because of the structural similarity of Yqey with the C-terminal domain of GatB—and with the mobile C-terminal extension in *D. radiodurans* GlnRS [363]—Yqey was renamed GatB/Yqey motif in the SCOP-hierarchy [http://supfam.org/SUPERFAMILY]). The larger D-loop structure of $tRNA^{Asp}$ prevents recognition by the Yqey domain of GatB and thereby contributes to the rejection of $tRNA^{Asp}$ from the transamidosome. In the bacterial-type *P. aeruginosa* asparagine transamidosome, the bacteria-specific GAD domain of ND-AspRS provokes a new architecture of the complex and both $tRNA^{Asn}$ molecules in the transamidosome simultaneously serve as substrates and scaffolds for the complex assembly (***4wj3***). This architecture rationalizes an elevated dynamic and a greater turnover of ND-AspRS within bacterial-type transamidosomes (629).

Since the same combination of nucleotides also determines specific $tRNA^{Gln}$-dependent formation of glutamine (189), it can be suggested that the glutamine transamidosome would operate as the asparagine transamidosome. If so, one is faced with the problem of a steric hindrance, since both class I ND-GluRS and GatCAB would recognize the acceptor stem of $tRNA^{Gln}$ by the minor groove side, implying that the transamidosome could not be formed. The crystal structure of the glutamine transamidosome from *T. maritima* shows how the dilemma is solved (***3al0***) (630). In contrast to the asparagine transamidosome where the accepting stem of $tRNA^{Asp}$ is contacted simultaneously by AspRS and GatCAB, it is the anticodon loop and

the outer corner of the L-shape of $tRNA^{Gln}$ that are simultaneously contacted by GluRS and GatCAB. This binding mode of $tRNA^{Gln}$ provides flexibility for alternative conformations that can flip from a productive glutamylation/nonproductive amidation state to a productive amidation/nonproductive glutamylation state, thereby evading the steric hindrance dilemma (630). Interestingly, a dynamic glutamine transamidosome was also characterized in the mesophilic *Bacteria H. pylori*, of which the assembly is consistent with the crystal structure of the *T. maritima* homolog (631). Hence, two strategies for asparaginylation are possible: either $tRNA^{Asn}$ binds GatCAB first, allowing aminoacylation and immediate transamidation once ND-AspRS joins the complex, or $tRNA^{Asn}$ is bound by ND-AspRS which releases the Asp-$tRNA^{Asn}$ product much slower than the cognate Asp-$tRNA^{Asp}$ (632). Formation of the *H. pylori* Asn-transamidosome is tRNA-independent and requires Hp0100, a protein component that stabilizes the dynamic transamidosome and additionally enhances the capacity of AdT to convert Asp-$tRNA^{Asn}$ into Asn-$tRNA^{Asn}$ (633).

Peculiar properties of SerRS in selenocysteine biology

Selenocysteine, isosteric to cysteine with the sulfur atom replaced by selenium, is the 21st amino acid in ribosome-mediated protein synthesis. Specific incorporation of selenocysteine is coded by UGA stop codons (under the control of SECIS elements [i.e., SElenoCysteine Insertion Sequences] within mRNAs coding for selenocysteine-containing proteins) that are read by an atypical tRNA called $tRNA^{Sec}$. Synthesis of selenocysteinyl-$tRNA^{Sec}$ occurs by an indirect pathway comprising $tRNA^{Sec}$ serylation by SerRS followed by conversion of serine to selenocysteine by a selenophosphate synthetase (634, 635). In *E. coli*, serylation efficiency of $tRNA^{Sec}$ is only ~1% that of the five canonical serine isoacceptors (636). This weak catalytic efficiency is explained by the atypical cloverleaf structure of $tRNA^{Sec}$ that differs from $tRNA^{Ser}$ by extended accepting- and D-arms as well as by a short D-loop with the consequence of an altered 3D-fold (637) that is explicitly seen in the crystal structure of human $tRNA^{Sec}$ (***3a3a***), particularly an absence of tertiary interactions between the D-stem and the long extra arm (638). A selenophosphate synthetase can than catalyze the tRNA-dependent conversion of serine to selenocysteine in a mechanism deciphered on the basis of the crystal structure of *A. aeolicus* selenophosphate synthetase (***2zod***) (630). In contrast to the situation of tRNA-dependent amidation, the serine to selenocysteine conversion in *Bacteria* likely proceeds in uncoupled reactions since $tRNA^{Sec}$ encompasses antideterminants in its acceptor arm (C_7–G_{66}/G_{49}•U_{65}/C_{50}–G_{64} box) that prevent interaction with elongation factor EF-Tu, but not with SelB, the specialized factor in *Bacteria* replacing EF-Tu in the selenocysteine pathway (639).

Aminoacylation of tmRNAs by AlaRSs

The transfer messenger RNA (tmRNA) system performs translational quality control in *Bacteria* and some organelles in a process called *trans*-translation (640). This system rescues stalled ribosomes and consists of AlaRS, EF-Tu/GTP, the SmpB factor (for Small protein B), and tmRNA. *E. coli* tmRNA is a chimera of ~360 nucleotides with modular and phylogenetically conserved structure (641). This chimera encompasses a 3′-$tRNA^{Ala}$-like domain connected via a pseudoknot-containing linker to a 5′-mRNA-like domain that encodes a short peptide used as a degradation signal. For rescuing stalled ribosomes alanyl-tmRNA enters the A site, thus allowing the incompletely synthesized protein to be transpeptidated on tmRNA and protein synthesis to be resumed on the mRNA-like moiety of the tmRNA. The tagged aberrant protein is then degraded by cellular proteases. The prerequisite is alanylation of tmRNA by AlaRS (642). Alanylation occurs because of the presence in the tRNA-like domain of tmRNA of residues homologous to the major G_3•U_{70} and A_{73} alanine identity determinants and of the modular arrangement of tmRNA allowing interaction with AlaRS. After alanylation, the charged tmRNA is complexed to EF-Tu (643) and is then ready for entering the ribosome. In this process, the SmpB factor (***1k8h***) (644) has a crucial role that is not entirely elucidated. It is a RNA-binding protein containing an OB-fold (a common structural motif present in many proteins with Oligonucleotide/oligosaccharide Binding capacity [218]) that interacts with tmRNA (***2czj***,***1p6v***) (645, 646) at each step of the rescue system, in particular, for increasing the alanylation capacity of AlaRS (640). Interestingly, the OB-fold of the SmpB factor resembles ribosomal protein S17, initiation factor IF1, and the N-terminal ACB domain of class IIb aaRSs (644).

BACTERIUM-LIKE AMINOACYL-tRNA SYNTHETASES

Organellar aaRSs

Mitochondria and chloroplasts are believed to be endosymbionts originating from *α-Proteobacteria* and

Cyanobacteria, which opens the question of the evolutionary origin of their aaRSs, since many of them (not all, e.g., human mt-GlyRS and mt-LysRS are eukaryal [647]) are considered to be bacterium-like (648). Organellar aaRSs are nuclear encoded and have intricate phylogenetic origins with gene duplication and horizontal gene transfer from bacterial endosymbionts to the *Eukarya* (649). This nuclear origin implies that their gene products are targeted toward the organelles. In plants, as documented for *Arabidopsis thaliana*, the same bacterium-like gene product is dual-targeted by different N-terminal signatures for delivery in either mitochondria or chloroplasts (650, 651). It is noteworthy that mt-aaRSs seem to exhibit a reduced catalytic efficiency for tRNA aminoacylation in comparison with that of the bacterial or cytosolic homologs, as explicitly shown, e.g., for several yeast (104) and mammalian mt-aaRSs (647, 652).

It is not within the scope of this essay to cover in depth the field of organellar aaRSs. However, peculiar structural and functional features of a few typical mt-aaRSs (mt-AspRS, mt-LeuRS, mt-PheRS, mt-ThrRS, and mt-TyrRS) should be pointed out. Thus, mt-PheRSs present atypical monomeric structures idiosyncratic to mitochondria as first observed for the *S. cerevisiae* mt-PheRS (103, 104) and confirmed by crystallography for the human enzyme (***3cmq***) (103), in sharp contrast with bacterial and other cytosolic PheRSs that are $(\alpha\beta)_2$ tetramers. On the other hand, *S. cerevisiae* mt-ThrRS recognizes two $tRNA^{Thr}$ isoacceptors with distinct anticodon loops. Remarkably, the crystal structure of the complex with $tRNA_2^{Thr}$ shows contacts with only the second and third anticodon base in contrast with the orthologous bacterial ThrRS that reads the entire anticodon sequence (***4yye***) (653). As to human mt-AspRS, functional studies have shown a greater sensitivity to adenylate analogs than bacterial AspRSs (one adenylate analog provides ~500-fold stronger competitive inhibition). Although the catalytic site of this mt-AspRS is almost identical to that of *E. coli* AspRS (654), it distinguishes by thermodynamic properties (655).

Furthermore, this mt-AspRS is not sensitive to identity position 73 and recognizes a minimalist identity set in cognate $tRNA^{Asp}$ restricted to the GUC anticodon. This result is in contrast to bacterial AspRSs, where G_{73} is a universally conserved major aspartate determinant (656). The reason for the relaxed specificity lies in two amino acid replacements in motif 2 within the AspRS catalytic site allowing accommodation of any base in the G_{73} binding pocket. Likewise, human and other mammalian mt-TyrRSs disobey the tyrosine identity rules (290) and rely more on A_{73} for specifying tyrosine than on the otherwise universal N_1–N_{72} identity pair. This result is in agreement with the crystal structure of human mt-TyrRS (***2pid***) (657). These restrictions in aminoacylation identity sets could result from reverse evolution of identity elements in mt-tRNAs because of the rapid mutagenetic rate of the mitochondrial genomes (656, 658). Another example of an uncommon property comes from the editing capacity of *Neurospora crassa* mt-LeuRS. In fact, this yeast enzyme is competent for posttransfer editing like other LeuRSs. However, mutants with altered LeuRS editing capacity do not dramatically affect yeast viability and mitochondrial function in the presence of high levels of nonleucine amino acids, in contrast to *E. coli*, in which the editing-defective mutations limit cell viability. It is possible that yeast mitochondria have evolved to tolerate lower fidelity in protein synthesis or have developed alternate correction mechanisms (659). On the other hand, this LeuRS, besides being an actor in mt-protein synthesis, acts in intron RNA splicing, an activity requiring the C terminus of the protein (660, 661). Likewise, another *N. crassa* mt-aaRS, namely TyrRS, is a splicing factor and, as shown by a cocrystal structure (***2rkj***), binds the intronic RNA across the two subunits of the TyrRS but at a side opposite from that which binds $tRNA^{Tyr}$ (662).

aaRSs and aaRS-Like Proteins in Cell-Wall Biogenesis and in Other Secondary Metabolic Pathways

It has been known since the 1960s that an atypical $tRNA^{Gly}$ species from *S. aureus* can be aminoacylated by canonical GlyRS and participates in cell-wall peptidoglycan synthesis (663, 664). Mirroring this finding, atypical aaRSs involved in nonribosomal peptide synthesis pathways were identified more recently in various *Bacteria*. Thus, a CysRS paralog is involved in mycothiol (1-D-*myo*-inosityl-2-[*N*-acetyl-L-cysteinyl]amido-2-deoxy-α-D-glucopyranoside) biosynthesis in *M. smegmatis* (***3c8z***) (665) and LysRS and AlaRS paralogs in *P. aeruginosa* or *Clostridium perfringens* participate in tRNA-dependent phospholipid aminoacylation (666, 667, 668). Another atypical aaRS-like protein, named MurM (Muropeptide factor M), present in *Streptococcus pneumoniae* attaches L-serine or L-alanine (provided by seryl-$tRNA^{Ala}$ and alanyl-$tRNA^{Ala}$) to the stem peptide lysine of Lipid II in cell-wall peptidoglycans (669).

Furthermore, peptide bond-forming systems that depend on tRNA and aaRS-like proteins (CDPSs or Cyclo DiPeptide Synthases) were identified in several *Bacteria*, such as *B. subtilis*, *Streptomyces noursei*, *Mycobacterium tuberculosis* (670), and *Bacillus licheniformis* (671). In addition, paralogous versions of MetRS are widespread in *Bacteria* (672). Remarkably, the CDPS from *M. tuberculosis* that uses tyrosyl-tRNA^{Tyr} as substrate to catalyze the formation of cyclo(L-Tyr–L-Tyr), demonstrates a structural resemblance with the catalytic domain of class Ic TyrRS (***2x9q***) (673). Likewise, AlbC from *S. noursei* (the protein coded by the third gene "C" of the antibacterial peptide Albonoursin biosynthesis operon acting at the first step of the biosynthetic pathway [674]) is one other member of the CDPS family and is similar to the catalytic domain of class Ic aaRSs (***3oqv***). Unprecedented, however, this monomeric protein uses phenylalanyl-tRNA^{Phe} and leucyl-tRNA^{Leu} to synthesize cyclo(L-Phe–L-Leu) (675) instead of tyrosyl-tRNA^{Tyr} that is typically formed by a dimeric TyrRS. In the case of *S. noursei* AlbC, the two charged tRNA substrates are accommodated in different binding sites and not all leucyl-tRNA^{Leu} isoacceptors are used as second substrate (676). The same mimicry with class Ic aaRSs exists in a CDPS from *B. licheniformis* that catalyzes cyclodileucine formation. The crystal structure of this CDPS (***3oqh***) (designated YvmC-Blic according to the name of the third gene "C" in the gene cluster *yvm* of the cyclodileucine biosynthetic pathway and its origin from *B. licheniformis*) suggests that all CDPS enzymes share a common aaRS-like architecture and a catalytic mechanism involving the formation of an enzyme-bound intermediate (671).

Other paralogous aaRSs were identified in a few peculiar *Bacteria*, where they have key roles in unexpected metabolic pathways. An example is a SerRS paralog from *Streptomyces viridifaciens* that provides seryl-tRNA^{Ser} in the biosynthetic pathway of valinimycin (a cyclic decapeptide antibiotic, so named because it contains valine residues) (677). Another example comes from the radiation-resistant bacterium *D. radiodurans* that codes for an auxiliary TrpRS that contains an N-terminal extension similar to that of proteins involved in stress responses (678, 679). This TrpRS-2 is induced during responses to radiation damage and binds stoichiometrically to nitric oxide synthase, and, furthermore, it efficiently charges tRNA^{Trp} with 4-nitrotryptophan and 5-hydroxytryptophan (84, 680). The crystal structures of this TrpRS, either the *apo*-form (***2a4m***) or ATP-bound (***1yid***) and tryptophan-bound (*1yi8*) versions show overall structure similarity to standard bacterial TrpRSs but reveal also several idiosyncrasies, such as smaller amplitude motions of the helical ACB domain upon substrate binding and atypical tryptophan recognition. Interestingly, the loop conformations of the ACB domain resemble more those of human TrpRS than those of *B. stearothermophilus* TrpRS, indicating different modes of tRNA recognition in TrpRSs (678). The biological function of *D. radiodurans* TrpRS-2, also found in six other bacterial genomes (678), remains elusive. The 4-nitrotryptophanyl-tRNA^{Trp} is probably not used in protein synthesis but rather participates in the biosynthesis of metabolites such as thaxtomin, a dipeptidic phytotoxin containing a nitrotryptophan moiety produced by *Streptomyces* species (681). This unusual activity of *D. radiodurans* TrpRS-2 is a nice example of an additional aaRS function that provides a link between tRNA and nitric oxide metabolism (84).

The existence of other bacterium-like aaRSs remains an open question since genomes have not been systematically screened for such proteins. From considerations on aaRS evolution, gene transfer processes, and metabolomics, it can be anticipated that such proteins exist and wait to be identified.

PARALOGS OF BACTERIAL AMINOACYL-tRNA SYNTHETASES

The modular architecture of aaRSs recapitulates how addition of novel functional domains to the primordial catalytic cores led to their modern structures. This modularity implies, first, that isolated domains within aaRSs can be produced as stable proteins that may have conserved functionality (this has been amply documented, especially for editing domains; see above) and, second, that paralogs of aaRS domains may be present in extant organisms (371, 682). On the other hand, because aaRSs are among the oldest proteins, one would expect that memory of structural elements present in aaRSs is imprinted in contemporary proteins that participate in functions differing from tRNA aminoacylation in protein synthesis (371, 682, 683, 684). Note that several such proteins were discovered in the frame of structural genomics programs aiming to understand proteins of unknown function, as was, e.g., the case of the YbaK (a *trans*-editing protein related with the editing domain of ProRS) and YadB (a paralog of the catalytic domain of GluRS) proteins (452, 533).

Mimicry of Catalytic Domains

At present, paralogs of the catalytic domain of 10 aaRS specificities (namely, 5 class I mimics [CysRS, GluRS, MetRS, TrpRS, and TyrRS] and 5 class II mimics [AlaRS, AspRS, HisRS, LysRS, and SerRS]) have been characterized in *Bacteria*. These proteins participate in a variety of functions that are seemingly unrelated, but that all necessitate activation of amino acids. For the paralogs participating in nonribosomal peptide synthesis (AlaRS, CysRS, LysRS, MetRS, and TyrRS), in antibiotic biogenesis (SerRS), and in tRNA mischarging by two tryptophan analogs (TrpRS), see "aaRSs and aaRS-like proteins in cell-wall biogenesis and in other secondary metabolic pathways," above.

Catalytic site mimicry of class I aaRSs

Bacterial YadB proteins are paralogs of the catalytic domain of GluRSs. These proteins were identified in various bacterial phyla, including proteobacterial, deinococcal, and cyanobacterial genera (239, 685). The *E. coli* protein has 34% sequence identity with *E. coli* GluRS, and its crystal structure (***1nzj***, ***2zlz***) (533, 686) shows the canonical fold of the GluRS catalytic domain (180). This protein activates glutamate and charges activated glutamate on tRNA (534), but, in contrast to canonical GluRSs, the activation step is tRNA independent, probably because of the replacement of a critical tryptophan in GluRS by a leucine (Leu_{194}) in YadB, thereby allowing proper binding of ATP without tRNA-aided adaptation of the catalytic pocket (533). The accepting tRNA, however, is not $tRNA^{Glu}$ but instead bacterial $tRNA^{Asp}$ where glutamylation occurs on a *cis*-hydroxyl group of a ribose mimic at position 34 of its anticodon, namely, the dihydroxycyclopentane ring of the Q-base (or queuosine, a 7-deazaG-derivative) forming a labile ester linkage (685, 687). Thus, YadB is a tRNA modification enzyme (renamed Glu-Q-RS for Glutamyl-Q-tRNA^{Asp}-Synthetase) and a new member of the group of enzymes that incorporate amino acid moieties in anticodon loops, such as lysine or threonine moieties, respectively, on C_{34} or A_{37} of certain tRNAs (319, 320, 688). It is likely that the function of Glu-Q-RSs is reminiscent of the early evolution of tRNA aminoacylation systems when primordial tRNA was restricted to minihelices. Although Glu-Q-RS and GluRS differ widely in function, they share strong structural resemblance within their catalytic core and quasi-equivalence of their zinc-binding sites (a SWIM domain [$Cys_{98}Cys_{100}Cys_{125}His_{127}$] in *E. coli* GluRS slightly altered [$Cys_{101}Cys_{103}Cys_{119}Tyr_{115}$] in *E. coli* Glu-Q-RS with His replaced by Tyr). While zinc is crucial for the positioning of $tRNA^{Glu}$ on GluRS, it contributes to enhanced protein solubility and decreased aggregation in Glu-Q-RS (689).

Other proteins related to the catalytic domain from class I aaRSs, in particular, that of TyrRSs, but containing only altered HIGH signature motifs (the HIGH sequence is one of the class I signature involved in ATP recognition), are the Glycerol 3-phosphate Cytidyl-Transferases (GCTs) and the ATPsulfurylases (ATPS). These enzymes catalyze reactions that are very different from tRNA aminoacylation (GCTs participate in the biosynthesis of lipids and intricate carbohydrates and use CTP instead ATP to activate their alcoholic or sugar substrates, and ATPSs catalyze the first step of the incorporation of inorganic sulfate into the precursors of cysteine and methionine). In addition they present various types of oligomeric structures in sharp contrast to monomeric class I aaRSs. Despite these peculiarities, both GCT and ATPS families recognize bound CTP or ATP via interactions with the conserved HxGH signature motif, as is the case of ATP recognition by class I aaRSs. Crystal structures of *B. subtilis* GCT (***1coz***) (690) and bacterium-like *S. cerevisiae* ATPS (***1g8h***) (691) support these assumptions. In summary, aaRSs, GCTs, and ATPSs utilize the same active site and catalytic strategy with virtually identical positions of their HxGH signature, suggesting that the three families diverged from a common ancestor (682).

Knowledge of the structure of class I aaRSs and the plausible assumption that primordial proteins had simplified structures provided the conceptual background for the design of a minimal TrpRS as a prototype of class I minimalist/ancestral aaRSs. The resulting 130-residue protein (embedding the HIGH and KMSKS signatures and a short inserted CP1 domain) retains substantial tryptophan-dependent PP_i exchange activity (28, 198). The nucleotide sequence of this minimalist catalytic domain based on the gene of *B. stearothermophilus* TrpRS (named TrpRS urzyme [198]) shows significant antisense complementarity with the sequence of class II *E. coli* HisRS. It is most interesting that an artificial construct of the anticipated HisRS urzyme shows catalytic activity for histidine activation (692). Altogether, this antisense complementarity gives support to the appealing hypothesis that class I and class II aaRSs arose opposite one another on the same ancestral gene (in other words, that primordial class I aaRSs would be complementary replicas of class II aaRSs) (31, 693, 694).

Catalytic site mimicry of class II aaRSs

An *E. coli* protein similar to an isolated catalytic domain of AspRS is an asparagine-biosynthesizing enzyme (***11as***) (695). Such asparagine synthetases (AsnA) present in *Bacteria* and *Archaea* have a reaction mechanism implying formation of an aspartyl-adenylate intermediate in agreement with the conserved architecture of the catalytic sites in AsnA and AspRS (695). It is likely that these proteins have evolved from a common AspRS ancestor even though their sequence similarities are small (696). However, in contrast with YadB (see above), AsnA proteins have lost the capacity to transfer the activated amino acid to tRNA.

Various *α-* and *γ-Proteobacteria*, such as *E. coli* and *S. enterica*, contain a gene (called *genX*, *poxA*, or *yjeA*) coding for the catalytic domain of class II LysRSs (86, 531, 532). In *S. enterica*, mutants of *poxA* are associated with multiple phenotypes, including decreased growth rate, hypersensitivity to herbicides and amino acid analogs, and decreased pathogenicity (86). Bacterial PoxA proteins (also referred to as YjeA and GenX) are paralogs of class IIb LysRSs (***3g1z***, ***3a5y***). Importantly and unexpectedly, they specifically aminoacylate bacterial EF-P with lysine via a lysyl-adenylate-dependent reaction, but are unable to aminoacylate $tRNA^{Lys}$ despite a great resemblance with the catalytic core of canonical LysRSs (229, 697). Lysylation occurs on a highly conserved Lys_{34} of EF-P and is followed by isomerization of Lys_{34}-α-Lysine catalyzed by YjeK that leads to Lys_{34}-β-Lysine. This pathway is analogous to the two-step modification of the eukaryal EF-P homolog, eIF5A that generates hypusine (a polyamide-derived unusual amino acid only found in *Eukarya* and a few *Archaea*) (698). Interestingly, the crystal structures of the free (with bound AMP [***3g1z***] or adenylate [***3a5y***]) and of the EF-P:adenylate bound (***3a5z***) paralog, together with a structure-based sequence comparison of the paralogs with the catalytic domain of bacterial LysRSs account well for the lysylation of EF-P at a position mimicking the tRNA-accepting end and for the lack of lysylation of tRNA as the result of amino acid replacements in the catalytic domain (229, 697). The Lys_{34} modification of EF-P is essential for cell survival and likely plays a regulatory role in translation (229, 697, 699).

HisZ is based on the catalytic domain of HisRS and is widely represented in *Bacteria* (but not in *E. coli*) and *Archaea*. This HisRS paralog lacks aminoacylation activity, but, in contrast, is the allosteric regulator of ATP PhosphoRibosyl-Transferase (ATP-PRT), the first enzyme of the histidine biosynthesis pathway where it joins ATP and 5-PhosphoRibosyl-1-PyroPhosphate (PRPP) (700). Structural and functional studies shed light on the evolution and activity of this enzyme of hetero-octameric organization with four HisG catalytic subunits and four HisZ regulatory subunits. Structures of ATP-PRTs under two functional states (with or without PRPP), respectively, from *Lactococcus lactis* (***1z7m***, ***1z7n***) (701) and of the HisG/HisZ complex from *T. maritima* (***1usy***) (702) reveal a phosphate ion located in the HisG/HisZ interface and a total of eight histidine binding sites located within these interfaces in a region highly conserved between HisZ and HisRS. Steady-state kinetics indicates that only the complete octameric complex is active and noncompetitively inhibited by the pathway product histidine (626). Crystal structures of *E. coli* ATP-PRT have been solved in complex with the inhibitor AMP and the product PR-ATP (PhosphoRibosyl-ATP, with the PR-moiety linked to adenosine N2 atom) (***1h3d***, ***1q1k***) (703). They clearly identify AMP in the PRPP-binding site, with the adenosine ring occupying the ATP-binding site. Comparison with two structures of the ATP-phosphoribosyltransferase from *M. tuberculosis* (*apo*-form [***1nh7***] and enzyme in complex with inhibitor histidine and AMP [***1nh8***] [704]) indicates that histidine is solely responsible for the large conformational changes observed between the hexameric forms of the enzyme (703).

BirA is a biotin ligase with regulatory properties of its own gene that comprises a domain having striking structural similarity with the catalytic domain of SerRSs (705) and more generally of the catalytic domain of class II aaRSs. Note also the similarity of BirA with domain B8 of the large and noncatalytic β-subunit of PheRSs (236). While the crystal structure of *E. coli* BirA (***1hxd***) (706) clearly shows a biotin adenylation site resembling the catalytic domain of class II aaRSs, it reveals also the absence of structural features associated with amino acid binding and monomer dimerization, thus explaining the monomeric state of BirA proteins in contrast to class II aaRSs that are dimers or multiples of dimers.

Intriguing is the discovery in various bacterial species of a novel group of enzymes that are similar to class II aaRSs and transfer activated amino acids to the phosphopantetheine group of small Carrier Proteins, and therefore were named aa:CP ligases. Some of them are truncated SerRSs homologous to the catalytic domain of the atypical SerRSs found in methanogenic *Archaea*, others derive from the catalytic domain of AlaRS and GlyRS (707, 708).

These proteins have relaxed amino acid specificity and lack tRNA aminoacylation activity. In contrast, they transfer activated amino acids to the phosphopantetheine prosthetic group of putative carrier proteins. A representative member from the bacterium *Bradyrhizobium japonicum* (a microsymbiotic nitrogen-fixing *Proteobacteria* in legume-root nodules) has been functionally and crystallographically characterized. It activates preferentially glycine instead of serine and deviates slightly from the canonical SerRS catalytic domain, although it presents the characteristic signature motifs of class II aaRSs and the canonical Zn-binding site of SerRSs (***3mf2***, ***3mey***, ***3mf1***) (707). A series of crystal structures of *B. japonicum* Gly:CP ligase in functional complexes with the carrier protein from *B. japonicum* (***4h2s***, ***4h2t***, ***4h2u***, ***4h2v***) and *Agrobacterium tumefaciens* (***4h2w***, ***4h2x***, ***4h2y***) show a fundamentally different binding of the carrier protein compared to the tRNA binding of the structurally related aaRSs (708). These structures reveal how a conserved class II aaRS catalytic core can adapt to another function through minor structural alterations (708).

Editing site mimicry of class II aaRSs

Ybak, ProX, and AlaX factors are discussed in "Error correction" under "Aminoacylation of tRNA," above.

Mimicry of tRNA Binding Domains

The OB-fold with β-barrel architecture is a widespread RNA binding motif, notably in aaRSs, where it is present under various versions in both class I and class II enzymes. For example, it was found in bacterial and eukaryal MetRSs and in class IIb aaRSs, where it is the canonical ACB domain. OB-folds are also present in aaRS-related yeast Arc1p and mammalian p43 proteins (Arc1p, a cofactor for MetRS and GluRS, is homologous to the auxiliary p43 protein of the multi-aaRS MARS complex [709]) and in EMAP II cytokines whose structure is conserved in the B2 domain of the β-subunit of heterotetrameric PheRSs. The structure of the isolated OB domain of *E. coli* LysRS (***1krs***, ***1krt***), either free or in complex with polyU, was solved by NMR (710). It is identical to that of native *E. coli* LysRS (224) and other class IIb aaRSs and shows striking similarity to the nuclear ribonucleoprotein U1A (711). Interestingly, the complex with polyU identified the amino acids important for binding the UUU anticodon of $tRNA^{Lys}$ (710).

Trbp111 and its ortholog CsaA are other proteins related to aaRSs that are present in various *Bacteria* and *Archaea*. Trbp111 is a dimeric protein (2x111 amino acids) discovered in the hyperthermophilic bacterium *A. aeolicus*. As pinpointed by its name, Trbp111 is a tRNA-binding protein interacting with any kind of tRNA (712). Its crystal structure and that of the *E. coli* ortholog show a classical OB-fold in the core of the monomer (***1pyb***, ***3ers***) (713). Docking and solution data are consistent with a 2:1 Trbp111:tRNA complex with tRNA recognition occurring through its T-stem opposite to the concave site recognized by the aaRSs (713, 714). Given this functional property, it is likely that the Trbp111 domain in MetRS (see "Structure of aminoacyl-tRNA synthetases," below) acts in *cis* to increase the affinity of cognate $tRNA^{Met}$.

Bacterial CsaA secretion chaperones are dimeric proteins (2x ~110 amino acids) with export-related activity that share sequence and conformational homology with Trbp111 and the C-terminal domain of archaeal MetRSs (715). Several crystal structures are available, notably from *T. thermophilus* (***1gd7***) (715) and *B. subtilis* (***2nzh***) (715) that show an OB-fold core with N- and C-terminal α-helical extensions involved in dimer formation. On the basis of the structural similarity with Trbp111, it has been proposed that CsaA proteins possess a tRNA-binding ability (716) that still remains elusive.

Given the ancestry of tRNA, it was not unreasonable to conjecture that many constitutive domains or subdomains of extant proteins including aaRSs, but also free-standing proteins recognizing tRNA (or tRNA domains) unrelated with the functioning of tRNAs in ribosome-mediated protein biosynthesis, should be present in proteomes. The few examples discussed above show the correctness of the anticipation. It is likely that many other examples will become available in the near future.

CONCLUSIONS AND PERSPECTIVES

The Present Status

The overview on aaRSs published in 1996 in the last printed EcoSal corpus already covered a large body of structural and functional results that beautifully and convincingly demonstrated the primordial role of aaRSs in translation (10). At that time, aaRS understanding became textbook knowledge and many scientists outside the field claimed that no paradigm shift would occur anymore with aaRSs so that only modest progress could be expected. However, since 1996, the science of aaRSs has made significant advances and has undergone great

mutations. The insight moved from essentially reductionist and classical genetics viewpoints toward more integrated cellular biology and physiology perspectives. Contrary to the common belief prophesying modest incremental progress, one has observed an abundance of new findings that amplified in the past decade. A number of anticipated new aaRS properties received robust experimental support, and new atypical and often unexpected structural and functional properties were discovered. On the other hand, unexplored topics became rejuvenated, as is the case of the biology of Ap_nA compounds produced and consumed by aaRSs (e.g., references 717 and 718). At present, aaRS research is invading all biology. Three issues deserve special comments. They are as follows.

(i) *The remarkable development of aaRS structural biology*. Indeed, a plethora of new X-ray structures (and some NMR structures) became accessible, particularly in the past 10 years (~20 structures known in 1996 corresponding to 6 amino acid specificities and 3 complexes with tRNA, compared with ~600 structures presently in the PDB). This trend was especially prominent in *Bacteria*, not only for canonical aaRSs but also for the wealth of paralogs discovered in an increasing number of sequenced genomes. The fact that structures of the same aaRS could be visualized under different conformational states and that structures from different organisms could be compared completely renewed our understanding of aaRS function and evolution. For several aaRSs, the mechanism of tRNA aminoacylation has reached a high degree of sophistication (18) (see details in "Aminoacylation of tRNA," above). In this respect, the example of the TrpRS from *B. stearothermophilus* is emblematic, since a precise structural and temporal description of the steps leading to tryptophan activation could be derived on the basis of a large panel of crystal structures (11 PDB entries in 2015 for the protein in complex with different small ligand associations) completed by molecular dynamics simulations and kinetic data on mutant proteins (328, 354, 365). Likewise, structural studies focusing on tRNA aminoacylation gave clues for more precise descriptions of the amino acid transfer on the 3′-terminal $-CCA_{OH}$ of tRNA (18) and, importantly, for explaining posttransfer editing, i.e., the aaRS-catalyzed hydrolysis of mischarged amino acids (e.g., references 248, 455, and 719). Also remarkable is the finding of dynamic coupling between editing and synthetic sites in class IIa bacterial-like ProRS (720). On the other hand, exploration of genomes demonstrated the universal presence of aaRS paralogs in living organisms and structural genomics established definitively the evolutionary origin of the modular architecture of aaRSs.

(ii) *The discovery of a broad variety of new functions for canonical aaRSs and their paralogs*. The new functions concern not only the translation machinery itself (e.g., for atypical tRNA or protein aminoacylation, for clearing errors) but also diverse metabolic pathways a priori unrelated to translation (e.g., for nonribosomal peptide bond synthesis, for atypical ester bond formations using activated amino acids) (see details and examples in the three preceding sections). In most cases, these new functions rely on subtle modifications of the catalytic sites of canonical aaRSs. This large diversity of functions illustrates how the invention of the two types of aaRS catalytic cores (class I and class II specific) and their tinkering during evolution was beneficial for life and how translation is intermingled with other cellular processes.

(iii) *Progress in aaRS gene regulation*. Various experimental evidence has indicated that quite a few *E. coli* aaRS genes may be inducible under cognate amino acid starvation conditions but at a level much lower than the corresponding amino acid biosynthetic operons under the same experimental conditions (reviewed in reference 479). In *E. coli*, the molecular mechanisms underlying these specific regulations are understood in only a limited number of cases and seem to vary from one aaRS to the other. For instance, the operon encoding PheRS is regulated by a leader peptide-dependent transcriptional attenuation mechanism similar to that of the *E. coli trp* operon, whereas the gene for ThrRS has been shown to be autoregulated at the level of translation in a way similar to that of ribosomal protein genes. In *E. coli*, a majority of the aaRS genes also seem to be under more global regulation, such as growth rate-dependent control. The situation is very different in *B. subtilis* and many other Gram-positive *Bacteria*, in which the induction levels after cognate amino acid starvation of several aaRS genes resemble those obtained with biosynthetic genes. Also, the control mechanism, common to most *B. subtilis* aaRS genes, is very different from those of *E. coli* aaRS genes.

In summary, the advances in aaRS structural biology, enzymology, and genomics, together with the deeper knowledge on aaRS genes, led to a more complete biology of bacterial aaRSs. These advances were also beneficial for a better understanding of the archaeal and eukaryal aaRSs, especially the human aaRSs in both their cytosolic

and mitochondrial versions. Moreover, progress in the aaRS field was paralleled by equally important progress in the tRNA field (e.g., references 264, 268, 721, and 722), both being the result of intensive interdisciplinary research efforts. From the viewpoint of applications, it is remarkable to realize how engineered aaRSs and aaRS domains are becoming important actors in biotechnology (e.g., as tools for the fabrication of proteins having incorporated nonnatural amino acids with focused chemical or biophysical/spectroscopic properties) and molecular medicine (e.g., for the search of new antipathogen drugs or for therapeutic use).

The Future of aaRS Research

It can be anticipated that the perspectives in the aaRS science will be flourishing, because they are founded on a state-of-the-art, robust theoretical, experimental, and methodological background. Interdisciplinary approaches will remain fundamental and will continue to combine reductionist and integrated approaches. Deciphering the systems biology of aaRSs will be one of the next challenges. The mechanisms underlying the expression and the regulation of aaRS genes in *Bacteria* are far from being understood in depth, and many questions remain unanswered, among others: Why is there such heterogeneity in *E. coli* control mechanisms? Why is the situation so different in *Enterobacteria* and *Firmicutes*? Why are the induction levels of aaRS genes so weak under starvation conditions in *E. coli*? Why is there a need for clearly inducible aaRSs in *B. subtilis*? Is there a general mechanism for global controls in *Enterobacteria*? What about global controls of aaRS synthesis in *Firmicutes*? If such controls exist, are they related, or not (723), to the mechanism of induction under starvation conditions? Likewise, understanding the biology of the aaRSs under normal and stress conditions presents other challenges. Finding answers implies understanding aaRS properties (e.g., enzymology, structural plasticity, supramolecular complexes, and characterization of partners, crowding—the effect of related or unrelated macromolecules on aaRS properties in crude mixtures/media—channeling, trafficking, regulation, degradation) under a variety of cellular conditions. Continuing to explore genomes will certainly reveal aaRSs and aaRS paralogs with new idiosyncratic properties and divulge novel connections with metabolic pathways. Moreover, deep sequencing of genomes will also enable establishment of the polymorphic sequence variations in specific aaRSs of given organisms and thus allow us to distinguish toxic from polymorphic mutations. Finally, and because of their ancient origin, aaRS sequences will remain markers of choice for refined structure-based taxonomies (14, 68) worth being compared with phenotype-based taxonomies. Studies of aaRS phylogenies will provide renewed understanding of the origin and evolutionary history of life on Earth. Despite countless speculations many questions remain essentially open. Thus, one would like to know the molecular identity of the progenitor of the first aaRS urzyme in a prebiotic world (RNA, proteic, or mixed?). How did this molecule acquire specificity? What is the connection between the proteic urzyme and ribozyme catalysts for RNA acylation? Why are there two classes of aaRSs? Was primordial life possible with aaRSs from only one class? Was class II before class I, or vice versa, or did the two classes emerge together? Class II aaRSs could have been first since they are specific for amino acids with broader chemical diversity than class I aaRSs and, therefore, better suited for catalysis and have kept memory of ancestral capacity to aminoacylate tRNA minihelices, but other arguments would favor the opposite possibility. Answering these questions will be challenging, but new experimental data are expected to insightfully enrich the ongoing debates on the emergence of aaRSs in life (2, 14, 27, 30, 34, 37, 198, 705, 724, 725, 726, 727).

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