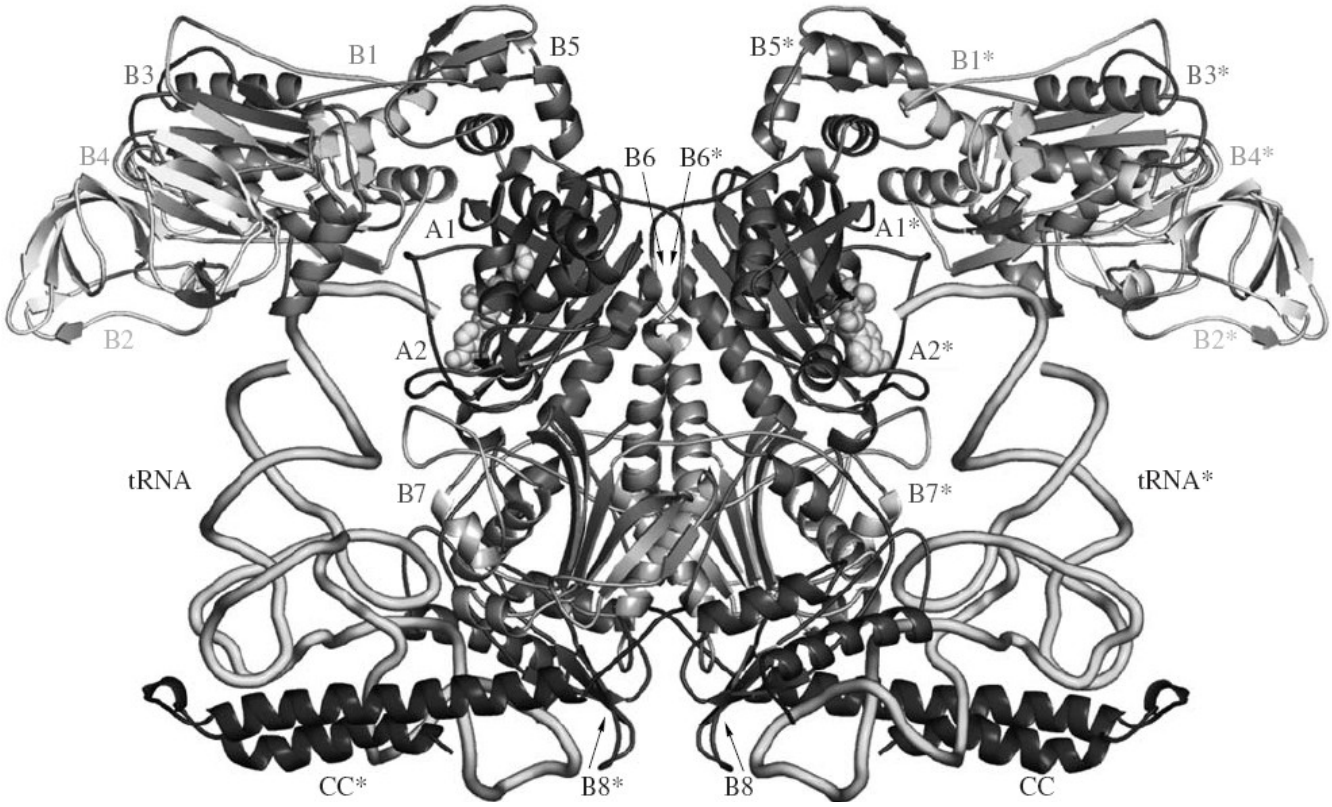

Multi-functionality of aminoacyl-tRNA synthetases

An example of biological complexity

Bachelor Scriptie
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Abstract

The role of aminoacyl-tRNA synthetases (aaRSs) in translation is known by everyone in the field of molecular biology. What people often do not realize, however, is that aaRSs are involved in a lot of processes apart from translation. AaRSs are involved in processes like transcriptional and translational regulation, antibiotic resistance, synthesis of unnatural amino acids, cell signaling and many more. This multi-functionality of aaRSs occurs in all three domains of life. Here I will discuss the extend of this multi-functionality in bacteria. I will compare the complexity of bacterial aaRS multi-functionality to that of eukaryotes. Finally I will describe new insights in bacterial complexity obtained from interactome studies. This literature study shows that bacterial aaRS multi-functionality is a lot less complex than that of eukaryotes. The most important point, however, is that the complexity of the functions of bacterial aaRSs may be drastically underestimated.

Contents

Picture on the Cover: *Thermus thermophilus* PheRS binding tRNA(Phe). Adapted from Safro and Moor, 2009 [1].

Introduction

Every molecular biologist is, or should be, familiar with the role that aminoacyl-tRNA synthetases (aaRSs) play in translation. What is unknown to most, however, is that besides their role in translation aaRSs, in all three domains of life, are involved in a wide variety of other processes. These are processes like transcriptional and translational regulation, cell cycle control, antibiotic resistance, synthesis of dipeptides, aminoacyl-tRNA editing, cell signaling and a lot more. Some of these processes are carried out by the aaRSs themselves. Others need additional proteins to form complexes with aaRSs.

In this text I will give an overview of the alternative aaRS functions and the complexes formed by aaRSs. First I will discuss all of the bacterial functions and complexes that exist (as far as I am aware). Following that I will discuss functions and complexes for eukaryotic aaRSs. The eukaryotic discussion won't be complete and merely serves to show the difference in complexity compared to the bacteria. Finally I want to make a case for a reconsideration of bacterial complexity using an interactome study done on the very simple bacterium *Mycoplasma pneumoniae*.

Knowledge about the different functions and interactions of aaRSs is probably of use to people that want to introduce unnatural amino acids (uaa's) into proteins. There are multiple ways to incorporate uaa's. This is usually done by introducing and overexpressing an aaRS from one species into another species. Then one switches the natural amino acid in the medium for the unnatural one. Alternatively a tRNA could be engineered to recognize a rare codon so the uaa can be genetically encoded [2]. In the discussion I will comment on the application of the knowledge presented in this text to uaa incorporation.

The questions I want to answer in this text are:

- What is the extend of the alternative functions of aaRSs in bacteria?
- How does this compare to the alternative functions of aaRSs in eukaryotes?
- As an example of the application of the knowledge presented here: What effect would these alternative functions of aaRSs have on the incorporation of unnatural amino acids?

The most important conclusion of this literature survey is that aaRSs are present in a vast network of alternative functions, linking the central process of translation to all kinds of other processes. This is definitely true for eukaryotes and also appears to be true for bacteria. It is at this moment impossible to predict the precise effect of changes made in the aminoacylation machinery. What can be said is that, because aaRSs are involved in many processes, a change in aaRS composition probably affects some other processes.

Background

Aminoacyl-tRNA synthetases (aaRSs) play a role in the proces called translation. In translation the sequence of nucleotides in the RNA is 'translated' into a sequence of amino acids. To understand the role that aaRSs are playing in translation first I will give an overview of the process of translation. As in this report the focus will be on bacteria I will explain only bacterial translation. After that I will explain the function of the aaRSs themselves.

Translation

The function of translation is to synthesize a polypeptide chain with the amino acids that make up that chain are in the sequence that is dictated by the mRNA. The whole proces is summarized in Figure 1. Translation starts with initiation. In the initiation phase a protein called initiation factor 2 (IF-2) forms a complex with a mRNA, GTP, and formylmethionine-tRNA (fMet-tRNA). Formylmethionine-tRNA is bound to a start codon on the mRNA, usually AUG. Two other initiation factors, IF-1 and IF-3, bind to the 30S ribosomal subunit to prevent premature association with the 50S subunit and to guide the IF-2 complex to the correct binding site (P site). After association of the IF-2 complex with the 30S subunit, the GTP bound to IF-2 is hydrolyzed and IF-1 and -3 dissociate. After that the full ribosome is formed by association of the 50S subunit. After the dissociation of IF-2 the elongation phase starts. In the elongation phase new amino acids are delivered to the ribosome aminoacyl site (A site) by an elongation factor called EF-Tu. The amino acids are delivered in the form of aa-tRNAs which are synthesized by aaRSs. When the anticodon on the aa-tRNA matches with the codon on the mRNA, EF-Tu dissociates. In the peptidyl transferase site (P site) a peptide bond is formed between formylmethionine and the other amino acid and in the process formylmethionine is released from its tRNA. The association of another elongation factor, EF-G, frees the A site so another round of elongation can take place. The peptide chain that is formed exits through a tunnel in the 50S subunit. This proces of elongation continues until a stopcodon is encountered. When the ribosome encounters a stop codon, no aa-tRNA binds. Instead a release factor binds which induces the release of the polypeptide chain from the ribosome. Finally the ribosome is recycled with the help from another group of proteins [3,4].

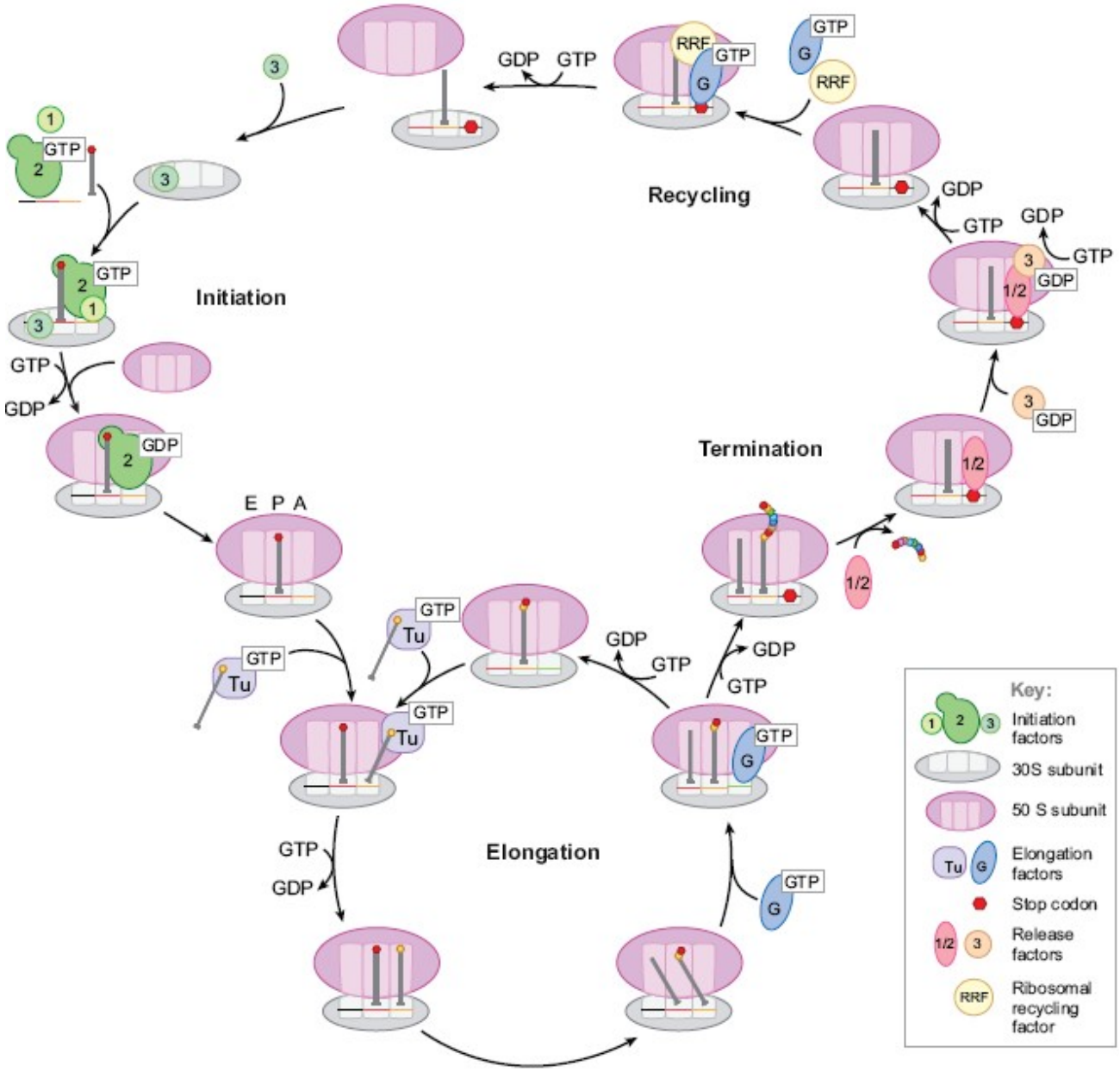

Figure 1: Schematic of prokaryotic translation. The figure is explained in the text. Adapted from Marshall, R.A., et al., 2008 [3].

Aminoacyl-tRNA Synthesis

Synthesis of the correct aa-tRNA, e.g. attaching amino acid Yyy to tRNA(Yyy), is catalyzed by the set of proteins called aminoacyl-tRNA synthetases (aaRSs). This process is what establishes the genetic code, it determines which amino acid is attached to which anticodon. In most bacteria there is an aaRS for every amino acid. AaRSs can be divided into two classes, class I and II. These classes have different folds and attach the amino acid (aa) to different positions on the tRNA. Class I aaRSs attach the aa's to the 3'-OH at the 3'-end and class II aaRSs attach he aa's to the 2'-OH at the 3'-end of the tRNA [5].

The reaction catalyzed by the aaRSs is called aminoacylation and consists of two steps. Both steps are carried out on the same aaRS. The first step is the activation of the amino acid using ATP. In this reaction an amino acid and ATP are converted to aa-AMP and PP_i. In the second step the amino acid is attached to the tRNA and AMP dissociates [5].

For a coupling of the specific tRNA to the correct amino acid the aaRS should be able to recognize specifically both the tRNA and the amino acid. TRNA's can be recognized, by the aaRSs, in a variety of ways. It are mainly the anticodon, acceptor stem and specific bases at the end of the acceptor stem (base 73) that are recognized. However, interactions at other places of the tRNA like the inside of the L-shape can also be important for recognition. In some cases it are modified nucleotides (e.g. methylated nucleotides) that are recognized. Not all aaRSs recognize all the parts just mentioned. In Figure 2 a tRNA molecule is depicted which recognition sites are highlighted [5].

To ensure that the correct amino acid is incorporated, which is rather difficult as some amino acids (e.g. isoleucine and leucine) are very similar, there exist two levels of selection. The first level is the recognition of the correct amino acid in the aminoacylation site and the second level is the recognition of the incorrect amino acid at a proofreading site on the same, or sometimes another, enzyme. An example of proofreading, or editing, is the case of Threonyl-tRNA synthetase (ThrRS). In the editing site of ThrRS Ser-tRNA(Thr), a misacylated aa-tRNA, is recognized and cleaved. Thr-tRNA(Thr), a correctly acylated aa-tRNA, is not cleaved [4, 5].

After the correct amino acid-tRNA has formed it can be transferred to EF-Tu that then associates with the ribosome [5].

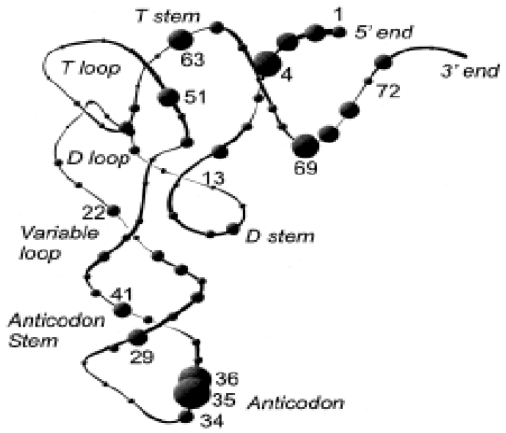

Figure 2: General architecture of a tRNA molecule. The size of the spheres indicates the frequency of involvement of the particular nucleotide in recognition by aaRSs. Adapted from Ibba and Soll, 2000 5.

AaRS multitasking in bacteria

Even in bacteria, thought to be very simple organisms, aminoacyl-tRNA synthetases have functions outside the realm of translation. Here I will discuss all functions and complexes found in the literature. A list is presented in Table 1. First I will discuss the alternative functions of aaRSs not associated with complex formation. After that I will discuss alternative functions associated with complex formation. Most aaRS functions described here are reported for only one or a few bacterial species. To what extend the different functions occur in other bacteria is in most cases not known. The aim of this section is to give a complete overview of alternative functions and complex formation of bacterial aaRSs.

Table 1: List of bacterial aaRS functions. All functions are discussed in the test.

Function	**Proteins(s)**	**Organism(s)**
Transcriptional regulation	AlaRS	*E. coli*
Translational regulation	ThrRS	*E. coli*
Cell cycle control (indirect)	CysRS	*B. subtilis*
Regulation of protein degradation	AlaRS	*E. coli* and a wide range of gram$^+$ and gram$^-$ bacteria
Synthesis of dipeptides	ArgRS, ValRS, IleRS	*E. coli*
Resistance against antibiotics	IleRS, TrpRS	*Streptomyces* (various species), *Pseudomonas fluorescens*
Using D-amino acids for aminoacylation	HisRS, TyrRS	*E. coli*
Editing of aa-tRNAs	ProRS, YbaK	*E. coli, H. influenzae*
Asn-tRNA synthesis	AsnRS, AdT	
Synthesis of 4-nitro-tryptophan	TrpRS, NOS	*D. radiodurans*
Multi-aaRS complex (function unknown)	Various aaRSs	*E. coli*

Alternative aaRS functions not associated with complexes

Transcriptional regulation Alanyl-tRNA Synthetase (AlaRS) from *E. coli* can inhibit the transcription of its own gene. This inhibition occurs when AlaRS binds to the promotor region. This transcriptional inhibition can be observed by looking at the mRNA concentration on gel after transcription in the presence of different concentrations of AlaRS. The higher the AlaRS concentration the smaller the amount of mRNA. A DNase protection experiment was done to verify that AlaRS binds to the promoter region of its own gene. The data shows that AlaRS is indeed able to protect parts of the promoter from cleavage. From the sequence and the DNase protection experiment it is clear that AlaRS binds at the same spot as RNA polymerase giving an explanation for the inhibitory effect. The inhibitory effect is enhanced by the

addition of alanine which enhances the affinity of AlaRS for the DNA [6]. This mechanism has only been found for AlaRS and only in E.coli.

Translational regulation Threonyl-tRNA synthetase (ThrRS) from *E. coli* is an inhibitor of the translation of its own mRNA. The mRNA, seen in Figure 3, mimics the tRNA substrate of ThrRS. The ThrRS dimer binds to two domains present on the mRNA each of which mimics a anticodon loop of tRNA(Thr). The ribosome binding site lies next to the ThrRS binding site. So ThrRS can be a competitive inhibitor of the ribosome. In crystal structures of ThrRS with tRNA(Thr) and of ThrRS with one of the, tRNA mimicking, mRNA domains you can see that the same residues are important for recognition in both substrates. Inhibition itself is mediated through the N-terminal domain of the ThrRS, probably by sterical hindrance. When the N-terminal domain is removed no inhibition is observed. When tRNA(Thr) concentrations are high the ThrRS will primarily bind to tRNA and not to the operator region on the mRNA. This means that ThrRS is synthesized when the levels of tRNA are increased. Thus levels of ThrRS and tRNA(Thr) are coupled. When tRNA(Thr) concentration goes up ThrRS concentration goes up and vice versa. Again like the mechanism for AlaRS this translational regulation is only found for this particular aaRS [7].

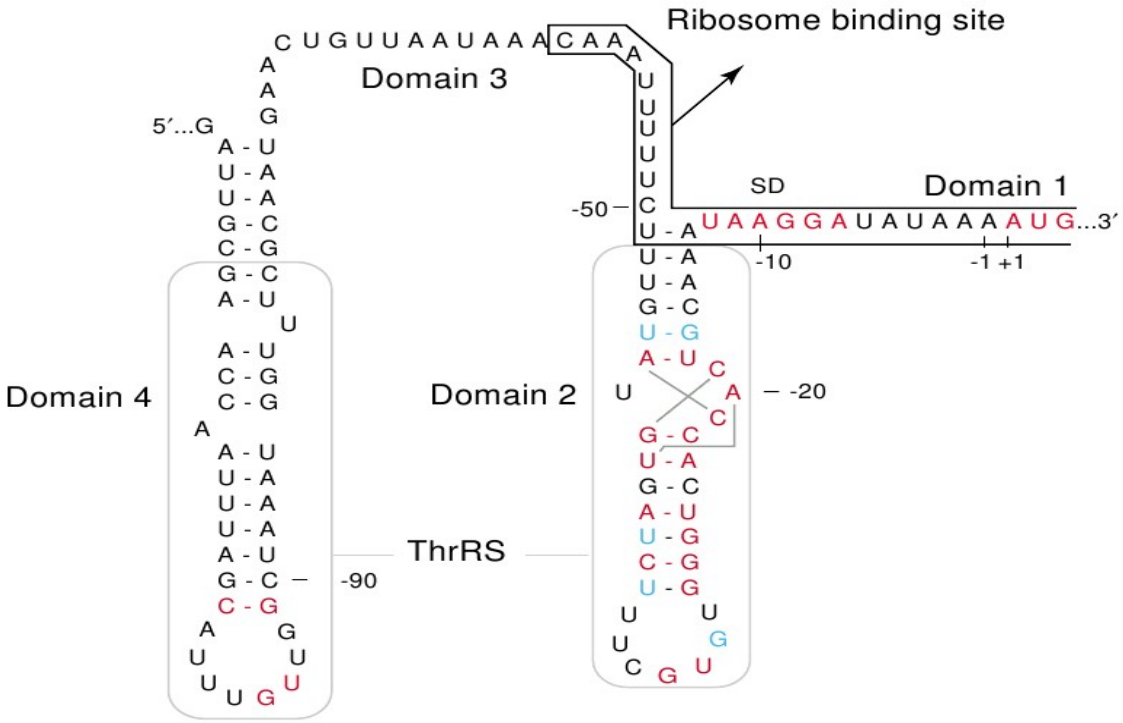

Figure 3: Operator region of the ThrRS encoding mRNA. Domain 2 and 4 mimic tRNA(tRNA), SD stands for shine dalgarno sequence. A box is drawn around the bases that form the ribosome binding site. Adapted from Romby, P., et al., 2003 [7].

Indirect cell cycle control Cysteinyl-tRNA synthetase (CysRS) from *Bacillus subtilis* appears to be involved, although indirectly, in the regulation of the cell cycle. Not much is known about how this works. A *B. subtilis* strain with a mutation in the CysRS gene has an altered cell cycle. The function of CysRS is probably the in the synthesis of a protein that is involved, directly, in cell cycle control. The concentration of this protein is lowered in the mutant strain [8]. This indicates the intimate connection the aaRSs have with the rest of the cell.

Regulation of protein degradation In *E. coli* a RNA, tmRNA, has been found that has both tRNA and mRNA properties. The tmRNA has a reading frame for a short peptide and a region which mimics the acceptor stem and one of the arms of the tRNA(Ala). It is thought that the tmRNA enters the ribosome when the ribosome has stopped translation at the 3'-end of a (truncated) mRNA. There the tmRNA adds a tag to the, already partly synthesized, peptide. The mRNA part of tmRNA serves as a template for the synthesis of the tag. The tag is a degradation signal, thus the tmRNA functions as a protection against, dysfunctional, truncated proteins. TmRNAs exist in a wide range of bacteria, both Gram positive and negative [9].

Synthesis of dipeptides Arginyl-tRNA synthetase (ArgRS), isoleucyl-tRNA synthetase (IleRS), and valyl-tRNA synthetase (ValRS) of *E. coli* are all able to deacylate their natural aminoacyl-tRNA. In this process a dipeptide is formed of arginine, isoleucine, or valine with cysteine. It has been suggested that the cysteine needed for the reaction can bind in the editing site of IleRS and ValRS. So a role of the aaRSs important for increasing the fidelity of translation can also be used to synthesize dipeptides. No physiological role of these dipeptides is reported [10].

Indolmycin resistance The antibiotics producing soil bacterium *Streptomyces coelicolor* contains two tryptophanyl-tRNA synthetase (TrpRS) enzymes. One of these enzymes offers resistance against the antibiotic indolmycin which is produced by *Streptomyces griseus*. Indolmycin selectively inhibits prokaryotic TrpRSs as it binds, competitively, to the tryptophan binding site. The TrpRS that offers resistance does not bind indolmycin and takes over the aminoacylation function when the cell is in a solution containing indolmycin. Without indolmycin in the medium only the normal TrpRS is expressed while in the presence of indolmycin the expression of the normal TrpRS is lowered and the resistant TrpRS is expressed [11]. The same TrpRS that offers resistance against indolmycin also offers resistance against another TrpRS inhibitor, chuangxinmycin [12]. The development of resistance against antibiotics by acquiring a second aaRS seems to be quite common. It has also been described for an IleRS in *Pseudomonas fluorescens* that offers resistance to pseudomonic acid [13] and for a SerRS in *Streptomyces sp.* that offers resistance against albomycin [14].

Using D-amino acids for aminoacylation It is often thought that no D-amino acids are present in the cell. This, however, is not the case as D-amino acids are used as a substrate by some proteins. It was shown for *E. coli* that HisRS and LysRS can attach D-amino acids to tRNAs. It is not known whether these D-amino acids are incorporated into proteins [36].

Functions of complexes containing aaRSs

A number of aaRSs associate with other proteins. For example a number of aaRSs in bacteria associate with the elongation factor EF-Tu which then transfers the aa-tRNA to the ribosome. Here a number of these interactions are described. Some of these interactions are important for translation others have a role other than translation. The first two functions discussed are related to translation but are different from the direct aminoacylation reaction that is seen as the main function of the aaRSs.

Deacylation of Cys-tRNA(Pro) As dicussed above some aaRSs have editing mechanisms to remove amino acids that have been attached to the wrong tRNA. In *E. coli* and *Haemophilus influenzae* ProRS can mischarge tRNA(Pro) with alanine and cysteine. It has an editing mechanism of itself though it is only able to deacylate Ala-tRNA(Pro). A non-aaRS editing protein, called YbaK, can deacylate Cys-tRNA(Pro). YbaK also deacylates other aaRSs, like Pro-tRNA(Pro), though much less efficient than Cys-tRNA(Pro). The hydrolysis of Cys-tRNA(Pro) is much faster than its synthesis. For Cys-tRNA(Cys), which is also

hydrolyzed by YbaK, the synthesis exceeds the hydrolysis. Cross-linking and fluorescence anisotropy experiments have shown that YbaK forms a complex with ProRS. It is likely that hydrolysis of Cys-tRNA(Pro) takes place when it is still bound to ProRS because EF-Tu protects Cys-tRNA(Pro) from hydrolysis and aa-tRNAs are always bound to protein [15-17].

Synthesis of Asn- and Gln-tRNA Generally, in a single organism, there is a specific aaRS for every amino acid. This however is not the case for a number of bacteria. Some bacteria don't have the asparagine- and glutamine-tRNA synthetases (AsnRS and GlnRS) and have to synthesize Asn-tRNA and Gln-tRNA via another mechanism. The mechanism adapted in a number of organisms is to first mis-acylate tRNA(Asn) with aspartate and then using an amidotransferase (AdT) to convert this Asp-tRNA(Asn) into Asn-tRNA(Asn).

The ester bond between the tRNA and the amino acid of the aa-tRNA is quite reactive so it quickly reacts if the aa-tRNA is unprotected. Binding of aa-tRNAs to EF-Tu can protect this ester linkage but EF-Tu doesn't have affinity for Asp-tRNA(Asn). So to protect the ester bond its was proposed that the AspRS, tRNA(Asn) and amidotransferase form a complex. Gel filtration, dynamic light scattering, static light scattering and native PAGE all indicate a complex is formed when AspRS, AdT and tRNA(Asn) are present. The stoichiometry of the complex is one AspRS dimer, two tRNA(Asn)s, and two AdT monomers. The complex formation did also occur in vivo [17, 18].

Synthesis of 4-nitro-tryptophan The bacterium *Deinococcus radiodurans* contains two tryptophanyl-tRNA synthetases, TrpRS I and TrpRS II. Both are able to aminoacylate tRNA(Trp) though TrpRS I is about five times as efficient. TrpRS II copurifies with nitric oxide synthase (NOS) which can synthesize NO by oxidizing L-arginine. Thus it was hypothesized that TrpRS II and NOS form a complex. NOS besides the synthesis of NO can bind and nitrate tryptophane to form 4-nitro-tryptophan.

A number of things indicate the formation of a TrpRs-NOS complex. The reaction rate of 4-nitro-tryptophan synthesis is increased about 3 to 4 times upon addition of TrpRS II. TrpRS II can also charge a tRNA(Trp) with 4-nitro-tryptophan. The affinity of the NOS for its substrate L-arginine increases when TrpRS II is added. Finally the fluorescence of fluorescent ATP analogues bound to TrpRS II is quenched upon addition of NOS. This quenching increases until a stoichiometry of 1:1 is reached for the proteins. All these things just mentioned indicate that a complex is formed and that the complex has a physiological function. Because a second TrpRS, which has a higher efficiency, is also present in *D. radiodurans* TrpRS II seems to be free for other functions than translation. The physiological role of the complex might be to perform one step in the synthesis of an unknown secondary metabolite [17, 19-21]. An interesting observation is that there are other bacteria that also contain two aaRSs for the same amino acid (see "Indolmycin resistance").

Multi aaRS complex In *E. coli* a multi-aaRS complex has been found using column chromatography. This complex dissociates quite easily and different complex sizes are obtained for different methods of breaking cells. The complex could be sedimented by centrifugation, just like eukaryotic complexes. This indicates that the complexes are not an artifact of the chromatography [17, 22]. The existence of a multi-aaRS complex in *E. coli* is still speculative and no further work on this complex is reported in the literature. In a recent interactome study, however, complexes containing of aaRSs were also observed (see "Existence of multi-aaRS complexes in bacteria").

AaRS multitasking in eukaryotes

Eukaryotes have an even more extensive repertoire of functions outside of translation than bacteria. This becomes especially evident when looking at the number of complexes containing aaRSs. The first part of this section is a summary of a number of complexes containing a single aaRS. Some of these complexes enhance certain aspects of translation, others have functions outside of translation. The second and third parts describe two complexes that contain more than one aaRS and that are in the center of a vast network of interactions and functions. No complete treatment nor a complete list is given of all aaRS functions or complexes found in eukaryotes. The main point of this section is to show that there are also alternative functions of aaRSs in eukaryotes and more importantly that the number and complexity of these functions is far greater than that observed for bacteria.

Simple functions

Pex21p In *Saccharomyces cerevisiae* pull-down experiments have shown that Seryl-tRNA synthetase (SerRS) and the protein Pex21p form a complex in vivo. Pex21p is important for peroxisome biosynthesis. There are three suggestions for the function of this aaRS containg complex. First, aminoacylation by SerRS is slightly enhanced by association with Pex21p. So Pex21p's function could be to regulate aminoacylation. Two other functions of this complex could be linking translation and peroxisome biosynthesis or to coordinate stress signaling. The idea that the SerRS-Pex21p complex plays a role in stress signaling gets credibility due to the fact that SerRS is also involved in the synthesis of stress signals (diadenosine oligophosphates) [17].

Knr4 In eukaryotes, as for bacteria [23], there seems to be a connection between aminoacylation and cell wall synthesis. In *S. cerevisiae* tyrosyl-tRNA synthetase (TyrRS) has been found to interact with and to be expressed at the same moment as Knr1 which is a protein that is involved in cell wall synthesis. It is suggested that because dityrosine is an important component of the cell wall that its synthesis is promoted by the interaction of TyrRS and Knr4 [17].

EF-1H The elongation factor EF-1H is thought to interact with a number of aaRSs. VaRS, LysRS, and MetRS are all thought to have enhanced aminoacylation activity when in complex with EF-1H. For PheRS and SerRS there is also thought to be an interaction though the function of this interaction seems to be chaperone-like [17].

Arc1p

In *S. cerevisiae* the protein Arc1p forms a ternary complex with both glutamyl- and methionyl-tRNA synthetase (GluRS and MetRS). Arc1p is a homologue of the p43 protein that is part of the mammalian multi-aaRS complex (see "multi-synthetase complex") and is thought to be a evolutionary intermediate of the multi-aaRS complex. The activity of GluRS and MetRS is increased upon binding Arc1p. Arc1p also increases the local tRNA concentration for GluRS and MetRS because it can bind to tRNA(Glu) and tRNA(Met). This tRNA binding capability makes Arc1p an important factor in tRNA trafficking between compartments and complexes in the cell.

The ternary complex can also be dissociated so the components can take part in other processes. When Arc1p is biotinylated it loses its capacity for binding GluRS and MetRS. It is thought that by releasing GluRS it can relocate into the mitochondria when the cell changes from fermentation to respiration. During respiration there is an increased need of translation in the mitochondria and GluRS could be the limiting factor. Arc1p also seems to be involved in telomere maintenance, transcriptional regulation and

ribosome biogenesis. So it is really in the middle of a whole number of processes as seen in Figure 4. GluRS itself also seems to have alternative functions as it can bind to damaged genomic DNA [24].

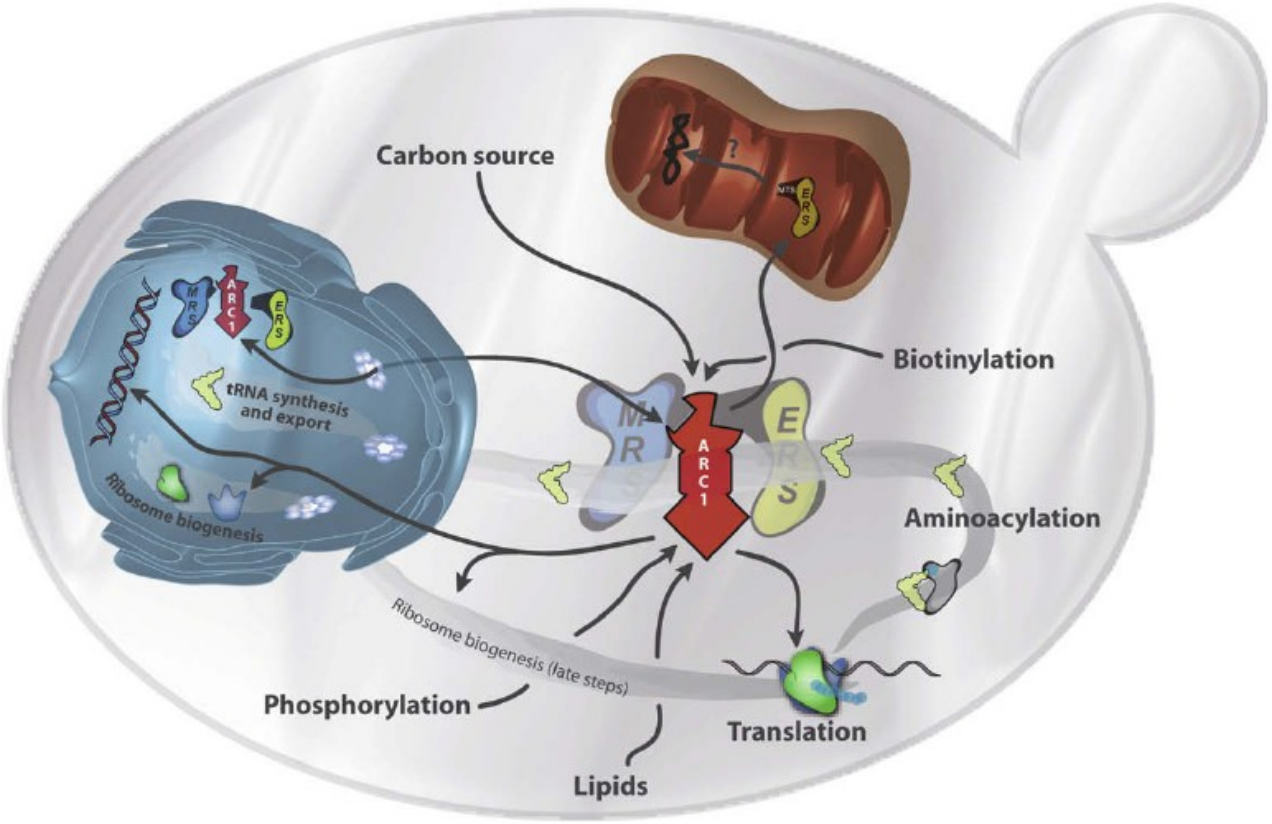

Figure 4: The central role of Arc1p. It is involved in telomere maintenance, transcriptional regulation, ribosome biogenesis, aminoacylation and a few other processes not mentioned in the text. Adapted from Frechin M, et al., 2010 24.

Multi-synthetase complex

The multi-synthetase complex (MSC), found in mammals, is a 1.4 Mda complex that consists of 11 protein subunits. Eight of these subunits are aaRSs, the remaining three are non-aaRS factors. The aaRSs present in the complex are leucyl-, isoleucil-, methionyl-, aspartyl-, glutaminyl-, lysyl-, arginyl-tRNA synthetase and the double aaRS glutamylprolyl-tRNA synthetase. The non-aaRS factors, which play roles in complex assembly and stability, are AIMP1 (also called p43), AIMP2 (p38), and AIMP3 (p18) [17, 25, 26].

A global structural model of the MSC has been made by combining electron microscopy with a number of other techniques. In this model the different subunits have all been assigned to a part of the structure. From this model it becomes clear that there is enough space for all the tRNA binding sites, which are spread out at the periphery of the complex. There is even space for association of additional proteins. This correlates well with the suggestion that perhaps more aaRSs are associated with the MSC than is visible with the techniques used today. AIMP2 interacts with most proteins in the complex but no details are known about these or the other interactions in the complex [17, 27].

The function of the MSC turns out to be rather complex and is not entirely clear. I will discuss the functions important for translation and those important for other processes separately.

Although the role of MSC in translation is not entirely understood there have been a number of suggestions. (1) It could be that the efficiencies of the different aminoacylation reactions increases, as is observed for the Arc1p-aaRS complex in *S. cerevisiae*. (2) It could be that the efficiency of delivery of aa-tRNAs to the ribosome is increased. Other suggestions are (3) that the association in a complex increases the stability of the subunits or (4) that it keeps the the aaRSs in the cytoplasm. These last two points were also observed for the *S. cerevisiae* Arc1p-aaRS complex. Of course it is also possible that the MSC performs a number or all of these functions. One could argue that incorporation of aaRSs in a complex causes sterical hinder, decreasing the efficiency of aa-tRNA delivery to the ribosome [17, 25]. However, this does not seem to be the case as the binding sites of the tRNAs are, according to a structural model of the MSC, positioned at the periphery of the complex [27]. Also, support has been found for the notion that MSC increases the efficiency of tRNA delivery to the translating ribosome. Aa-tRNAs that were synthesized outside of the cell and introduced into CHO (Chinese Hamster Ovary) cells weren't incorporated as efficiently as the tRNAs synthesized in the MSC by the CHO cells [17].

The most interesting fact about MSC is depicted in Figure 5. In this figure you can see that the MSC, or at least its components, are in the middle of a vast network of processes. Components of the MSC can dissociate and participate in other processes.

For example, lysyl-tRNA synthetase (LysRS) can be secreted by human cells in response to signaling via the transmembrane protein TNF-α. Outside the cell, LysRS is able to bind to immune cells and enhances immune cell migration and the production of TNF-α. This forms a positive feedback loop and induces an immune response. Besides being secreted, LysRS can also translocate to the nucleus to form complexes with proteins that do not associate with the MSC. Finally, it also has a transcriptional function. LysRS produces Ap_4A which can bind to an inhibitor of transcription. Upon binding the inhibitor is released from the DNA and the genes can be transcribed.

Methionyl-tRNA synthetase (MetRS) can also be translocated to the nucleus, like lysRS. This translocation is induced by a growth factor and when present in the nucleus MetRS is involved in rRNA synthesis.

Glutaminyl-tRNA synthetase (GlnRS) also has a signaling function, like LysRS. It can associate with the protein ASK1, or apoptosis signal-regulating kinase 1, which is a kinase involved in apoptosis. This interactions leads to an inhibition of apoptosis.

GluProRS can be released from the MSC by signal from interferon-γ. After release it can form another complex called the GAIT complex. GAIT stands for gamma-interferon-activated inhibitor of translation and it contains three other components besides GluProRS. This complex inhibits translation by binding to specific elements on target mRNAs.

The three non-aaRS factors, AIMP1,2, and 3, also have a variety of functions, as can be seen in Figure 5. To keep the discussion to the aaRSs I will not discuss these functions here [26].

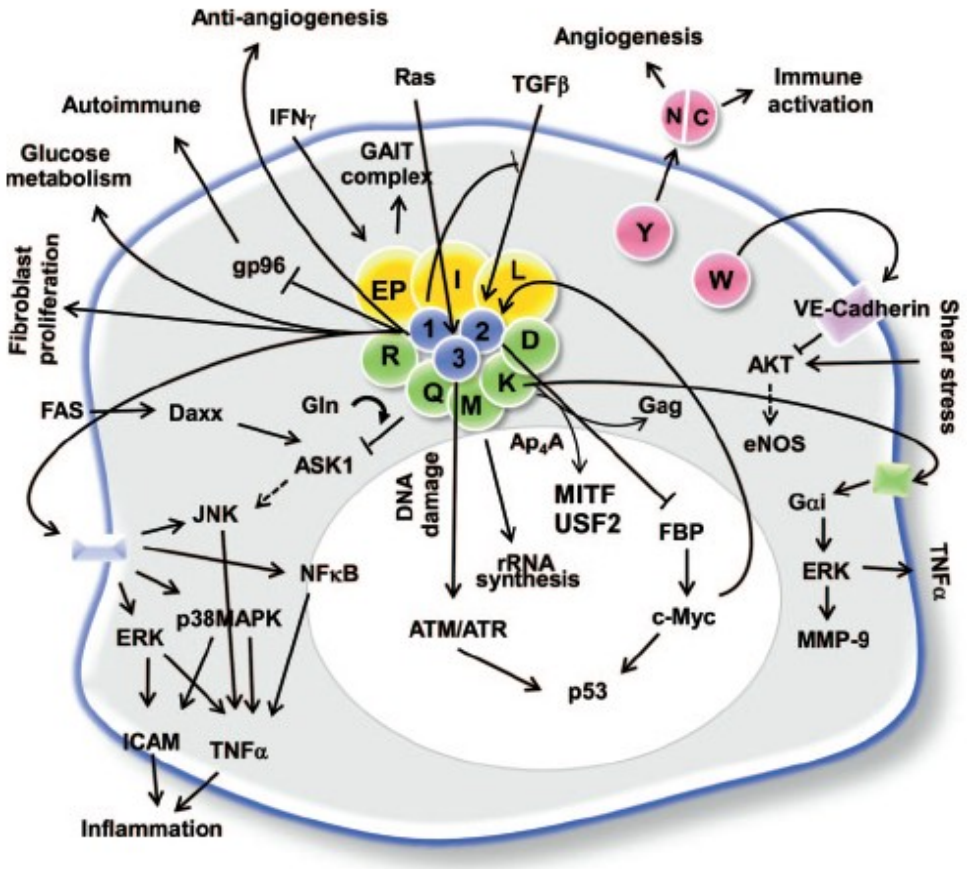

Figure 5: Signaling network of mammalian MSC. The components of the MSC can dissociate and participate in the other processes that are depicted in the figure. The functions of K (LysRS), M (MetRS), Q (GlnRS), and EP (GluProRS) are explained in the text. Adapted from Park, S.G., et al., 2008 [28].

Existence of multi-aaRS complexes in bacteria

In recent years a number of interactome studies have been published. These studies elucidated a large part of the interactome of *Campylobacter jejuni* [29], *Escherichia coli* [30, 31], *Treponema pallidum* [32], *Helicobacter pylori* [33], and *Mycoplasma pneumoniae* [34]. The interactome study on *M. pneumoniae* suggests an even broader palette of functions for aaRSs (see Figure 6). *M. pneumoniae* is a very simple bacterium with a genome size of only 0.82 Mb. The technique used to find interactions is tandem affinity purification-mass spectrometry (TAP-MS) and by analysis 116 heteromultimeric and 62 homomultimeric soluble protein complexes were found [34].

In this section I will first summarize a number of the interactions and presumed complexes of aaRSs that were found. After that I will discuss the implications of this. AlaRS, ArgRS, SerRS, and ThrRS are all present in complexes with sizes ranging between 2 and 11 subunits. AlaRS is even present in two of these complexes. The function of these complexes is unknown. ThrRS is also associated with the ~60 subunit ribosome complex, a 4 subunit complex also involved in the category of translation, ribosomal structure and ribosomal biogenesis and the 13 subunit pyruvate dehydrogenase complex. MetRS is also present in the pyruvate dehydrogenase complex and also in a heterodimer complex involved in the category DNA replication, recombination and repair. TrpRS, GlnRS, AsnRS, and GlyRS are all associated with the ~25

subunit RNA polymerase complex. Finally PheRS, TyrRS, MetRS, GluRS and ThrRS also together with 5 other proteins form a multi-aaRS protein. A total of 16 complexes containing aaRSs have been found [34].

The most striking about all these associations is that there are a number of aaRSs, like ThrRS and MetRS, that are present in multiple, five in the case of ThrRS, complexes. Also the amazing total of 16 complexes is quite large compared to the 4 complexes described before (see "AaRS multitasking in bacteria"). Especially when considering that *M. pneumoniae* is a very simple bacterium. Its genome is 5-6 times smaller than that of *E. coli*. Important to realize, however, is that the complexes just mentioned do not have to contain all subunits all the time. It could be that the interactions are of a much more transient nature. For example, it was observed for the putative *E. coli* multi-aaRS complex that it dissociates quite easily [22]. Indicating that perhaps complexes in bacteria are not as robust as complexes in eukaryotes. Although the interactions are transient they are of course still there and imply alternative functions for aaRSs.

Figure 6 summarizes the different processes aaRS were found to participate in (in *M. pneumoniae*). AaRSs are involved in metabolism, transcription, DNA replication, Translation, various other known and unknown processes. The roles of the aaRSs in these complexes are not understood. The most important insight from this study on *M. pneumoniae* is that the complexity of bacterial cells, at least in the case of the aaRSs, is drastically underestimated [34].

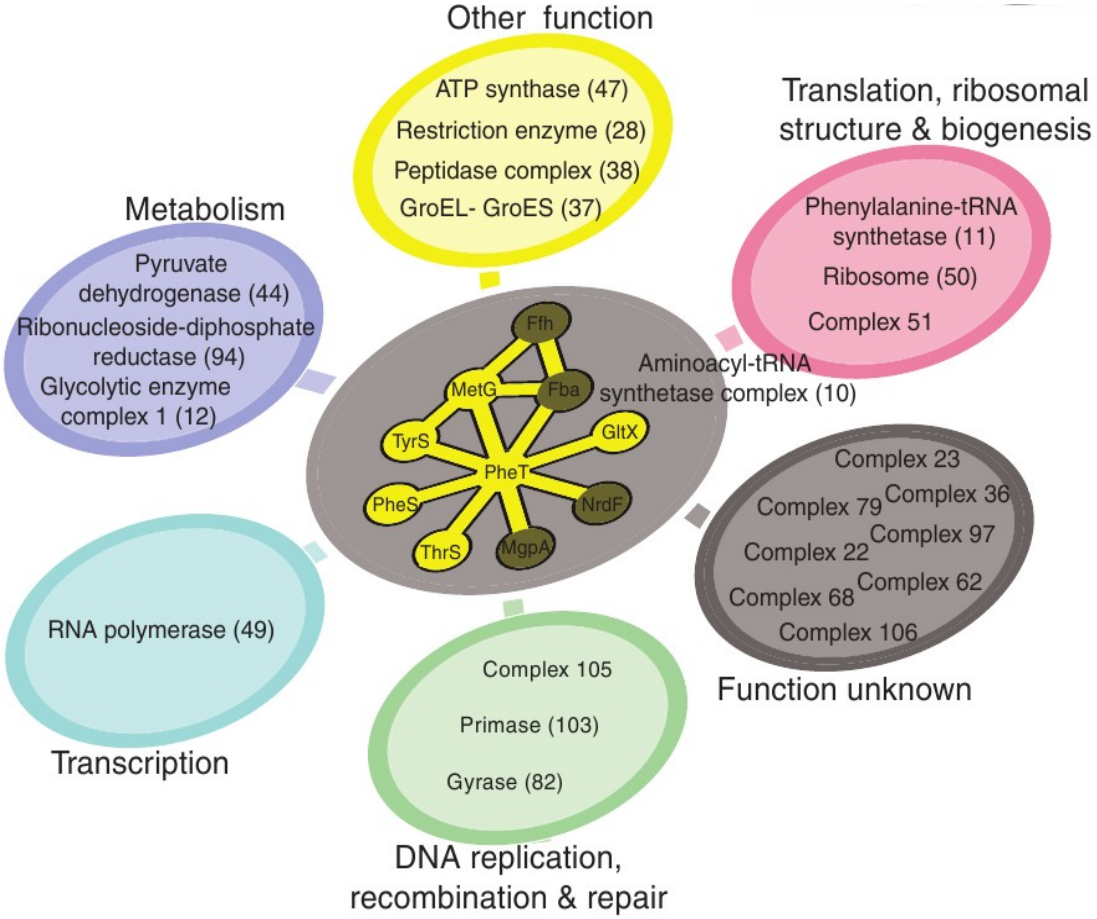

Figure 6: The figure shows the involvement of aaRSs in various processes outside of translation. Adapted from Kühner, S., 2009 [34].

Discussion

Besides being key players in translation, aminoacyl-tRNA synthetases participate in a lot of other processes. This is true for all three domains of life. Bacterial aaRSs can carry out functions as diverse as transcriptional regulation, translational regulation, cell cycle control (indirect), antibiotics resistance, synthesis of non-cognate amino acids, regulation of protein degradation, synthesis of dipeptides and aa-tRNA editing. Alternative aaRS functions only present in eukaryotes are cell signaling and organelle biosynthesis.

In bacteria most of the alternative functions seem to be carried out by single aaRSs and only a few functions need the formation of the complex. This seems to be in contrast with the situation in eukaryotes in which aaRSs play roles in multiple complexes. With some aaRSs even participating in more than one complex. However, I think that in this view the complexity of bacteria is drastically underestimated. The interactome study on *M. pneumoniae*, described above, indicates that bacterial aaRSs do interact with a lot of other proteins. These interactions could be rather transient, but they probably do have a function. Indeed, aaRSs found in the multi-aaRS structure found in *E. coli* are thought to dissociate quite easily. This transient nature of interactions also allows the aaRSs to associate with a variety of other proteins. This is the case for ThrRS in *M. pneumoniae* which is present in five complexes.

In eukaryotes complex formation is thought to occur by association of certain domains. These domains are often not present in bacteria [35]. This fact speaks against there being aaRS complexes in bacteria. However, some interactions between aaRSs and non-aaRS factors, in eukaryotes, are mediated by contacts between catalytic domains. So domains are not an absolute necessity for complex formation. Of course, aaRS interaction in bacteria don't have to be governed by exactly the same principles as in eukaryotes. In archaea a complex of three aaRSs has been found indicating that complexes do occur in simple prokaryotic organisms [17].

The thing that eukaryotic and bacterial complexes have in common is that in both cases not much is known about their function(s). This is especially the case for bacterial complexes. A question one also has to ask when considering transient interactions is: How much influence can transient interactions have on the different processes in the cell. The answer to this question is probably different for every complex and the answer has to await further research on the aaRS containing complexes.

With the knowledge obtained in this literature survey it is not possible to predict precisely the effect of introducing and overexpressing aaRSs. This has two main reasons. The first is that all proteins and substrates are part of networks containing all kinds of feedback mechanisms and connections to other processes. This makes it very hard to predict the exact effect of the presence of a certain level of aaRS. The second reason is that the aaRSs that are introduced usually are from another organism. It is at the moment unknown how conserved the interactions and complexes involving aaRSs are. It is thus also unknown whether the complexes found in *M. pneumoniae* are present in other bacteria. Although it would be impossible at the moment to predict what certain changes in aaRSs would do to the cell the cases discussed in this report at least give a conceptual framework from which observations could be explained. The discussion shows what kind of effects one might expect, e.g. antibiotics resistance and synthesis of dipeptides, and it shows that many aaRSs have multiple functions in a large variety of processes.

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