

Aminoacyl-tRNA synthetase complexes: beyond translation

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Summary

Although aminoacyl-tRNA synthetases (ARSs) are housekeeping enzymes essential for protein synthesis, they can play non-catalytic roles in diverse biological processes. Some ARSs are capable of forming complexes with each other and additional proteins. This characteristic is most pronounced in mammals, which produce a macromolecular complex comprising nine different ARSs and three additional factors: p43, p38 and p18. We have been aware of the existence of this complex for a long time, but its structure and function have not been well understood. The only apparent distinction between the complex-forming ARSs and those that do not form

complexes is their ability to interact with the three non-enzymatic factors. These factors are required not only for the catalytic activity and stability of the associated ARSs, such as isoleucyl-, methionyl-, and arginyl-tRNA synthetase, but also for diverse signal transduction pathways. They may thus have joined the ARS community to coordinate protein synthesis with other biological processes.

Key words: Aminoacyl-tRNA synthetase, Macromolecular protein complex, Multi-functionality, Protein network, Protein synthesis

Introduction

The human genome project bewildered us by revealing that our cells produce fewer proteins than expected. However, we are increasingly realizing that the problem of defining the roles of proteins is not less complex, because they are structurally and functionally more intertwined than we thought. Thus, dissecting the network of multi-functional cellular proteins has become one of the major tasks of the post-genome-project era. Since the activity of a protein can be regulated by systems involving subcellular location, expression, secretion, oligomerization or complex formation, the determination of protein function is a formidable task (Jeffery, 1999). In this line of work, proteins that can have multiple distinct functions represent a particularly interesting challenge.

Translation is one of the most complex biological processes, involving diverse protein factors and enzymes as well as messenger and transfer RNAs. As this process is required for the basic operation of cells, many translational factors and enzymes are considered to be housekeeping proteins. Aminoacyl-tRNA synthetases (ARSs) catalyze the ligation of specific amino acids to their cognate tRNAs, which is the initial step in protein synthesis. The aminoacylation reaction proceeds in two stages. First, ARSs activate their substrate amino acids by forming aminoacyl adenylate. Second, the enzyme-bound reaction intermediates are transferred to the 3' acceptor end of the tRNAs docking onto their active sites. Because tRNAs cannot distinguish amino acids conjugated to their ends, the correct recognition of amino acids and tRNAs by these enzymes is a crucial determinant to maintain the fidelity of protein synthesis.

Moonlighting functions of ARS proteins

Since ARSs play a crucial part in the flow of genetic

information from nucleic acids to proteins, they are thought to have emerged early in evolution and to be structurally highly tailored to specific recognition of substrate amino acids and tRNAs. Although the catalytic activities of these enzymes represent their essential role in maintenance of cell viability, accumulating evidence demonstrates that they in fact are versatile, multi-functional proteins regulated by a diverse set of control mechanisms. This functional flexibility appears to be extended through physical interactions with each other, as well as with additional cofactors, to areas not directly related to protein synthesis. These include RNA processing and trafficking, apoptosis, rRNA synthesis, angiogenesis and inflammation (Martinis et al., 1999; Ibba and Soll, 2001; Ko et al., 2002) (Table 1). These non-canonical and non-catalytic functions depend on their ability to engage in transcriptional control, DNA and RNA binding and signal-dependent protein-protein interactions, and be regulated by cellular localization, alternative splicing and extracellular secretion. For instance, *Escherichia coli* TRS[†] binds to its own mRNA leader sequence, which mimics tRNA structure. Binding of TRS to the mRNA prevents the 30S ribosomal subunit from docking onto the ribosome-binding sequence of the mRNA, thereby blocking the synthesis of the enzyme (Brunel et al., 1993; Romby et al., 1996). In addition, *E. coli* AlaRS[†] can bind to a palindromic sequence flanking the transcriptional start site of its own gene, repressing its expression. Interestingly, the DNA-binding capacity of this enzyme is enhanced by elevated concentrations of the cognate amino acid (Putney and Schimmel, 1981).

Mitochondrial YRS of *Neurospora crassa* and

[†]ARSs are generally defined using the single-letter code. Thus, TRS is the aminoacyl-tRNA synthetase for threonine. AlaRS is used for the aminoacyl-tRNA synthetase responsible for alanine.

Table 1. Non-canonical activities of ARSs

Species	ARSs	Function	References
<i>Escherichia coli</i>	TRS	Translational control	Romby et al., 1996; Brunel et al., 1993
<i>E. coli</i>	AlaRS	Transcription control	Putney et al., 1981
<i>Neurospora crassa</i>	Mitochondrial YRS	Splicing	Akins et al., 1987; Cherniack et al., 1990
<i>Saccharomyces cerevisiae</i>	Mitochondrial LRS	Splicing	Herbert et al., 1988; Labouesse, 1990
<i>Homo sapiens</i>	Cytoplasmic YRS	Angiogenic cytokine	Wakasugi et al., 2002a; Wakasugi et al., 2002b
<i>H. sapiens</i>	Cytoplasmic WRS	Angiostatic cytokine	Otani et al., 2002; Wakasugi et al., 2002b
<i>H. sapiens</i>	Cytoplasmic MRS	Transcription of rRNA	Ko et al., 2000
<i>H. sapiens</i>	Cytoplasmic QRS	Anti-apoptosis	Ko et al., 2001a

mitochondrial LRS of yeast species are required for the splicing of group I introns (Herbert et al., 1988; Cherniack et al., 1990; Labouesse, 1990). By contrast, human YRS can be split by polymorphonuclear (PMN) elastase into two fragments that display distinct cytokine activities (Wakasugi and Schimmel, 1999a; Wakasugi and Schimmel, 1999b). One of the fragments (mini-YRS) contains a conserved ELR motif identical to that found in CXC chemokines such as interleukin 8 (IL-8), Gro- α , Gro- β , Gro- γ and NAP-2, which act as angiogenic factors (Herbert et al., 1988; Clark-Lewis et al., 1991; Clark-Lewis et al., 1993; Arenberg et al., 1997). As expected, mini-YRS induces angiogenesis (Wakasugi et al., 2002a). Whereas human YRS is converted into two distinct cytokines by proteolytic processing (Wakasugi and Schimmel, 1999a; Wakasugi and Schimmel, 1999b), an N-terminally truncated form of WRS, possibly generated through alternative splicing, works as an anti-angiogenic cytokine (Otani et al., 2002; Wakasugi et al., 2002b; Kise et al., 2004).

Human MRS represents another example. It is translocated to the nucleus under proliferative conditions to augment rRNA synthesis in nucleoli, and the presence of MRS in nucleoli depends on the integrity of rRNA and the activity of RNA polymerase I. The addition of MRS stimulates rRNA synthesis, which indicates that it plays a role in rRNA synthesis in nucleoli, although the underlying mechanism is not clearly understood (Ko et al., 2000). Human QRS is recruited to apoptosis signal-regulating kinase 1 (ASK1) to block its kinase activity. The interaction of the two proteins is stimulated by the QRS substrate, glutamine, which can suppress cell death (Ko et al., 2001a). Note that, in general, the non-canonical activities of ARSs appear to be more prevalent in mammalian systems.

Mammalian multi-ARS complexes

The activity of a protein is often controlled through formation of transient or stable complexes with other proteins; thus, understanding the molecular assembly and structural organization of protein complexes helps us to determine the functions of their components. The formation of macromolecular complexes that function in DNA replication (Benkovic et al., 2001), transcription (Conaway et al., 1993) and translation (Kerjan et al., 1994; Asano et al., 1997), signal transduction (Ramakrishnan and White, 1998) and protein degradation (Glickman et al., 1998) is well established, and we know much about the regulation of the individual components.

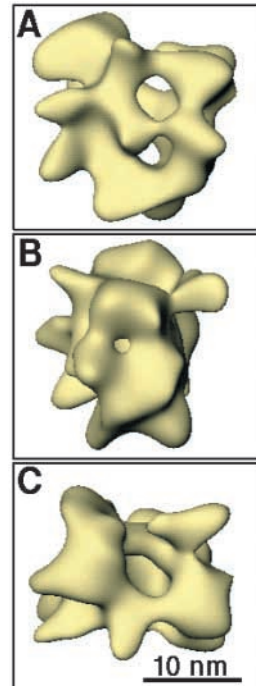
Several lines of evidence have suggested that the translational apparatus in mammalian cells is highly organized. In particular, association of translational components such as tRNA, ARSs and elongation factors with the cytoskeletal framework (Dang

et al., 1983; Mirande et al., 1985a; Sanders et al., 1996) and colocalization of these components have been described (Barbarese et al., 1995). ARSs can be classified into two groups based on their structural features (Eriani et al., 1990; Burbaum and Schimmel, 1991; Cusack et al., 1991). Class I ARSs each possess a Rossman fold in their catalytic domains, whereas class II enzymes contain three homologous motifs with degenerate sequence similarity. ARSs can also be grouped on the basis of their ability to form complexes with each other and non-enzymatic factors. Among the complexes formed by ARSs, the mammalian ARS complex is the most intriguing (Robinson et al., 2000; Kim et al., 2002; Ko et al., 2002; Han et al., 2003). This complex is distinctive compared with other macromolecular protein complexes in that its components are enzymes that carry out similar catalytic reactions simultaneously, and only a subset of ARSs are involved.

Although there is still some ambiguity about the stoichiometry and total number of components, at least nine different ARSs, including both class I and class II enzymes, have been consistently found in the mammalian complex: EPRS, IRS, LRS, MRS, QRS, RRS, KRS and DRS. Among these, IRS, LRS, MRS, QRS and RRS are monomers, whereas KRS and DRS are dimers. The largest component – EPRS – harbors two catalytic activities in a single polypeptide. The complex also contains three auxiliary factors of p43, p38 and p18 (Quevillon and Mirande, 1996; Quevillon et al., 1997; Quevillon et al., 1999). The complex has been purified to homogeneity from various mammalian tissues, including rat liver, rabbit liver and reticulocytes, sheep liver and spleen (Brevet et al., 1982; Kellermann et al., 1982; Cirakoglu and Waller, 1985; Venema and Traugh, 1991), as well as from cultured cells such as Chinese hamster ovary (CHO) cells (Mirande et al., 1985b) and murine erythroleukemia cells (Norcum, 1989). The complexes purified in these experiments display very similar patterns following gel electrophoresis, comprising 11 polypeptides with molecular weights from 18 kDa to 150 kDa (Kerjan et al., 1992; Kerjan et al., 1994). Although all of the enzymatic activities present can be assigned to their corresponding polypeptides, with the exception of PRS (Mirande et al., 1982; Cirakoglu and Waller, 1985), the structural organization of this complex has not yet been completely deciphered.

Several approaches have probed the structural organization of this complex. Its components can be partially dissociated under a variety of conditions, such as repeated centrifugation in the presence of high concentrations of phosphate (Dang and Yang, 1979), hydrophobic chromatography (Johnson et al., 1980) and incubation with chaotropic salts or detergents (Sihag and Deutscher, 1983; Norcum, 1991). The gross morphology

Fig. 1. Three-dimensional structure of the human multi-synthetase complex. (A) 'Front' view. (B) 'Side' view created by -90° rotation about the vertical axis. (C) 'Top' view created by -90° rotation about the horizontal axis. The multi-synthetase complex was isolated from cultured human cells and prepared for electron microscopy by negatively staining with NanoVan (Wolfe et al., 2003). The volume was calculated from 8437 images, filtered to its resolution limit of 33 Å and presented at a threshold corresponding to a particle mass of 1.2×10^6 Da.



of the complex has been explored by electron microscopy (Norcum, 1989; Norcum and Boisset, 2002; Wolfe et al., 2003) (Fig. 1), and the nearest neighbors among the component parts have been determined by chemical crosslinking (Norcum and Warrington, 1998) and genetic approaches (Rho et al., 1996; Quevillon et al., 1999).

The complex-forming and non-complex-forming human ARSs do not display distinct size distributions, structural features, post-translational modifications, expression profiles or chromosomal locations (Table 2 and data not shown). Moreover, comparison of functional motifs present in ARSs provides few clues as to what is responsible for complex formation. Interestingly, domains homologous to glutathione S-transferase (GST) are present in the N-terminal extensions of MRS and EPRS (Quevillon and Mirande, 1996; Quevillon et al., 1999) among the complex-forming enzymes, as well as the C-terminal regions of p18 and p38 among the non-enzymatic cofactors (Galani et al., 2001) (Fig. 2). Although these domains are also observed in other ARSs, such as mammalian VRS (Fig. 2) and the putative ERSs of *Schizosaccharomyces pombe* and *Arabidopsis thaliana*, these enzymes are also likely to be involved in different types of complex. Mammalian VRS is associated with elongation factor subunits that also contain the GST homology domain (Bec et al., 1989; Bec et al., 1994; Brandsma et al., 1995), and a CRS isoform can potentially associate with elongation factor subunits (Kim, J. E. et al., 2000). In yeast, ERS forms a primitive complex with MRS and Arc1p, and the importance of the GST-homology domain for complex formation has been shown experimentally (Deinert et al., 2001; Galani et al., 2001). Thus, the presence of the GST domain might be a crucial determinant for complex formation.

Multi-functionality of non-enzymatic components

As mentioned above, the human multi-ARS complex also

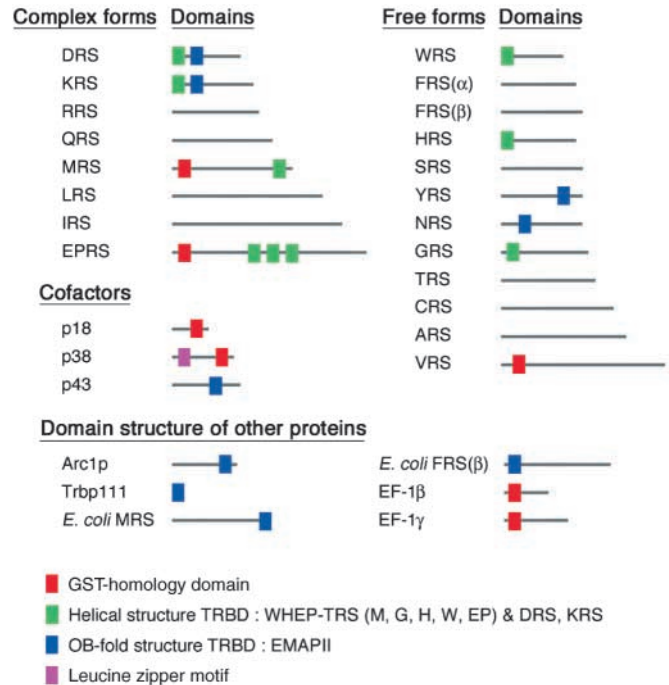


Fig. 2. Functional domains in ARSs and ARS-related factors. The domains homologous to glutathione S-transferase (GST; red boxes) are shown in the N-terminal regions of MRS, EPRS and VRS, as well as in the C-terminal regions of p18 and p38. p38 also contains a leucine zipper motif (violet box) and is involved in macromolecular assembly of ARSs (Quevillon et al., 1999; Ahn et al., 2003). The sequence similarity between the helical tRNA-binding domain (green boxes) of MRS, GRS, HRS, WRS and the three repeated domains of EPRS was revealed by sequence alignment (Kaminska et al., 2001). Interestingly, these motifs are also involved in protein-protein interactions (Rho et al., 1996; Rho et al., 1998). DRS and KRS also contain helical tRNA-binding domains (TRBD; blue boxes), although they are not related to the motif mentioned above (Frugier et al., 2000; Francin et al., 2002). By contrast, the oligonucleotide-binding (OB) fold domains (blue boxes) in p43 and YRS are related (Renault et al., 2001). The similar RNA-binding OB folds can also be detected in some ARSs (human DRS, KRS and NRS; *Escherichia coli* MRS and FRS β -subunit) and other proteins (Arc1p, Trbp111, EF-1 β and EF-1 γ).

contains the non-enzymatic factors p43, p38 and p18 (Fig. 2, Fig. 3A). Like ARSs, these ARS-associated factors also play diverse roles in processes other than protein synthesis. p43 is secreted as an active cytokine (Kao et al., 1992; Knies et al., 1998; Barnett et al., 2000; Ko et al., 2001b). The secreted p43 induces synthesis of various pro-inflammatory cytokines and chemokines, such as tumor necrosis factor (TNF)- α , IL-8, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 and IL-1 β from monocytes (Ko et al., 2001a), as well as intercellular adhesion molecule (ICAM)-1 (Park, H. et al., 2002). Synthesis of the latter promotes cell adhesion in a variety of physiological and pathological processes, including inflammation and atherosclerosis (Gimbrone et al., 1997).

p43 plays a complex role in angiogenesis. Although it induces migration of endothelial cells at low concentration, it suppresses angiogenesis by blocking the proliferation and triggering apoptosis of endothelial cells at high concentrations

Table 2. Size and structural classification of human ARSs and chromosomal locations of their encoding genes

Complex forms	Size (aa)	Class	Chromosome location
DRS	500	II	2q22.1
KRS	597	II	16q23-q24
RRS	660	I	5q35.1
QRS	775	I	3p21.3-p21.1
MRS	900	I	12q13.2
LRS	1176	I	5q32
IRS	1262	I	9q21
EPRS	1440	I, II	1q41-q42

Free forms	Size (aa)	Class	Chromosome location
WRS	471	I	14q32.31
FRS (α)	508	II	19p13.2
FRS (β)	589	II	2q36.1-q36.2
HRS	509	II	5q31.3
SRS	514	II	1p13.3-p13.1
YRS	528	I	1p34.3
NRS	548	II	18q21.2-q21.3
GRS	685	II	7p15
TRS	712	II	5p13.2
CRS	831	I	11p15.5
ARS	968	II	16q22
VRS	1264	I	6p21.3

ARSs are classified into two groups (I and II) depending on their structural features. The chromosomal locations of the ARS-encoding genes were obtained from LocusLink of the National Center for Biotechnology Information.

(Park, S. G. et al., 2002). p43 contains a caspase-cleavage site, which is targeted upon apoptosis; this releases the C-terminal domain of p43 (previously known as EMAPII) from the complex. The process was thought to trigger the secretion of the cytokine component from p43, causing the disintegration of the multi-ARS complex to block protein synthesis. However, p43 processing does not appear to affect the function of the complex, and it turns out that the uncleaved form of the p43 is the active cytokine (Ko et al., 2001b). The role of proteolytic cleavage of p43 at apoptosis is thus unclear at this point.

p38 also has an unexpected additional role. It can bind to FUSE-binding protein (FBP), a transcriptional activator of the gene encoding Myc, which promotes its ubiquitylation and proteasome-dependent degradation (Kim et al., 2003). When the expression of endogenous p38 is abolished by insertion of a gene-trap vector in the p38-encoding gene, Myc is overexpressed owing to the lack of p38-mediated suppression, which causes hyperproliferation of lung cells. The consequent malfunction of the lung causes p38^{-/-} mice to die at birth, although they survive development through the prenatal stage.

It is not known yet whether the smallest cofactor, p18, is also multi-functional. It shares limited sequence similarity with elongation factor subunits (Quevillon and Mirande, 1996), thus it could have a role connecting the aminoacylation and translational machineries. Considering the functional diversity of the two other factors, it would not be surprising to find other crucial activities of this factor.

The evolution of ARS complexes

ARS complexes much less complex than the mammalian one may exist in lower organisms such as *Haloarcula marismortui*

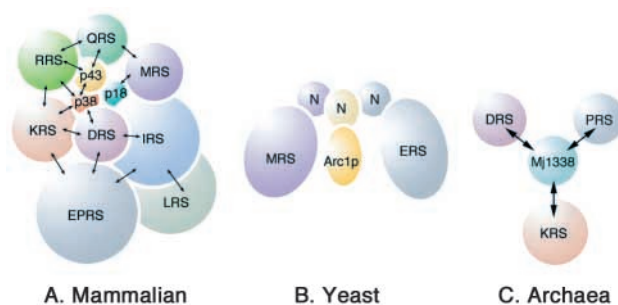
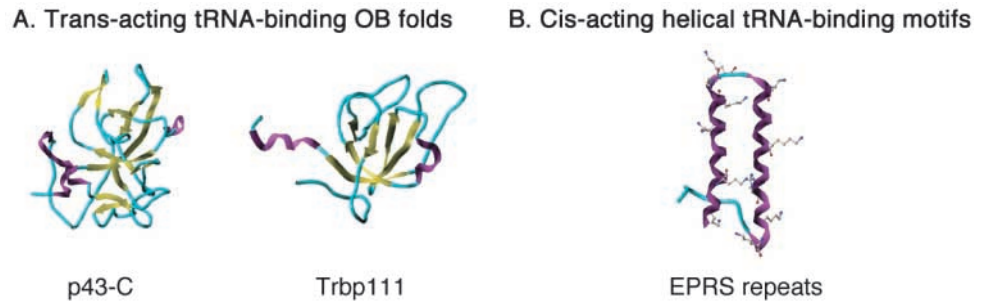


Fig. 3. A schematic hypothetical model for the organization of mammalian, yeast and archaea tRNA synthetase complexes. (A) The two-dimensional arrangement of the components in the mammalian multi-ARS synthetase complex. p38 is a scaffold protein for the assembly of the components. Some of the interactions, which have been determined by two-hybrid analyses (Rho et al., 1996; Quevillon et al., 1999) and crosslinking methods (Norcum and Warrington, 1998), are shown as arrows in this diagram. Owing to the limits of two-dimensional display, some interactions are not shown. (B) The yeast ARS complex consisting of two ARSs (MRS and ERS) and Arc1p, the yeast homolog of mammalian p43. Both ARSs interact directly with the N-terminal domain of Arc1p through their N-terminal appended domains (Galani et al., 2001). (C) The putative metabolic protein Mj1338 copurified with PRS from *Methanococcus jannaschii* interacts with KRSs from human and *Methanobacterium thermoautotrophicum* in pull-down assays. Its interaction with DRS was also confirmed through identification of aminoacylation activity in a DEAE fraction obtained from total cell lysate of *M. jannaschii* (Lipman et al., 2000; Lipman et al., 2003). Although some components in these complexes have the potential for homodimerization, for simplicity this is not shown. The spatial arrangements and sizes of the components do not necessarily reflect their relative positions in the complexes.

(Goldgur and Safro, 1994), *Methanococcus jannaschii* (Lipman et al., 2000; Lipman et al., 2003) and *Saccharomyces cerevisiae* (Simos et al., 1996). In yeast, cytoplasmic MRS and ERS form a complex with Arc1p – the yeast homolog of mammalian p43 – through their N-terminal extensions (Galani et al., 2001) (Fig. 3B). Arc1p binds preferentially to tRNA^{Met} and tRNA^{Glu}, facilitating the delivery of the substrate tRNAs to their cognate enzymes, although it has a general affinity for all tRNAs (Simos et al., 1996; Simos et al., 1998; Deinert et al., 2001). Although Arc1p itself is not essential for yeast viability (Simos et al., 1996), it becomes crucial when its absence is combined with the depletion of Los1p, which is involved in the nuclear export of tRNAs (Grosshans et al., 2000b; Mucha, 2002). This indicates that Arc1p is also involved in nuclear transport of tRNA (Grosshans et al., 2000a; Grosshans et al., 2000b; Galani et al., 2001). However, whether Arc1p actually delivers nascent tRNAs from the nucleus to cytoplasmic ARSs has yet to be shown.

Trbp111, a homolog of Arc1p, is present in the extreme thermophile *Aquifex aeolicus* (Morales et al., 1999) and binds to single tRNA molecules as a dimer (Swairjo et al., 2000). However, it is not yet known whether this protein forms any specific complex with ARSs. Recently, Lipman et al. copurified a novel protein, Mj1338, with PRS from the archaeon *M. jannaschii*. Mj1338 also has a general affinity for tRNA and the potential to interact with KRS and DRS, in addition to PRS (Lipman et al., 2000; Lipman et al., 2003) (Fig. 3C).

Fig. 4. The three-dimensional structure of trans- and cis-acting tRNA-binding domains present in ARSs and ARS-associated factors. (A) The peptides A147 to E314 of p43 (Kim, Y. et al., 2000; Renault et al., 2001) and M1 to A111 of Trbp111 (Morales et al., 1999). Two domains contain the typical oligonucleotide-binding (OB) fold, consisting of a five-stranded β -barrel that is known to have RNA-binding capability. (B) The 57 aa peptide region from D677 to P733 of human EPRS (Cahuzac et al., 2000; Jeong et al., 2000). Notice that the basic residues are exposed from the two helices arranged in anti-parallel mode.



Interestingly, it is predicted to be a metabolic protein related to the members of the H₂-forming N⁵, N¹⁰-methylene tetrahydromethanopterin (5,10-CH₂-H₄MP) dehydrogenase family, which catalyze an intermediate step in the C1 unit metabolism of the methanogen.

p43 shares structural similarity with Trbp111 and other tRNA-binding proteins present in lower organisms (Renault et al., 2001), and is capable of binding to tRNA (Shalak et al., 2001) to help the tRNA dock onto the bound ARS (Park et al., 1999) (Fig. 4A). Clear homologs of p38 and p18 have not been found in lower eukaryotes or bacteria. However, the C-terminal domains of p18 and p38 share significant sequence similarity with the N-terminus of Arc1p (Galani et al., 2001), which might thus combine features of the three non-enzymatic members of the mammalian complex.

Interestingly, many ARSs also have cis-acting domains that appear to facilitate the recruitment of tRNAs to their catalytic sites (Cahuzac et al., 2000; Frugier et al., 2000; Kaminska et al., 2001; Francin et al., 2002; Francin and Mirande, 2003). For example, the C-terminus of *E. coli* MRS (Morales et al., 1999), and the N-terminus of the *E. coli* FRS β -subunit (Simos et al., 1996) (Fig. 2) share structural similarity with the nonenzymatic factors in the mammalian ARS complex, and thus they probably function similarly to their trans-acting counterparts (Valenzuela and Schulman, 1986; Kim et al., 1993; Mosyak et al., 1995; Goldgur et al., 1997). It is thus likely that the trans-acting tRNA-binding proteins derive from the ARSs (Fig. 2, Fig. 4A) but have acquired more functional flexibility during evolution. However, we cannot exclude the alternative possibility – that they were inserted into the ARS structure to augment the catalytic efficiency of the enzymes. Some ARSs also contain cis-acting motifs that are structurally unrelated to the common domains found in Arc1p, p43 and Trbp111 (Cahuzac et al., 2000; Frugier et al., 2000; Kaminska et al., 2001; Francin et al., 2002; Francin and Mirande, 2003) (Figs 2, 4). They usually form amphiphatic helices in which one side of the helix displays an array of basic residues for interaction with tRNAs (Fig. 4B).

Assembly and disassembly of ARSs and cofactors

In the mammalian multi-ARS complex, ARSs engage in multiple interactions with each other, using their non-catalytic and catalytic core domains (Rho et al., 1996; Rho et al., 1998; Rho et al., 1999; Kim, T. et al., 2000). These probably contribute to the stability of the complex in a cooperative manner. However, studies show that ARS-binding factors have

a pivotal role in the assembly of the multi-ARS complex. p38 contains putative leucine zipper motifs (Quevillon et al., 1999; Ahn et al., 2003) (Fig. 2) and is involved in multiple interactions with different components within the multi-ARS complex (Fig. 3A). Deletion of p38 causes the complex to disintegrate into individual components, which are then degraded, proving its crucial role in maintaining the structural integrity of the whole complex (Kim et al., 2002). Although the importance of the other two factors in the complex formation has not been evaluated, p43 and p18 are specifically bound to RRS and MRS, respectively (Park et al., 1999; Quevillon et al., 1999). Analysis of the multi-ARS complex by electron microscopy demonstrated that p43 is located centrally within the complex, implicating it in the assembly of the complex (Norcum and Warrington, 2000). Thus, p43 and p18 might be involved in the assembly of a subcomplex structure, even if they are not as crucial as p38.

The function of the multi-ARS complex

Why do ARSs form a complex? Channeling is clearly one possibility. Channeling has been suggested as an efficient way to utilize substrate for sequential reactions (Srere, 1987). For example, for sequential metabolic enzymes, stimulation of the first enzyme induced by a protein-protein interaction with the next provides a structural basis for channeling. The supramolecular assemblies of ARSs and elongation factors (Mirande, 1991; Kisselev and Wolfson, 1994; Yang, 1996) represent structural evidence for the subcellular organization of the protein synthesis machinery. Moreover, the existence of a channeled tRNA cycle during mammalian protein synthesis provides functional evidence for cellular compartmentalization of translation (Negrutskii and Deutscher, 1991; Negrutskii et al., 1994; Stapulionis and Deutscher, 1995). According to the proposed channeling scheme, aminoacyl-tRNAs are vectorially transferred from ARSs to ribosomes as ternary complexes of EF-1 α , GTP and aminoacyl-tRNA (Negrutskii and Deutscher, 1991; Stapulionis and Deutscher, 1995). The GDP-bound form of EF-1 α could be involved in the capture of deacylated tRNA at the exit site of ribosomes and its delivery to ARSs (Petrushenko et al., 1997). Nascently produced tRNAs can also be delivered from the nucleus to ARSs for aminoacylation.

Components of the EF-1 complex could be attracted to the charged tRNAs by their direct affinity for ARSs as well as tRNAs (Sivaram and Deutscher, 1990; Negrutskii and Deutscher, 1991; Negrutskii and Deutscher, 1992; Stapulionis and Deutscher, 1995). ARSs such as DRS, FRS, LRS, HRS,

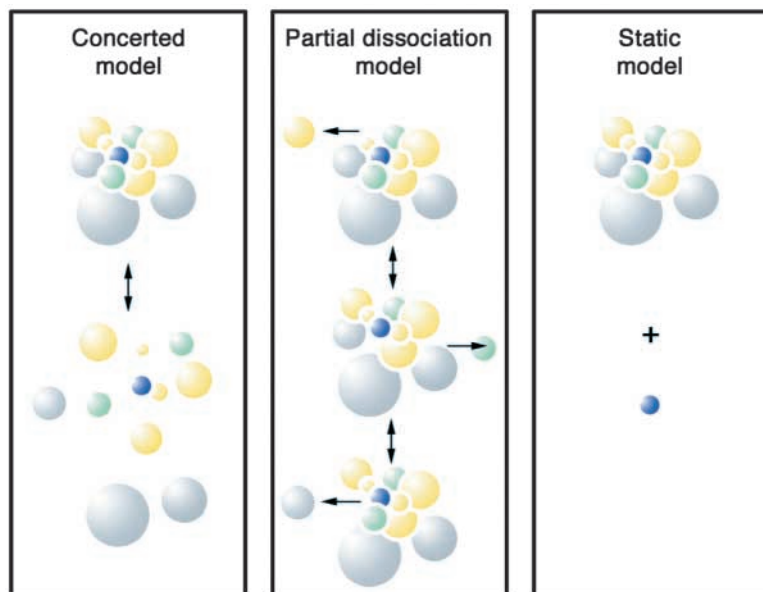


Fig. 5. Three hypothetical models for dynamic movement of complex components. In the concerted model, all the components associate and dissociate simultaneously. In the partial association/dissociation model, each component associates or dissociates on an individual basis. In this case, there would be several different subcomplexes. In the static model, each component would be synthesized whenever necessary for other activities. In this case, the complex would be maintained stably once it is formed.

EPRS, ARS, KRS and WRS have been observed to interact with subunits of EF-1H (Reed and Yang, 1994; Negrutskii et al., 1996; Lee et al., 2002). The multi-ARS complex could thus generate a channel for the delivery of tRNAs (Calado et al., 2002; Simos et al., 2002; Hopper and Phizicky, 2003).

Another complex, in which VRS is associated with the four subunits of EF-1H, is also present (Bec et al., 1989; Bec et al., 1994; Brandsma et al., 1995; Negrutskii et al., 1999; Galani et al., 2001). In this complex, the N-terminal extension of VRS is bound to the EF-1H subunits (β , γ and δ) that are responsible for guanine nucleotide exchange. Careful kinetic analyses of the VRS EF-1H complex demonstrated that the catalytic activity of VRS is enhanced about twofold by the addition of the α subunit (Negrutskii et al., 1999).

A systematic trafficking network involving mammalian ARSs and the translational machinery might thus exist (Negrutskii and Deutscher, 1991). Indeed, the primitive complex consisting of Arc1p, MRS and ERS found in yeast provides supporting evidence for this model (Simos et al., 1996; Simos et al., 1998). However, clustering of different ARSs within a complex might not necessarily positively affect the flow of tRNAs. The macromolecular assembly might sterically hinder efficient movement of large tRNA substrates. Thus, it will be interesting to see how ARSs are spatially arranged within the complex so that different tRNAs can move into and out of their cognate catalytic cores without colliding.

Alternatively, complex formation might contribute to the subcellular localization of ARSs. The ARS complex has been found in the nucleus (Nathanson and Deutscher, 2000), and the catalytic activities of the ARSs are thought to help the proofreading of newly synthesized nuclear tRNAs (Lund and Dahlberg, 1998). Another function of the complex might be to control the cellular turnover of the components. The chaperone Hsp90 facilitates assembly of multi-ARS complexes (Kang et al., 2000). Blocking its activity suppresses the incorporation of nascent ARSs, which are subsequently degraded. This indicates that their association is required to protect the components from degradation. The finding that dissociation of the components by depletion of the p38 protein results in their

rapid degradation further supports this idea (Kim et al., 2002). Thus, their association to form a complex might control the cellular turnover of ARSs.

Last, complex formation might be used to control non-canonical activities of the components. As already mentioned, several ARSs have additional functions (Table 1). Similarly, two of the non-enzymatic factors, p43 and p38, also play unique moonlighting roles. Thus, cells must somehow control the dynamic equilibrium between ARSs and cofactors used for protein synthesis and those used for novel regulatory activities. The multi-ARS complex may thus function as a molecular reservoir for distribution of these enzymes and cofactors. Further work is clearly needed if we are to understand the functions of this complex. Note that the roles suggested above need not be mutually exclusive, and additional functions are of course possible.

The dynamic balance of complex components

How are the diverse activities of components of the multi-ARS complex physically regulated while they are tied together within the complex? There are a few different possibilities (Fig. 5). First is a 'concerted association/dissociation model', in which all of the components are associated and dissociated in a concerted manner. A second possibility is a 'partial association/dissociation model', in which the components behave independently and different subcomplexes exist, depending on the conditions. Finally, there is the 'static complex model', in which the complex is dynamically inert and each component is synthesized *de novo* whenever its additional functions are needed.

Protein-protein interactions can be regulated by post-translational modifications such as phosphorylation. There are many phosphorylation sites for different kinases in ARSs. Damuni et al. have shown that the catalytic activities of the complex-forming ARSs can be modulated by phosphorylation *in vivo* and *in vitro* (Damuni et al., 1982). Five enzymes (DRS, QRS, ERS, MRS and KRS) are phosphorylated in reticulocytes. QRS and DRS are selectively phosphorylated in response to 8-bromo-cAMP (Pendergast et al., 1987), and DRS, ERS, MRS and KRS are phosphorylated *in vitro* by casein kinase I (Pendergast and Traugh, 1985). Phosphorylation by casein kinase I reduces the aminoacylation activity and alters the binding of the ARS complex to tRNA-Sepharose. Protein kinase C selectively phosphorylates QRS in rabbit reticulates stimulated by tumor-promoting phorbol esters (Venema and Traugh, 1991). Phosphorylation by protein kinase C *in vivo* also inhibits aminoacylation activity. However, no solid evidence is yet available that the assembly

or disassembly of the multi-ARS complex is regulated by phosphorylation (Pendergast et al., 1987).

Since p38 is indispensable for the maintenance of the multi-ARS complex, it is unlikely that p38 embedded in the multi-ARS complex is dispatched to other sites, since this would destabilize the complex. To prevent a shortage of p38 in the multi-ARS complex when it needs to be delivered to other target sites, the level of p38 should be dynamically regulated. Indeed, the level of p38 is significantly increased by transforming growth factor (TGF)- β , which generates additional p38 that is not bound to the multi-ARS complex (Kim et al., 2003). This fraction of p38 localizes to the nucleus, as determined by cell fractionation and immunofluorescence staining to control Myc expression (Kim et al., 2003). Thus, at least the 'static model' seems to apply to p38. However, because p38 is the main switch for assembly of the complex, it can also be used to control complex formation.

As mentioned above, one of the complex-forming ARSs, QRS, can bind to ASK1, and this interaction is enhanced by increases in the level of glutamine without changing the total cellular level of QRS (Ko et al., 2001a). In this case, glutamine might control the dynamic equilibrium between QRS in the ARS complex and at its alternative target site. Perhaps QRS shuttles between the multi-ARS complex and ASK1, depending on the conditions or glutamine concentration. These observations favor the partial association/dissociation model. Thus, different models appear to apply, depending on the component.

Conclusions and perspectives

The ARS-binding non-enzymatic cofactors seem to play a crucial role in the assembly of multi-ARS complexes. Among three mammalian ARS cofactors, p43 has relatives in prokaryotes as well as yeast and archaea. By contrast, homologs of p38 and p18 are present in the fly but not in other lower eukaryotes and bacteria – although a domain of p18 and p38 has significant homology with the N-terminal domains of yeast Arc1p, ERS, EF-1 β and EF-1 γ (Galani et al., 2001). From a structural point of view, Arc1p can be considered to be an ancestor of mammalian ARS cofactors, which appear to have adopted additional activities required for multicellular life. The cytokine activity of p43 is presumably necessary for the communication between different cells and for responses to exogenous stresses. In this regard, the cytokine activities shown by some mammalian ARSs provide further evidence for the need to link protein synthesis with signal transduction. Another ARS-binding factor, p38, is essential for the viability of multicellular organisms but not survival of their cells in culture and is required for the functional differentiation of alveolar type II cells during lung development (Kim et al., 2003). Although additional activities of p18 have not yet been revealed, we expect that it also plays a role in the control of cell fate, like the other two factors.

Why did these multi-functional factors become part of the ARS community? It could simply be an evolutionary coincidence: they happened to associate with the ARSs, through their GST-homology domains (Deinert et al., 2001; Galani et al., 2001), and subsequently other enzymes joined the primitive complex. In fact, p38 and p18 interact with each other, and p18 specifically interacts with MRS (Quevillon

et al., 1999). In addition, the deletion of the GST-homology domain in the C-terminal region of p38 results in the dissociation of EPRS and MRS from the complex (Kim et al., 2002).

Alternatively, these cofactors might have become linked to ARSs for functional reasons. ARSs can be good sensors of cellular conditions because they use amino acids as their reaction substrates. Interestingly, many ARSs can undergo conformational changes following binding to amino acids (Kornelyuk et al., 1995; Onesti et al., 2000). In the case of QRS, its anti-apoptotic interaction with ASK1 is stimulated by the increase in glutamine levels (Ko et al., 2001a). In addition, the concentration of charged/uncharged tRNAs is a crucial determinant for protein synthesis (Rojiani et al., 1990; Kimball, 2001). Therefore, these factors might monitor the condition of the cell by being physically linked to ARSs, in addition to enhancing the stability and catalytic capability of ARSs or controlling their cellular trafficking.

The evolutionary paradox in gene evolution is that higher eukaryotic cells harbor much more DNA than necessary. Conversely, the number of encoded proteins in these organisms appears to be much smaller than we used to predict. How can this limited number of proteins meet the demand for functional diversity that highly differentiated multicellular organisms require? Mammalian systems appear to take advantage of subcellular compartments, in which the same protein can be placed in a different physical environment or combined with a different repertoire of proteins. Perhaps higher organisms have evolved to maximize the safety and flexibility of genetic information by having extra DNA, yet economize by using one protein for many different purposes. In this regard, the mammalian ARSs and their associated factors provide a fascinating example of such multi-functionality.

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