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Breakthroughs and Views

Molecular network and functional implications of macromolecular tRNA synthetase complex

Jung Min Han,[a] Jin Young Kim,[b] and Sunghoon Kim[b,*]

[a] *Imagene Co. Biotechnology Incubating Center, Golden Helix, Seoul National University, San 56-1, Shillim-dong, Kwanak-Gu, Seoul 151-741, Republic of Korea*

[b] *National Creative Research Initiative Center for ARS Network, College of Pharmacy, Seoul National University, San 56-1, Shillim-dong, Kwanak-Gu, Seoul 151-741, Republic of Korea*

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Abstract

Understanding the complex network and multi-functionality of proteins is one of the main objectives of post-genome research. Aminoacyl-tRNA synthetases (ARSs) are the family of enzymes that are essential for cellular protein synthesis and viability that catalyze the attachment of specific amino acids to their cognate tRNAs. However, a lot of evidence has shown that these enzymes are multi-functional proteins that are involved in diverse cellular processes, such as tRNA processing, RNA splicing and trafficking, rRNA synthesis, apoptosis, angiogenesis, and inflammation. In addition, mammalian ARSs form a macromolecular complex with three auxiliary factors or with the elongation factor complex. Although the functional meaning and physiological significance of these complexes are poorly understood, recent data on the molecular interactions among the components for the multi-ARS complex are beginning to provide insights into the structural organization and cellular functions. In this review, the molecular mechanism for the assembly and functional implications of the multi-ARS complex will be discussed.

Keywords: Network; Aminoacyl-tRNA synthetase; Complex; Protein synthesis; Elongation factor; Protein–protein interaction; Review

Elucidation of the structure and dynamic control of protein networks is a central problem in the post-genome era because most of the proteins exert their activities in the context of a complex network. Proteins can communicate with each other in a different mode depending on their dynamic relationship. They can either form tight and static complexes, or loosely or dynamically linked networks. Macromolecular protein complexes can be as simple as homo- or heterodimer, or contain multiple components with highly organized structures. The well-known examples include those that are involved in the basic processes of DNA replication, transcription, and translation as well as in the regulation of signal transduction and protein turnover (Table 1). The functional reason for the macromolecular complex formation can be found in the reaction efficiency, coordinated control of the processes, cellular framework, and molecular transport. Within the complex, the subunits or components are often differentiated to play complementary roles in terms of their structure and function. For example, ribosome is divided into two subunits. The large subunit binds tRNA and mediates peptidyl transfer, while the small subunit controls mRNA binding, decoding, and fidelity. Therefore, in order to promote the accurate decoding of mRNA and rapid formation of peptide bonds, coordinated functions of components of the ribosomal complex are required. The COP9 signalosome (CSN) complex functions at the interface between signal transduction and ubiquitin-dependent proteolysis. CSN has two activities—a protein kinase and a deneddylase [1,11]. The CSN-associated kinase phosphorylates transcription factors, which determines their stability towards the ubiquitin system while the CSN-associated deneddylase regulates the activity of specific SCF E3 ubiquitin ligases. Therefore, the CSN complex appears to be a platform that links signaling with ubiquitin-dependent proteolysis. The

* Corresponding author. Fax: +82-2-875-2621.
E-mail address: sungkim@snu.ac.kr (S. Kim).

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Table 1
Examples of macromolecular complexes in the cell

Complex	Function	kDa	Subunit	Reference
COP9 signalosome (CSN)	Signal transduction	450 kDa	8	[1]
eIF3	Initiation of translation	600 kDa	10	[2,3]
RNA polymerase II transcription initiation	mRNA transcription	1000 kDa	6	[4]
Escherichia coli DNA polymerase III holoenzyme	Replication	432.6 kDa	10	[5]
Survival of motor neurons (SMN)	snRNP assembly	50S particle	5	[6]
Aminoacyl-tRNA synthetase (ARS)	Aminoacyl-tRNA synthesis	1000 kDa	11	[7,45]
Cohesin complex	Mitosis	385 kDa	4	[8]
Bacterial ribosome	Protein synthesis	2300 kDa	58	[9]
Proteasome 19S regulatory particle (RP)	Protein degradation	400 kDa	8	[10]

eukaryotic translation initiation reactions are promoted by eukaryotic initiation factors (eIFs). The eIF3 is a multiprotein complex of ~600 kDa that plays a central role in the translation initiation reaction, influencing ribosomal association, the formation of the 40S initiation complex by interacting with the ternary complex of eIF2–GTP–Met–tRNAi, and mRNA binding [12,13]. Thus, the components of the eIF3 complex should have coordinated relationships in order to promote the accurate initiation of protein synthesis.

Although it has been proposed that protein synthesis would proceed via well-organized higher-order molecular machinery in vivo, the details of the molecular networks among the involved factors and enzymes are largely unknown. Particularly intriguing among the translational complexes is the macromolecular complex consisting of nine different aminoacyl-tRNA synthetases (ARSs) and non-enzyme factors. Knowledge of the structural and functional details of this multi-component ARS complex is necessary, not only to understand the mechanism of eukaryotic protein synthesis, but also to elucidate the principle of the intracellular organization of the protein network. Moreover, because the existence of the multi-ARS complex appears to be restricted to multicellular eukaryotes, understanding the functional reason for the formation of this complex may give insight into the distinction in the regulation of protein synthesis and other functionally linked biological processes between the prokaryotic and eukaryotic systems.

Aminoacyl-tRNA synthetase

Specific recognition between ARSs and tRNAs is critical for the precise translation of the genetic code into the protein sequence. In spite of their common function in protein synthesis, ARSs have been diversified in their molecular weight, primary sequence, and quaternary structure. Based on amino acid sequence alignments and structural features, ARSs have been divided into two classes (Table 2) [14–16]. The class I synthetases are generally monomers or dimers and share two consensus sequences, the HIGH (His–Ile–Gly–His) and KMSKS (Lys–Met–Ser–Lys–Ser) motifs, that form a Rossmann nucleotide-binding fold. The class II synthetases are generally dimers or tetramers and are characterized by three specific sequence motifs. The motif 1 has a conserved Pro residue and is involved in dimer formation. The class II synthetases do not contain the Rossmann nucleotide-binding fold, but share a very different catalytic domain that consists of an antiparallel β-sheet structure. The motifs 2 and 3 contain a conserved Arg residue and constitute the active site that is involved in the formation of the aminoacyl adenylate intermediate and binding of tRNA. The vast amount of information on the nucleotide sequences and three-dimensional structures provide insights into the evolution of ARSs. In contrast to the 20 standard amino acids, which are conserved in all organisms, both synthetases and their cognate tRNAs have changed significantly throughout evolution. From several biochemical and

Table 2
Classification of aminoacyl-tRNA synthetases

Class I		Class II	
Ia	IRS (a), LRS (a), VRS (a)	IIa	ARS (a, a4), PRS (a2)
	CRS (a), MRS (a, a2)		HRS (a2), SRS (a2), TRS (a2)
	RRS (a)		GRS (a2,a2b2)
Ib	QRS (a)	IIb	NRS (a2)
	ERS (a), KRS-I (a)		DRS (a2), KRS-II (a2)
Ic	YRS (a2), WRS (a2)	IIc	FRS (ab)2

These enzymes can be divided into two classes, based on their structural features. Each class is further grouped into three subclasses, according to the chemical properties of the substrate amino acids. The two lysyl-tRNA synthetases are found in both the classes.

phylogenetic analyses of ARSs and tRNAs, it has become clear that co-adaptations of these enzymes and tRNAs must have occurred through evolution in order to maintain the genetic code and translate the genetic code into protein sequence.

Complex formation of aminoacyl-tRNA synthetases

ARSs can also be distinguished, based on their property to form a macromolecular complex. A primitive form of these complexes is present in yeast. The yeast ARS complex is composed of glutamyl- (ERS) and methionyl-tRNA synthetases (MRS), and the non-enzyme component called Arc1p which is homologous to mammalian p43 that is also bound to the multi-ARS complex [17]. Due to its relative simplicity, its structural organization and function have been well understood [18]. This complex is stable in vivo and is dependent on the association between the N-terminal domains of Arc1p and the enzymes [19]. It was shown that the complex formation was necessary to enhance the catalytic activity of the bound enzymes and the export of nuclear tRNA to cytosol [18]. Presently, two different types of ARS complexes have been reported in the mammalian system. One is the complex that consists of valyl-tRNA synthetase (VRS) and the translational elongation factor complex. The proposed role of this complex is to facilitate the delivery of the charged tRNAs to ribosome. VRS forms a complex with EF-1H [20–22]. This complex has also been shown to functionally interact with EF-1α [23]. Similar interactions with the elongation factor complex were also reported in phenylalanyl- (FRS), aspartyl- (DRS), histidyl- (HRS), and leucyl- (LRS) tRNA synthetases [24–26]. The protein moieties that contribute to the assembly of this multi-enzyme structure have been partially identified. The association of VRS with EF-1H is mediated by the N-terminal polypeptide extension that characterizes the higher eukaryotic enzyme, as compared with its prokaryotic or lower eukaryotic counterparts [22]. The second type of ARS complex has been found from flies to humans and consists of eight different enzyme polypeptides. The identified core enzymes include bifunctional glutamylprolyl-tRNA synthetase (EPRS), isoleucyl- (Ile), leucyl- (Leu), glutaminyl- (Gln), methionyl- (Met), lysyl- (Lys), arginyl- (Arg), and aspartyl- (Asp) tRNA synthetases. Three auxiliary proteins (apparent molecular weights of 18, 38, and 43 kDa) are invariably associated with the complex [17,27,28]. Genetic, biochemical, and structural approaches have provided a picture of the interactions between the various components of the larger complex and implicated many of the idiosyncratic insertion and extension domains for the complex formation [28–33]. The possible roles of the accessory proteins have also been unveiled to some extent. The p18 sequence suggests that it is responsible for the transient interaction of the complex with EF-1H [26]. p38 is important for the assembly of the whole complex [34], indicating its role as a scaffold protein within the complex. p43, which is homologous to Arc1p, probably helps tRNA binding by the complex [19,35]. It may also play a role in the assembly of the multi-ARS complex.

Structural organization of aminoacyl-tRNA synthetase complex

Although the multi-ARS complex has been known for the last two decades, the structural organization and functional significance of this multi-enzyme complex are not yet completely delineated. Mammalian ARSs have extra peptide appendices that are absent in their prokaryotic counterparts. Because prokaryotic ARSs exist as free forms and do not form macromolecular complexes, eukaryotic extra peptide appendices were considered as responsible for the molecular assembly of the enzymes or in other novel functions that are unique to the eukaryotic system.

To define the physical locations of the components in three-dimensional space, structural details will be needed at a higher resolution. Although the structures of many prokaryotic ARSs have been solved by X-ray crystallography, still structural information on mammalian ARSs is limited. Recently, the X-ray crystal structures of human mini-tyrosyl-tRNA synthetase (YRS) and the C-terminal domain of the ARS-associating factor, p43, were solved [36,37], and the solution structure of the peptide appendix that is present in bifunctional glu-prolyl-tRNA synthetase (EPRS) was solved by NMR [38,39].

Multi-ARS complexes have been isolated from a number of mammalian sources, including animal tissues [40,41] and cultured cell lines [42]. Overall, the molecular weight of the complex was approximately $1.0–1.2 \times 10^6$ Da and sedimented as an 18–30S particle [43]. In negatively stained electron micrographs, the particle appears in several orientations including triangular, rectangular, and square shapes [29,44]. A working structural model of the complex was proposed as a cup or elongated 'U' shape based on low resolution electron microscopic images [29]. The earliest electron micrographs of density gradient fractions of the ARS preparations from rat liver showed negatively stained "dumbbell"-shaped and circular particles of 11–14 nm in size [45]. A study of the purified high molecular weight ARS complex from the rabbit reticulocytes presented primarily metal-shadowed preparations in which only irregular globular masses that are composed of several small loosely associated spheres could be seen [46]. After cross-linking experiments, more compact, but

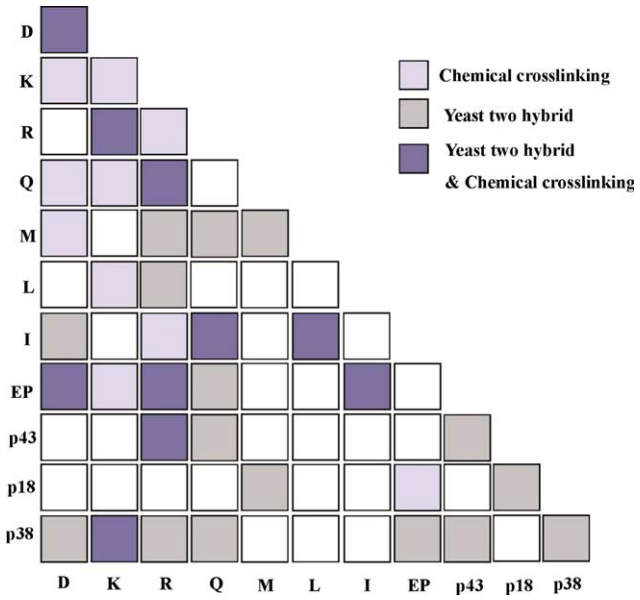

Fig. 1. The pairs between the components for the multi-ARS complex identified by two-hybrid analysis and chemical crosslinking methods. The potential 55 heterologous and 11 homologous pairs of the complex-forming ARSs are displayed as rectangles. The two-hybrid data from two independent studies [28,33] were combined. The interacting pairs that were determined by the two-hybrid analyses (gray) were compared with the neighboring pairs, suggested by the chemical crosslinking method (pale blue). The interacting pairs that were detected by both of the two methods are indicated as dark blue.

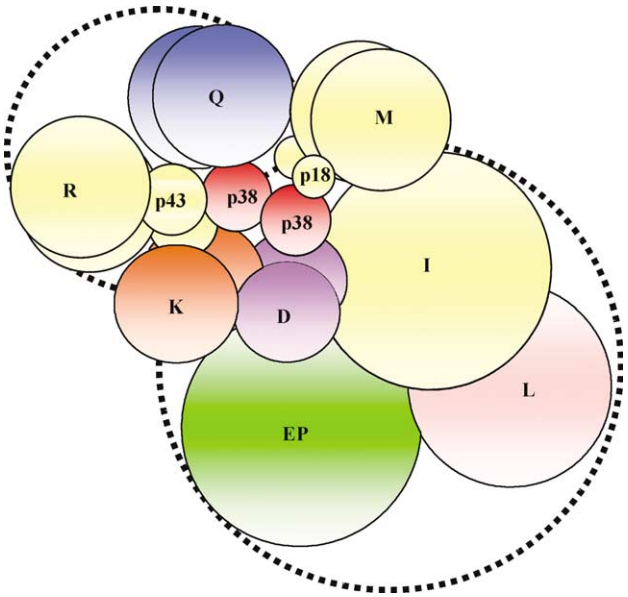

Fig. 2. The proposed two-dimensional arrangement of the components in the multi-ARS complex. In this model, the components are arranged into two subdomains with respect to their anchorage sites in p38. One subdomain of QRS, RRS, and p43 is linked to the N-terminal region of p38 and the other including KRS, DRS, MRS, p18, EPRS, IRS, and LRS is bound to the C-terminal peptide of p38. RRS, KRS, DRS, MRS, QRS, p38, and p43 were drawn as dimers as proposed in the previous reports [28,31,33,75,76]. Each of p18 makes specific interaction with MRS. The five components (IRS, MRS, RRS, p43, and p18) depend on p38 for their cellular stability (marked yellow). The two-dimensional arrangement of the components does not necessarily reflect their relative positions in the three-dimensional structure.

still highly amorphous images, with maximum dimensions of 30–40 nm were observed.

Several approaches were undertaken to probe the structural organization of the ARS complex. Stepwise dissociation of the components from the complex was carried out by using non-ionic detergent [44,47] and changing salt concentration [48]. The particle shape was appraised by electron microscopy studies [29]. All of these studies have suggested that this multi-enzyme complex has a discrete and well-defined architecture. To solve the structural details of ARS complex, the neighboring proteins of the complex-forming ARSs were analyzed by chemical cross-linking [31,49] and a yeast two-hybrid analysis [28,33] (Fig. 1). Interestingly, only a portion of the possible interaction pairs was supported by both of these approaches (Fig. 1, dark blue), probably due to the difference in the working principles of the two techniques. On the basis of these results as well as our previous report [34], the components of the ARS complex can be displayed two-dimensionally and grouped into two subdomains by their association with p38 (Fig. 2). In this model, one subdomain, containing QRS, RRS, and p43, is linked to the N-terminal 83 aa region of p38, while the other subdomain, consisting of MRS, KRS, DRS, p18, and three high molecular weight enzymes, EPRS, IRS, and LRS [31], is anchored to the C-terminal region of p38. Although the detailed spatial arrangement of the components and oligomerization of each component are still unclear, it is obvious that the p38 protein is an essential scaffold molecule for the assembly of the ARS complex. Among ARS complex components, IRS, MRS, RRS, p18, and p43 depend on p38 for their protein stability [34] (Fig. 2). It is very interesting that the stability and assembly of the whole macromolecular protein complex depend on a single component.

Multi-functionality of ARS and functional implications for the complex formation of ARSs

Recent evidence showed that the components of the ARS complex participate in various biological processes in addition to protein synthesis (Fig. 3). Human QRS was shown to have an anti-apoptotic function [50]. QRS specifically interacts with the apoptosis signal-regulating kinase 1 (ASK1) in a glutamine-dependent manner and inhibits the ASK1-mediated apoptosis by inhibiting the kinase activity of ASK1. Since Fas-induced ASK1 and c-Jun N-terminal kinase (JNK) activities are inhibited by the glutamine supply, it was suggested that QRS might mediate the anti-apoptotic effect of glutamine. By using a yeast two-hybrid analysis, KRS was identified as the human immunodeficiency virus type 1 (HIV-1) viral

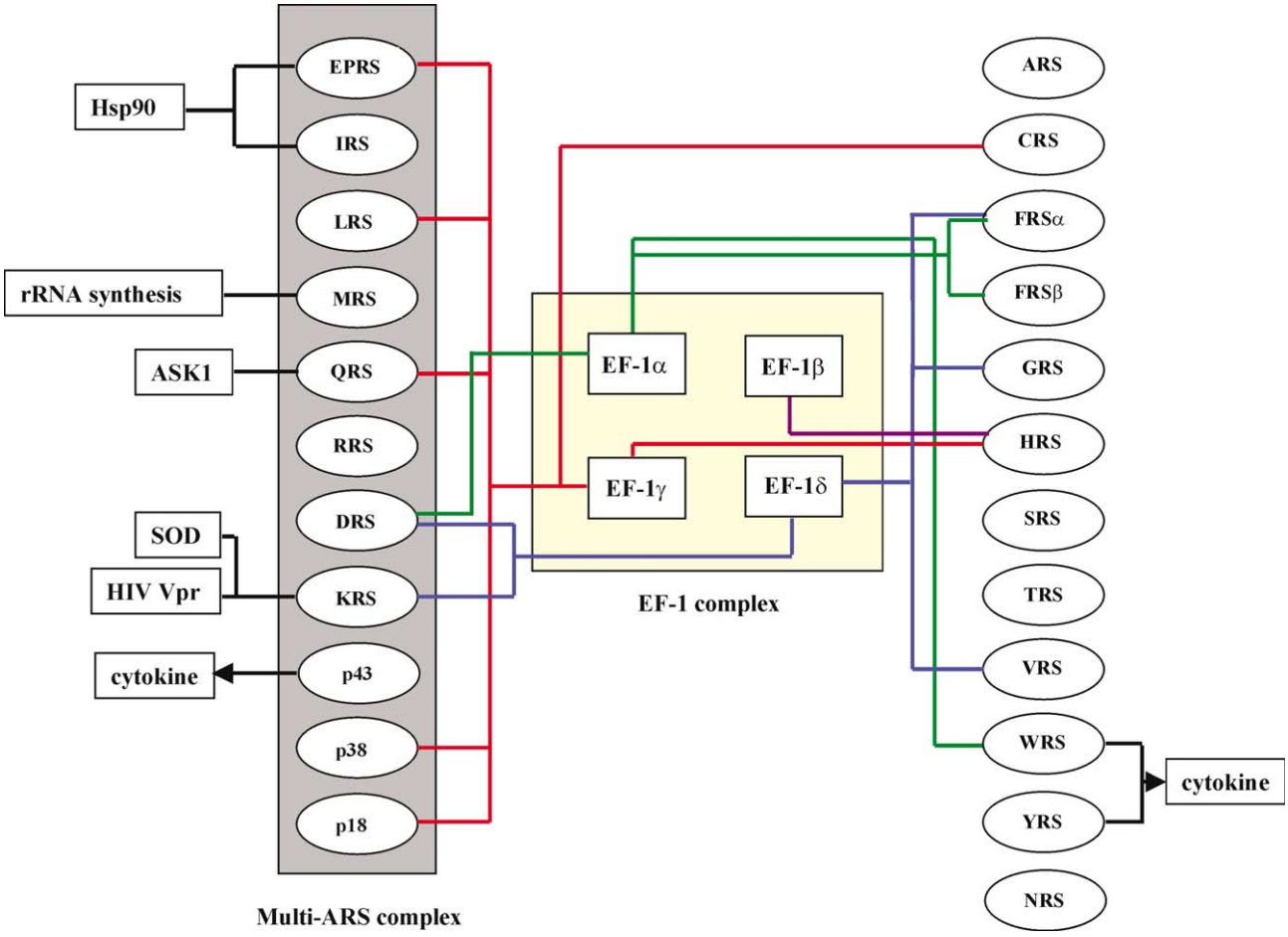

Fig. 3. Schematic diagram of the novel activities and interactions of ARSs. The complex-forming ARSs are vertically arrayed in the gray box. Functional or physical interactions of ARSs are indicated with lines. ARSs that interact with the subunits of the elongation factor complex are marked with the lines of a different color. WRS, YRS, and p43 are secreted and work as cytokines (arrows).

protein R (Vpr)-interacting protein [51]. The KRS-mediated aminoacylation of $tRNA^{Lys}$ is inhibited by the interaction with Vpr. Since $tRNA^{Lys}$ is the primer for reverse transcription of the HIV-1 genome, it is thought that the interaction between Vpr and KRS may influence the initiation of HIV-1 reverse transcription. In this regard, it is interesting that KRS is also found in the virion of HIV [52]. KRS also interacts with mutant forms of Cu, Zn superoxide dismutase (SOD1), which are observed in familial and sporadic amyotrophic lateral sclerosis cases, but not with the wild-type SOD1, suggesting the relevance of this interaction to the motor neuron disease [53]. Recently, it was reported that TIP-15, which was identified as a cellular protein that can bind to the C-terminal end of the HTLV-1 Tax protein via its two PDZ domains, interacts with KRS using the same domain [54], suggesting the presence of a PDZ domain binding site (PDZ-BS) in KRS. The sequence at the C-terminal end of KRS matches the X-S/T-X-V-COOH consensus motif that was previously defined as the PDZ domain-binding site. However, the functional significance and physiological binding partner of PDZ-BS in KRS are still unknown. Human MRS was found at the nucleolus as well as in the cytoplasm [55]. The cellular localization of MRS is regulated by various growth factors, such as insulin, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). It was also proposed that the nucleolar MRS may play a role in ribosomal RNA synthesis [56]. The yeast protein Arc1p interacts with Los1p, the homolog of human exportin-t and a nuclear pore-associated protein that is involved in tRNA export [18]. Therefore, the mammalian p43 protein of the ARS complex may also be involved in the nuclear export of tRNA. Another interesting function of p43 is focused on its cytokine activity. p43 is secreted in several cell lines [57–59] and activates mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB) to induce cytokines and chemokines, such as tumor necrosis factor (TNF), interleukin-8 (IL-8), macrophage chemotatic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and IL-1β [60]. p43 induces monocytic cell adhesion [61] and apoptosis of endothelial cells [62,63]. However, the multi-functionality does not appear to be the unique

signature of the complex-forming enzymes and co-factors. For instance, the non-complex forming enzyme, YRS, is also secreted in the apoptotic condition and split into two distinct polypeptides by leukocyte elastase, generating pro-inflammatory cytokine activity [64]. The N-terminal polypeptide of YRS binds to the IL-8 type A receptor and functions as an IL-8-like cytokine. The C-terminal polypeptide of YRS has a sequence homology (~50%) with p43 and also shows pro-inflammatory cytokine activity. Another free form enzyme, WRS, exerts angiostatic activity since it inhibits endothelial cell proliferation as well as new blood vessel formation [65]. In addition, there are reports showing that even prokaryotic or unicellular eukaryotic ARSs, which do not form a macromolecular complex, are also non-catalytically involved in other biological processes. In bacteria, alanyl-tRNA synthetase is responsible for the aminoacylation of both $tRNA^{Ala}$ and a tRNA–mRNA hybrid molecule, transfer-messenger RNA (tmRNA). The functional role of Ala-tmRNA is to direct the C-terminal tagging with a protease targeting sequence of prematurely truncated proteins and ribosome rescue [66–68]. A structural analysis of the threonyl-tRNA synthetase (TRS)-$tRNA^{Thr}$ crystal provided evidence that ARS is involved in the regulation of its own mRNA expression. This regulation is achieved via the binding of the enzyme to the leader sequence of its own mRNA, which partially mimics the structure of $tRNA^{Thr}$ [69]. In *Neurospora crassa* and some other fungi, the mitochondrial tyrosyl-tRNA synthetase (YRS) is a splicing factor for group I introns [70]. A similar function is also attributed to yeast mitochondrial leucyl-tRNA synthetase (LRS) [71].

Although the modulation of the catalytic activities of ARSs by the associated co-factors, such as p43 [72] or arc1p [18], has been suggested as one of the functional reasons for the complex formation, a more functional reason for the complex formation was expected. Another indication came from the effect of heat shock protein 90 on the complex formation. It was shown that hsp90 facilitated the molecular assembly of the nascent ARS polypeptides. Also, the inactivation of hsp90 resulted in the degradation of the components. This suggests that the complex formation is required for the cellular stability of the component proteins [73]. This possibility was further supported by the effect of the p38 depletion on the cellular concentration of the components for the complex [34]. In the absence of p38, many components, such as MRS, QRS, IRS, p43, and p18, lost their cellular stability. Based on these results, another possible function for the molecular assembly of ARSs is to maintain the cellular stability of the components. Since the overall protein synthesis does not appear to stringently depend on the cellular concentration of ARSs [74], the rapid turnover of the component proteins may be related more to their roles in biological processes that are not directly associated with their catalytic activities or protein synthesis. The dynamic control of the component proteins between the multi-ARS complex and other biological processes needs further investigation.

Conclusions and future prospects

Since many of the complex-forming ARSs are active in the free state in vitro, it is clear that the complex formation is not essential for their catalytic activity. In the multi-ARS complex, the aminoacylation reactions that are catalyzed by the component enzymes would proceed in parallel. Considering the size of the substrate tRNAs, a significant traffic jam during the entrance and exit of the substrates and steric hindrance between them are expected within the complex. Also, the mouse embryonic fibroblast cells that are deficient in p38, which is an essential scaffold for the multi-ARS complex formation, showed normal viability and proliferation (data not shown), despite the fact that many components lost their cellular stability [34]. This result further supports the supposition that the complex formation is not essential for protein synthesis and proliferation at the cellular level. Nonetheless, the 38-deficient mice showed neonatal lethality which suggests that the presence of p38 and the complex formation of ARSs are essential for the viability at the organism level. Combined together, it seems that the complex formation should be functionally associated more with the non-canonical activities of the complex-forming enzymes and co-factors rather than with their roles in protein synthesis. To understand the function and mechanism for the molecular assembly and dynamic regulation, it is essential to unravel all of the activities of the component enzymes and co-factors and also to understand the dynamic regulation of the complex formation and dissociation of the components. When the human genome structure was announced, we were surprised that the expected protein numbers were much less than expected. However, we now begin to realize that the complexity of life is mainly determined by a sophisticated network of proteins and their multi-functionality rather than the number of genes. In this context, the molecular interactions of ARSs and their multi-functionality would warrant a good example in the post-genome research.

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