Review

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Novel regulatory interactions and activities of mammalian tRNA synthetases

Aminoacyl-tRNA synthetases (ARSs) catalyze the attachment of specific amino acids to their cognate tRNAs, thereby ensuring the faithful translation of genetic code. In addition to their enzymatic function, these enzymes have been discovered to regulate various cellular functions such as tRNA export, ribosomal RNA synthesis, apoptosis, inflammation and angiogenesis in mammalian. The insights into the noncanonical activities of these enzymes have been obtained from their unique cellular localization, interacting partners, isoform generation and expression control. Mammalian ARSs also form a macromolecular protein complex with a few auxiliary factors. Although the physiological significance of this complex is poorly understood, it also supports the potential of mammalian ARSs as sophisticated multifunctional proteins for regulating various cellular procedures. In this review, the novel regulatory activities of mammalian ARSs will be discussed in different biological processes.

Keywords: Aminoacyl-tRNA synthetase / Cellular localization / Noncanonical activities / Protein-protein interactions / Review PRO 0266

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1 Introduction

Many biological researches have been established based on the idea that one gene encodes one protein with one function since George Beagle and Edward Tatum proposed one gene-one protein hypothesis in 1943. However, the one protein-one function hypothesis appears to be too naive to explain the complexity of life. Through extensive studies, we now understand that cellular proteins exert their activities *via* a fine network with neighboring proteins through which they are coordinated with, and responded to, diverse internal and external conditions. A recent well-known example of a multifunctional protein is cytochrome c. It is not only a mitochondrial protein involved in ATP generation but also a cytoplasmic protein triggering programmed cell death [1]. The multifunctionality of a protein can be achieved depending on its cellular localization, secretion, expression, oligomerization, ligand/substrate concentration, binding sites and complex formation [2].

Aminoacyl-tRNA synthetases (ARSs) can be viewed as multifunctional proteins based on their roles in a number of critical cellular activities such as tRNA processing, RNA splicing and trafficking, apoptosis, and transcriptional and translational regulation (see reviews in [3, 4]). These noncanonical activities have been found in ARSs of different phylogenetic lineages, suggesting that these enzymes have evolved to adopt extra activities to link protein synthesis with other biological processes. Here we will just focus on the novel regulatory interactions and activities mediated by mammalian ARSs.

2 Nuclear and nucleolar function of ARSs

Although ARSs are normally involved in cytoplasmic protein synthesis, they have been also found in the nucleus. For example, mammalian arginyl-tRNA synthetase (RRS),

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Abbreviations: ARS, aminoacyl-tRNA synthetase; **EPRS**, glutanyl-prolyl-tRNA synthetase; **IRS**, isoleucyl-tRNA synthetase; **KRS**, lysyl-tRNA synthetase; **MRS**, methionyl-tRNA synthetase; **RRS**, arginyl-tRNA synthetase; **WRS**, tryptophanyl-tRNA synthetase; **QRS**, glutaninyl-tRNA synthetase

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glutamyl-prolyl-tRNA synthetase (EPRS), and tryptophanyl-tRNA synthetase (WRS) were detected in the nucleus by using an immunogold technique [5, 6] and enzymatic activities of ARSs could be detected in crude nuclear fractions [7]. The presence of the putative nuclear localization signals was reported in yeast ARSs and human methionyl-tRNA synthetase (MRS) [8, 9], suggesting that many eukaryotic ARSs could be generally localized in the nucleus. Since nuclear export of eukaryotic tRNAs is retarded if they are not charged with amino acids [10, 11], ARSs in the nucleus might play important roles in proof-reading and exporting tRNAs. Using oocytes from *Xenopus laevis*, Lund *et al.* [10] showed that aminoacylation of tRNA occurs in the nucleus prior to its moving to the cytoplasm, where proteins are assembled. It implies that ARSs must somehow get into the nucleus from the cytoplasm. The complex involved in *Xenopus* tRNA export contains tRNA, the tRNA export receptor exportin-t, and the guanosine 5'-triphosphate GTP-bound form of Ran (Ran-GTP), a GTPase required for nucleocytoplasmic transport of both RNAs and proteins [12]. *Saccharomyces* Los1p, the homolog of human exportin-t, mediates nuclear export of tRNA and interacts with Arc1p which functions as a cofactor for MRS and glutamyl-tRNA synthetase (ERS) [13]. Thus, aminoacylation of ARSs appears to be tightly coupled with the nuclear export of nascent tRNA.

Human MRS provides an example showing its different functions depending on its cellular localization. Mammalian MRS was found at the nucleolus as well as in the cytoplasm [14] but the function of nucleolar MRS had not been explained until Ko *et al.* [9] reported its role in ribosomal RNA synthesis. Human MRS was found in the nucleoli of the highly proliferating but not in the quiescent cells, and the nucleolar localization was triggered by various growth factors such as insulin, platelet-derived growth factor, and fibroblast growth factor (Fig. 1), suggesting that the localization of MRS in the nucleolus is regulated by cellular proliferation signals. Since the treatment of anti-MRS antibody decreased ribosomal RNA synthesis, human MRS might play a role in the biogenesis of rRNA in the nucleoli, while it is catalytically involved in protein synthesis in the cytoplasm. Thus, mammalian MRS may be dually involved in protein synthesis as an enzyme and as a positive regulator for ribosome biogenesis (Fig. 1).

3 Regulation of apoptosis by ARSs

Monocytes and macrophages should be rapidly recruited for removing apoptotic cells because cellular contents released from apoptotic cells are harmful for surrounding cells. Thus, apoptotic cells secrete factors with leukocyte

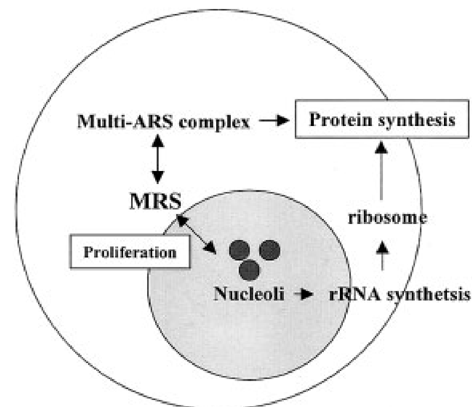

Figure 1. Growth dependent nucleolar localization of human MRS. Human MRS is normally associated with a macromolecular protein complex consisting of different ARSs and cofactors. However, it is also translocated into nucleoli to enhance ribosomal RNA synthesis by growth factors. Thus, MRS can be involved in protein synthesis as an enzyme to generate charged tRNA and as a positive regulator of ribosome biogenesis.

and monocyte chemotaxis activity. Interestingly, one of ARSs, tyrosyl-tRNA synthetase (YRS) can work as a chemoattractant to recruit leukocytes and monocytes [15]. Under apoptotic conditions, human YRS is secreted and split into two distinct cytokines by leukocyte elastase that is an extracellular protease [15, 16] (Fig. 2). The catalytic amino terminal domain of YRS binds to the interleukin-8 type A receptor and functions as an interleukin-8 like cytokine. In principle, secretion of an essential component of the translational apparatus as an early event in apoptosis would be expected to arrest translation and thereby accelerate cell death. The secreted YRS could function as intercellular signal transducers, attracting polymorphonuclear leukocytes (PMNs) and amplifying the local concentration of PMN elastase. This recursive cycle could enhance the cleavage of secreted human YRS, thereby recruiting more macrophages to the sites of apoptosis, which would promote removal of cell corpses.

The *C*-terminal domain of YRS shows a homology to p43 that is one of the ARS associating factors (Fig. 2). p43 is also secreted in methylcholanthrene fibrosarcoma cells, 32D myeloid precursor cells, and human prostatic adenocarcinoma cells (Fig. 2) [17–19]. It also shows the cytokine activity, activating mitogen activated protein kinases (MAPKs) and nuclear factor kappa B (NFκB) to induce cytokines and chemokines such as TNF, IL-8, macrophage chemotactic protein-1, macrophage inflammatory protein-1α and IL-1β [20, 21]. Interestingly, it is cleaved to release its *C*-terminal domain from the multi-ARS complex by caspase 7 activated upon apoptosis [22]. Since

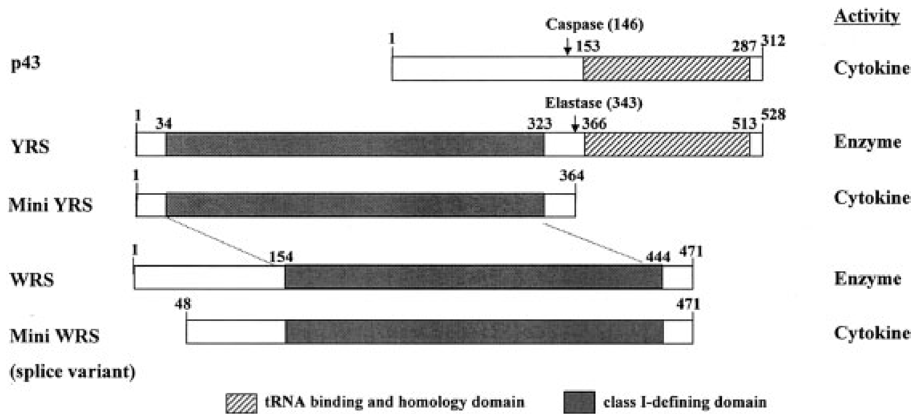

Figure 2. Sequence structure and activities of human tyrosyl-tRNA synthetase and tryptophanyl-tRNA synthetase and ARS associating factor, p43. The *C*-terminal domains of human YRS and p43 share about 50% sequence homology and contain pro-inflammatory cytokine activity. YRS and p43 are cleaved by extracellular elastase and caspase-7, respectively. WRS and YRS both belong to class I that was determined by the structural feature of the catalytic domains of ARSs [79]. The *N*-terminal truncated mini WRS can be produced by alternative splicing [80, 81]. The mini-WRS can work as an angiostatic cytokine.

the *C*-terminal domain of p43 is capable of binding to tRNA [23, 24] to facilitate the catalytic activity of the associated ARS [25], the proteolytic cleavage of p43 upon apoptosis would inactivate its function as a positive regulator of ARS. p43 also induced apoptosis of endothelial cells *via* its interaction with the alpha subunit of ATP synthase [26]. In addition to p43, WRS was suggested to be angiostatic since it inhibits endothelial cell proliferation, as well as preventing new blood vessel formation in chicken chorioallantoic membrane assay, a murine matrigel model, and a postnatal mouse retinal model [27, 28]. Interestingly, the truncation of YRS and WRS is required for the maturation of the active cytokine, while the cleavage of p43 is not essential for the cytokine activity (Fig. 2).

Glutaminyl-tRNA synthetase (QRS) charges glutamine to its cognate tRNA for protein synthesis. Interestingly, human QRS was also shown to work as an apoptosis suppressor [29]. QRS makes a specific interaction with apoptosis signal-regulating kinase 1 (ASK1) in a glutamine-dependent manner and inhibits the ASK1-mediated apoptosis by suppressing the activation of ASK1 (Fig. 3). Since glutamine supplementation inhibits Fas induced ASK1 and c-Jun *N*-terminal kinase (JNK) [30, 31], QRS is likely to provide a possible mechanism for the anti-apoptotic effect of glutamine. With all these data, a few different ARSs appear to be involved in the control of cell death *via* their idiosyncratic mechanism.

4 Expression control of ARSs

Although ARSs and their cofactors are universally expressed, their expression levels are varied temporally and spatially in the developing fruit fly and mouse. For example, Seshaiah and Andrew [32] demonstrated that each ARS is uniquely expressed in different tissues and developmental stages of *Drosophila*. In particular, WRS is highly induced upon cellular exposure to interferon because its promoter contains an interferon responsive region [33, 34]. However, there is no direct evidence explaining the functional reason for the up-regulation of WRS by interferon.

Expression levels of p43 also varies depending on tissues and developmental stages. For instance, there is a significant surge in the expression of p43 within the lungs on postnatal days 8–16 of the mouse [35]. It is produced throughout the lung, predominantly in the myoepithelium that lines the bronchioles. In addition, p43 is highly expressed in microglial cells within lesions of experimental autoimmune encephalomyelitis, neuritis, and uveitis [36]. Interestingly, the high level of p43 is also observed in the foam cells of atherosclerotic lesions and prostate

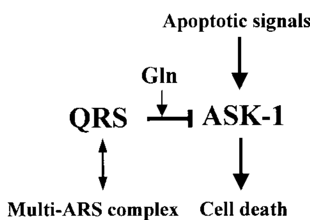

Figure 3. Glutamine-dependent anti-apoptotic activity of human glutaminyl-tRNA synthetase *via* its interaction with apoptotic signal-regulating kinase-1 (ASK1). ASK1 plays a pivotal role in the control of apoptosis [82]. The kinase activity of ASK1 is positively or negatively regulated by various signals [83]. Human glutaminyl-tRNA synthetase is associated with other ARSs to form a macromolecular protein complex [58]. However, it also makes the interaction with ASK1 in a glutamine-dependent manner to repress ASK1, thereby blocking cell death.

adenocarcinoma cells [19, 20]. The high expression of p43 in specific developmental stages and tissues suggests that p43 could have functions that still have to be unveiled in more detail.

Generation of different ARS isoforms by alternative splicing have been reported. Four and two different isoforms generated by alternative splicing have been reported for mammalian WRS and isoleucyl-tRNA synthetase (IRS), respectively [37, 38]. Interestingly, sequences homologous to an interferon-stimulated response element have commonly been found in the regulatory regions of these genes, although the relationship between this element and alternative splicing is not understood. Two recent reports revealed alternative splicing of cysteinyl-tRNA synthetase (CRS), and lysyl-tRNA synthetase (KRS) [39, 40], using a combination of bioinformatics and molecular biological approaches. Kim *et al.* [39] employed two exon prediction programs GENSCAN [41] and FGENE [42], to analyze the genomic sequences of five ARSs (cysteinyl-, valyl-, histidyl-, glycyl-tRNA synthetases and phenylalanyl-tRNA synthetase β subunit). In addition, EST fragments were searched for possible alternative splicing. Consequently, four different exon combinations were suggested from either side of 5' and 3' ends of human CRS mRNA. From these analyses, a new exon for human CRS encoding an 83-residue domain was identified. This exon encodes the domain homologous to elongation factor subunits 1β and 1γ as well as other proteins involved in protein synthesis. This domain was specifically expressed in testis and is responsible for the specific interaction of CRS with elongation factor-1γ [39].

Alternative splicing of ARSs is also used to control cellular localization between cytoplasm and mitochondria. The mitochondrial form of human KRS is generated by the insertion of a novel exon [40]. The similar observations have been also reported in yeast and *Arabidopsis* [43, 44]. Therefore, the novel activity and cellular localization of ARSs may be also controlled at the level of RNA processing. The expression control of these enzymes by alternative splicing would add more sophistication to their novel biological activities that would be also controlled at the level of transcription and translation.

5 Molecular interactions of ARSs

In higher eukaryotes, at least eight different aminoacyl tRNA synthetases form a complex with three enzymatic proteins of about 43, 38 and 18 kDa [45–47]. Although the complex has been known for the last two decades, the structural organization and interactions between the components have not been well understood. Mammalian ARSs have extra peptide appendices that are absent in the corresponding prokaryotic enzymes. Interestingly, prokaryotic ARSs exist as free forms, and not as a complex, suggesting that complex formation may not be essential for their catalytic activities. Thus, the eukaryotic peptide appendices have been thought to be involved in protein-protein interactions within the multi-ARS complex or in other novel cellular functions.

Various approaches have been applied to determine the structure of the ARS complex. Chemical crosslinking of the components for the complex identified the nearest neighbors [48, 49]. Stepwise dissociation of the components from the complex [48–51], and electron microscopy of the complex were also carried out [52, 53]. Based on the results of these approaches, the complex has been proposed to consist of three subdomains. The base subdomain (domain III) consists of three high molecular weight enzymes, EPRS, IRS and leucyl-tRNA synthetase (LRS). Domain I contains monomers of MRS and QRS and a dimer of aspartyl-tRNA synthetase (DRS), and domain II is made of dimers of KRS and RRS [50, 54].

However, these biochemical methods uncovered only relative physical location of protein components in the complex. To determine specific interactions of the components for the assembly or maintenance of the complex, it was necessary to apply other methods. A yeast two-hybrid method has been used to identify such interactions [25, 47, 55–57]. In one study, the full-length genes for DRS, RRS, KRS, QRS, MRS, EPRS, p43, p38 and p18 were subjected to the yeast two-hybrid assay [47]. The result showed that p38 is a core scaffold protein for protein-protein interaction in the complex and interacts with most of the ARS components. In addition, mapping of the interaction domains of p38 for several partners showed that each component of the complex has a discrete binding site for the interaction with p38. While this study used the full-lengths of ARS genes for the interaction assays, the peptide appendices unique for ARSs of the higher eukaryotes were also tested [55, 57]. In this study, the unique appendices of ARSs in the complex were determined by a multiple sequence alignment of prokaryotic and higher eukaryotic ARSs and used for a yeast two-hybrid assay. The result showed that all of the components for the complex are interconnected using their unique peptide appendices.

In addition to the appended domains of ARSs, their catalytic core domains are also necessary for targeting them to the ARS complex, implying that the structural organization of the ARS complex would be complicated. Recently, the complex targeting domain was determined in human QRS by monitoring its peptide fragments incorporated to the complex by co-immunoprecipitation. Interestingly, the peptide of QRS responsible for the assembly of the com-

plex is located in the catalytic domain of the enzyme [58]. This finding poses an intriguing question how ARSs within the complex would execute their catalytic activity, while their catalytic domains are involved in protein-protein interactions. EPRS makes a specific interaction with heat shock protein 90 (Hsp90), and their interaction is dependent on Hsp90 activity. Inactivation of Hsp90 prevents the incorporation of newly synthesized ARSs into the multi-ARS complex, implying that Hsp90 mediates protein-protein interactions with mammalian ARSs during complex formation [59].

To understand the interaction and function of the multi-ARS complex, solving its three dimensional structure is very important. Although the structures of many prokaryotic ARSs have been solved by X-ray crystallography, not one of the human ARS structures has been reported yet. Recently, the X-ray crystal structure of the *C*-terminal domain of p43 was solved [23, 60] and the solution structure of the multifunctional peptide appendix of EPRS was solved by NMR [61, 62]. Although much progress has been made during the last decade, the structure of the multi-ARS complex still remains unsolved.

Mammalian ARSs also make stable or transient interactions with the subunits of elongation factor [EF] complex to facilitate the delivery of the charged tRNAs to the ribosome. A well-known example is valyl-tRNA synthetase (VRS) that forms a stable complex with EF-1$\beta\gamma\delta$ complex [63–65]. Its activity was enhanced by EF-1α and GTP [66]. Similar functional interactions with the EF complex were also reported in other ARSs such as phenylalanyl- (FRS), aspartyl- (DRS), histidyl- (HRS) and leucyl- (LRS) tRNA synthetases [67–69].

6 Perspectives

The catalytic aspect of aminoacyl-tRNA synthetases as the enzymes for protein synthesis is well understood, although their three dimensional structures and mechanisms for the specific recognition of their cognate RNAs are still under intensive investigation. Various lines of evidence have demonstrated their noncanonical functions in addition to their catalytic role as some of the examples were reviewed here. In addition to their novel functions in normal physiological condition, these enzymes may be involved in various pathological processes. A high proportion of patients with autoimmune disease such as myositis contains autoantibodies against different ARSs [70–73]. Interestingly, myositis was induced by DNA immunization with the gene encoding HRS [74]. A recent report showed that amylotrophic lateral sclerosis (ALS) patients have a mutation in lysyl-tRNA synthetase (KRS) that gained the ability to bind to superoxide dismutase (SOD) [75]. In addition, KRS is also selectively packaged into human immunodeficiency virus-1 (HIV-1) [76]. These reports suggest that ARSs and their association factors can be an interesting target for biomedical research as well.

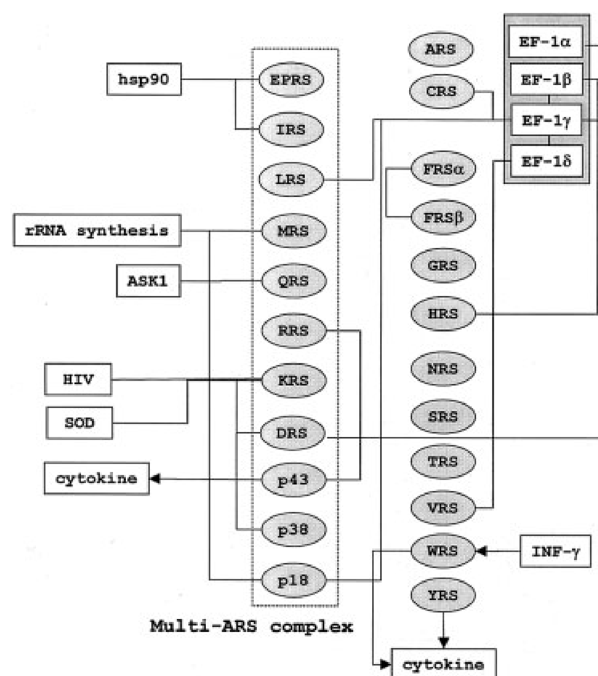

Figure 4. Schematic summary of the novel activities and interactions of mammalian ARSs. The components for the multi-ARS complex were included in the dotted box. Functional interactions and functions of ARSs are connected with lines. YRS, WRS and p43 are secreted to work as cytokines in the extracellular matrix. WRS is induced by INFγ. Several ARSs are associated with the subunits of EF-1 complex (gray box).

It is a long-standing question why eukaryotic ARSs form a macromolecular protein complex. ARSs excluded from this complex still maintain their catatalytic activities. For example, RRS can exist as free and complex forms while maintaining its catalytic activity [77]. The free form of QRS or RRS still possesses aminoacylation activity [58, 78], suggesting that association with the multi-ARS complex is not essential for enzyme catalysis. Instead of helping their enzymatic activity, the complex formation could be necessary to localize the ARSs near the endoplasmic reticulum-bound ribosomes or to maintain the cellular stability of the proteins. In summary, mammalian ARSs should be reconsidered as a novel example of proteins with a broad spectrum of cellular functions. This multifunctionality of mammalian ARSs would constitute a novel functional network linking various biological processes with protein synthesis (Fig. 4).

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