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Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers

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Aminoacyl-tRNA synthetases (ARSs) are essential enzymes that join amino acids to tRNAs, thereby linking the genetic code to specific amino acids. Once considered a class of 'housekeeping' enzymes, ARSs are now known to participate in a wide variety of functions, including transcription, translation, splicing, inflammation, angiogenesis and apoptosis. Three nonenzymatic proteins – ARS-interacting multi-functional proteins (AIMPs) – associate with ARSs in a multi-synthetase complex of higher eukaryotes. Similarly to ARSs, AIMPs have novel functions unrelated to their support role in protein synthesis, acting as a cytokine to control angiogenesis, immune response and wound repair, and as a crucial regulator for cell proliferation and DNA repair. Evaluation of the functional roles of individual ARSs and AIMPs might help to elucidate why these proteins as a whole contribute such varied functions and interactions in complex systems.

Introduction

Aminoacyl-tRNA synthetases (ARSs; individual enzymes are denoted by the one letter amino acid abbreviation followed by RS, with the exception of alanyl-tRNA synthetase, which is denoted by 'AlaRS') are ancient proteins that arose during the transition from the RNA world to the theater of proteins, to establish the genetic code by catalyzing the joining of specific amino acids to their cognate tRNAs. The ARS-catalyzed aminoacylation reaction proceeds in two steps (Figure 1). In the first step, ARS catalyzes the condensation of its substrate amino acid and ATP to form an aminoacyladenylate. In the second step, the aminoacyl moiety is transferred to the 3′ terminal adenosine of the cognate tRNA. ARSs also catalyze a secondary chemical reaction to synthesize diadenosine oligophosphates (Ap_nA) using ATP or ADP instead of tRNA. Ap_4A has been proposed as a second messenger for cell regulation [1]. ARSs are distinguished into two classes, depending on the architecture of their catalytic domains. The class I and II ARSs contain a Rossman nucleotide binding fold with alternating β-strands and α-helices, and a seven-stranded β-sheet with flanking α–helices in their active sites, respectively. The 11 class I and ten class II enzymes (KRS is found in both classes [2]) are further subdivided to three subgroups (a, b and c), based on the chemical nature of their substrate amino acids [3]. Whereas the core structures of the class I and class II enzymes have an ancient origin, the modern enzymes are structurally more complex owing to domain fusions and associations with nonenzymatic proteins. ARSs from many organisms are now known to participate in diverse regulatory roles [4], expanding their functions well beyond protein synthesis. In some cases, structural adaptations that gave rise to extra functions have been deciphered, whereas in others it is still a matter of speculation.

The large number of regulatory activities of ARSs and their associated factors suggests that they should be considered as a new family of signaling molecules, rather than as individual proteins with anomalous adaptations. It also poses the question as to why components of the 'housekeeping' machinery might have been selected for cellular regulatory processes and signal transduction. In this review, we address the current knowledge on

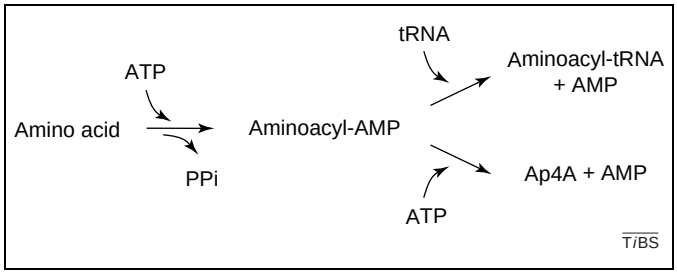

Figure 1. Two catalytic reactions of ARSs. The cognate amino acid and ATP form the enzyme-bound reaction intermediate aminoacyl adenylate. When the cognate tRNA is docked into the catalytic site, the activated amino acid is transferred to the accepter end of tRNA, releasing AMP. Aminoacyl adenylate can be also attacked by the pyrophosphate moiety of an additional ATP, resulting in the formation of Ap_4A. Abbreviation: PPi, inorganic pyrophosphate.

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noncanonical activities of ARSs and their linkage to diverse signaling pathways.

ARSs for noncanonical activities

Many ARSs are equipped with extra activities in addition to their catalytic activity. Some control the efficiency and fidelity of the catalytic reaction. For example, specialized tRNA binding domains attached to the N- or C-terminal ends guide the productive docking of cognate tRNAs in some ARSs [5–7]. Editing domains, appended to the ends or inserted into the core domains, catalyze the tRNA-dependent hydrolysis of incorrectly attached amino acids [8]. The catalytic efficiency of ARSs is further enhanced by association with RNA-binding *trans*-acting factors [9] or elongation factor subunits [10,11] that facilitate the trafficking of tRNAs. ARSs also have activities unrelated to their catalytic functions. These noncanonical activities vary depending on the type of ARS and its cellular location (Table 1).

Extracellular

Some mammalian ARSs are secreted to trigger signaling pathways. Human YRS is secreted and processed into two protein fragments. The resulting N-terminal fragment, mini-YRS, becomes a proangiogenic factor, and the carboxy-terminal protein becomes an immune cell stimulant for migration and production of tumor necrosis factor (TNF), tissue factor and myeloperoxidase [12,13]. Mini-YRS pro-angiogenic activity depends on a three amino acid Glu-Leu-Arg (ELR) motif within the Rossman-fold domain [12–15].

WRS is structurally related to YRS, but embeds an antiangiogenic activity [16,17]. The structural and evolutionary relationship between WRS and YRS suggests that the cell-signaling activities in these two ARSs might have developed together [17]. Alternative mRNA splicing or protein proteolysis is thought to be involved in the removal of an N-terminal helix–turn–helix domain to produce active antiangiogenic factors (mini- and T2-WRS), which inhibit mini-YRS and vascular endothelial growth factor-induced blood vessel development and endothelial cell activation [16,18]. A short peptide inserted into the anticodon-binding region was proposed to contribute to cytokine activity [19]. Cell binding is dependent on the vascular endothelial cell-specific cadherin, vascular endothelial cadherin [20], which is required for angiogenesis [21].

KRS has a complex evolutionary profile and exhibits a diversity of functions. Human KRS is involved in the packaging of HIV virion via the interaction of its N-terminal motif with the C-terminal capsid region of Gag protein of HIV [22]. Human KRS is also secreted from various cell lines in response to TNF-α, and stimulates macrophages and peripheral blood mononuclear cells to enhance migration and TNF-α production [23]. Thus, KRS and TNF-α seem to form a positive feedback loop to amplify the secretion of both factors. Several other ARSs, such as HRS, NRS and SRS, also stimulate immune cells through their interactions with cell surface chemokine receptors [24].

Cytoplasmic

ARSs also express their noncanonical activities in the cytoplasm. EPRS is a fusion of the two enzymes specific for glutamic acid and proline into a single polypeptide. Three repeats of a tRNA-binding motif are inserted as a linker for the two enzymes in human EPRS [25,26]. Although EPRS is one of the components of a multi-ARS complex [4] (see later), it was separately identified as a component of the interferon-gamma (IFN-γ)-activated inhibitor of translation complex [27]. In response to IFN-γ, EPRS is phosphorylated and released from the multi-ARS complex to bind to the 3′ untranslated region (UTR) of ceruloplasmin transcripts, at a stem-loop structure in the mRNA ribonucleoprotein complex, to silence translation. Ceruloplasmin is a copper-containing plasma protein that is a multifunctional oxidase with important roles in inflammation and iron homeostasis [28]. The suppression of its expression could help to terminate inflammation and prevent injury caused by accumulation of this enzyme. Another component of the multi-ARS complex, QRS, inhibits apoptosis through an interaction with apoptosis signal-regulating kinase 1 (ASK1), which mediates various apoptotic stimuli, such as Fas, TNF-α and reactive oxygen species [29]. The interaction between QRS and ASK1 is enhanced by its substrate, glutamine, which is known to be an antiapoptotic amino acid.

Nuclear

Human KRS was shown to exert transcriptional control in mast cells via its secondary catalytic product, Ap_4A [30]. Microphthalmia transcription factor (MITF) is a basic helix–loop–helix leucine zipper DNA binding protein and its activity is inhibited by its interaction with a tumor suppressor protein, Hint (previously known as protein kinase C-interacting protein 1). In quiescent leukemia cells, KRS forms a trimeric complex with MITF and Hint but upon immunological activation, KRS-synthesized

Table 1. Noncanonical activities of ARSs

Species	ARSs (classes)	Location	Target	Activities
Homo sapiens	YRS (I)	Extracellular	Endothelial cell	Angiogenic cytokine
H. sapiens	WRS (I)	Extracellular	Endothelial cell	Angiostatic cytokine
H. sapiens	KRS (II)	Extracellular	Macrophage	Inflammatory cytokine
H. sapiens	KRS (II)	Plasma membrane	HIV Gag	Viral assembly
H. sapiens	EPRS (I, II)	Cytoplasmic	3′ UTR	Translational silencing
H. sapiens	QRS (I)	Cytoplasmic	ASK1	Anti-apoptosis
H. sapiens	KRS (II)	Nuclear	MITF	Transcriptional control
H. sapiens	MRS (I)	Nuclear	Nucleoli	rRNA transcription
N. crassa	YRS (I)	Mitochondrial	Group I intron	Splicing
Saccharomyces cerevisiae	LRS (I)	Mitochondrial	Group I intron	Splicing
E. coli	TRS (II)	Bacterial	5′UTR	Translational control

Ap_4A binds to Hint, which liberates MITF. The released MITF then activates target gene expression, leading to the activation of mast cells. Thus, KRS has another role that is distinguished from its activities in the extracellular matrix and plasma membrane described above. Human MRS contains two putative nuclear localization signals in its C-terminal region, and is translocated to nucleoli when the cells are in a proliferative stage [31]. Nucleolar MRS was suggested to have a positive role in the biogenesis of rRNA, although its working mechanism is not clearly understood. Many other ARSs have been found in the nucleus [32] without an obvious explanation.

Mitochondrial

Eukaryotic cells have a separate pool of ARSs for mitochondrial protein synthesis. The noncanonical activities are also observed in this set of enzymes. Mitochondrial YRS of *Neurospora crassa* works as a splicing factor through an interaction with a conserved tRNA-like structural motif in the group I intron [33]. Yeast mitochondrial LRS binds to the bI4 intron and collaborates with bI4 maturase to excise the group I intron [34]. In this case, splicing activity crucially depends on the LRS CP1 (connective polypeptide 1) domain, which is also responsible for proofreading aminoacyl-tRNAs [35].

Bacterial

Although most of the noncanonical activities have been found in eukaryotic ARSs, translational control activity has been found in *Escherichia coli* TRS. It binds the 5′ UTR of its own transcript upstream of the Shine Dalgarno sequence to repress translation initiation by competitive binding with the ribosome [36]. Based on these diverse activities found in different ARSs, the multifunctionality seems to be ubiquitous, regardless of the enzyme type and species.

ARS-interacting multifunctional proteins

The canonical and noncanonical activities of ARSs are further refined by their association with nonenzymatic factors. In mammalian systems, three ARS-interacting factors are known to form a macromolecular complex with nine different ARSs (details are described in next section). Although these factors are tied together within this multienzyme complex, each has a unique role distinct from protein synthesis. Each protein was originally known by its molecular mass designation, p43, p38 or p18. However, this nomenclature has been confusing, particularly in the case of p38, which is a commonly used designation for p38 mitogen-activated protein kinase, so we propose a new naming system for these three proteins, as ARS-interacting multifunctional proteins (AIMPs).

AIMP1 (previously known as p43) is secreted from various mammalian cells to exert an action on different target cells [37] (Figure 2). AIMP1 has limited homology with interleukin (IL)-8, IL-1 and von Willebrand factor antigen II in its C-terminal domain but lacks a classical hydrophobic signal peptide [38]. The secreted AIMP1 acts on acute monocytic leukemia cells to activate mitogen-activated protein kinases via phospholipase C-γ, protein kinase C and nuclear factor κB. Ultimately, AIMP1 induces production of TNF-α, IL-8, macrophage chemotactic protein 1, macrophage inflammatory protein 1α and IL-1β [39,40]. In addition, AIMP1 stimulates monocyte adhesion via the induction of intercellular adhesion molecule 1 [41], which is widely expressed on the surface of vascular endothelium, monocytes, lymphocytes and leukocytes [42,43] and has an important role as a surface-adhesion molecule in lymphocyte extravasation [44]. AIMP1 is abundant in tissues undergoing apoptosis in the mouse embryo and at atherosclerotic lesions of the human aorta [40,45].

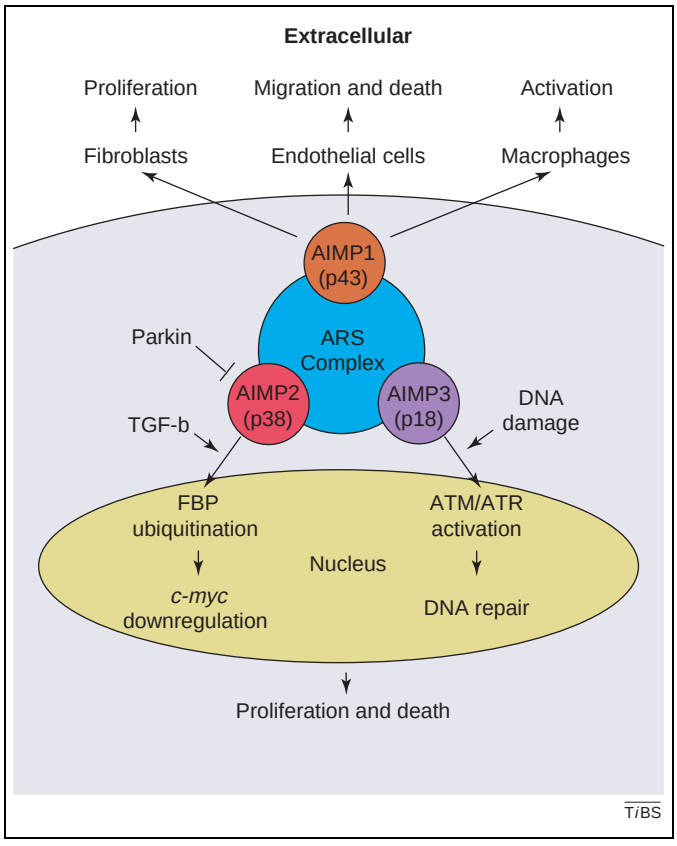

Figure 2. Activities of AIMPs. Three factors, AIMP1 (p43), AIMP2 (p38) and AIMP3 (p18), are tightly associated with a macromolecular protein complex consisting of nine different ARSs. AIMP1 is secreted as a cytokine. It enhances proliferation and collagen production of fibroblasts, induces migration and death of endothelial cells and activates macrophages. AIMP2 is the target of parkin for ubiquitination. It is induced by transforming growth factor-β and is translocated to the nucleus to mediate the proteasome-dependent degradation of FBP, a transcriptional activator of *c-myc*. Thus, it has a crucial role in lung cell differentiation. AIMP3 is induced by DNA damage or cell proliferation and is translocated to the nucleus to activate ATM/ATR, which are necessary for p53 induction and DNA repair.

AIMP1 also shows a dual activity in angiogenesis, promoting migration of endothelial cells at low concentration, and inducing apoptosis at high concentration [46]. It binds to the α subunit of ATP synthase [47], which was previously suggested to be a cellular mediator of the antiangiogenic activity of angiostatin [48]. The 146 amino acid N-terminal domain of AIMP1 was shown to enhance the proliferation of fibroblasts and collagen synthesis [37]. Its secretion was found to be enhanced by TNF-α released from macrophages that are recruited to wound regions. Thus, AIMP1 is a signaling factor with multiple activities, depending on the context and cell target.

AIMP2 (previously known as both p38 and JTV-1) is associated with the multi-ARS complex and interacts with

AIMP1 via its coiled-coil leucine zipper motif [49]. However, it is also implicated in the control of cell fate and pathogenesis. Upon transforming growth factor-β treatment, AIMP2 is induced and translocated to the nucleus to bind to FUSE (far upstream element)-binding protein (FBP) [50], a transcriptional activator of the *c-myc* gene [51] (Figure 2). AIMP2 binding stimulates ubiquitination and proteasomal-dependent degradation of FBP. These events lead to downregulation of c-myc, which is required for differentiation of functional alveolar type II cells [50]. Loss-of-function mutations in mouse AIMP2 was shown to cause neonatal lethality as a result of respiratory distress syndrome resulting from the hyperplasia of lung epithelial cells [50]. It was also proposed to be a substrate for parkin [52], the E3 ubiquitin–protein ligase involved in ubiquitination and proteasomal degradation of specific protein substrates. Pathogenic mutations of parkin induce abnormal accumulation of toxic proteins, leading to cellular apoptosis and progressive degeneration of motor neurons [53]. AIMP2 forms aggregates in neurons upon overexpression. Aggresome-like inclusions of AIMP2 recruit the proteasomal 20S subunit, as well as the molecular chaperones Hsp70 and Hdj-2, and then become ubiquitinated by parkin for degradation. It is postulated that uncontrolled overexpression of AIMP2 induces aggregation and contributes to dopaminergic neuronal cell death in Parkinson's disease. Thus, AIMP2 is not only a mediator but also a target for ubiquitination.

AIMP3 (previously known as p18) is the smallest protein in the multi-ARS complex (see later). Although its direct association with AIMP1 and AIMP2 has not been clearly demonstrated, it contains a glutathione S-transferase-homology domain similar to that of AIMP2, and shares homology with the β, γ subunit of elongation factor 1 [4,54], which is involved in the interaction with elongation factor-1γ [11]. Although the role of AIMP3 within the multi-ARS complex is not yet clear, its unique role has been demonstrated in the maintenance of chromosomal DNA. Upon DNA damage, AIMP3 was induced and translocated to the nucleus, where it upregulated p53 by direct activation of ataxia-telangiectasia mutated (ATM)/ATM and Rad3 related (ATR) (serine/threonine kinases involved in the DNA-repair process) [55] (Figure 2). Deletion mapping showed that AIMP3 directly binds to the FRAP/ATM/TRRAP (FKBP-rapamycin-associated protein/ATM/transactivation–transformation-domain associated protein) conserved domain of ATM and ATR. Although the loss of function of AIMP3 caused early embryonic lethality, heterozygous mice were born alive but developed various tumors spontaneously. In human cancer cells and patient tissues, a reduction in AIMP3 levels was frequently observed, suggesting that AIMP3 is a haploinsufficient tumor suppressor. Thus, AIMP1, AIMP2 and AIMP3 seem to have their idiosyncratic functions in the control of cell fate, although they might have a similar function in the control of ARSs.

Macromolecular complex consisting of ARSs and AIMPs

As mentioned above, a macromolecular complex called the multi-ARS complex consists of nine different ARSs (ERS, PRS, IRS, LRS, MRS, QRS, RRS, KRS and DRS) and three AIMPs. It has been isolated from diverse higher eukaryotic organisms. To understand its structural organization, comprehensive approaches have been conducted, such as partial dissociation, chromatography, electron microscopy, chemical crosslinking and genetic mapping [56]. Although the three-dimensional structure of the complex has not yet been elucidated, the interaction network between the components was suggested based on these analyses [4]. The assembly of the complex seems to involve multi-directional protein–protein interactions among catalytic [57] and extra domains [58] of the component enzymes. The three AIMPs seem to have specific interaction partners. AIMP1 is located in the center of the complex [59] and makes a high-affinity interaction with RRS. AIMP2 makes a high-affinity interaction with DRS and KRS [60] (Figure 3), and AIMP2 depletion completely disintegrates the whole complex [61]. A specific interaction between AIMP3 and MRS has also been demonstrated [62] (Figure 3). The components are divided into two subdomains based on their association with the different regions of AIMP2 [61] (Figure 3). One subdomain, consisting of AIMP1, RRS and QRS, is anchored to the N-terminal region of AIMP2, and the association of this domain was also shown by an *in vitro* reconstitution experiment [60]. Based on these data, three AIMPs seem to have scaffolding roles, holding their target enzymes in place.

Two different functions can be considered for the multi-ARS complex. Firstly, it brings together the enzymes for

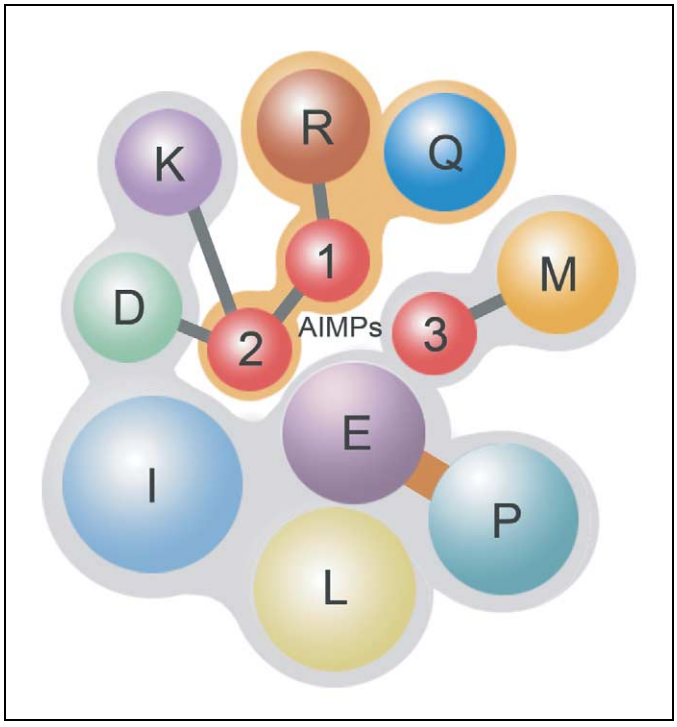

Figure 3. Schematic representation of a macromolecular complex, consisting of nine ARSs and three AIMPs, in a mammalian system. ARSs and AIMPs are represented by their substrate amino acids and numbers, respectively. Three AIMPs seem to be paired with their specific target ARSs (solid lines). AIMP1, RRS and QRS are anchored to the 83 amino acid N-terminal region of AIMP2 (orange region), and the other components are linked to the C-terminal region of AIMP2 (gray region). AIMP1 interacts with AIMP2 via a leucine zipper motif. In addition to these interactions, the complex is further stabilized by multidirectional interactions between the components, although, for simplicity, these interactions are not shown here.

the efficiency and control of translation by providing a channel for the flow of tRNAs and localization of translation in different cellular places. In this regard, it is worth noting that AIMP1 facilitates the docking of tRNA to the catalytic site of RRS [63], and the whole multi-ARS complex has even been found in the nucleus [30]. In addition, enzyme turnover can be controlled [61,64] through association and dissociation [4]. Secondly, the complex could serve as a molecular reservoir that controls the noncanonical activities of ARSs. The pre-existing ARSs or AIMPs in the complex can rapidly respond to various cellular stimuli until they are refilled by *de novo* synthesis. In this regard, it should be noted that EPRS is dissociated from the complex by its IFN-γ-dependent phosphorylation [27], and AIMP3 is translocated to nuclei within ten minutes upon DNA damage [55].

Perspectives

Although the additional functions described here are idiosyncratic to these enzymes, the class as a whole shows that these proteins are repeatedly adapted and used by cells. This unusual versatility is attributed to the structural and functional pliability of the ARS framework. The multi-functionality of ARSs and AIMPs might be, in part, an economic strategy to maximize the functional diversity of proteins with a limited genetic resource. The expanded functions of these enzymes are probably related to a persistent need for new functions in the development of complex systems and the distribution of the proteins in each and every cell type, where they are exposed to, and must adapt to, an array of new selective pressures. The expansion of these adaptations is seen in examples such as human KRS, which exhibits the broadest range of activities, including Ap_4A formation, HIV packaging, transcription regulation and cell signaling. Yet another example, YRS, exemplifies how the core structure has been adapted in different organisms to achieve different specific functions, from intron splicing in mitochondrial *N. crassa* YRS to cell signaling by human YRS.

Apart from Ap_4A synthesis, most of the extra functions are not related to catalysis of aminoacylation. When an ARS is translocated from the cytoplasm, its capacity for intracellular aminoacylation is lost, through the new functional assignment of the synthetase. In addition, some forms of cytokine-active synthetase fragments, such as T2-WRS, have lost their aminoacylation function, again suggesting that, in at least some instances, the new function comes at the cost of loss of aminoacylation capacity. Whether these losses significantly disturb protein synthesis is not known. If this is the case, then cytokine activities of synthetases would be directly connected to protein synthesis.

ARSs might be considered as 'hub proteins' that have a central role in protein networks. Cellular protein networks are scale free. Proteins with a high degree of functional diversity serve as hub proteins and make functional connections to other hub proteins and to proteins with fewer or single functions [65]. Although clear criteria for hub proteins have not been established, ARSs and other translational factors have functional connections with splicing, apoptosis, transcription, protein folding and trafficking, transcription and diseases [66,67]. Thus, regardless of the expanded function associated with each individual ARS, this set of proteins serves as a family of hub proteins that has a wide impact on cellular mechanisms beyond translation.

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