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Is There an Answer?

Do Aminoacyl-tRNA Synthetases have Biological Functions other than in Protein Biosynthesis?

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Is There An Answer? is intended to serve as a forum in which readers to *IUBMB Life* may pose questions of the type that intrigue biochemists but for which there may be no obvious answer or one may be available but not widely known or easily accessible.

Readers are invited to e-mail f.vella@sasktel.net if they have questions to contribute or if they can provide answers to questions that are provided here from time to time. In the latter case, instructions will be sent to interested readers. Answers should be, whenever possible, evidence-based and provide relevant references.

Frank Vella

The decoding system for the genetic code for protein synthesis is well conserved from bacteria to humans during evolution. Aminoacyl-tRNA synthetases (ARSs) are key components for protein synthesis since they link specific amino acids to their cognate tRNAs. Recent studies reveal that ARSs have a variety of functions other than protein synthesis.

Multifunctionality of ARSs is achieved through their promiscuity in molecular interactions. For instance, their ability to interact with tRNAs has expanded to other cellular nucleic acids so as to regulate such processes as transcription, splicing and translation. Alanyl-tRNA synthetase of *E. coli* inhibits the transcription of its own gene, *ala*S, through binding to the palindromic sequence in the promoter near the transcription initiation site (*1*). Mitochondrial tyrosyl-tRNA synthetase (YRS) of *N. crassa* functions as a splicing factor (*2*) by recognizing a conserved tRNA-like structural motif in group I introns (*3–6*). Likewise, yeast mitochondrial leucyl-tRNA synthetase binds to the bI4 intron forming a complex with bI4 maturase to remove the group I intron (*7*, *8*). *E. coli* threonyl-tRNA synthetase binds to the 5′-untranslated region (UTR) of its own transcript at a stem loop-like secondary structure upstream of the Shine-Dalgarno sequence (*9*, *10*) and competes with ribosome to suppress translational initiation (*11–13*).

Glutamyl-prolyl-tRNA synthetase (EPRS) is a bifunctional polypeptide specific for glutamic acid and proline (*14*, *15*). The two specific enzymes are bridged by a linker sequence composed of 3 repeats of the tRNA-binding motif. EPRS is also a component of the interferon gamma IFN γ-activated inhibitor of translation (GAIT) complex (*16*). IFN γ induces phosphorylation of EPRS, which then represses translation by binding to the 3′-UTR of the gene transcript for the copper plasma protein ceruloplasmin (Cp) that is secreted from hepatocytes and activates macrophages. Accumulation of Cp causes prolonged inflammation and induces tissue injury. So repression of its transcription could help to terminate the exaggerated inflammation and prevent injury caused by Cp.

ARSs are also secreted and function as extracellular signaling molecules. This secretion does not seem to utilize the classical ER/Golgi export pathway since ARSs lack the typical signal peptides for secretion. YRS is secreted and processed by elastase into an N-terminal fragment, mini-YRS, which is pro-angiogenic, and a C-terminal fragment that stimulates immune cells to migrate and produce TNFα, tissue factor, and myeloperoxidase (*17*). The pro-angiogenic activity of mini-YRS resembles that of CXC-chemokines such as interleukin 8, and depends on the three amino acid ELR motif located within the Rossmann-fold catalytic domain (*17*, *18*). In contrast to YRS, two truncated isoforms of tryptophanyl-tRNA synthetase (WRS), mini-WRS and T2 WRS have potent anti-angiogenic activity (*19–21*). They inhibit mini-YRS as well as growth factor-induced blood vessel development. Several endothelial cell signaling pathways induced by shear stress are also inhibited by T2-WRS (*22*). Its cell binding

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depends on the vascular endothelial cell specific VE-cadherin which is required for angiogenesis (*23*, *24*). Recently, lysyl-tRNA synthetase (KRS) was shown to be secreted by various cancer cell lines in response to TNFα (*25*). The secreted KRS stimulates macrophages to induce matrix metalloproteinase-9 secretion for migration and to produce TNFα. Several ARSs, such as histidyl-, asparaginyl-, and seryl-tRNA synthetase, also stimulate immune cells through their interactions with cell surface chemokine receptors (*26*) and mediate inflammatory disease.

ARSs also catalyze a secondary reaction in which the aminoacyl-AMP reaction intermediate reacts with ATP to produce diadenosine tetraphosphate (Ap_4A) (*27*, *28*). Protein kinase C-dependent phosphorylation of ARSs increases Ap_4A synthesis by several fold (*29*) without affecting the aminoacylation reaction. Ap_4A binds to DNA polymerase α (*30*), which is enhanced by tryptophan (*31*). Ap_4A can also be used as a primer for DNA synthesis (*31*). Since DNA replication in G1-arrested hamster kidney cells is induced by Ap_4A (*32*), it has been proposed that the intracellular concentration of Ap_4A correlates with proliferation in mammalian and *E. coli* cells (*33*). Human KRS forms a trimeric complex with microphthalmia transcription factor (MITF), basic helix-loop-helix leucine zipper and the ubiquitous member of the histidine triad protein family, Hint. The Ap_4A synthesized by KRS binds to Hint, and causes dissociation of MITF from the complex, and activates the target gene expression (*34*). Thus, ARSs appear to regulate biological processes via their secondary catalytic reaction.

Glutaminyl-tRNA synthetase inhibits the pro-apoptotic signaling pathway through glutamine-dependent interaction with apoptosis signal-regulating kinase 1 (*35*). KRS is a component of the human immunodeficiency virus type 1 (HIV-1) particle by interacting with its protein Gag (*36*, *37*) and suppresses pre-maturation of viral proteins by inhibiting HIV-1 protease (*38*). Methionyl-tRNA synthetase was observed in nucleoli where it enhances rRNA synthesis under proliferative conditions (*39*). The functional diversity of ARSs is expanded further through their association with **A**RS-**i**nteracting **m**ulti-functional **p**roteins (AIMP1, AIMP2, AIMP3) (*40*).

Why have ARSs acquired functions additional to their catalytic activities? The answer cannot be simply attributed to the outcome of evolutionary coincidence involving only a few ARSs since accumulating evidence suggests that functional pleiotrophy could be universal throughout the ARSs. Multifunctionality of ARSs could be the result of their evolutionary age. Since they must have emerged early in evolution and thus had a higher chance to acquire additional functions than younger proteins, ARSs may have been selected to acquire additional functions because of their crucial role in metabolism. Whatever is the reason, ARSs should be recognized as core signal mediators in addition to their catalytic role in protein synthesis.

NOTE

We apologize to those authors whose work is relevant but who could not be included because of space limitations.

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New Question

Do aerobic, anaerobic and facultative bacterial species differ in their content or expression of key enzymes of glycolysis and of the tricarboxylic acid cycle?