IUBMB *Life*, 62(4): 296–302, April 2010

Critical Review

Aminoacyl-tRNA Synthetase–Interacting Multifunctional Proteins (AIMPs): A Triad for Cellular Homeostasis

Sang Gyu Park[1], Eung-Chil Choi[2] and Sunghoon Kim[2,3]

[1]*Department of Biomedical Science, CHA University, Yeoksam-dong, Kangnam-gu, Seoul, Korea*
[2]*Center for Medicinal Protein Network and Systems Biology, College of Pharmacy, Seoul National University, Seoul, Korea*
[3]*Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Technology, Seoul National University, Suwon, Korea*

Summary

Aminoacyl-tRNA synthetases (ARSs) are highly conserved for efficient and precise translation of genetic codes. In higher eukaryotic systems, several different ARSs including glutamyl-prolyl-, isoelucyl-, leucyl-, methionyl-, glutaminyl-, lysyl-, arginyl-, and aspartyl-tRNA synthetase form a macromolecular protein complex with three nonenzymatic cofactors (AIMP1/p43, AIMP2/p38, and AIMP3/p18). Although the structure and functional implications for this complex formation are not completely understood, rapidly accumulating evidences suggest that this complex would work as a molecular hub linked to the multiple signaling pathways that involve the components of enzymes and cofactors. In this article, the roles of three nonenzymatic components of the multi-tRNA synthetase complex in the assembly of the components and in cell regulation are addressed. © 2010 IUBMB

IUBMB *Life*, 62(4): 296–302, 2010

Keywords transfer RNAs; aminoacyl-tRNA synthetases.

INTRODUCTION

Aminoacyl-tRNA synthetases (ARSs) are ancient enzymes linking amino acids to their specific tRNAs consuming ATP and the specific recognition of their substrates is critical for the fidelity of protein synthesis. Although these catalytic activities are common among these enzymes throughout different species, there are distinct features that distinguish higher eukaryotic ARSs from their prokaryotic counterparts. Among them, the most captivating one is the macromolecular protein complex comprising nine different ARSs and three nonenzymatic factors. Although this complex has been known for decades, its three-dimensional structure and functional implications have not been clearly determined. However, rapidly accumulating evidences suggest that this complex may play a role as a molecular hub to coordinate protein synthesis and diverse regulatory signal pathways involving the components for this complex since many component ARSs have been shown to exert multiple roles as a signal mediator in addition to their canonical roles as catalysis.

Among the components of the multi-tRNA synthetase complex, lysyl-tRNA synthetase (KRS) is the enzyme that shows the most diverse activities so far. First, KRS is secreted as a proinflammatory cytokine (*1*). Second, it is also translocated into nucleus to catalytically generate the second messenger molecule, Ap_4A, which is then used to control the transcription of the genes involved in immune control (*2–6*). Third, it is also incorporated into human immunodeficiency virus virion (*7*). The bifunctional enzyme, glutamyl-prolyl-tRNA synthetase (EPRS), is the largest component of the multi-tRNA synthetase complex. It is also phophorylated by IFN-γ treatment in mast cells and forms a new RNA–protein complex with a few other cellular factors in the 3′ UTR region of the specific set of mRNA to silence translation (*8*). In addition, glutaminyl-tRNA synthetase (QRS) appears to mediate antiapoptotic activity of its substrate amino acid, glutamine, via the interaction with apoptosis signal kinase 1 (*9*). Methionyl-tRNA synthetase (MRS) seems to be also located in the nucleus to coordinate ribosomal RNA synthesis on growth stimulating condition. By considering that nearly half of the complex-forming ARSs play noncanonical activities apart from the multi-tRNA synthetase complex, other ARSs existing within the complex are also expected to play additional roles other than protein synthesis. Among the components of the multi-tRNA synthetase complex, the three nonenzymatic factors are particularly intriguing in terms of their significance for the assembly of the complex and pathophysiological implications.

Received 11 January 2010; accepted 17 February 2010

Address correspondence to: Sunghoon Kim, Center for Medicinal Protein Network and Systems Biology, College of Pharmacy, Seoul National University, Seoul 151-742, Korea. E-mail: sungkim@snu.ac.kr

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.324

STRUCTURAL FEATURES OF MULTI-tRNA SYNTHETASE COMPLEX

Many approaches have been tried to get the insights into the architecture of the multi-tRNA synthetase complex such as chemical crosslinking (*10*), yeast two-hybrid analysis (*11, 12*), pull-down assay (*13*), and systematic depletion studies (*14*). Besides, an overall outlook of the complex was suggested by cryoelectron microscopy using immunogold-labeled antibodies and structural reconstitution (*15*). The model generated by the combination of the results obtained by different approaches consists of three domains (*10*). Domains 1, 2, and 3 harbor MRS-QRS-DRS, KRS-RRS, and EPRS-IRS-LRS (LRS), respectively (Fig. 1A). The linkage pattern analyses by specific depletion of each component with its siRNA showed that the cellular stability of the components are interdependent in multiple direction but there seems to be a hierarchy between the components (*14*) (Fig. 1A). These three subdomains are thought to be linked by three AIMPs. In particular, the components can be grouped into two subdomains based on their associations with AIMP2 that is thought to be a scaffolding protein for the complex assembly. Namely, RRS, QRS, and AIMP1 form one subdomain through the interactions with the N-terminal region of AIMP2, whereas the rest of the components are linked to the C-terminal domain of AIMP2 (*13*) (Fig. 1B).

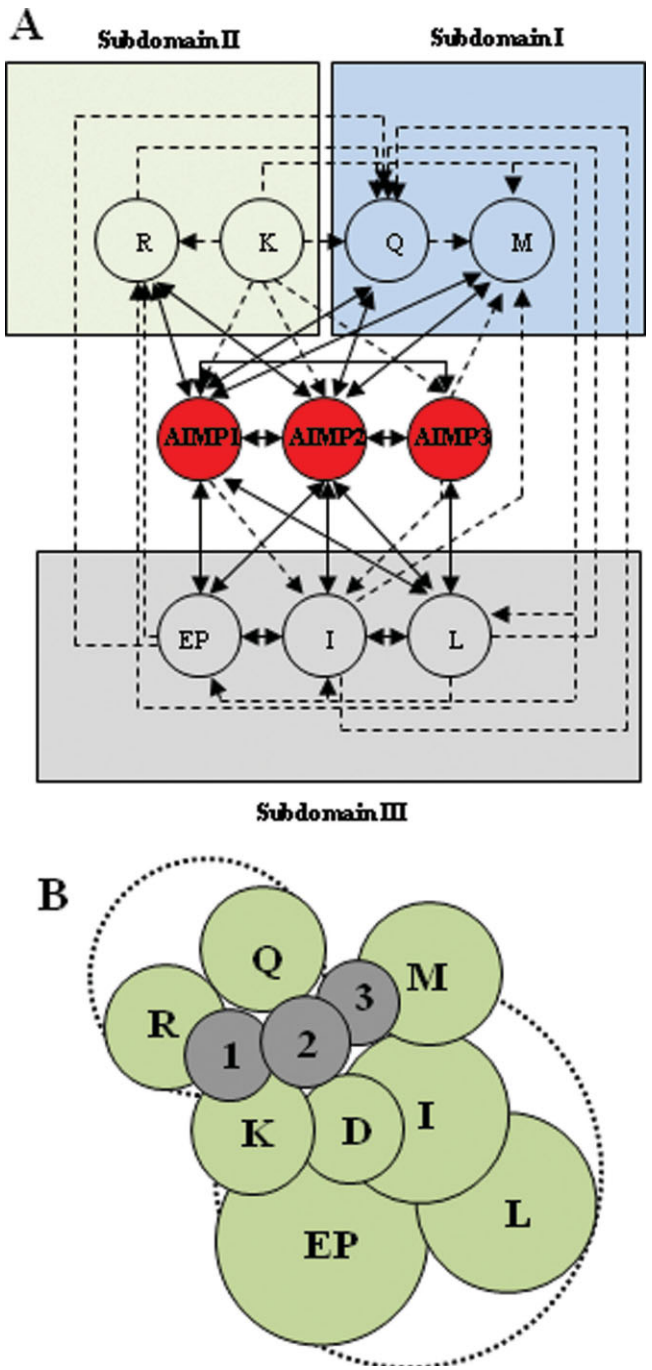

Figure 1. Interaction network and molecular arrangement of complex-forming ARSs and AIMPs. A: The interaction network between the components is indicated by arrows. The pairs of the proteins whose stability are mutually dependent are marked by double-headed arrows. If the stability of one component depends on the other partner, it is linked by single arrow dotted line. Three subdomains were assigned from the model previously proposed (*10*). Three AIMPs are multiply linked to most of the enzyme components. B: The components of the multi-tRNA synthetase complex can be also grouped into two subdomains based on their affiliation to AIMP2. RRS, QRS, and AIMP1 form one subdomain through the interactions with the N-domain of AIMP2. The rest of the components of the complex MRS, IRS, DRS, KRS, EPRS, and LRS are clustered with the C-domain of AIMP2 (*13*).

ROLES OF AIMPs IN THE ASSEMBLY AND STABILITY OF MULTI-tRNA SYNTHETASE COMPLEX

Although three AIMPs are the smaller components in comparison to the enzyme components in the multi-tRNA synthetase complex, they appear to play critical roles in the assembly of the complex-forming enzymes based on the following evidences. First, AIMPs are tightly linked with each other and their cellular stabilities appear to be interdependent (*14*). Second, immunogold tracing of AIMP1 with its specific antibody in electron microscopy localized it at the center of the multi-tRNA synthetase (*16*). Third, each of the three components appears to have its preferably interacting enzymes although they are also linked to other enzymes less tightly (*11, 17–19*). Among three AIMPs, AIMP2 appears to interact with the majority of the component proteins (*19*).

Although the reason for the specific preference of these factors to their enzyme counterparts is not fully understood, they appear to be required both for the cellular stability and catalytic activities of the bound enzymes. In the case of the pair of

AIMP1 and RRS, AIMP1 appears to facilitate tRNA delivery to the catalytic site of the bound RRS, and the deletion of the interacting domains from AIMP1 and RRS abolished the stimulatory effect of AIMP1 (*18*). Although it is not yet determined whether AIMP2 and AIMP3 would also enhance the catalytic activities of their partner enzymes, the depletion of each of AIMP2 and AIMP3 gave significant destabilization effect on the bound enzymes (*14*).

STRUCTURE AND REGULATORY FUNCTIONS OF AIMP1

The three-dimensional structure of its 95 aa C-terminal domain harbors oligonucleotide-binding fold, and this domain is capable of interacting with tRNA (*20, 21*). Although the structure of the 146 aa N-terminal domain AIMP1 has not yet been solved, it was shown to make specific protein–protein interaction with the N-terminal noncatalytic extension of RRS, a component of the multi-tRNA synthetase complex (*18*).

AIMP1 is most prominent in its functional diversity among the components of the multi-tRNA synthetase complex (*22*). The functions of AIMP1 appear to vary depending on its cellular location and interacting partners (Fig. 2A). Although AIMP1 is mainly bound to the multi-tRNA synthetase complex, it is also secreted on hypoxia or cytokine stimulation and works as a cytokine on various target cells such as endothelial cells, monocyte/macrophage, dendritic cells (DCs), fibroblasts, and pancreatic α cells (*23–28*). The secreted AIMP1 controls angiogenesis depending on its local concentration. While it promotes angiogenesis by inducing migration of endothelial cells via ERK activation, it also inhibits endothelial proliferation via JNK-dependent apoptosis as its level is increased (*23*). AIMP1 stimulates monocytes/macrophages via activation of p38 MAPK, ERK, and NFκB, inducing secretion of proinflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-8 (IL-8), and macrophage chemotactic protein-1 (*24, 29, 30*). AIMP1 also induces maturation of DCs and enhances production of IL-6 and IL-12 which are critical cytokines for the suppression of cancer prevalence (*31*). With this immune stimulatory activity, systemic infusion of AIMP1 into nude mice harboring human cancer cell line suppressed tumor growth through activation of immune system (*32*). AIMP1 is secreted from macrophages by stimulation of TNFα at wound lesion and enhances fibroblast proliferation and collagen synthesis (*26*). Genetic depletion of AIMP1 shows retardation of wound healing, decreased fibroblast proliferation, and collagen synthesis, suggesting AIMP1 as a critical molecule in dermal tissue regeneration (*26*). AIMP1 was found to be secreted from pancreatic α cells under low blood glucose level below 100 mg/dL (*27*) and induces the secretion of glucagon for blood glucose homeostasis. AIMP1 is also targeted to liver and adipose tissue, inducing glycogenolysis for blood glucose supply and degradation of triacylglyceol into glycerol and free fatty acid, respectively. AIMP1 knock-out mice show low blood glucose level, defect in glucagon secretion, increased glycogen in liver, and decreased glucose tolerance compared with the wild-type mice.

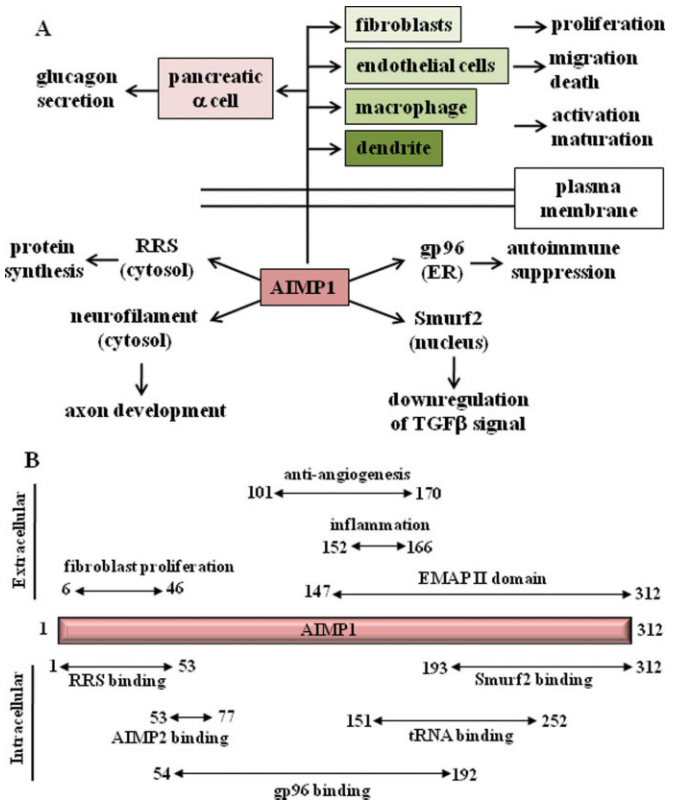

Figure 2. Functional diversity of AIMPs. A: The secreted AIMP1 works to diverse target cells with distinct activities (*22*). In cytoplasm, AIMP1 mainly associates with RRS within the multi-tRNA synthetase complex. However, it is located in ER to bind gp96 to suppress autoimmune responses and Smurf2 in nucleus to downregulate TGF*β* signaling. Recently, its association with neurofilament was shown to be important for axon development. B: Structural separation of AIMP1 activities. The peptide regions responsible for the indicated activities are marked along the 312 aa polypeptide of AIMP1.

AIMP1 can be located in endoplasmic reticulum and bound to gp96, a regulator of DC maturation through interaction with CD91 and Toll-like receptor 2/4 (*33*). AIMP1 enhances the interaction of gp96 with KDEL receptor, which inhibits translocation of gp96 to cell surface. Thus, AIMP1 depletion resulted in the increased surface levels of gp96, leading to hyperactivation of DCs and development of lupus-like autoimmune phenotypes in mice. Thus, AIMP1 should be critical for the prevention of autoimmune diseases. In TGF*β* signaling, AIMP1 is targeted to Smurf2, E3 ubiquitin ligase, which mediates turnover of TGF*β* receptor, and induces stabilization of Smurf2 by inhibiting ubiquitin-dependent degradation, leading to reduced phosphorylation of regulatory Smad (*34*). The absence of AIMP1 resulted in the accumulation of TGF*β* receptor, showing the

enhanced TGFβ signaling. Thus, AIMP1 plays a negative regulator of TGFβ signaling to prevent prolonged suppression of cell cycle. AIMP1-deficient mice also show the prominent motor neuron degeneration (*35*). Mechanistic studies revealed that AIMP1 is involved in the assembly of axonal neurofilament network through the interaction with neurofilament light subunit. It is not known yet how the different activities of AIMP1 can be controlled. At least deletion mapping determined the different peptide regions of AIMP1 that are primarily involved in distinct activities (*22*) (Fig. 2B). Alternatively, AIMP1 may have very flexible conformation that can be adjusted to fit for the interactions with different partner proteins. Additionally, post-translational modification may guide its cellular localization or control the affinity of AIMP1 to different target proteins.

ROLES OF AIMP2 IN UBIQUITIN DELIVERY AND TUMOR SUPPRESSION

AIMP2 can also execute additional activities apart from the multi-tRNA synthetase complex and determine cell fate via antiproliferative and proapoptotic activities. On TGFβ stimulation, AIMP2 is translocated into nucleus to downregulate FUSE-binding protein (FBP), transcriptional activator of c-myc proto-oncogene, resulting in the transcriptional suppression of c-myc (*36*). This activity was shown to be critical to arrest cell proliferation and initiate differentiation of lung epithelial cells during lung development.

AIMP2 can promote cell death via multiple directions. For instance, it can exert proapoptotic activity in response to DNA damage via p53 (*37*). In this case, it is activated and binds to p53 in nucleus. The binding of AIMP2 to p53 may protect p53 from MDM2 attack. AIMP2 can also shift TNFα signaling to apoptosis via downregulation of TNF receptor–associated factor 2 (TRAF2), a critical mediator of TNFα signaling (*38*). Thus, the combined roles of AIMP2 in the control of cell proliferation and death via these mechanisms suggest it as potent tumor suppressor. In fact, AIMP2 heterozygous mice expressing AIMP2 lower than normal become highly susceptible to various tumorigenesis (*39*) although AIMP2 homozygous mice showed neonatal lethality due to lung dysfunction resulting from overproliferation of lung epithelial cells (*36*).

Although the working mechanism of AIMP2 looks complex, its action involves the control of ubiquitin delivery. For instance, in the cases of FBP (*36*) and TRAF2 (*38*), it enhances ubiquitination of these target proteins (Fig. 3A). On the contrary, AIMP2 blocks MDM2-mediated ubiquitination of p53 (*37*) (Fig. 3B). In other lines of work, AIMP2 itself was identified as a substrate of Parkin, one of the E3 ubiquitin ligase (*40, 41*) (Fig. 3C). Parkin rescues neuronal apoptosis by induction of ubiquitin-dependent degradation of AIMP2. If this interaction is broken, the cells will accumulate AIMP2, which may cause undesirable neuronal cell death. Although its working mechanism in the ubiquitin delivery system is not completely understood, it is clear that AIMP2 is a novel regulator of cell fate through the control of ubiquitin delivery among many target sites.

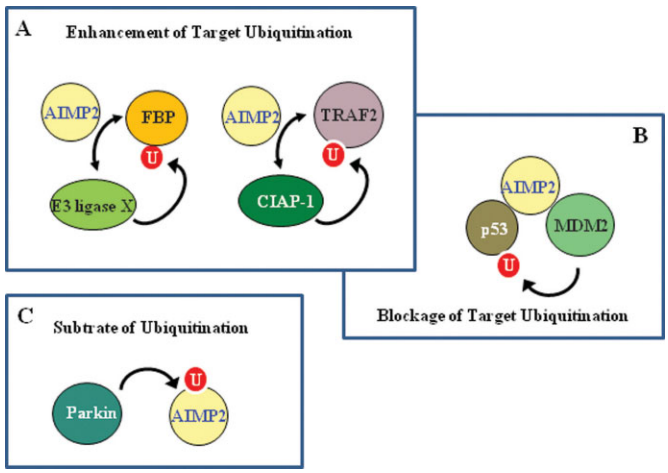

Figure 3. Diverse roles of AIMP2 in ubiquitin delivery. A: AIMP2 promotes ubiquitination of FBP and TRAF2 on TGFβ (*36*) and TNFα signals (*38*), respectively. Although cIAP-1 was suggested to be E3 ligase for ubiquitination of TRAF2, the enzyme responsible for the ubiquitination of FBP is still to be determined. B: On DNA damage, AIMP2 is activated to bind p53 to block MDM2-mediated ubiquitination (*37*). C: AIMP2 itself was suggested to be a substrate for Parkin, ubiquitin ligase (*40, 41*).

FUNCTIONAL SIGNIFICANCE OF AIMP3 FOR CHROMOSOME STABILITY AND TUMOR SUPPRESSION

AIMP3/p18, the smallest molecule in multi-ARS complex, shows sequence homology with subunits of elongation factor (EF), implying a potential role in linking the aminoacylation of tRNA and protein synthesis in ribosome (*17*). Recent X-ray analysis of AIMP3 revealed structural homology to the proteins with GST fold such as yeast glutamyl-tRNA synthetase, Arc1p, EF-1$\beta\gamma$, and glutathione S-transferase (*42*) (Fig. 4A). AIMP3 is translocated to nucleus during DNA synthesis phase or in response to DNA damage and oncogenic stresses (*43, 44*) (Fig. 4B). In nucleus, AIMP3 is shown to interact with ATM and ATR, the upstream kinases of p53. Thus, AIMP3 appears to work against DNA damage via p53 in cooperation with AIMP2, although its working mechanism is distinct from AIMP2 (Fig. 4C). The mutations that ablate its ability to interact with ATM were found in leukemia patients (*42*) (Fig. 4A).

AIMP3-deficient mice caused embryonic lethality demonstrating the functional importance of AIMP3 in embryonic development (*43*). Although AIMP3 heterozygous mice ($AIMP3^{+/-}$) were born alive with normal anatomical and morphological shape, they showed higher susceptibility to spontaneous tumor development perhaps due to the reduced activity of AIMP3 in the response against DNA damage. In fact, AIMP3 heterozygous cells are also highly susceptible to cell transformation

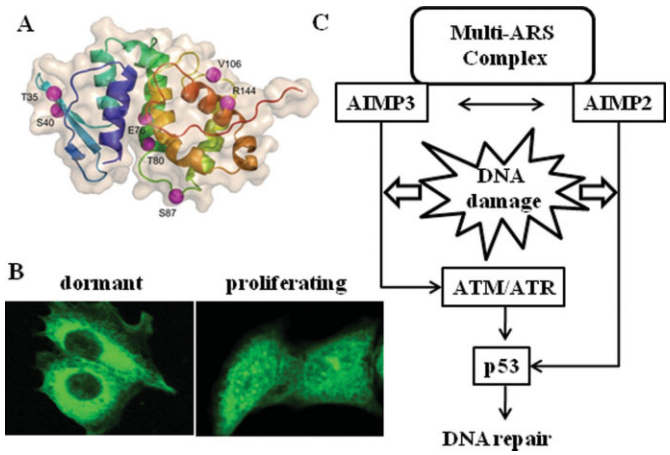

Figure 4. Three-dimensional structure and nuclear function of AIMP3. A: Three-dimensional structure of AIMP3 and mutations was found in leukemia patients (*42*). The point mutations at S87, V106, and R144 ablate the ability of AIMP3 to bind ATM. B: The cellular distribution of AIMP3 in different cell status. In dormant DU145 cells in serum-free medium, majority of AIMP3 is found in cytosol (left panel), whereas a significant portion of AIMP3 is observed as nuclear foci in proliferating cells [right panel (*43*)]. C: AIMP3 and AIMP2 bound to the multi-tRNA synthetase complex respond to DNA damage and work together via p53 for DNA repair or apoptosis (*37, 43*).

induced by oncogenic stimulation such as Ras or Myc when compared with AIMP3 wild-type cells. These transformed AIMP3$^{+/-}$ cells showed severe abnormal cell division and nuclear structure and instability in chromosome structure (*44*). Thus, AIMP3 is also a haploinsufficient tumor suppressor as AIMP2, both of which are harbored within translational machinery.

PERSPECTIVES

Although a few decades had passed since three AIMPs were found to reside within multi-tRNA synthetase complex, their functional implications remained unsolved. The functional implications for these factors can be seen from a few different perspectives. From evolutionary point of view, these factors may have been adopted to highly differentiated and multicellular systems to coordinate protein synthesis with other regulatory processes. The tight communication between different molecules and cells would be more important as the complexity of biological systems is increased. Lower eukaryotes and prokaryotes appear to have relatively simpler forms of ARS complexes with nonenzymatic factors. For instance, yeast contains Arc1p that shows limited homology to AIMP3 and forms a trimeric complex with MRS and ERS (*45*). Yeast cells also contain a factor, pex21p, that binds to SRS. In both complexes, the nonenzymatic components appear to help the aminoacylation activities of the bound enzymes (*46*). In prokaryotes, a factor, Knr4, interacts with YRS and is thought to play a role in dityrosine formation during sporulation (*47*). Nitric oxide synthase binds to WRS to enhance the enzymatic activity (*48*). Ybak and GatCAB interacts with PRS and DRS, respectively, to edit the misacylated tRNA (*49, 50*). As the complexity of organisms is increased, more factors may have been needed to work as liaison between protein synthesis and other regulatory processes and to expand the functions of ARSs. Perhaps, three AIMPs may have been selected to play such roles in highly differentiated eukaryotic systems.

Second, AIMPs control cellular turnover of the bound ARSs. Since the catalytic activities of ARSs are constitutively necessary for cellular protein synthesis, it would be more reasonable to have these enzymes to be stable in cells. However, the human complex-forming ARSs appear to be unstable in the absence of AIMPs. Perhaps, these enzymes are made with low intrinsic stability. When they are involved in protein synthesis as catalysis, they need to be stable through the association with AIMPs. However, when they are dissociated from the complex and recruited to mediate their target sites, they may have to be rapidly removed after finishing their jobs. Thus, AIMPs may have been adopted to meet these two conflicting goals of ARSs. Third, AIMPs may play a role in efficient delivery of tRNA substrates for catalysis. By considering the size of tRNA, complex formation between ARSs may hinder the free access of tRNA substrates to the active sites of the corresponding enzymes. Nonetheless, higher order structure formed between the enzymes and AIMPs may facilitate ordered trafficking of macromolecular enzyme substrates in regulated manner. In this regard, it is worth noting that AIMP1 facilitates aminoacylation of the bound enzyme, RRS (*18*) and Arg-tRNA produced by RRS within the multi-tRNA synthetase complex is more efficiently used for protein synthesis than that by free form RRS (*51*).

As aminoacylation of tRNAs is an initial and essential step in protein synthesis, this process should proceed with high stability and fidelity. Yet, the enzymes and machinery responsible for this process may have to be linked to other processes for overall coordination and homeostasis of the cells and organisms. The three ARS-interacting multifunctional proteins appear to have been selected to play dual roles in housekeeping and signaling.

ACKNOWLEDGEMENTS

This work was supported by the Korea Science and Engineering Foundation through Acceleration Research Grant (2009-0063498) and the grants of the 21th Frontier Functional Proteomics Research (FPR0881-250) and the WCU project of the MEST and the KOSEF (R31-2008-000-10103-0).

REFERENCES

1. Park, S. G., Kim, H. J., Min, Y. H., Choi, E. C., Shin, Y. K., Park, B. J., Lee, S. W., and Kim, S. (2005) Human lysyl-tRNA synthetase is

secreted to trigger proinflammatory response. *Proc. Natl. Acad. Sci. USA* **102**, 6356–6361.
2. Yannay-Cohen, N., Kay, G., Yang, C. M., Han, J. M., Kemeny, D. M., Kim, S., Nechushtan, H., and Razin, E. (2009) LysRS serves as a key signaling molecule in the immune response by regulating gene expression. *Mol. Cell* **34**, 603–611.
3. Levy, I. C., Cohen, N. Y., Kay, G., Razin, E., and Nechushtan, H. (2008) Ap4A hydrolase is part of the transcriptional regulation network in immunologically activated mast cells. *Mol. Cell. Biol.* **28**, 5777–5784.
4. Yannay-Cohen, N. and Razin, E. (2006) Translation and transcription: the dual functionality of LysRS in mast cells. *Mol. Cells* **22**, 127–132.
5. Wright, M., Boonyalai, N., Tanner, J. A., Hindley, A. D., and Miller, A. D. (2006) The duality of LysU, a catalyst for both Ap4A and Ap3A formation. *FEBS J.* **273**, 3534–3544.
6. Lee, Y. N., Nechushtan, H., Figov, N., and Razin, E. (2004) The function of lysyl-tRNA synthetase and Ap4A as signaling regulators of MITF activity in FcepsilonRI-activated mast cells. *Immunity* **20**, 145–151.
7. Cen, S., Javanbakht, H., Kim, S., Shiba, K., Craven, R., Rein, A., Ewalt, K., Schimmel, P., Musier-Forsyth, K., and Kleiman, L. (2002) Retrovirus-specific packaging of aminoacyl-tRNA synthetases with cognate primer tRNAs. *J. Virol.* **76**, 13111–13115.
8. Sampath, P., Mazumder, B., Seshadri, V., Gerber, C. A., Chavatte, L., Kinter, M., Ting, S. M., Dignam, J. D., Kim, S., Driscoll, D. M., and Fox, P. L. (2004) Noncanonical function of glutamyl-prolyl-tRNA synthetase: gene-specific silencing of translation. *Cell* **119**, 195–208.
9. Ko, Y. G., Kim, E. Y., Kim, T., Park, H., Park, H. S., Choi, E. J., and Kim, S. (2001) Glutamine-dependent antiapoptotic interaction of human glutaminyl-tRNA synthetase with apoptosis signal-regulating kinase 1. *J. Biol. Chem.* **276**, 6030–6036.
10. Norcum, M. T. and Warrington, J. A. (1998) Structural analysis of the multienzyme aminoacyl-tRNA synthetase complex: a three-domain model based on reversible chemical crosslinking. *Protein Sci.* **7**, 79–87.
11. Quevillon, S., Robinson, J. C., Berthonneau, E., Siatecka, M., and Mirande, M. (1999) Macromolecular assemblage of aminoacyl-tRNA synthetases: identification of protein-protein interactions and characterization of a core protein. *J. Mol. Biol.* **285**, 183–195.
12. Rho, S. B., Kim, M. J., Lee, J. S., Seol, W., Motegi, H., Kim, S., and Shiba, K. (1999) Genetic dissection of protein–protein interactions in multi-tRNA synthetase complex. *Proc. Natl. Acad. Sci. USA* **96**, 4488–4493.
13. Kim, J. Y., Kang, Y. S., Lee, J. W., Kim, H. J., Ahn, Y. H., Park, H., Ko, Y. G., and Kim, S. (2002) p38 is essential for the assembly and stability of macromolecular tRNA synthetase complex: implications for its physiological significance. *Proc. Natl. Acad. Sci. USA* **99**, 7912–7916.
14. Han, J. M., Park, S. G., Lee, Y., and Kim, S. (2006) Structural separation of different extracellular activities in aminoacyl-tRNA synthetase-interacting multifunctional protein, p43/AIMP1. *Biochem. Biophys. Res. Commun.* **342**, 113–118.
15. Wolfe, C. L., Warrington, J. A., Treadwell, L., and Norcum, M. T. (2005) A three-dimensional working model of the multienzyme complex of aminoacyl-tRNA synthetases based on electron microscopic placements of tRNA and proteins. *J. Biol. Chem.* **280**, 38870–38878.
16. Norcum, M. T. and Warrington, J. A. (2000) The cytokine portion of p43 occupies a central position within the eukaryotic multisynthetase complex. *J. Biol. Chem.* **275**, 17921–17924.
17. Quevillon, S. and Mirande, M. (1996) The p18 component of the multisynthetase complex shares a protein motif with the beta and gamma subunits of eukaryotic elongation factor 1. *FEBS Lett.* **395**, 63–67.
18. Park, S. G., Jung, K. H., Lee, J. S., Jo, Y. J., Motegi, H., Kim, S., and Shiba, K. (1999) Precursor of pro-apoptotic cytokine modulates aminoacylation activity of tRNA synthetase. *J. Biol. Chem.* **274**, 16673–16676.
19. Robinson, J. C., Kerjan, P., and Mirande, M. (2000) Macromolecular assemblage of aminoacyl-tRNA synthetases: quantitative analysis of protein–protein interactions and mechanism of complex assembly. *J. Mol. Biol.* **304**, 983–994.
20. Kim, Y., Shin, J., Li, R., Cheong, C., Kim, K., and Kim, S. (2000) A novel anti-tumor cytokine contains an RNA binding motif present in aminoacyl-tRNA synthetases. *J. Biol. Chem.* **275**, 27062–27068.
21. Kaminska, M., Deniziak, M., Kerjan, P., Barciszewski, J., and Mirande, M. (2000) A recurrent general RNA binding domain appended to plant methionyl-tRNA synthetase acts as a cis-acting cofactor for aminoacylation. *EMBO J.* **19**, 6908–6917.
22. Lee, S. W., Kim, G., and Kim, S. (2008) Aminoacyl-tRNA synthetase-interacting multifunctional protein 1/p43: an emerging therapeutic protein working at systems level. *Exp. Opin. Drug Discov.* **3**, 945–957.
23. Park, S. G., Kang, Y. S., Ahn, Y. H., Lee, S. H., Kim, K. R., Kim, K. W., Koh, G. Y., Ko, Y. G., and Kim, S. (2002) Dose-dependent biphasic activity of tRNA synthetase-associating factor, p43, in angiogenesis. *J. Biol. Chem.* **277**, 45243–45248.
24. Park, H., Park, S. G., Kim, J., Ko, Y. G., and Kim, S. (2002) Signaling pathways for TNF production induced by human aminoacyl-tRNA synthetase-associating factor, p43. *Cytokine* **20**, 148–153.
25. Matschurat, S., Knies, U. E., Person, V., Fink, L., Stoelcker, B., Ebenebe, C., Behrensdorf, H. A., Schaper, J., and Clauss, M. (2003) Regulation of EMAP II by hypoxia. *Am. J. Pathol.* **162**, 93–103.
26. Park, S. G., Shin, H., Shin, Y. K., Lee, Y., Choi, E. C., Park, B. J., and Kim, S. (2005) The novel cytokine p43 stimulates dermal fibroblast proliferation and wound repair. *Am. J. Pathol.* **166**, 387–398.
27. Park, S. G., Kang, Y. S., Kim, J. Y., Lee, C. S., Ko, Y. G., Lee, W. J., Lee, K. U., Yeom, Y. I., and Kim, S. (2006) Hormonal activity of AIMP1/p43 for glucose homeostasis. *Proc. Natl. Acad. Sci. USA* **103**, 14913–14918.
28. Kim, E., Kim, S. H., Kim, S., Cho, D., and Kim, T. S. (2008) AIMP1/p43 protein induces the maturation of bone marrow-derived dendritic cells with T helper type 1-polarizing ability. *J. Immunol.* **180**, 2894–2902.
29. Ko, Y. G., Park, H., Kim, T., Lee, J. W., Park, S. G., Seol, W., Kim, J. E., Lee, W. H., Kim, S. H., Park, J. E., and Kim, S. (2001) A cofactor of tRNA synthetase, p43, is secreted to up-regulate proinflammatory genes. *J. Biol. Chem.* **276**, 23028–23033.
30. Park, H., Park, S. G., Lee, J.-W., Kim, T., Kim, G., Ko, Y.-G., and Kim, S. (2002) Monocyte cell adhesion induced by a human aminoacyl-tRNA synthetase-associated factor, p43: identification of the related adhesion molecules and signal pathways. *J. Leukoc. Biol.* **71**, 223–230.
31. Kim, E., Kim, S. H., Kim, S., and Kim, T. S. (2006) The novel cytokine p43 induces IL-12 production in macrophages via NF-kappaB activation, leading to enhanced IFN-gamma production in CD4+ T cells. *J. Immunol.* **176**, 256–264.
32. Han, J. M., Myung, H., and Kim, S. Antitumor activity and pharmacokinetic properties of ARS-interacting multifunctional protein 1 (AIMP1/p43). *Cancer Lett.* **287**, 157–164.
33. Han, J. M., Park, S. G., Liu, B., Park, B. J., Kim, J. Y., Jin, C. H., Song, Y. W., Li, Z., and Kim, S. (2007) Aminoacyl-tRNA synthetase-interacting multifunctional protein 1/p43 controls endoplasmic reticulum retention of heat shock protein gp96. Its pathological implications in lupus-like autoimmune diseases. *Am. J. Pathol.* **170**, 2042–2054.
34. Lee, Y. S, Han, J. M., Son, S. H., Choi, J. W., Jeon, E. J., Bae, S. C., Park, Y. I., and Kim, S. (2008) AIMP1/p43 downregulates TGF-beta signaling via stabilization of smurf2. *Biochem. Biophys. Res. Commun.* **371**, 395–400.
35. Zhu, X., Yin, Y., Shao, A., Zhang, B., Kim, S., and Zhou, J. (2009) MSC p43 required for axonal development in motor neurons. *Proc. Natl. Acad. Sci. USA* **106**, 15944–15949.
36. Kim, M. J., Park, B. J., Kang, Y. S., Kim, H. J., Park, J. H. Kang, J. W., Lee, S. W., Han, J. M., Lee, H. W., and Kim, S. (2003) Downregulation of FUSE-binding protein and c-myc by tRNA synthetase cofactor p38 is required for lung cell differentiation. *Nat Genet.* **34**, 330–336.

37. Han, J. M., Park, B. J., Park, S. G., Oh, Y. S., Choi, S. J., Lee, S. W., Hwang, S. K., Chang, S. H., Cho, M. H., and Kim, S. (2008) AIMP2/p38, the scaffold for the multi-tRNA synthetase complex, responds to genotoxic stresses via p53. *Proc. Natl. Acad. Sci. USA* **105**, 11206–11211.
38. Choi, J. W., Kim, D. G., Park, M. C., Um, J. Y., Han, J. M., Park, S. G., Choi, E. C., and Kim, S. (2009) AIMP2 promotes TNFalpha-dependent apoptosis via ubiquitin-mediated degradation of TRAF2. *J. Cell Sci.* **122**, 2710–2715.
39. Choi, J. W., Um, J. Y., Kundu, J. K., Surh, Y. J., and Kim, S. (2009) Multidirectional tumor-suppressive activity of AIMP2/p38 and the enhanced susceptibility of AIMP2 heterozygous mice to carcinogenesis. *Carcinogenesis* **30**, 1638–1644.
40. Corti, O., Hampe, C., Koutnikova, H., Darios, F., Jacquier, S., Prigent, A., Robinson, J. C., Pradier, L., Ruberg, M., Mirande, M., Hirsch, E., Rooney, T., Fournier, A., and Brice, A. (2003) The p38 subunit of the aminoacyl-tRNA synthetase complex is a Parkin substrate: linking protein biosynthesis and neurodegeneration. *Hum. Mol. Genet.* **12**, 1427–1437.
41. Ko, H. S., von Coelln, R., Sriram, S. R., Kim, S. W., Chung, K. K., Pletnikova, O., Troncoso, J., Johnson, B., Saffary, R., Goh, E. L., Song, H., Park, B. J., Kim, M. J., Kim, S., Dawson, V. L., and Dawson, T. M. (2005) Accumulation of the authentic Parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. *J. Neurosci.* **25**, 7968–7978.
42. Kim, K. J., Park, M. C., Choi, S. J., Oh, Y. S., Choi, E. C., Cho, H. J., Kim, M. H., Kim, S. H., Kim, D. W., Kim, S., and Kang, B. S. (2008) Determination of three-dimensional structure and residues of the novel tumor suppressor AIMP3/p18 required for the interaction with ATM. *J. Biol. Chem.* **283**, 14032–14040.
43. Park, B. J., Kang, J. W., Lee, S. W., Choi, S. J., Shin, Y. K., Ahn, Y. H., Choi, Y. H., Choi, D., Lee, K. S., and Kim, S. (2005) The haploinsufficient tumor suppressor p18 upregulates p53 via interactions with ATM/ATR. *Cell* **120**, 209–221.
44. Park, B. J., Oh, Y. S., Park, S. Y., Choi, S. J., Rudolph, C., Schlegelberger, B., and Kim, S. (2006) AIMP3 haploinsufficiency disrupts oncogene-induced p53 activation and genomic stability. *Cancer Res.* **66**, 6913–6918.
45. Karanasios, E., Simader, H., Panayotou, G., Suck, D., and Simos, G. (2007) Molecular determinants of the yeast Arc1p-aminoacyl-tRNA synthetase complex assembly. *J. Mol. Biol.* **374**, 1077–1090.
46. Rocak, S., Landeka, I., and Weygand-Durasevic, I. (2002) Identifying Pex21p as a protein that specifically interacts with yeast seryl-tRNA synthetase. *FEMS Microbiol. Lett.* **214**, 101–106.
47. Dagkessamanskaia, A., Martin-Yken, H., Basmaji, F., Briza, P., and Francois, J. (2001) Interaction of Knr4 protein, a protein involved in cell wall synthesis, with tyrosine tRNA synthetase encoded by TYS1 in Saccharomyces cerevisiae. *FEMS Microbiol. Lett.* **200**, 53–58.
48. Buddha, M. R., Keery, K. M., and Crane, B. R. (2004) An unusual tryptophanyl tRNA synthetase interacts with nitric oxide synthase in Deinococcus radiodurans. *Proc. Natl. Acad. Sci. USA* **101**, 15881–15886.
49. An, S. and Musier-Forsyth, K. (2004) Trans-editing of Cys-tRNAPro by Haemophilus influenzae YbaK protein. *J. Biol. Chem.* **279**, 42359–42362.
50. Bailly, M., Blaise, M., Lorber, B., Becker, H. D., and Kern, D. (2007) The transamidosome: a dynamic ribonucleoprotein particle dedicated to prokaryotic tRNA-dependent asparagine biosynthesis. *Mol. Cell* **28**, 228–239.
51. Kyriacou, S. V. and Deutscher, M. P. (2008) An important role for the multienzyme aminoacyl-tRNA synthetase complex in mammalian translation and cell growth. *Mol. Cell* **29**, 419–427.