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Homeostatic mechanisms by alternative forms of tRNA synthetases

Min Guo¹ and Paul Schimmel²

¹Department of Cancer Biology, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458

²Department of Chemical Physiology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Abstract

Although aminoacyl tRNA synthetases (aaRSs) act in translation, numerous examples demonstrate ex-translational functions in fundamental networks of higher organisms. These functions involve modified versions of aaRSs and natural fragments. The interplay of aaRS-derived entities appears to be at the heart of homeostatic mechanisms controlling angiogenesis, inflammation, metabolism and tumorigenesis.

Homeostasis is robust in animals and arises from interconnections within complex webs of macromolecular interactions of genes and their products. Although aminoacyl tRNA synthetases (aaRSs) have long been known for their translation function--the attachment of amino acids to their cognate tRNAs--recent results solidify that they are also regulatory proteins deeply embedded in web-like cellular networks.

While an early example of ex-translational functions of aaRSs is the ying-yang interplay between natural extracellular fragments of human TrpRS and TyrRS in angiogenesis, a more recent example further illustrates the multiple pathways in which aaRSs participate in a balanced framework. Interestingly, TyrRS was found to contain a surprising nuclear localization signal that is regulated by its cognate tRNA [1]. In this instance, a reduction in the level of tRNA^{Tyr} resulted in a higher level of nuclear import of the synthetase, where it is believed to be involved in one or more gene regulatory mechanisms. Conversely, an increase in the level of tRNA^{Tyr} diminished the nuclear concentration of TyrRS. Thus, the subcellular distribution of TyrRS is directly controlled by the demands of protein synthesis. This control is itself an example of a homeostatic mechanism that balances a translational with an ex-translational function. The balancing of these roles is possibly connected to the stress response [1].

Two further and recent examples highlight an unanticipated mechanism by which the ex-translational function of a synthetase is regulated. This regulation, in contrast to the examples discussed above, involves 'auto-balancing', in which a natural fragment regulates the activity of the same protein from which it originated. This homeostatic 'auto-balance' mechanism is broadly outlined in Figure 1a.

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The first example is provided in Figure 1b. Interferon (IFN)- γ treatment of human myeloid cells induces phosphorylation of a tRNA synthetase fusion protein, known as Glu-ProRS (or EPRS), which is unique to higher organisms. This bi-functional synthetase resides in a large complex of nine synthetases and three scaffold proteins that is designated as the multi-tRNA synthetase complex (MSC). EPRS contains a functional ERS and PRS (GluRS and ProRS), which are fused by three repeats of ~60 amino acid helix-loop-helix WHEP domains. IFN- γ -induced phosphorylation of EPRS occurs at two serine residues close to the third WHEP domain, and provokes the release of EPRS from the MSC. Next, phospho-EPRS forms a heterotetrameric GAIT (gamma-interferon-activated inhibitor of translation) complex with NSAP1 (NS1 associated protein), GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and phosphorylated ribosomal protein L13a. This GAIT complex then recognizes GAIT elements in the 3' UTRs of specific mRNAs and thereby exerts transcript-selective translational repression by blocking the recruitment of the translational pre-initiation complex [2]. This translational inhibition happens 16–24 h after IFN- γ treatment and is thought to be important to 'switch off' production of proinflammatory proteins (such as VEGF-A) and therefore facilitate the resolution of the inflammatory response [3].

However, completely switching off VEGF-A could have adverse effects. Interestingly, a GAIT element-binding fragment of EPRS counters the GAIT complex itself, to assure basal translation of VEGF-A mRNA [4]. The fragment is produced through an unusual alternative polyadenylation, in which the UAU codon of Tyr⁸⁶⁴ in EPRS is cleaved (to UA) and then polyadenylated to generate a C-terminal truncated EPRS^{N1}. Interestingly, Tyr⁸⁶⁴ is at the end of the second WHEP domain, so that EPRS^{N1} loses the third WHEP domain and the sites for phosphorylation. The result is that EPRS^{N1} cannot form the GAIT complex; instead, through its first two WHEP domains, EPRS^{N1} retains the capacity to bind to the GAIT element of VEGF-A mRNA. In fact, EPRS^{N1} binds to GAIT-element-containing RNA at a higher binding affinity than does full-length EPRS. Thus, EPRS has developed a sophisticated homeostatic auto-balancing mechanism for regulating production of VEGF-A [4].

The second example is depicted in Figure 1c. One of the three scaffold proteins in the MSC is known as MSC p38 or, alternatively, AIMP2 (aaRS interacting multifunctional protein 2). As the core protein of the MSC, it interacts with most of the MSC components; therefore, depletion of MSC p38 led to complete disruption of the MSC [5]. Significantly, heterozygous MSC p38-deficient mice had increased susceptibility to various tumor types, suggesting a role for MSC p38 as a haplo-insufficient tumor suppressor. Also supporting this role is the demonstration that MSC p38 binds to and regulates the ubiquitylation of FBP, TRAF2, and p53, key players in apoptotic signaling in response to DNA damage, oxidative stress and TNF- α treatment [6–8]. These functions of MSC p38 are MSC-independent. For example, DNA damage triggers phosphorylation of MSC p38 and stimulates its release from the MSC. Then, it enters the nucleus and directly binds p53 through its GST domain, disrupts the MDM2-p53 association, and thereby blocks MDM2-mediated ubiquitylation and degradation of p53 [7].

Remarkably, an internally deleted alternative splice variant of MSC p38, DX2, does not contain residues 46 to 114, including its leucine zipper (LZ), and therefore does not associate with the MSC [9]. However, all interactions of MSC p38 with FBP, TRAF2, and p53 are through the first half of the GST domain. By retaining the GST domain, the DX2 variant retains the ability to bind p53, but does not inhibit the p53-MDM2 association. Therefore, DX2 competes with MSC p38 for p53 binding and promotes MDM2-mediated degradation of p53. Through this function, together with a similar interaction with TRAF2, DX2 counters the pro-apoptotic activity of released MSC p38 [9, 10]. Importantly, DX2 and

the ratio of DX2:full length MSC p38 have been implicated in cancer development and therapy [9, 10].

The exciting findings in these papers raise several intriguing questions. In the case of the nine tRNA synthetases bound together in the MSC, would splice variants that retained the catalytic domain but lost their MSC-associating sequence be part of a general mechanism to generate another functional pool of aaRSs? Similar to EPRS and MSC p38, would splice variants of other synthetases pair with their full-length counterparts, or with other splice variants, and thereby regulate the novel aaRS-associated functions? Finally, how do these pairs contribute to control of homeostasis, so that the response is kept in the optimal range? It will be interesting to learn whether DX2 is created to ensure a p53 'trickle', and whether a proper ratio of EPRS/EPRS^{N1} is needed to link VEGF-A expression with vasculature development and tumorigenesis in animals.

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Figure 1.

Mechanisms for aaRSs maintaining homeostatic balance in animals.

(a) A general homeostatic mechanism for regulation of homeostasis by tRNA synthetases and associated proteins. Environmental insults activate the generation of modified forms of aminoacyl-tRNA synthetases (aaRSs, yellow), such as phosphorylated variants with novel activities. Although the modified aaRSs implement a variety of responses through interactions with signaling proteins (blue), cells also produce splice variants that counter the newly presented activities and modulate the overall output. (b) When released from the multi-tRNA synthetase complex (MSC) after treatment with interferon (IFN)- γ , EPRS recruits three other proteins to form the GAIT complex, which binds and silences translation of vascular endothelial growth factor (VEGF)-A mRNA [3]. A truncated form, EPRS^{N1}, counters the translational repression by competing for binding to VEGF-A mRNA, and thereby helps to maintain a low but constant basal level of VEGF-A expression [4]. (c) A splice variant of MSC scaffold protein p38/AIMP2, designated as DX2, counters the pro-apoptotic activity of released MSC p38 by competing for association with p53 and thereby promoting p53 degradation. An imbalance of DX2 and MSC p38 can lead to increased tumorigenesis, suggesting a crucial role for the homeostatic balance in prevention of tumor formation [9]. LZ, leucine zipper motif; GST, glutathione S-transferase homology domain.