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Cytosolic aminoacyl-tRNA synthetases: Unanticipated relocations for unexpected functions

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ABSTRACT

Prokaryotic and eukaryotic cytosolic aminoacyl-tRNA synthetases (aaRSs) are essentially known for their conventional function of generating the full set of aminoacyl-tRNA species that are needed to incorporate each organism's repertoire of genetically-encoded amino acids during ribosomal translation of messenger RNAs. However, bacterial and eukaryotic cytosolic aaRSs have been shown to exhibit other essential nonconventional functions. Here we review all the subcellular compartments that prokaryotic and eukaryotic cytosolic aaRSs can reach to exert either a conventional or nontranslational role. We describe the physiological and stress conditions, the mechanisms and the signaling pathways that trigger their relocation and the new functions associated with these relocating cytosolic aaRS. Finally, given that these relocating pools of cytosolic aaRSs participate to a wide range of cellular pathways beyond translation, but equally important for cellular homeostasis, we mention some of the pathologies and diseases associated with the dis-regulation or malfunctioning of these nontranslational functions.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) belong to a family of ubiquitous and essential enzymes. Their primary task is to supply the protein synthesis machineries (cytosolic and organellar) with the full set of aminoacyl-tRNAs (aa-tRNAs) necessary to translate all messenger RNA codons into their corresponding amino acids (aa) [1]. In theory, a given organism has to encode at least one aaRS for each genetically-encoded aa, which means that a prokaryotic species that uses for protein synthesis the standard genetic code composed of 20 aa, would need 20 genes encoding the 20 required aaRSs. However, depending on the organism's origin and genetic code repertoire, the number of aaRSs that can be found in a given organism ranges from 18 to 23 in prokaryotes [2] to nearly 45 in eukaryotes [3]. Despite their essential biochemical function the majority of prokaryotes and all eukaryotic organelles have less unique aaRSs than genetically-encoded aa, because these organisms (or compartments) can compensate the lack of an essential aaRS by alternative routes to generate the aa-tRNA species corresponding to the orphan tRNA/codon pair ([4–6] and reviewed in [7,8]). Paradoxically, prokaryotes and mainly bacteria also quasi-systematically encode more aaRSs than the necessary set for decoding the genetic code repertoire because the majority of bacterial genomes harbor more than one gene for a given aaRS [2]. Although the majority of the bacteria present one or several duplicated aaRSs (and even triplicated ones), the reason for the presence of 2 aaRSs of the same aa and tRNA-charging specificity in a given species has rarely been studied. However, in the few cases that were studied, it was found that one of the two forms is usually expressed under specific environmental or stress conditions and that this stress-induced aaRS possesses specific catalytic traits allowing formation of the cognate aa-tRNA in these particular physiological conditions [9–14].

In eukaryotes, the presence of at least 2 different translationally-active and membrane-separated compartments, the cytosol and mitochondria, makes already the situation more complex with respect to the number of aaRSs that these organisms have to possess; and becomes even more complicated if the organism possesses additional organelles besides the mitochondria. Because the organellar and cytosolic translation machineries are of different phylogenetic origins, each eukaryote carries several compartment-specific pools of aaRSs, one for cytosolic translation and one for each organelle (reviewed in [15]). While both sets of genes are encoded by the nuclear genome and translated by the cytosolic protein synthesis machinery, the organellar pools are easily distinguishable from the cytosolic one because they are of prokaryotic origins and because these aaRSs possess, usually in the N-terminus, an organelle-specific targeting sequence that directs their import into the corresponding compartment (reviewed in [15]). However, even if in theory, a eukaryotic species using the standard 20 aa-containing genetic code and that possesses mitochondria would have to encode 40 aaRS

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genes, there is not a single eukaryotic species that has been proposed so far to encode 2 complete and unique compartment-specific sets of 20 aaRSs [5,15]. Depending on the species that was studied, up to 17 mitochondria-specific aaRS genes can be missing (reviewed in [15]) from the nuclear genome in certain species. To compensate this apparent lack of mitochondrial aaRSs, eukaryotes essentially utilize two different strategies: i) they use an alternate two-steps route to generate the aa-tRNA species for which the cognate aaRS is missing [6,16,17] or ii) they import the corresponding cytosolic aaRS making this enzyme a dual-localized aaRS both cytosolic and mitochondrial (reviewed in [15,18]). When both the cytosolic and mitochondrial aaRS are encoded by a single gene, both forms are either translated from two mRNAs of different length generated through different processes (see below). While using a dual-localized aaRS encoded from a single gene makes perfect sense when the gene encoding the mitochondria-specific aaRS is missing, there are examples in higher plants and *S. cerevisiae* (*Sce*), where a cytosolic aaRS can be targeted to mitochondria even if the corresponding mitochondrial ortholog encoded by a separate gene already exists (reviewed in [15]). The rational for having duplicated mitochondrial aaRS (one strictly organellar and one dual-localized) has been clarified in yeast [6] but has so far not been ruled out in higher plants. However, it was shown that some of these plant dual-localized aaRSs are not able to charge tRNA inside the mitochondria [19], suggesting that they might have a nonconventional role inside this compartment that has yet to be identified. Interestingly, in plants the cytosolic aaRSs that are dual-localized are strictly shared with the mitochondria and never with the chloroplast, however the majority of the mitochondrial aaRSs are dual-targeted both in the mitochondria and the chloroplasts because they harbor an ambiguous organellar targeting sequence [20].

The other common trait of eukaryotic cytosolic aaRSs that influences their subcellular localization is the capacity of some of them to form so-called Multi-Synthetase Complexes (MSCs). These complexes were found in all species studied so far ranging from fungi to mammals ([21–24] and reviewed in [25]). They are all composed of cytosolic aaRS and 1 to 3 auxiliary assembly factors also called Aminoacyl-tRNA synthetase Interacting Multifunctional Proteins (AIMPs). The number of aaRSs and AIMPs that build up these MSCs varies from 2 aaRSs and 1 AIMP in the *S. cerevisiae* AME complex to 9 aaRSs and 3 AIMPs (AIMP1, AIMP2 AIMP3 also called p43, p38 and p18 respectively) in the human, MARS complex. With the exception of the yeast AME MSC (reviewed in [26]), the association of cytosolic aaRSs into MSC does not impact the catalytic efficiency of the participating aaRSs but rather regulates the alternative subcellular localization and non-conventional function that some of them have been shown to display. The studies that have been published over the past 2 decades uniformly show that these MSCs can be considered as cytosolic retention platforms for aaRSs that can be released from these complexes under specific physiological or stress conditions to relocate into other subcellular compartments in which they can exert nonconventional functions (reviewed in [27–30]). Additionally, the AIMPs can also be released from MSCs to be targeted to other compartments (reviewed in [31]). The additional compartments that these MSC-released cytosolic aaRSs or AIMPs can reach and the repertoire of atypical physiological processes to which these proteins can participate is in constant expansion ([29], reviewed in [32]). Studies show that MSCs-released aaRSs can i) stay in the cytosol by building other non-MSC complexes, ii) be imported into mitochondria or nuclei, iii) bind to membranes or even iv) be secreted (reviewed in [18]). Once relocated, they can exert a myriad of regulatory or signaling functions that impact processes among which gene expression, angiogenesis, apoptosis, inflammation, adiposity, nutrient signaling, cell-to-cell crosstalk or even resveratrol-mediated poly(ADP-ribose) polymerase 1 (PARP1) activation (reviewed in [28–31], [33]). Their involvement in these processes is far from being trivial since in human, malfunctioning of these aaRSs' additional roles, inevitably leads to diseases [31,34].

At the molecular level, the release of an aaRS or AIMP from the MSC is usually mediated by posttranslational modification of the protein that includes chemical modification or proteolytic cleavage of the aaRSs at a specific residue [35]. More rarely, the release of an aaRS can be initiated by decreasing the quantity of the AIMP binding the aaRS [36]. There are 2 types of molecular mechanism by which these aaRSs exert their atypical function, either they bind to one or several new protein partners that deviates the aaRS from aa-tRNA formation or they accumulate a by-product or intermediate of the tRNA aminoacylation reaction (reviewed in [27–30]) or both. Note that in human and yeast some of the cytosolic aaRSs that do not belong to the MSCs have also been shown or predicted to be able to relocate to other subcellular compartments and, in some cases, their atypical role has been characterized.

In the present review we give a detailed overview of all the subcellular compartments that free-standing or MSC-participating cytosolic aaRSs can reach. We mention the mechanisms, strategies and and physiological or stress conditions that trigger their relocation and the unexpected cellular processes to which relocated cytosolic aaRSs participate. We have subdivided relocating aaRSs into two categories: those who keep their canonical function in their new subcellular destination, meaning formation of an aa-tRNA intermediate or end-product that supplies the translation machinery and those who exert a non-conventional function that does not require formation of an aa-tRNA species.

2. Membrane-localized aaRSs in bacteria

Prokaryotic cells are composed of distinct compartments that differ according to their phylogenetic origin. In all cases, the cytoplasm, in which transcription and translation of genetic information occur, is engulfed by a negatively charged plasma membrane made up of various lipid species, ranging from phospholipids to hopanoids [37,38]. The structure of the outer shell of prokaryotes differs between Gram-positive [39] and Gram-negative [40] bacteria, and between phylogenetical groups of archaea [41], but to date, no aaRS has been detected outside the cytoplasm, where the 20 enzymes find their substrates (ATP, aa, tRNAs) and perform their canonical aminoacylation function. Several studies, however, have shown that aaRS can sometimes be localized at membranous structures (see below).

Central and essential biological functions depend on the integrity of plasma membranes, such as respiration, electron transport, proton translocation, mechanical stresses resistance, nutrients and exogenous molecules selection and transport, interaction with the environment or host macromolecules, etc. [37–41]. Because of their relatively uniform negative charge, bacterial membranes are targeted by and sensitive to cationic antimicrobial peptides (CAMPs) synthesized and secreted by other bacteria or fungi, or by immune cells within a host [42]. Various CAMPs exist, but they often target bacteria through charge interaction, leading to membrane disruption and ultimately to bacterial death [42]. Bacteria possess CAMPs resistance factors of various types [43,44], and among them, aminoacyl-phosphatidylglycerol synthases (aaPGSs) [45,46]. AaPGSs are composed of two separated domains, an integral membrane N-terminal domain with 2 to 14 transmembrane helices, with a phospholipid flippase activity, and a C-terminal soluble domain of the DUF2156 family that has an aa-tRNA transferase activity. AaPGSs catalyze the transfer of an aa from an aa-tRNA to a second substrate, phosphatidylglycerol (PG) or cardiolipin (CL) [45]. For example, LysPGS transfers Lys from K-tRNA^{K} to PG, leading to a lysylated PG (K-PG), whose overall charge is + 1, in comparison to PG (− 1). AaPGSs can be specific of one aa (KPGS, APGS), bispecific (A/KPGS) or multispecific (R/A/KPGS) and transfer corresponding amino acids onto PG or CL, providing aminoacylated phospholipids with modified charge properties. Such modifications change the overall properties of membranes such as permeability to various compounds (metals, antimicrobials, *etc.*), rigidity and, of course, global charge. AaPGSs have