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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 nonconventional 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

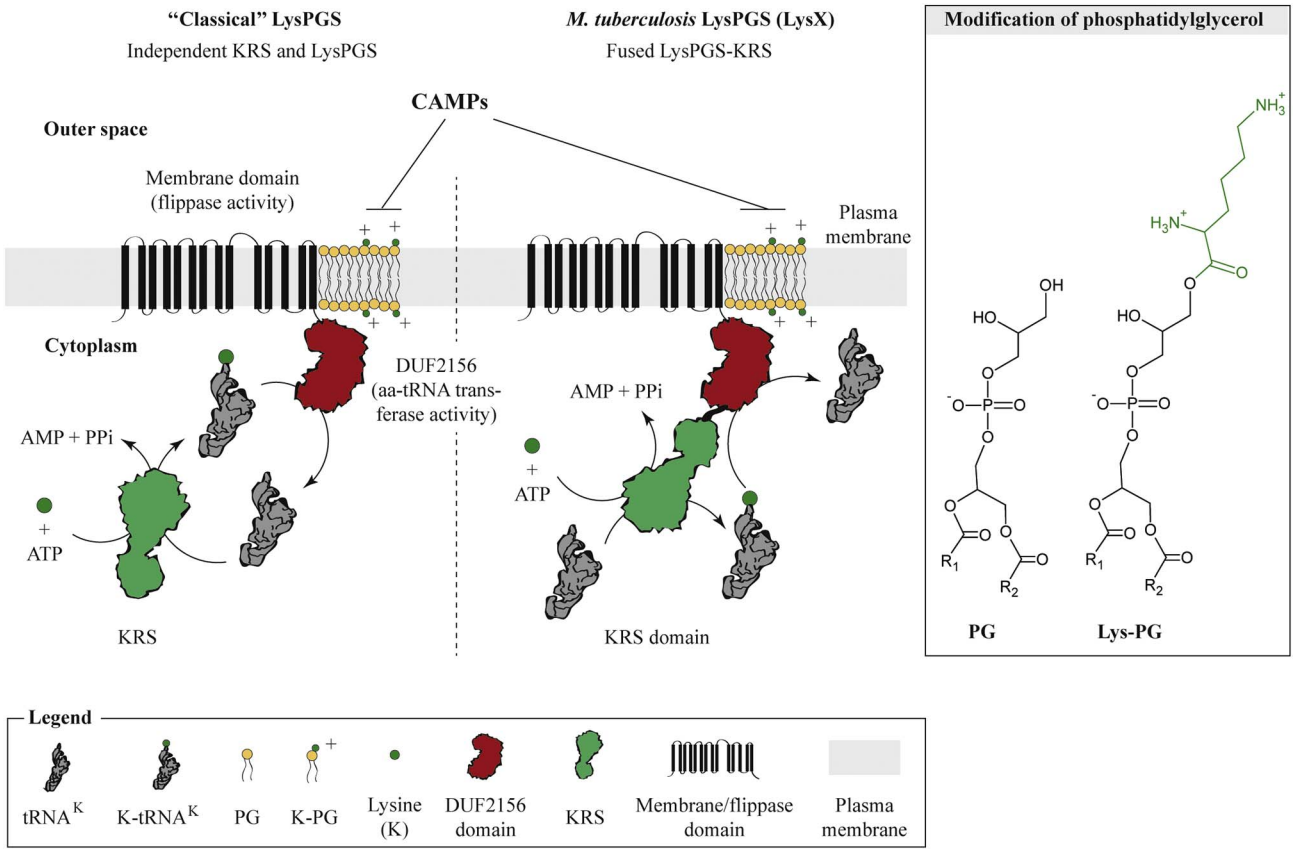

Fig. 1. Aminoacyl-phosphatidylglycerol synthases. "Classical" aaPGS, like the LysPGS from *S. aureus* is composed of an N-terminal integral membrane domain with 14 transmembrane helices (TMH, black), that targets the enzyme to the plasma membrane, and a C-terminal soluble domain, facing the cytoplasm (red), which belongs to the DUF2156 family of aa-tRNA transferases. The DUF2156 domain utilizes K-tRNA^K, produced independently by a "canonical" KRS (green), and catalyzes the transfer of K (green cicle) from tRNA^K to a hydroxyl group of phosphatidylglycerol (PG). Resulting K-PG can then be transferred to the outer leaflet of the plasma membrane by the N-terminal membrane domain that displays a phospholipid flippase activity. Changes in membrane charge properties participate in resistance against CAMPs. In *M. tuberculosis*, the LysX gene product is a LysPGS-KRS fusion protein, with the N-terminal flippase/membrane (black) and middle DUF2156 (red) domains of classical aaPGSs, followed by a KRS C-terminal domain (green). This domain is responsible for the synthesis of K-tRNA^K that is subsequently used by the DUF2156 and flippase domains to produce surface exposed K-PG molecules. The aaPGS part of the protein cannot scavenge K-tRNA^K produced by the second "canonical" KRS in *M. tuberculosis*. The right panel presents structures of PG and K-PG. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

been demonstrated to increase resistance against CAMPs in various model pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and others and have been related to virulence phenotypes. Those virulence factors have thus been named Multiple Peptide Resistance Factors [45,46].

In *Mycobacterium tuberculosis* and numerous Actinobacteria [45], two KRS genes are detected. One of them (*lysS*, Rv3598c) encodes a canonical KRS with an N-terminal anticodon-binding domain (ABD) and a C-terminal catalytic core, while the second (*lysX*, Rv1640c) is composed of an N-terminal MprF-like domain (flippase and DUF2156 domains) followed by a KRS domain (ABD and catalytic domains) ([47], Fig. 1 and Table 1).

This KRS localizes at the plasma membrane, through the flippase-like domain, and has been shown to be a *bona fide* KPGS [47,49]. Interestingly, the "classical" MprF part of the protein cannot scavenge K-tRNA^K synthesized by the second, canonical KRS, when deprived of the KRS appended domain, in contrast to other "classical" aaPGS. The KRS domain thus specifically synthesizes K-tRNA^K for use in membrane modification. The LysX protein is, like other aaPGSs, involved in membrane remodeling, CAMPs resistance and virulence [47].

In cyanobacteria, specialized membrane structures separated from the plasma membrane, called thylakoids, are involved in oxygenic photosynthesis, in particular in the assembly and functioning of photosystems. Proteins and protein complexes can be targeted within the thylakoid lumen, or at the surface of the organelle-like compartment, and perform various functions related to oxygenic photosynthesis. For example, F_1F_0 ATP synthase is assembled and functions at the surface of thylakoids [50,51]. Recent work highlighted that among 279 sequenced cyanobacteria, 102 contain one or several aaRSs that are anchored to the outer leaflet of thylakoid membranes [52]. Thylakoid-specific targeting of aaRSs depends on the presence of a CAAD (for Cyanobacterial Aminoacyl-tRNA synthetases Appended Domain) domain that has been detected in 7 different aaRSs (ERS, VRS, LRS, IRS, CRS, MRS, RRS) at various positions within the protein sequences. CAAD is found at the very C-terminus of ERS, but in a C-terminal internal region in other aaRSs [52]. In all cases, no other paralogs of CAAD-containing aaRSs is found in proteomes, which suggested that they are all active in spite of unusual membrane localization [52–54]. Phylogenetic studies suggest that only cyanobacteria contain aaRSs with a CAAD domain, and that insertion of this module in aaRSs corresponds to several independent evolutionary events [52,53]. The CAAD domain shares homology with the CURT1/TMP14/PSI-P-family proteins, found in chloroplasts of plants. They are all membrane proteins involved in the organization of thylakoids and grana in chloroplasts, by influencing curvature of lipid bilayers [53]. AaRS-CAAD domains have not been shown to be involved in modulating thylakoid curvature and/or ultrastructure, but have been experimentally demonstrated to anchor aaRSs in thylakoid membranes [52,54]. It is to be noted that VRS-CAAD, that is thylakoid-bound, relocates at cell poles of mature heterocysts (specialized and differentiated cyanobacterial cells) upon nitrogen starvation [52]. A combination of *in vivo* and *in vitro* experiments showed that VRS-CAAD is active and found only in membrane fractions of cyanobacteria, while

Table 1
Non-canonical functions and relocalizations of aaRS: AaRSs that exert nontranslational functions are grouped as a function of the subcellular compartment to which they relocate: (C, cytoplasm; N, nucleus; M, mitochondria; C. mb, cytoplasmic membrane; L, lysosome; V, vacuole), or when they are extracellularly secreted (HIV, human immunodeficiency virus; RSV, Rous sarcoma virus; Exo, exosomes; EC, extracellular (secreted); S, (host) serum). Organisms or groups of organisms in which the aaRS relocalizations where reported in this review are abbreviated as follows: Mam, mammalian; Vert, vertebrates; High. Euk, higher eukaryotes; *Hsa*, *Homo sapiens*; *Sce*, *Saccharomyces cerevisiae*; Zf, zebrafish; *Bma*, *Brugia malayi*; *Mtu*, *Mycobacterium tuberculosis*. The schematized structural organization of each aaRS was designed based on pfam 31.0 (http://pfam.xfam.org/), NCBI blastp, recent reviews [29,48] and the cited references (domains are not at scale). Orange and blue boxes correspond to anticodon/tRNA binding domains and catalytic core respectively, gray boxes correspond to additional domains and green boxes to WHEP domains. Red marks indicate the position of the residue that is post-translationally modified and/or domains involved in relocalization. p, phosphorylation; ac, acetylation; n.d., not determined; Nt ext., N-terminal extension; Ct, C-terminal; Transmb, transmembrane domain; cat, Catalytic; tRNA bd, tRNA binding domain.

Comp.	Organism	aaRS	Structural organisation	(Re)loc. mec	domain(s) involved	Physiological funtion	References
C.	*Hsa*	EPRS		pS886/pS999	WHEP	Translation regulation (GAIT element)	[32], [35], [83-87]
	Hsa, Mmu	EPRS		pS990	WHEP	antiviral immunity	[88]
	Hsa	QRS		MARS released	Catalytic	Anti-apoptotic	[89-92]
	Mam	RRS		Alternative AUG	Nt. Zn finger	Protein degradation	[93-98]
N.	*Hsa*	KRS		pS207	tRNA bd.	Transcription activation (MITF)	[103-105]
	Mam. Zf	YRS		acK244	NLS tRNA bd.	DNA damage response	[109-112]
	Vert.	WRS		NLS	WHEP	p53 signaling	[113-115]
	Vert.	SRS		NLS	UNE-S	Anti-angiogenic	[28], [116], [120], [121]
	Vert.	TRS		n.d.	not UNE-T	Anti-angiogenic	[122]
	Hsa	MRS		NLS	Nt ext. (GST)	rRNA transcription	[123]
	Sce	MRS		NLS	GST-like	*ATP1* transcription regulation	[36]
	Sce	DRS		NLS (Nt)	Nt ext.	DRS-mRNA translational regulation	[116], [125]
M.	*Sce*	ERS		MTS	GST-like	ATP9 translation regulation	[36]
C. mb.	*Hsa*	KRS		pT52	ABD + ext.(1-72)	Promotes Cell-migration	[126-128]
	Hsa, Mmu	EPRS		pS999	WHEP	Adiposity and lifespan regulation	[32]
	Mtu	KRS		Transmb.	Flippase, Duf2156	Virulence and cAMP resistance	[45-47]
L.	Mam.	LRS		VPS34 (cat. site)	catalytic, UNE-L	Lysosomal mTORC1 activation	[136], [137]
V.	*Sce*	LRS		Gtr1	editing domain	Vacuolar mTORC1 activation	[139]
HIV	*Hsa*	KRS		pS207 / Gag	Helix H7	Primer $tRNA^{K}$ packaging	[140-144],[145]
RSV	*Hsa*	WRS		n.d.	n.d.	Primer $tRNA^{W}$ packaging	[146]
E.	*Hsa*	KRS		Syntenin	cleavage site, PDZ d.	TNFa and cell migration in macrophages	[147], [148]
EC.	High. Euk.	YRS		n.d.	Mini-YRS	Pro-angiogenic (IL8 like)	[151], [152], [154-156]
	High. Euk.	YRS		n.d.	EMAPII like Ct d.	Pro-inflammatory cytokine	[148], [153], [163]
	High. Euk.	WRS		n.d.	Mini-WRS	Angiostatic	[153], [154] [158-162]
	Hsa	WRS		n.d.	WHEP	Early immune response	[164]
	Hsa	TRS		n.d.	n.d.	Endothelial cell migration	[165], [166]
S.	*Hsa*	GRS		n.d.	n.d.	Defense against tumor formation (ERK)	[173], [174]
S.	*Bma*	NRS		secreted	UNE-N	Host Pro Inflammatory	[167-172]

deletion of the CAAD domain renders the protein soluble without impacting its kinetic activity. Importantly, a series of pull-down experiments coupled with LC-MS/MS characterizations conducted in *Anabaena* sp. PCC 7120 strongly support that VRS-CAAD directly interacts with the F_1F_0-ATP synthase in thylakoid membranes in a CAAD-dependent manner. Interaction between VRS-CAAD and F_1F_0-ATP synthase does not influence aminoacylation activity, and no impact on the overall ATP production, used as a proxy of ATP synthase activity, could be evidenced. However, authors propose several hypotheses relating CAAD-containing aaRSs and nutrient sensing (in particular nitrogen through aa sensing), or possible moonlighting functions that have yet still to be experimentally addressed [52].

3. Eukaryotic relocating aaRSs with conventional functions

The intracellular space of eukaryotic cells has a complex organization and displays more than one translationally active compartment. It is therefore questionable to ask if there is a specific aaRS for each compartment or if a single polypeptide chain can be found at multiple localizations within a cell. In this section, we examine how each translationally-active compartment is supplied with a complete set of aa-tRNA synthesizing activities and how cytosolic aaRS are distributed throughout the cell. The latter are generally considered as soluble enzymes but it is known for decades that they may be associated with ribosomes, organelles or lipids ([55] and references therein) and nowadays a growing number of publications lead to the conclusion that cytosolic aaRSs exert their canonical functions at multiple locations within the cell.

3.1. Nuclear translocation of aaRS and tRNA nuclear export

Eukaryotic precursors tRNAs are transcribed in the nucleus and undergo several maturation steps before being fully functional for aminoacylation in the form of L-shaped and fully modified tRNAs. Those include 5′ and 3′ processing, CCA addition and post-transcriptional base modifications [56]. A majority of maturation steps take place within the nucleus. Classically, all tRNAs are then transported through nuclear pores to reach the cytosol where they are aminoacylated by cognate cytosolic aaRSs, and participate in ribosomal protein synthesis [57,58]. Experiments conducted in the mid-eighties surprisingly showed that a fraction of the Phenylalanyl-tRNA-synthetase pool (FRS) relocalizes into the nucleus, although its function inside the

organelle was not clearly understood [59]. The presence of an aaRS in the nucleus, where tRNAs are synthesized and processed led to the attractive hypothesis that aminoacylation of nuclear tRNAs by aaRSs could be a quality control step to assess their structural and functional integrity prior to export [60,61]. In correlation to that, aminoacylation has been experimentally demonstrated for $tRNA^{Y}$ and initiator $tRNA^{M}$ ($tRNA_i^{M}$) in *X. laevis* oocytes [60]. However, for a putative quality control mechanism involving aminoacylation to occur in the nucleus for all tRNAs, the corresponding aaRSs should be all able to enter the nucleus as well. This hypothesis was reinforced by the detection of nuclear localization sequences (NLS) in most of these enzymes [18,62]. Incidentally, results obtained in the yeast *S. cerevisiae* showed nuclear tRNA aminoacylation activity for MRS, IRS and YRS [61,63]; and more recently 19 tRNA species have been detected in the form of aa-tRNAs in the nucleus [64]. Most of aa-tRNA are exported [65] to fuel cytosolic protein synthesis, but it has been demonstrated that 30% are retained inside the nucleus, where they might be involved in protein modification, especially in the case of R-$tRNA^{R}$, that participates in the N-end rule degradation pathway [66]. Several authors also suggested that nuclear pools of cytosolic aaRSs might assemble into molecular complexes [67,68]. Finally, nuclear relocalization of many cytosolic aaRSs seems to be a frequent feature across organisms and translocation of cytosolic aaRSs through the nuclear pores is accomplished by the classical import machinery (NLS/importins), although various other strategies to reach the nucleus [69]. Finally, their canonical function is only one of the many roles they exhibit inside this compartment.

3.2. Addressing cytosolic aaRS to mitochondria through MTS acquisition

In all organisms studied so far, no gene encoding mitochondrial aaRSs was found in any of the mitochondrial genomic DNAs. All mitochondrial aaRSs are nuclear-encoded [70,71], aside the 20 cytosolic aaRS genes. Importantly, the full set of the 20 mitochondrial aaRS genes has never been detected in any nuclear genomes so far [15,72,73]. Indeed, some aaRSs genes originating from mitochondria (or plastids in photosynthetic organisms and apicomplexans) have been lost during evolution. Consequently, mitochondrial aminoacylation activities have to be fulfilled by other aaRS, namely the cytosolic aaRSs. In this case, mitochondrial targeting sequences (MTS) are found at the N-terminus of the mitochondrial-targeted cytosolic aaRSs (Fig. 2). Addition of MTS can be achieved through different mechanisms such as alternative splicing of the aaRS transcript (human KRS) [74] or from an alternative translation start (AUG) site within the same transcript (human GRS) [75]. Moreover, both forms can be translated from mRNA transcripts that differ only in the 5′ regions. For example, the yeast *HTS1* gene, coding for the sole HRS, has two in-frame translational starts separated by an alternative transcriptional start leading, upon translation, to two isoforms of HRS, a longer (mitochondrial) and a shorter (cytosolic) one [76]. A similar strategy has been evidenced for yeast VRS [77]. Alternatively, experiments showed that the gene encoding the two isoforms of yeast ARS is translated from a single mRNA, where the mitochondrial isoform is initiated from an upstream atypical ACG start codon, and the cytoplasmic isoform from a classical AUG start codon [78]. This dual-targeting mechanism, that involves synthesis of two protein isoforms from a single mRNA seems to be the rule in multi organellar organisms like *A. thaliana* [3,79] or apicomplexan parasites [80].

3.3. Mitochondrial-cytosolic dual-localization of a single aaRS

Apart from synthesizing two protein isoforms (one with and one without an MTS) from expression of a single gene, another strategy to generate a cytosolic/mitochondrial dual-localized protein is to produce a mitochondrial protein (harboring an MTS) and to retain it within the cytosol through interaction with an anchoring protein. For example, the yeast cytosolic ERS is imported into the mitochondria even if it does not harbor an identifiable targeting signal [6]. Experiments demonstrated that mitochondrial import of cytosolic ERS compensates the absence of mitochondrial QRS. Indeed, mitochondrial Q-$tRNA^{Q}$ cannot be synthesized directly, but *via* the indirect transamidation route. In this pathway, aminoacylation of mt-$tRNA^{Q}$ is specifically performed by the mitochondria-localized cytosolic ERS, which produces a physiologically misacylated E-$_{mt}tRNA^{Q}$. This intermediate is further converted to

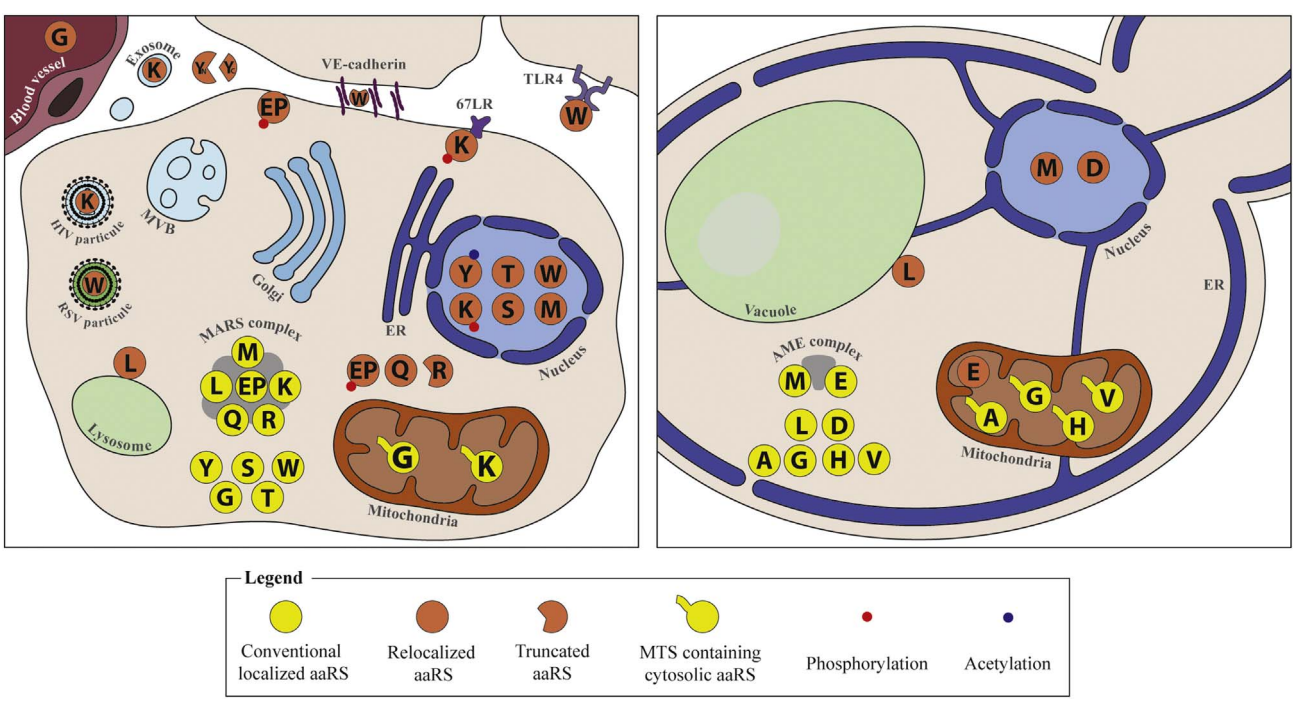

Fig. 2. Multiple subcellular localizations of mammalian (left) and yeast cytosolic aaRSs (right). Only mammalian and *S. cerevisiae* cytosolic aaRSs that relocate from the cytosol to a different compartment or that can be released from the MARS MSC and stay in the cytosol are shown. Only aaRSs that relocate to the nucleus and which are exerting non-conventional functions are represented on the figure.

Q-$_{mt}$tRNA^{Q} by the tRNA-dependent GatFAB amidotransferase in yeast [6]. In *A. thaliana* and mammals [16,17], a *bona fide* mitochondrial ERS, called non-specific, produces *E*-$_{mt}$tRNA^{Q} in addition to the cognate E-$_{mt}$tRNA^{E}, and the misacylated intermediate is converted to Q-$_{mt}$tRNA^{Q} by the mitochondrial GatCAB amidotransferase. Mitochondrial translocation of cytosolic ERS is triggered by a non-canonical MTS located after the first 190 residues [6]. Yeast cytosolic ERS is essential in both the cytosol and mitochondria, and regulation of its localization has been proven to be crucial during diauxic shift (transition from fermentation to respiration). Both yeast ERS and MRS are associated to the cytosolic anchoring protein Arc1p [22], preventing nuclear accumulation of the two aaRSs [81]. Upon diauxic shift, levels of soluble Arc1p decrease significantly, which triggers simultaneous release of both MRS and ERS [36]. While cytosolic MRS relocates into the nucleus (see Section 4.2.7), cytosolic ERS translocates into mitochondria, where it generates Q-tRNA^{Q} and controls the overall rate of mitochondrial protein synthesis.

4. Eukaryotic relocating cytosolic aaRSs with nonconventional functions

As mentioned in the introduction, the primary function of an aaRS is to aminoacylate its cognate isoacceptor tRNAs in the translationally active compartment where the aaRS is supposed to be localized. In eukaryotes, where several organelles and compartments are found, defining the exact subcellular localization of a given aaRS is not an easy task, since some aaRSs are free-standing enzymes while other are found in an MSC (Multi-"Synthetase" Complex), *i.e.* the human MARS-complex or the yeast AME complex. Furthermore, MSC-associated aaRSs can be released from the complex under specific physiological or stress conditions, and/or upon posttranslational modification. One of the remarkable feature of aaRSs is that they are modular enzymes. Some of them inherited additional domains, that are sometimes only be found in aaRSs, upon various evolutionary events, [29]. Some of those additional domains are necessary to ensure the specificity of the tRNA aminoacylation ("conventional") reaction (*i.e.* Connective Peptide of the editing domains), whereas other domains are involved in "non-canonical" (and sometimes non-translational) functions that these enzymes can carry out. Interestingly, some additional domains are required for both conventional and non-canonical functions in a single aaRS.

In this context, the additional domain can harbor the non-canonical function by itself or can be the target of a post-translational modification, required for aaRS relocalization. Indeed, to fulfil their non-canonical function(s), aaRSs often have to relocate from the cytoplasm (that is, their "canonical" localization) towards a new compartment. Alternatively, an MSC-associated aaRS can stay within the cytosol but be released from the MSC. In this section, we sorted the cytosolic aaRSs as a function of the subcellular compartment in which they exert their non-canonical functions. All the aaRS relocalizations reported in this section are summarized in Table 1 and Fig. 2.

4.1. aaRSs released from the MSC but staying in the cytoplasm

4.1.1. Human glutamyl-prolyl-tRNA-synthetase (EPRS) in response to IFNγ stimulation

In higher eukaryotes and notably human, ERS is fused to PRS by a non-catalytic linker that contains 3 so-called WHEP repeats. The phylogenetic origins of these WHEP domains (present in tryptophanyl-(W) histidinyl-(H) glycyl-(G) and glutamyl-prolyl-tRNA synthetase (EP) where explored by Ray et al. [82] who focused their work on this unique case of fused bi-functional aaRSs called EPRS. In the human MARS complex these WHEP repeats interact with AIMP3 and lysyl-tRNA-synthetase (KRS). However, EPRS can be released from the MARS complex upon sequential bi-phosphorylation of serine residues within the WHEP domain. For instance, in myeloid cells Serine 886 (S886) is phosphorylated by the Cyclin-dependent kinase Cdk5/p35 kinase [83] and Serine 999 (S999) is phosphorylated by the mammalian Target Of Rapamycin 1 (mTORC1) activated p70 ribosomal protein S6 kinase 1 (S6K1) [32]. These phosphorylations occur upon interferon gamma (IFNγ) stimulation and trigger the release of EPRS. The phosphorylated WHEP repeats then sequentially interact with NSAP1, GAPDH, phosphorylated ribosomal L13a and the Gamma Interferon Inhibitor of Translation (GAIT) elements that can be found in the 3′-end of some mRNAs leading to the assembly of the so-called GAIT complex. This cytoplasmic GAIT complex then interacts with the translation initiation factor eIF4G, which inhibits translation of the mRNAs targeted by the GAIT complex [35,83–86].

Because, this translation inhibition was shown to be suppressed 14–16 h after IFNγ stimulation, Yao and coworkers [87] investigated the mechanism underlying the recovery of the translation of the mRNAs targeted by the GAIT complex. They discovered an alternative route which allows mRNAs that display GAIT-elements to be basally translated even after GAIT-complex assembly. This mechanism involves an EPRS isoform called EPRS^{N1} which is translated from a truncated EPRS mRNA. This shorter EPRS mRNA is generated through an alternative polyadenylation pathway that changes an UAU (encoding tyrosine 864) to a stop codon (UAA). This EPRS^{N1} isoform is composed of the ERS core followed by approximatively two WHEP domains. Consequently, the region of the WHEP domain that can be biphosphorylated is missing in EPRS^{N1}, preventing association of this EPRS variant to the GAIT complex. However, EPRS^{N1} being still able to bind to the mRNA GAIT-elements, it competes with the GAIT-complex for binding to these mRNAs, leading to a basal expression of pro-inflammatory mRNA.

4.1.2. Human glutamyl-prolyl tRNA-synthetase (EPRS) in response to viral infection

The mitochondrial antiviral signaling protein (MAVS) is considered as a central regulator of responses to viral infections through its capacity to block virus replication. MAVS is inhibited by poly(rC)-binding protein 2 (PCBP2)-dependent ubiquitination and is activated through viral RNA genome sensors such as RIG-I and MDA5. Lee and coworkers [88] showed that following viral infection, EPRS is phosphorylated at S990 which triggers its release from the MSC and promotes its interaction with PCBP2. This interaction prevents MAVS ubiquitination and sustains in this way antiviral immunity. Thus, EPRS is also an important factor of antiviral immunity.

4.1.3. Human glutaminyl-tRNA-synthetase (QRS)

The human QRS, which participates to the MARS MSC by binding to AIMP1 and arginyl-tRNA-synthetase (RRS) has been shown to possess an anti-apoptotic action. Apoptosis can be triggered by different factors (*i.e.* Tumor Necrosis Factor (TNF), Fasl, Reactive Oxygen Species (ROS)) and different pathways (*i.e.* c-Jun N-terminal kinase (JNK) and p38), and an essential factor for apoptosis is the Apoptosis Signal-regulating Kinase 1 (ASK1). To prevent apoptosis and to enhance cell survival, the QRS catalytic domain interacts directly with the ASK1 catalytic domain, in a glutamine-dependent manner. The QRS unique N-terminal domain (UNE-Q) does not seem to be involved in this aaRS-dependent anti-apoptotic pathway. Furthermore, because QRS can be released from the MARS MSC [89], Ko et al. [90] proposed that there is an equilibrium between the interaction of QRS with the MARS complex and with ASK1. On the opposite, Hwang et al. [91] showed that the translocation of ASK1 into the nucleus through its interaction with the Apoptosis-Linked Gene 2 (ALG2), also prevents apoptosis. However, no functional link between the QRS- and ALG2-dependent anti-apoptotic pathways was further revealed so far. Finally, Shao et al. [92] demonstrated that the microRNA miR-US4-1 encoded by the Human Cytomegalovirus (CMV) genome targets human QRS and induces cell apoptosis.

4.1.4. Mammalian RRS

During evolution, mammalian RRSs have inherited two leucine-

zippers in their N-terminal extension, and like for other aaRSs, these leucine-zippers were shown to be involved in binding with AIMPs or other aaRSs in order to participate to the MARS complex. However, beside the full-length RRS, an alternative AUG translation start allows synthesis of a 74-residues shorter RRS isoform, in which the leucine zippers are missing [93–95]. Consequently, this isoform cannot bind to the MSC and is found in free form in the cytoplasm [96–98]. Both RRS isoforms present similar aminoacylation activities [99] but it was suggested that R-tRNA^{R}s produced by the shorter isoform are rerouted from protein synthesis to provide activated arginyl moiety for the Arg-dependent protein degradation pathway ("N-end rule") [100–102].

4.2. Cytosolic aaRSs translocating to the nucleus

4.2.1. Human lysyl-tRNA-synthetase KRS (Mast Cells)

Many non-canonical functions were discovered for cytosolic KRS this past decade. For example, mast cells are known to be implicated in allergenic and anaphylaxis responses and also in some immune disorders. Through allergen recognition by the immunoglobulin E high affinity receptor, IgE-FcεRI, activated mast cells accumulate diadenosine tetraphosphate (Ap4A) also called "alarmone" or "second messenger". This second messenger binds to Histidine triad nucleotide-binding protein 1 (Hint1) which in turn releases the Microphthalmia-associated Transcription Factor (MITF) leading to its activation and the subsequent upregulation of the transcription of subset of genes (*i.e.* tryptophan hydroxylase and mast-cell protease 5) [103–106]. Recent structural investigations revealed that the pathway that activates MITF involves the phosphorylation of KRS at S207 (located at the ABD) by the MAPK/ERK kinase which is itself activated after allergen stimulation. The subsequent KRS conformational change induces *i*) its dissociation from the MARS complex (AIMP2) *ii*) inhibition of its aminoacylation capacity and *iii*) its translocation into the nucleus. Inside the nucleus, the exposed C-terminal domain of KRS interacts with the MITF which in turn dissociates from Hint1. Furthermore, KRS synthetizes Ap4A (KRS is responsible for the synthesis of about 70–80% of cellular Ap4A) which inhibits Hint1, leading to MITF-KRS-dependent upregulation of gene transcription under allergenic stimulation [107,108]. Interestingly, a very recent study reported that HIV1 infection in human cells induces similar LysRS translocation into the nucleus (see below Section 4.5.1).

4.2.2. Zebrafish and mammalian tyrosyl-tRNA-synthetase (YRS)

Such an aaRS-dependent transcription-regulation was also shown for mammalian and zebrafish YRS. Angiogenin, nicotinamide and oxidative stress were shown to trigger YRS translocation into the nucleus. This relocalization occurs trough a hexapeptide NLS which is located into a tRNA binding domain which is not the Anticodon Binding Domaine (ABD) of YRS [109]. Under stress conditions, resveratrol fits into the YRS active site by mimicking tyrosine and triggers *i*) inhibition of YRS's capacity to aminoacylate tRNA^{Y} (YRS null mutant like) [110] and *ii*) an increase in acetylation of YRS's lysine 244 (K244) by the p300/CBP-associated factor (PCAF acetylase *vs*. SIRT1 deacetylase) [111]. This acetylation induces a conformational change that exposes YRS's NLS and triggers its nuclear translocation. Interestingly, Fu et al., [109] showed that binding of tRNA^{Y} prevents nuclear relocation of YRS, which is in agreement with the fact that early acetylation occurs into the YRS tRNA-binding domain (K244). Following nuclear import, YRS binds to TRIM28 (which notably represses the transcription factor E2F1) and to its associated Histone Deacetylase 1 (HDAC1) [112]. Trough acetylation, E2F1 is activated and improves expression of genes involved in DNA damage repair (*i.e.* Breast Cancer 1 BRCA1 and the recombinase RAD51). Furthermore, two studies showed that YRS is able to stimulate NAD^{+}-dependent PARP1 activity which is well known for its participation to the DNA damage response [110,112]. Taken altogether, nuclear YRS seems to be an important factor to prevent DNA damages.

4.2.3. Vertebrate tryptophanyl-tRNA-synthetase (WRS)

Liu and coworkers [113] showed, in 2004, the presence of 5 WRS mRNA splice variants in human cells which are translated into 2 different WRS isoforms: the full length (residues 1 to 471) and the mini-WRS (residues 48–471). Both, WRS and mini-WRS can be further processed by extracellular elastases to produce T1-WRS (residues 71–471) and T2-WRS (residues 94–471). For instance, the full-length WRS (component of the MARS complex) harbors an additional N-terminal WHEP domain which is either in a "locked" conformation (presence of Trp∼AMP into the active site) or "open" conformation (absence of W∼AMP into the active site). Furthermore, WRS is a class 1 aaRS that homodimerizes (α2) and harbors a C-terminal NLS. WRS was already shown in 1993 [114] to be imported into the nucleus and later studies [115] showed that nuclear WRS increases upon IFN-γ treatment (anti-angiogenic and anti-proliferative). Upon IFN-γ stimulation, the WHEP domain from the nuclear WRS homodimer "opens" and serves as a bridge between PARP1 and DNA-dependent protein kinase (DNA-PKc), which is essential for DNA-PKc-ADP-ribosylation by PARP1 and p53 phosphorylation (Ser15). Consequently, phosphorylated p53 triggers cell death.

4.2.4. Vertebrate seryl-tRNA-synthetase (SRS)

SRS, that belongs to class 2 aaRSs, was also shown to be imported into the nucleus [116]. This import requires a robust NLS located into a unique C-terminal domain called UNE-S [28,117] which is conserved in all vertebrate SRSs from fish to human. In human cells (HUVEC = endothelial cells, HEK293T cells), the amount of nuclear SRS was estimated to be 10% of the total SRS content [116]. In 2009, two studies [118,119] identified different SRS mutations that could lead to deletion or sequestration of the NLS (into the UNE-S domain). These mutants have deficiencies in SRS nuclear import and anomalies in angiogenesis. This phenotype was explained by an original "Yin-Yang" mechanism whereby nuclear SRS competes with c-Myc for binding to the promoter of Vascular Endothelial Growth Factor (VEGFa); c-Myc binds to the promoter of VEGFa and recruits a histone acetyltransferase which increases chromatin decondensation at VEGFa locus and its expression. Contrariwise, nuclear SRS binding to the VEGFa promoter can recruit Sirtuin 2 (SIRT2) which deacetylates histones at the VEGFa locus and represses its expression [120]. Beside this Yin-Yang mechanism that acts on the proximal *cis* regulation element (CRE) which avoids abnormal vascularization, a recent study proposed the existence of a distal CRE regulation mechanism. Notably, they demonstrated that nuclear SRS binds a transcription factor Yin-Yang1 (YY1), building a complex that represses VEGFa expression. As described for the SRS-SIRT2 complex, the recently identified SRS-YY1 complex competes with the Nuclear Factor kappa B (NFκB) which binds the same distal CRE to enhance VEGFa expression [121].

4.2.5. Vertebrates from zebrafish to human threonyl-tRNA-synthetase (TRS)

Recently Cao and coworkers [122] reported the capacity of human (HUVEC) and zebrafish TRSs to relocate to the nucleus. Based on a newly identified *taARS^{cq16}* mutant in zebrafish, they show that TRS down-regulates VEGFa expression to prevent abnormal vascular development. Interestingly, this regulatory function resembles that of the human SRS described in the previous section. The fact that *taARS^{cq16}* mutant phenotype could not be abolished by SRS mRNA injection suggests that TRS-mediated regulation of angiogenesis proceeds through a different mechanism. However, there were able to swap the Zebrafish TRS by its human ortholog, indicating that this new non-canonical regulatory function is conserved in human.

4.2.6. Human methionyl-tRNA synthetase (MRS)

MRS is another MSC-participating aaRS capable of relocating to the nucleus and more precisely to the nucleolus where ribosomal RNA (rRNA) are transcribed. Compared to prokaryotic MRSs, human MRS

possesses an N-(267 amino-acids) and a C-terminal extension, in the latter of which a NLS was found. Ko and coworkers [123] showed that nucleolar translocation of human MRS requires rRNA and RNA polymerase I activity and that this relocation upregulates rRNA biogenesis when cells are proliferating upon treatment with growth factors like insulin, Platelet-Derived Growth Factor (PDGF) or Epidermal Growth Factor (EGF). Finally, the mechanism that triggers release of MRS from the MARS complex remains unclear but Ko and coworkers anticipated that phosphorylation of the MRS N-terminal extension through casein kinase II could be the event initiating nucleolar relocation of MRS. This hypothesis is supported by the fact that this kinase is involved in cell growth and rRNA biogenesis. However, to our knowledge the exact pathway and mechanism of MRS nucleolar relocation still needs to be verified experimentally.

4.2.7. S. cerevisiae MRS

As mentioned above, the yeast AME composed of Arc1 ERS and MRS has been shown to constitute a cytosolic retention platform for the two participating aaRSs. In fermenting yeast cells (high glucose concentration), the majority of MRS and ERS are found bound onto Arc1 that enhances their cognate tRNA aminoacylation efficiency [22]. However, upon switch to the respiratory metabolism (low glucose concentration), *ARC1* transcription is downregulated by the Snf1/4 kinase pathway (glucose-sensing pathway), which leads to synchronous release of MRS and ERS from Arc1. Released ERS then relocates to the mitochondria and indirectly controls accumulation of the Atp9 subunit of the F_O domain of the mitochondrial ATP synthase (ATPase). Simultaneously, dissociation of MRS from the AME complex unmasks a bipartite NLS located in the MRS N-terminus and its subsequent nuclear translocation. Nuclear MRS then regulates transcription of Atp1 subunit of the F_1 domain of ATPase. Taken together the AME complex dissociation allows a synchronous synthesis and assembly of a functional ATPase and thus mitochondrial ATP synthesis [36].

4.2.8. S. cerevisiae aspartyl-tRNA-synthetase (DRS)

It was first suggested that, *in vivo*, DRS could interact with its own mRNA ($mRNA^{DRS}$) through the 70-amino-acid N-terminal extension that contains a lysine-rich RNA binding motif [124]. A second study by Frugier and coworkers [125] confirmed the nuclear translocation of DRS through a NLS that was shown to be located in this N-terminal extension. They proposed a mechanism by which the superfluous amount of DRS that is produced by yeast cells, enters into the nucleus and bind its own mRNA thereby inhibiting its expression and stopping further accumulation of the enzyme.

4.3. Cytoplasmic aaRSs that are targeted to the cytoplasmic membrane

4.3.1. Human lysyl-tRNA synthetase (KRS)

In most human organs, laminin is found as an extracellular matrix support for surrounding cells, which interact with laminin through membranous integrins. However, in cancer cells, laminin bound to integrin induces p38-MAPK (Mitogen Activated Protein Kinase) activation which in turn phosphorylates the threonine 52 (T52) located in the N-terminal extension of KRS. T52 phosphorylation induces a conformational change of this part of KRS that triggers its release from the MARS complex and allows its interaction with the membranous 67LR laminin receptor. This interaction occurs between the KRS N-terminal domain composed of the ABD and N-terminal extension (1–72) and the C-terminal domain of the 67LR. By doing so, KRS inhibits Nedd4-mediated ubiquitination of 67LR, enhancing laminin-dependent cell migration and cell dissemination which is considered as a marker of metastasis [126]. Kim and coworkers [127] also identified a compound (BC-KYH16899) that inhibits the KRS-dependent 67LR-mediated cell migration without affecting the aminoacylation capacity of KRS. This BC-KYH16899 compound is thus a promising molecule that could selectively inhibit cancer cells dissemination without affecting the proteinogenic role of KRS. Finally, Nam and coworkers [128] investigated the molecular steps occurring downstream this KRS – 67LR interaction and showed that KRS-dependent cell-dissemination is done through cell-cell and cell-extracellular matrix interactions.

4.3.2. Mouse and human EPRS

In higher eukaryotes, ERS is fused to PRS by a non-catalytical linker that contains 3 WHEP repeats. This bifunctional EPRS was well characterized notably in human cells in which it participates to the MARS complex by interacting with AIMP2, KRS and DRS [30,129,130]. We already described in a preceding section the non-canonical function that EPRS exhibits in myeloid cells. However, Arif et al. [32] recently reported another relocation for MARS-released EPRS and a new non-translational function of this aaRS. In contrast to myeloid cells in which EPRS WHEP repeats are phosphorylated at S886 by Cdk5/p35 kinase, and at S999 by S6 kinase 1 (S6K1), in adipocytes EPRS was only shown to be phosphorylated at S999 making unclear whether S886 phosphorylation is also required in this cell type. Furthermore, contrary to mast cells, in adipocytes, the mTORC1-dependent S6K1 kinase that belongs to AGC kinases, is activated under insulin stimulation (mTORC1: mammalian Target Of Rapamycin Complex 1). Phosphorylated EPRS (at S999) interacts then with the cytoplasmic Fatty Acid Transport Protein 1 (FATP1). Formation of the EPRS-FATP1 complex is followed by its relocation to plasma membrane where it induces Long Chain Fatty Acid (LCFA) uptake. Thus, in adipocytes (but not in myeloid cells), and under insulin stimulation (but not under IFNγ stimulation), EPRS regulates adiposity and lifespan in a S6K1-dependent manner.

4.4. Yeast and human cytoplasmic aaRSs that are targeted to the vacuole and the lysosome

4.4.1. Mammalian leucyl-tRNA-synthetase (LRS)

In eukaryotes, TOR complexes (TORC) were shown to be involved in many processes such as cell growth, differentiation and metabolism, but increasing evidences suggest that its regulation is far from being fully understood. Indeed, different studies showed that amino acids and especially leucine [131–133], glutamine [133–135] and arginine [131] participate to activation of mTORC1. To be activated, mTORC1 requires a combination of Rag GTPases (component of the RAGULATOR complex) that are themselves regulated by a set of Guanine nucleotide Exchange Factors (GEFs) and GTPase-Activating Proteins (GAPs). It was suggested that LRS serves as a GAP for one of these Rag GTPases [136,137]. Beside these combinations of Rag-GTPases, mTORC1 activation at the lysosomes requires phosphatidic acid which is obtained by phospholipase D1-dependent (PLD1) hydrolysis of phosphatidylcholine. PLD1 recruitment to the lysosome is done by direct interaction with phosphatidylinositol-3P (PI3P) which is synthetized at the lysosome through the vacuolar protein sorting 34 (VPS34), a class 3 PI3-kinase which phosphorylates phosphatidylinositol and which is known to be involved in autophagy [138]. Yoon and coworkers [137] demonstrated that LRS interacts directly *via* its catalytic domain with VPS34 and activates it *via* its unique C-terminal extension called UNE-L. Furthermore, leucine binding but not tRNA binging nor tRNA aminoacylation is required for mTORC1 activation. Finally, Yoon and coworkers performed immunoprecipitation assays which suggest that this non canonical role of LRS requires the release of the enzyme from the MARS complex and its relocation at the lysosomal surface.

4.4.2. S. cerevisiae leucyl-tRNA synthetase (LRS)

In the Yeast *S. cerevisiae* two TOR complexes (TORC1 and TORC2) are also found and TORC1 is regulated by GTPases. Like for mammals, LRS was shown to be involved in vacuolar TORC1 regulation but this regulation is achieved through a different mechanism than the one detailed in the previous section. In yeast, LRS interacts with one of the GTPases (Gtr1) from the EGO complex (corresponding to the

mammalian RAGULATOR) *via* its non-essential connective peptide that corresponds to the editing domain (a domain involved in non-cognate aa-tRNA hydrolysis). When a non-canonical amino-acid is charged on $tRNA^L$, a conformational change occurs that disrupts the LRS-Gtr1 interaction and inhibits vacuolar TORC1. Bonfils and coworkers [139] proposed that in *S.cerevisiae*, LRS serves as GAP for Gtr1 GTPase and thus participates to the inactivation of TORC1.

4.5. Cytoplasmic aaRS that are targeted outside the cell

Many aaRSs were reported to be targeted/secreted outside the intracellular space. In the present section we decided to describe aaRSs that are either incorporated into viruses or secreted. For secreted aaRSs, we describe those which are secreted to the intercellular space *via* the Multi Vesicular Body MVB/exosome pathway, *via* the Endoplasmic Reticulum RE/Golgi pathway or *via* a yet unidentified pathway. Finally, we mention the aaRSs that have been found in the human serum.

4.5.1. Human KRS is encapsidated into HIV virions

During HIV1 infection, a key step for viral replication is the reverse transcription (RT) of the genomic RNA. This step requires a primer which has been shown to be the human $tRNA^{Lys3}$ (tRK3). Cen and coworkers [140] showed that, in addition to tRK3, the human KRS is also encapsidated into HIV1 particles by interacting with the viral Gag precursor. Whereas other studies [141–143] brought evidences that tRK3 incorporation is regulated by KRS, Liu and coworkers [Anticodon-like binding of the HIV-1 tRNA-like element to human lysyl-tRNA synthetase] investigated the interaction of LysRS with the viral genome. Furthermore Dewan et al. [144] demonstrated that the H7 helix located in the class II motif 1of human KRS, and which is responsible for KRS dimerization, is also responsible for the interaction with HIV1 Gag protein.

As mentioned in a previous section (Section 4.2.1), a very recent study [145] investigated further the implication of KRS after HIV1 infection in human cells. This study shows that following HIV1 infection, phosphorylated KRS (S207P) is released from the MSC and translocates into the nucleus. Like in mast cells, phosphorylated KRS is deprived of its capacity to aminoacylate its cognate tRNA but retains its ability to bind the tRNA. Although nuclear LysRS function was not yet clearly established, this study strongly suggests that translocation into the nucleus of the phosphorylated LysRS-tRK3 complex *i*) occurs before the reverse transcription of HIV1 genome and *ii*) is important for tRK3 packaging and for HIV1 infectivity. Finally, in agreement with the previously described EPRS antiviral function (Section 4.1.2), Duchon and coworkers could detect a low amount of released EPRS upon HIV1 infection.

4.5.2. Human WRS is encapsidated into Rous sarcoma virus (RSV)

In the previous paragraph, we reported that KRS and the tRK3 are incorporated into HIV1 particles. Cen and coworkers [146] described a similar situation concerning the Rous sarcoma virus, except that $tRNA^W$ is used as primer for reverse transcription of the viral genomic RNA. Like for HIV1 the aaRS homologous to the tRNA species used as a primer, in this case human WRS, is also encapsidated. Interestingly, the Moloney Murine Leukemia Virus that uses $tRNA^P$ as primer for reverse transcription of its genomic RNA, does not encapsidate the cognate PRS into the viral particles. The reason why certain viruses encapsidate a given aaRS alone or together with its cognate tRNA, has so far, not been ruled out.

4.5.3. Human KRS is secreted via exosomes

Park and coworkers [147] showed that under starvation and stimulation by Tumor Necrosis Factor α (TNFα), the human KRS can be secreted from human cells and interacts with macrophages to induce their TNFα secretion and cell migration. However, the KRS secretion pathway and its detailed molecular steps remained unclear until recently. Kim and coworkers [148] showed that, during evolution human KRS inherited a unique N-terminal extension which harbors a Caspase 8-specific cleavage site, and a C-terminal PDZ-binding domain. Whereas many proteins that harbor a localization signal are secreted by the ER-Golgi pathway, the human KRS interacts *via* its PDZ-binding domain to syntenin which guides KRS from de MARS complex into exosomes and more precisely in their lumen, prior to its release from the cell. Note that this KRS-syntenin interaction had already been suggested previously by Meershaert and coworkers [149] and Baietti and coworkers [150]. Kim and coworkers also showed the importance of the N-terminal caspase 8-specific cleavage site in this KRS secretion pathway and proposed a mechanism in agreement with the published 3D structure of KRS [107]; KRS is a class II aaRS that forms homodimers in which the caspase 8 cleavage site that is located in the N-terminal extension of one of the monomers masks the syntenin interaction site located in the C-terminal domain from the second monomer, thus inhibiting exosomal KRS secretion. Under starvation conditions, Caspase 8 cleaves the KRS N-terminal domain which allows KRS–syntenin interaction and subsequent incorporation of the enzyme into exosomes.

4.5.4. From insect to vertebrates, the balance between mini-YRS and -WRS regulates angiogenesis

We already reported in the previous sections that nuclear imported WRS, SRS and TRS are important angiogenesis regulators. However, secreted forms of YRS and WRS are also key players of angiogenesis regulation. In comparison to prokaryotic YRS, insect and vertebrates YRS have inherited an C-terminal Endothelial-monocyte-activating polypeptide II (EMAPII) like domain that can be cleaved by extracellular proteases (elastases) from the remaining core of YRS called “mini-YRS” [151]. This mini-YRS has similarities with the CXC chemotactic factor interleukin 8 (IL8), in particular with the ELR motif (Glu-Leu-Arg) harbored by pro-angiogenic chemokines. The evolutionary close WRS can similarly be cleaved into a “mini-WRS” (where translation starts at M48 due to alternative splicing or extracellular cleavage inside the WHEP domain) but the cleaved product doesn't harbor the ELR motif. As such, it was shown that in endothelial cells (HUVEC) and in polymorphonuclear leukocytes (PMN), extracellular mini-YRS induces cell migration and angiogenesis [152], whereas extracellular WRS has opposite effects [153,154]. Extracellular mini-YRS is increased through TNFα secretion and in endothelial cells mini YRS induces phosphorylation of Src, Akt, ERK (Extracellular Signal Regulated Kinase), and VEGF-receptor 2, required for angiogenesis [155,156]. Because IL8 is known to act on the CXCR1/2 receptor, My-Nuong Vo et al., [157] verified whether this is also the case for mini-YRS. This study showed, that under low mini-YRS secreted concentrations, mini-YRS is monomeric and induces cell migration *via* CXCR1/2 whereas under high mini-YRS secreted concentrations, mini-YRS is dimeric and inhibits cell migration (antagonistic effect). On the other hand, mini-WRS exerts its angiostatic activity by binding the first extracellular (EC1) domain of VE-cadherin (vascular endothelial cadherin). Recent studies [158,159] showed that mini-WRS K153 residue binds VE-cadherin and that VE-cadherin residues W2 and W4 are docked into the empty W-AMP pocket. Through this interaction, *i*) VE-cadherin interaction with activated VEGF receptor 2 (VEGFR2) is disrupted and *ii*) the subsequent activation of Akt that, in the absence of mini-WRS leads to angiogenesis, is inhibited [160,161]. However, the mechanism by which VEGFR2 is activated by mini-YRS remains unclear and Zeng et al. [162] in human umbilical vein endothelial cells in hypoxia suggested a yet unknown trans-activation mechanism. Thus, mini-WRS and mini-YRS cytokine-like antagonistic effects belongs to a pathway that fine tunes cell migration and angiogenesis.

4.5.5. From insect to vertebrates, YRS EMAPII-like domain

Compared to their prokaryotic homologs, insects and vertebrates YRSs inherited a additional C-terminal EMAPII-like domain. When YRS is secreted, this domain is cleaved by extracellular elastases from the

remaining "mini-YRS". This cleavage occurs during the apoptotic state and the EMAPII-like peptide will exert 2 different functions *i*) it carries chemo-attractive capacities which allows the recruitment of phagocytes to the apoptotic site and phagocytosis in order to prevent damages for surrounding cells; and *ii*) it induces TNFα secretion by leucocytes [151,153,163]. Finally, it was suggested that in the full-length YRS, EMAPII-like blocks IL-8 proangiogenic activity by preventing it recognition by IL8 receptor.

4.5.6. Secreted human full-length WRS (but not the mini-WRS) induces early innate immune response

Human WRS contains a unique 154 aa-long N-terminal domain that includes a WHEP domain (residues 8–64). Recently Ahn and coworkers [164] discovered a new function for the full-length WRS (FL-WRS) but not for the mini-WRS that we described previously. They showed that early after infection by pathogens, FL-WRS is secreted from monocytes and interacts with macrophages to induce innate immune stimulation (phagocytosis and cytokine production). Based on the available WRS crystal structure, Ahn et al. showed that in order to activate this type of immune response, FL-WRS binds to Toll-Like-Receptor 4 (TLR4) - MD2 complexes and probably also to TLR2 *via* its N-terminal extension. Finally, binding and activation was also showed with the 154 aa-long N-terminal domain alone.

4.5.7. Secreted human TRS regulates also angiogenesis

Similar to the above-mentioned mini-YRS angiogenic function, human TRS is also secreted under TNFα or VEGF stimulation and induces endothelial cell migration. However, it has not been elucidated if TRS cleavage or posttranslational modification is necessary for this non-canonical function [165]. Furthermore, Wellman and coworkers [166] found a link between stress-induced overexpression of TRS, angiogenesis and ovarian cancer.

4.5.8. Brugia malayi asparaginyl-tRNA-synthetase (NRS)

Brugia malayi is a filarial nematode which is a human parasite responsible for filariasis that causes chronic infections and elephantiasis. *Brugia malayi* NRS (BmNRS) is secreted from this intravascular worm parasite into patient's serum [167,168] and acts as immunodominant antigen. *Bm*NRS presents a 110 aa-long N-terminal extension (compared to prokaryotic NRSs) that harbors a 80-long IL8-like domain. Different studies demonstrated that *Bm*NRS induces several physiological responses *via* binding to 2 different types of receptors: IL8 CXCR1/2 receptors and purine receptors [168–172]. Recently Jothi and coworkers added to this list: endothelial cell proliferation, migration, ring and tube formation, vasodilation and angiogenesis which are also usually induced by VEGF.

4.5.9. Mouse and human glycyl-tRNA-synthetase GRS

Concerning GRS, Park and coworkers [173] recently showed that Fas-ligand secreted from cancer cells induces GRS secretion which can then circulate in the serum. Normally, phosphatase 2 (PP2A)-dependent ERK inhibition is blocked by its interaction to the membrane protein cadherin 6 (CDH6). However, binding of GRS to CDH6 *via* its extracellular domain, releases PP2A which inhibits ERK and provokes tumor cell death. Furthermore, this study provides *in vivo* data (mouse models) on GRS-mediated antitumor effects and suggests that this aaRS, by having this cytokine-like function, is a key player in an endogenous tumor surveillance mechanism. Finally, the mechanism by which GRS is secreted remains so far unknown, but He and coworkers [174] suggested that GRS is secreted by the exosomal pathway.

5. Concluding remarks

As described herein, the functional plasticity of relocating cytosolic aaRS is really remarkable for enzymes once considered to be restricted to the production of the ribosomes' aa-tRNA substrates. However, the repertoire of nontranslational functions carried by these enzymes is probably even greater, especially in human, if one considers all the possible isoforms that that have been predicted to be produced by alternative splicing or initiation of translation [175]. The majority of these splice variants are catalytic nulls meaning that the alternative splicing event partially or entirely removed the functional parts responsible for aa-tRNA synthesis, leaving, usually, only domains that were added to the aaRS core in the course of evolution. These pieces of aaRSs have been found to have a tissue-specific expression profile and do not contribute or participate to the aminoacylation reaction. However, a strong selection pressure led to their conservation throughout evolution most probably because they exert a specific biological function that has nothing to do with protein synthesis but nevertheless equally essential. When tested in cell-based assay designed to check their involvement in cytoprotection, immunomodulation, acute inflammatory response, transcriptional regulation, regenerative responses, cell differentiation and cholesterol transport, almost 90% of these catalytically null pieces of aaRSs where shown to exert at least one specific biological activity. Given the type of physiological role they might be playing and their tissue-specific expression one can easily imagine that malfunctioning of their functions or a change in their expression will very likely be associated with diseases. Indeed, it has already been shown that malfunctioning of the nontranslational functions that cytosolic relocating aaRSs exert, triggers a wide spectrum of diseases in human [31,34]. The pathologies to which these aaRSs have been connected to, include for example *i*) neuronal syndromes like type-2 Charcot Mary Tooth [174,176–178] and Amyotrophic Lateral Sclerosis [179], *ii*) susceptibility to cancers [180–182], diabetes [183,184] or other abnormal metabolic conditions [32] and *iii*) autoimmune disorders [185–189]. The latter example is particularly interesting since auto-antibodies against 9 cytosolic aaRSs have been found in patients with autoimmune disorders among which inflammatory myopathies, interstitial lung diseases and rheumatoic arthritis [190,191]. Despite a growing number of reports describing the clinical features of these subtypes of autoimmune diseases also called the "antisynthetase syndrome" the physiological conditions and molecular mechanism by which these aaRSs become an antigen are still unknown.

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