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Review

Processivity of translation in the eukaryote cell: Role of aminoacyl-tRNA synthetases

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ABSTRACT

Several lines of evidence led to the conclusion that mammalian ribosomal protein synthesis is a highly organized biological process in vivo. A wealth of data support the concept according to which tRNA aminoacylation, formation of the ternary complex on EF1A and delivery of aminoacyl-tRNA to the ribosome is a processive mechanism where tRNA is vectorially transferred from one component to another. Polypeptide extensions, referred to as tRBDs (tRNA binding domains), are appended to mammalian and yeast aminoacyl-tRNA synthetases. The involvement of these domains in the capture of deacylated tRNA and in the sequestration of aminoacylated tRNA, suggests that cycling of tRNA in translation is mediated by the processivity of the consecutive steps. The possible origin of the tRBDs is discussed.

1. Introduction

Translation of genetic information into proteins involves a definite set of nucleic acids and proteins. A cell free translation system has been reconstituted with 46 tRNA species and 32 protein components isolated from *Escherichia coli* (initiation, elongation and release factors, aminoacyl-tRNA synthetases, ribosome and formylase) [1]. An essentially similar array of molecules is required for ribosomal protein synthesis in eubacteria, archaea and eukaryotes. The substrates of the protein-making machine, the ribosome, are aminoacylated tRNAs that should be delivered in the form of a ternary complex with elongation factor EF1A and GTP. Accordingly, the EF1A·GTP·aa-tRNA should be non-limiting and represents the major form of EF1A and tRNA in growing cells. After transfer of the amino acid to the growing polypeptide chain on the ribosome, deacylated tRNA replenishes the pool of EF1A·GTP·aa-tRNA following aminoacylation by a family of 20 enzymes, one per amino acid, the aminoacyl-tRNA synthetases. Several lines of evidence suggest that during the elongation step of translation in the cytoplasm of eukaryote cells tRNAs flow in a closed circuit referred to as the tRNA cycle (Fig. 1).

2. tRNA cycling

The concept of tRNA cycling in translation was proposed by Smith in 1975 [2] and further refined by Deutscher and co-workers [3–6]. Using a permeabilized Chinese hamster ovary cell system, they observed that exogenously added aminoacyl-tRNAs are poor substrates for protein synthesis [3], that endogenous aminoacyl-tRNAs are sequestered within the cell [4] and that there is no leakage of tRNA in the cellular fluid after dissociating from the ribosomal E-site [6]. They concluded that tRNA is never freely diffusing in the cytoplasm of mammalian cells, and should be always associated with components of the translation machinery. It was shown that cytoplasmic confinement of tRNA requires interaction with components of the translation apparatus in yeast [7], but that retrograde accumulation of tRNAs in the nucleus of *Saccharomyces cerevisiae* [8] or of rat hepatoma cells [9] is observed in response to amino acid deprivation. Thus, when the flow of the translation circuit is closed, tRNA is no more sequestered in the cytoplasm. How a tRNA is vectorially transferred from one component to the other at each step of the translational process, and is sequestered in the cytosol, is not entirely understood. Recent functional and structural data on mammalian aminoacyl-tRNA synthetases provided some clues on how this family of enzymes has evolved to ensure efficient capture and sequestration of tRNA at the aminoacylation step of the tRNA cycle in translation.

3. General RNA binding domains

As a general rule, eukaryotic aminoacyl-tRNA synthetases have polypeptide chain extensions appended to the N- or C-terminal extremity of their prokaryotic-like catalytic domains [10]. The appended domains of yeast aspartyl- [11], glutaminyl- [12] or valyl- [13] tRNA synthetases are tRNA-binding domains (tRBD) that act in *cis* of the catalytic domains to improve tRNA binding. Additional tRBD can also be provided in *trans*, as in the case of the Arc1p

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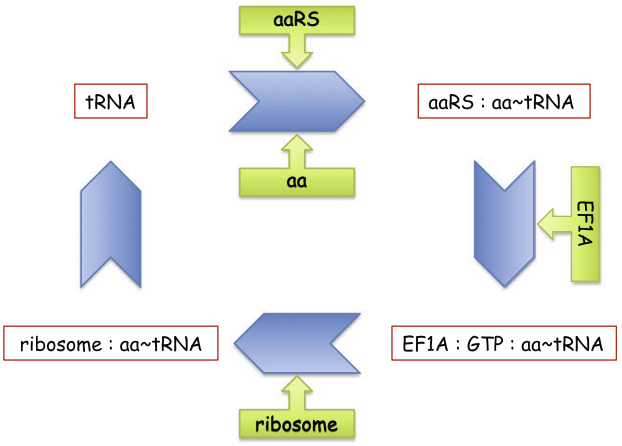

Fig. 1. The translation cycle. Along translation, tRNA is aminoacylated with a cognate amino acid (aa) by an aminoacyl-tRNA synthetase (aaRS) to form an aminoacyl-tRNA (aa ~ tRNA) which is delivered to the ribosome as a ternary complex with elongation factor EF1A (EF1A:GTP:aa ~ tRNA). After transfer of the amino acid to the growing polypeptide chain in response to codon recognition on the ribosome, tRNA is released and enters a new translation cycle.

protein that associates with glutamyl- and methionyl-tRNA synthetases in yeast [14], and of the Pex21p protein that associates with yeast seryl-tRNA synthetase [15].

Because yeast generally contains two distinct sets of aminoacyl-tRNA synthetases, a eukaryotic cytosolic and a prokaryotic mitochondrial enzyme, it was interesting to analyze the requirement for an additional tRBD in the two compartments. In the case of valyl-tRNA synthetase, the yeast *VAS1* gene encodes the two forms through alternative initiation of translation [16]. Whereas valyl-tRNA synthetase from *E. coli* complements the yeast cytoplasmic enzyme only when a tRBD is appended in *cis* of its catalytic domain, complementation of the yeast mitochondrial enzyme does not require an additional tRBD [13]. Similarly, the yeast cytosolic glutamyl-tRNA synthetase is also the source of the mitochondrial, non-discriminating enzyme when Arc1p is depleted [17]. Thus, the functioning of glutamyl-tRNA synthetase in mitochondria does not require its association with a tRBD. These results exemplify the major role played by the appended tRBD in the context of translation in a eukaryotic environment, the cytosol, and show that these domains are dispensable in the context of translation in a prokaryotic environment, the mitochondria.

Many human cytosolic aminoacyl-tRNA synthetases also possess additional tRBD appended either in *cis* or in *trans* of their catalytic domains. Methionyl-tRNA synthetase, which has been extensively studied in a large variety of organisms, from bacteria to mammals, revealed different scenario adopted along evolution to link a tRBD to the catalytic domain (Fig. 2a). The minimum enzyme is found in some bacteria, such as *Aquifex aeolicus*, or in mitochondria. The crystal structure of the minimal enzyme has been determined as a complex with $tRNA^{Met}$ (Fig. 2b) [18]. In several bacteria, an OB-fold based dimerization domain is appended at the C-terminus and improves tRNA binding [19]. In plants, such as the rice *Oryza sativa* [20], an additional pseudo dimerization domain (Fig. 2d) is appended to this dimerization domain [21] and leads to a monomeric enzyme which displays potent tRNA-binding capacities (K_d of 0.08 μM for the native enzyme and of >2 μM for the deleted enzyme) [20]. This tRBD associated in *cis* to plant methionyl-tRNA synthetase is similar to the tRBD of Arc1p which is associated in *trans* to *S. cerevisiae* methionyl-tRNA synthetase (Fig. 2a). This association is mediated by the N-terminal domains of Arc1p and methionyl-tRNA synthetase which display GST-like folds [22]. Interestingly, human methionyl-tRNA synthetase also possesses a C-terminally appended tRBD domain, but completely unrelated to the OB-fold domain of the plant enzyme [23]. The short C-terminal polypeptide extension of human methionyl-tRNA synthetase displays a helix-turn-helix fold (Fig. 2c), rich in lysine residues, also encountered appended to a variety of eukaryotic aminoacyl-tRNA synthetases with different specificities [24,25]. After deletion of this domain, the affinity for $tRNA^{Met}$ is strongly decreased (K_d of 0.1 μM for the native enzyme and of 4 μM for the deleted enzyme) [23].

Another well-studied example of tRBD appended to a human synthetase is lysyl-tRNA synthetase [26–28]. Systematic site-directed mutagenesis of positively charged amino acid residues located in the N-terminal appended tRBD of that enzyme, led to the identification of a motif KxxxK(K/R)xxK which contributes the tRNA-binding site [27]. The native enzyme has a potent tRNA-binding capacity (K_d of 0.06 μM) and a deletion of the tRBD led to a 100-fold decrease of the affinity for $tRNA^{Lys}$ [27]. Human valyl-tRNA synthetase, which displays a N-terminal tRBD containing the tRNA-binding motif identified in human lysyl-tRNA synthetase, also binds tRNA with high affinity [29].

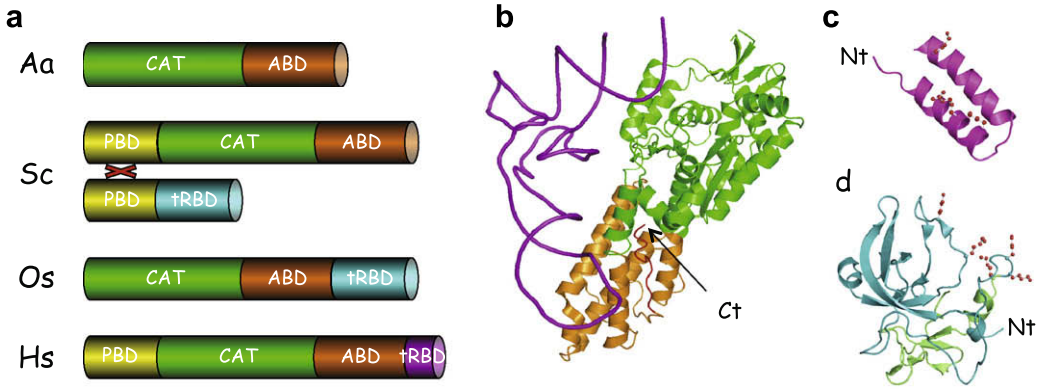

Fig. 2. RNA-binding appendices conferring processivity on methionyl-tRNA synthetase. (a) The smallest methionyl-tRNA synthetase, made of a catalytic (CAT) and anticodon-binding domain (ABD), is known in *Aquifex aeolicus* (Aa). In *Saccharomyces cerevisiae* (Sc) a N-terminal polypeptide extension contributes a protein-binding domain (PBD) that mediates association with Arc1p, a factor containing a tRNA-binding domain (tRBD). In the plant *Oryza sativa* (Os), a homologous tRBD is appended in *cis* at the C-terminus of the protein. In *Homo sapiens* (Hs), methionyl-tRNA synthetase contains a N-terminal PBD that mediates association of this enzyme within the multi-synthetase complex MARS, and a specific C-terminal tRBD. (b) The crystal structure of methionyl-tRNA synthetase from *A. aeolicus* in complex with $tRNA^{Met}$ [18]. CAT is in green, ABD in orange. (c) The solution structure of human tRBD [24]. The side-chains of the amino acid residues involved in tRNA-binding [23] are indicated. (d) The crystal structure of EMAPII [21], homologous to the tRBD of Arc1p and of the plant synthetase.