Chapter 2

On the Emergence and Evolution of the Eukaryotic Translation Apparatus

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1. Introduction

Proteins are one of the elementary components of life and account for a large fraction of mass in the biosphere. They catalyze the big majority of reactions sustaining life, and play structural, transport, and regulatory roles in all living organisms. Hence, "Translation", i.e. the process of decoding a messenger (m)RNA by the ribosome to synthesize a protein, is a fundamental process for all forms of life (*1, 2*). Accordingly, many mechanisms to control gene expression at the translational level have evolved. They allow organisms to *i*) rapidly and reversibly respond to different stresses or sudden environmental changes; *ii*) quickly produce proteins in tissues and developmental processes where transcription is absent or limited; and *iii*) elicit asymmetric localization of proteins when is required (*1-3*). For instance, gametogenesis, early embryogenesis, memory and neurogenesis are processes where translational control plays a prominent role (*4-8*).

The knowledge of the basic processes of translation was established some decades ago, and many regulatory mechanisms have been subsequently elucidated in different organisms (*9-11*). In recent years, the use of powerful genome-wide sequencing, proteomics and bioinformatics-based technologies in both model and non-model organisms, has shown that a number of components of the translation apparatus has undergone a large diversification across eukaryotes, and that many of them emerged at different times on evolution (*11, 12*). Moreover, universal and lineage-specific mechanisms regulating translation have been described, and evidence supports the notion that some of them might have emerged at different times during evolution (*11, 12*). Evidence supports the notion that some of these mechanisms might have appeared by tinkering, i.e. co-opting and assembling molecules and regulatory mechanisms from other cellular processes (*11*). Overall, the emerging view suggests two general principles. On one hand, that while the fundaments of translation are well conserved across all forms of life, in eukaryotes the initiation step has undergone

substantial increase in complexity as compared to prokaryotes (*11, 13-18*); on the other hand, that after eukaryotes emerged the translation apparatus continued evolving to certain degree during eukaryotic diversification (*12*). The continue divergence of eukaryotes led to the diversification of metabolic requirements, to the appearance of different levels of body plans and organismal complexity, and to the arousal of novel developmental programs and behavioural patterns. Altogether, these changes led to the invasion of novel ecological niches. These events most probably were both the causes and effects of a parallel diversification and specialization, to different levels in different taxa, of components and mechanisms of the translation apparatus. Here I will review current knowledge on how this apparatus might have originated and further evolved in eukaryotes, making emphasis on the initiation step of translation.

2. An overview of the translation process in eukaryotes

Eukaryotic translation is a sophisticated and tightly regulated process, the basic steps of which are conserved in all eukaryotes. It is performed by the ribosome together with multiple 'translation' factors and aminoacyl-tRNA synthetases (aaRSs). It is divided into four major groups of steps: initiation, elongation, termination and recycling (Fig. 1).

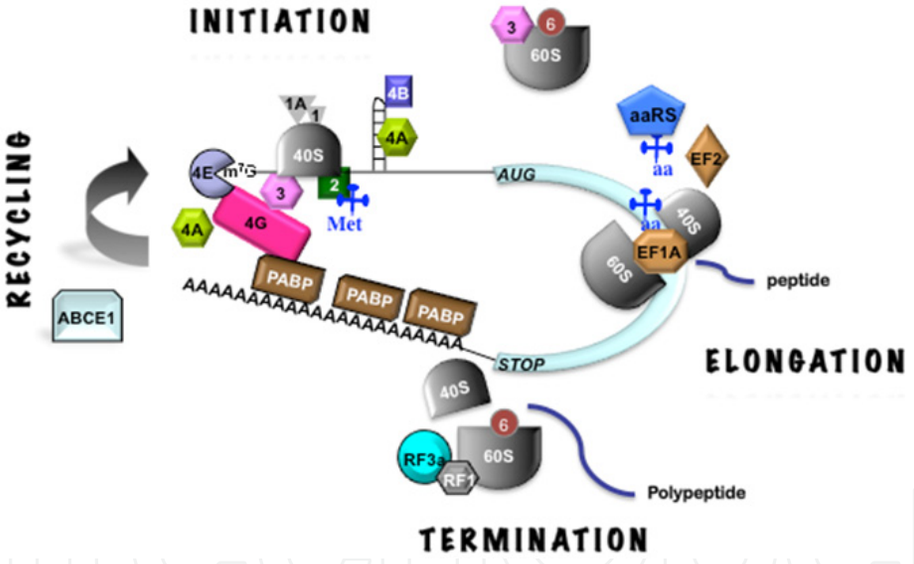

Figure 1. The general process of translation in eukaryotes. A typical eukaryotic mRNA is represented. The cap structure (m^7G), the open reading frame (light blue box) and the poly(A) tail are depicted. During *Initiation*, most eukaryotic mRNAs are translated by the cap-dependent mechanism, which requires recognition by eIF4E (light purple) complexed with eIF4G (red) and eIF4A (light green) –the so-called eIF4F complex– of the cap structure at the 5′ end. A 43S pre-initiation complex (consisting in a 40S ribosomal subunit (dark gray) loaded with eIF3 (pink), eIF1 and eIF1A (light grey), initiator Met-$tRNA_i^{Met}$ (blue clover), eIF2 (dark green) and GTP binds the eIF4F-mRNA complex and scans along the 5′-UTR of the mRNA to reach the start codon (usually an *AUG* triplet). During the scanning eIF4A, stimulated by eIF4B (dark blue), unwinds secondary RNA structure in an ATP-dependent manner. The poly A-binding protein (PABP, dark brown) binds both the poly(A) tail and eIF4G promoting mRNA circularization. Free 60S ribosomal subunit is stabilized by eIF6 and eIF3. *Elongation* is assisted by elongation factors eEF1A and eEF2 (light brown). During this step, aminoacyl-tRNA synthetases (aaRSs,

blue) catalyze the binding of amino acids (*aa*) to cognate tRNAs. *Termination* is mediated by the release factors eRF1 (gray) and eRF3 (light blue), and happens when a termination codon (*STOP*) of the mRNA is exposed in the A-site of the ribosome. In this step, the completed polypeptide (blue line) is released. During *Recycling*, which is required to allow further rounds of translation, both ribosomal subunits dissociate from the mRNA. Recycling is assisted by ABCE1 (light blue). eRF1 remains associated with the post-termination complexes after polypeptide release.

2.1. Initiation

Translation initiation is mediated by eukaryotic initiation factors (eIF). For the big majority of mRNAs, translation initiation happens by the so-called cap-dependent mechanism (*19-23*). It begins with the dissociation of the ribosome into its 60S and 40S subunits by eIF6. Free 40S subunit, which is stabilized by eIF3, eIF1 and eIF1A, binds to a ternary complex (consisting of eIF2 bound to an initiator Met-tRNA$_i^{Met}$ and GTP) to form a 43S pre-initiation complex. In separate events, the cap structure (m^{7}GpppN, where N is any nucleotide) present in the 5′ end of the mRNA is recognized by eIF4E in complex with eIF4G (forming the so-called eIF4F complex). Then the 43S pre-initiation complex is recruited to the 5′ end of the mRNA, a process that is coordinated by eIF4G through its interactions with eIF4E and the 40S ribosomal subunit-associated eIF3. eIF4G also interacts with the poly A-binding protein (PABP) which interacts with the mRNA 3′-poly(A) tail, thereby promoting circularization of the mRNA and increasing its stability. The closed-loop model proposes that during translation, cross-talk occurs between both mRNA ends due to this circularity. The ribosomal complex then scans in a 5′ —> 3′ direction along the 5′-untranslated region (UTR) of the mRNA to reach the start codon, usually an AUG. During scanning, eIF4B stimulates the activity of eIF4A, which unwinds secondary RNA structures in the mRNA. eIF1, eIF1A, and eIF5 assist in the positioning of the 40S ribosomal subunit at the correct start codon so that eIF2 can deliver the anti-codon of the initiator Met-tRNA$_i^{Met}$ as the cognate partner for the start codon, directly to the peptidyl (P)-site of the 40S ribosomal subunit. Once the ribosomal subunit is placed on the start codon, a 48S pre-initiation complex is formed. Then eIF5 promotes GTP hydrolysis by eIF2 to release the eIFs. Finally, the 60S ribosomal subunit joins the 40S subunit in a eIF5B-dependent manner to form an 80S initiation complex. The outcome of the initiation process is a 80S ribosomal complex assembled at the start codon of the mRNA containing a Met-tRNA$_i^{Met}$ in the P-site (*19-22*).

In some mRNAs, 5′-UTR recognition by the 40S ribosomal subunit is driven by RNA structures located in *cis* within the mRNA itself. Such structures are defined as Internal Ribosome Entry Site (IRES) and are located nearby the start codon. This mechanism takes place without involvement of the cap structure and eIF4E and is called an IRES-dependent initiation of translation (*13, 24, 25*).

2.2. Elongation

Translation elongation is assisted by elongation factors (eEF). During this step, mRNA codons are decoded and peptide bonds are formed sequentially to add amino acid residues

to the carboxy-terminal end of the nascent, mRNA-encoded, peptide (*21, 26-28*). Elongation involves four major steps: *i*) formation of the ternary complex eEF1A·GTP·aminoacyl-tRNA and delivery of the first elongator aminoacyl-tRNAs to an empty ribosomal tRNA-binding site called the A (acceptor)-site. It is in the A-site where codon/anticodon decoding takes place; *ii*) Interaction of the ribosome with the mRNA-tRNA. This duplex activates eEF1A·GTP hydrolysis and guanine nucleotide exchange on eEF1A; *iii*) Peptide bond formation then occurs between the P-site peptidyl-tRNA and the incoming aminoacyl moiety of an A-site aminoacyl-tRNA. This reaction is catalyzed by the peptidyl transferase center of the 60S ribosomal subunit, and the products comprise of a new peptidyl-tRNA that is one amino acid residue longer and a deacylated (discharged) tRNA. *iv*) Binding of eEF2·GTP and GTP hydrolysis promote the translocation of the mRNA such that the deacylated tRNA moves to the E (exit)-site, the peptidyl-tRNA is in the P-site, and the mRNA moves by three nucleotides to place the next mRNA codon into the A-site. The deacylated tRNA in E-site is then ejected from the ribosome. The whole process is repeated along the mRNA sequence. When a stop codon is reached the process of termination is initiated (*21, 26-28*).

2.3. Termination

Translation termination is mediated by two polypeptide chain-release factors, eRF1 and eRF3. When any of the termination codons is exposed in the A-site, eRF1 recognizes the codon, binds the A-site, and triggers the release of the nascent polypeptide from the ribosome by hydrolysing the ester bond linking the polypeptide chain to the P-site tRNA. This reaction leaves the P-site tRNA in a deacylated state, leaving it to be catalyzed by the peptidyl transferase center of the ribosome. eRF1 recognizes stop signals and functionally acts as a tRNA-mimic, whereas eRF3 is a ribosome- and eRF1-dependent GTPase that, by forming a stable complex with eRF1, stimulates the termination process (*21, 29, 30*).

2.4. Recycling

In the recycling step, both ribosomal subunits are dissociated releasing the mRNA and deacetylated tRNA, so that both ribosomal subunits can be used for another round of initiation (*21, 29, 30*) (Fig.1). Evidence suggests that the ABC-type ATPase ABCE1 is probably the general ribosome recycling factor which coordinates termination with recycling (*31*). ABCE1 establishes multiple contacts with both ribosomal subunits as well as with the release factors and stimulates ribosome dissociation. ABCE1 also influences eRF1 function during stop-codon recognition and peptidyl-tRNA hydrolysis. During ribosome recycling, eRF3 dissociates from ribosomal complexes after GTP hydrolysis, whereas eRF1 remains associated with posttermination ribosomal complexes after peptide release (*31-33*).

According to the closed-loop model, termination and recycling may not release the 40S ribosomal subunit back into the cytoplasm. Instead, this subunit may be passed from the poly(A) tail back to the 5'-end of the mRNA, so that a new round of initiation can be started on the same mRNA (*21, 29, 34*)

3. The emergence of eukaryotic translation

The emergence of eukaryotes from prokaryotic ancestors led toward novel, higher levels of cell organization. A plethora of new features emerged at the cellular level, including the acquisition of a nucleus, an endoplasmic reticulocyte and endosymbiotic bacteria, the formation of split genes sorted out in chromosomes, an actin-based cytoskeleton and, in many phyla, the emergence of multicellularity, behavioural patterns and developmental programs. In this new type of cell, novel features appeared in the translation process, including the spatio-temporal separation between transcription and translation, and the increase of the number of events occurring during the initiation step that led to the establishment of the cap-dependent mechanism.

During the emergence of eukaryotes, the translation apparatus itself also underwent profound changes, including the evolution of the 40S and 60S ribosomal subunits from the prokaryotic 30S and 50S, respectively. This was due to the addition of several rRNA expansion segments, peptide additions to most ribosomal proteins, and the addition of extra eukaryotic-specific components, including novel ribosomal proteins and the 5.8S rRNA (*26*, *35-38*). Moreover, the number of initiation factors increased. While in prokaryotes translation initiation is assisted by three factors, in eukaryotes initiation needs the interplay of at least fourteen factors. Thus, novel, eukaryotic-specific translation factors evolved (including eIF3, eIF4B, eIF4E, eIF4G, eIF4H and eIF5). The mRNA also underwent profound changes which can be summarized as follows: *i*) it acquired a novel molecular structure, from polycistronic to monocistronic, capped, and polyadenylated transcripts; *ii*) it acquired a novel life cycle, from simultaneous transcription/translation, to be transcribed, spliced and exported from the nucleus, to be stored, translated and degraded in the cytoplasm; and *iii*) it acquired a novel functional conformation when engaged in translation, displaying a functional cross-talk between the 5'- and 3'-ends. Finally, with the emergence of eukaryotes the number of different mechanisms that regulate translation was expanded (*11-15*, *17*, *39*).

How the transition from prokaryotic to eukaryotic translation occurred remains still unresolved. I will discuss some ideas that have been set forward to try to address this question.

4. The transition from prokaryotic to eukaryotic translation initiation

4.1. Translation initiation in the prokaryotic world

It was established in the 1970s that in eubacteria the recruitment of the small ribosomal subunit to the mRNA occurs by a direct interaction. This happens via the complementary base pairing between the Shine-Dalgarno (SD) sequence of the mRNA, which is a purine-rich region located at around 10 nucleotides upstream the start codon, and a sequence at the 3' end of the 16S rRNA on the ribosome (referred to as anti-Shine-Dalgarno sequence, ASD) (*40*, *41*). The importance of the SD sequence to initiate translation was later experimentally corroborated in different eubacteria and archaea (*42-44*), and has been retained in some cell organelles that evolved from eubacteria over a billion years ago (*45*). This, together with the

large proportion of genes having the SD sequence in the well studied bacteria, led to the general idea that for the vast majority of prokaryotic mRNAs the SD sequence was the essential (although not necessarily the sole) element to select the correct initiation codon, and that the SD/ASD interaction during initiation is conserved in most prokaryotes (*16, 46, 47*). However, in recent years a large number of mRNAs lacking a SD sequence have been discovered widespread in a variety of different eubacterial and archaeal lineages. These include mRNAs devoid of 5'-UTR (and hence referred to as "leaderless" mRNAs) (*15, 16, 39, 45, 48-54*), and mRNAs that possess a 5'-UTR and lack a SD sequence (*45, 48-53, 55*). For leaderless mRNAs the start codon itself was found to serve as the most important signal for ribosome recruitment and for translation initiation. Here the initiator tRNA and IF2 are critical for complex formation between the start codon and the ribosome. It is noteworthy that translation initiation of leaderless mRNAs involves the undissociated ribosome 70S instead of the 30S ribosomal subunit (*15, 16, 39, 51, 56-59*). mRNAs with a 5'-UTR devoid of a SD sequence exhibit a pronounced minimum in secondary structure and AUG start codons reside in single-stranded regions of the mRNAs. For these mRNAs, ribosome binding to the start codon is a sequence-independent event, but is strictly dependent on the local absence of RNA secondary (*45*). Translation initiation of these transcripts is promoted by the ribosomal protein S1 (RPS1), which is a component of the 30S ribosomal subunit. RPS1 interacts with the 5'-UTR of an mRNA initiating translation efficiently, regardless of the presence of a SD sequence. Intriguingly, neither archaeobacteria nor eukaryotes contain a RPS1 gene, raising the question of how leadered mRNAs devoid of a SD sequence are translated in Archaea (*39, 46, 49, 51*). Finally, evidence suggests that alternative, unknown mechanisms might be used to initiate translation in Cyanobacteria (*49*) and in haloarchaea (*60*).

Overall, the emerging view indicates that in the prokaryotic world, both SD-dependent and SD-independent translation mechanisms are present in all major groups of prokaryotes. Indeed, evidence suggests that the leaderless mechanism might represent the major pathway to initiate translation in Archaea (*52-54*). Thus, it has been suggested that the last common ancestor of existing life already possessed an established fundamental translational apparatus, but the mechanisms to initiate translation initiation further diversified in the bacterial and archaeal lineages (*17, 49, 50, 60*).

4.2. What was the mechanism to recruit mRNAs in the last common ancestor of existing life?

Despite the presence of leaderless and leadered, SD-lacking mRNAs across prokaryotes, a recent study using the genomes of 277 prokaryote species, both eubacteria and archaea, showed that the anti-SD sequence at the 3' end of the 16S rRNA on the ribosome is highly conserved among all species, and that loss of the SD sequence seems to have occurred multiple times, independently, in different phyla (*49*). These observations strongly suggest that the SD/ASD interaction plays an important role in translation initiation in essentially all prokaryote species that are descended from the last universal common ancestor. Thus, the SD-based mechanism of ribosome recruiting might have driven translation initiation in the last common ancestor of existing organisms, but was further lost in different phyla (*49*). For

those phyla which lost the SD sequence, RSP1-mediated or leaderless mRNA-used mechanisms of translation initiation work to great extent (*49*). The evolutionary pressures that led to the loss of SD sequences, however, are completely unknown.

4.3. The transition to eukaryotes

As mentioned above, while the fundaments of translation are well conserved in all forms of life, in eukaryotes the initiation step underwent substantial increase in complexity and in number of initiation factors as compared to prokaryotes.

Although it is established that eukaryotes evolved from archaeal ancestors, we still don't know what lineage they evolved from. Thus, we don't know what type of mRNA (i.e. SD-containing, leaderless, or SD-lacking transcript) the first eukaryotes possessed. Nevertheless, all eukaryotic mRNAs lack SD sequences and ribosomes have no RPS1. I have previously suggested that three were the most important evolutionary forces that led to the emergence of the cap-dependent initiation mechanism in eukaryotes, namely (*i*) the need to adjust to the emergence of the nuclear membrane and interrupted genes, (*ii*) the subsequent requirement to splice and export intron-less mRNAs to the cytoplasm, and (*iii*) the absence of SD sequence and RSP1 in eukaryotic mRNAs (*11, 13, 14*). Because eukaryotic mRNAs lack both SD sequences and RPS1 protein, they cannot efficiently recruit the small ribosomal subunit directly to the initiation codon. This, together with the fact that most initiation factors that evolved only within the eukaryotes (including eIF4E, eIF4G, eIF4B, eIF4H and eIF3) are involved in the cap-binding and the scanning processes, indicates that the absence of both SD sequences and RSP1 protein was one of the crucial selection pressures that led to early eukaryotes to develop a novel mechanism to ensure the correct landing of the ribosome at the 5'-end of mRNAs, namely the cap-dependent initiation (*11, 13, 14*).

I have discussed that during eukaryogenesis and before the time when the cap-dependent initiation was developed, it is possible that there would have been a transition period where the mRNAs of the early eukaryotes were translated in a cap-independent, IRES-driven manner. In this period, 5'-UTRs lacking SD motifs that were able to passively recruit the 40S ribosomal subunit would have been positively selected for and could, therefore, have become the first examples of IRESs (Fig. 2). In this scenario, the cap structure, a proto-eIF4G, the poly(A) tail and an ancestral PABP, might have appeared for functions in RNA metabolism that emerged among the primary adaptive responses to the emergence of split genes and the need for nucleocytoplasmic RNA export, but initially had no role in translation (*11, 13, 14*). As a consequence of the absence of the SD sequence in eukaryotic mRNAs, the scaffold proteins eIF4G, eIF3 and eIF4B, as well as the 5'-end cap structure, eIF4E, and RNA helicases were later incorporated into the already established but incipient eukaryotic translation machinery because they ensured a more efficient recruitment of the 40S ribosomal subunit by the mRNA. Altogether, these events led the "scanning" process to evolve and to the establishment of the current cap-dependent translation initiation. Mutations in PABP, which allowed binding to eIF4G and promoted mRNA circularization underwent a strong positive selection because they ensured a more efficient recruitment of

the 40S ribosomal subunit by the mRNA, stimulating translation and increasing mRNA stability. mRNA circularization provides an effective means for the protein synthesis apparatus to selectively translate only intact mRNAs, i.e., those that harbor both a cap and a poly(A) tail. Thus, mRNA circularization also (and perhaps primarily) underwent a strong positive selection because it represents a checkpoint that determines whether or not to initiate translation (*11*, *13*, *14*).

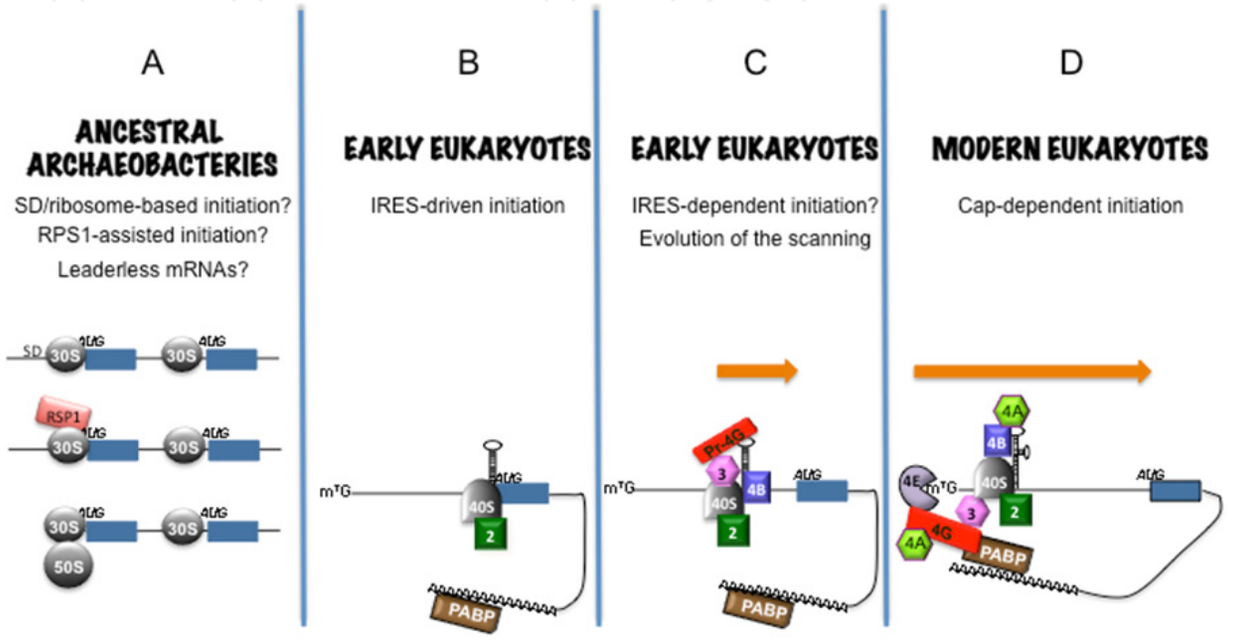

Figure 2. A proposed model for the evolution of the cap-dependent.
initiation of translation. (A) Ancestral archaeal cells had polycistronic (blue boxes) mRNAs. It is not known what was the mechanism used by the prokaryotic ancestors of eukaryotes to recruit the mRNA by the 30S ribosomal subunit. (B) The evolutionary transition from ancestral archaeal cells to early eukaryotes is represented. Due to the appearance of the nucleus and split genes, transcription and translation were decoupled, and a need to splice, export and protect the transcripts during nucleus-cytoplasm export emerged. The cap structure might have first appeared at this evolutionary stage to provide a "platform" to assemble the splicing, export and RNA protection mechanisms (see reference *14* for details). The arousal of PABP might have happened at this stage as part of the mechanisms to protect mRNAs (see reference *11* for details). The lack of SD sequences in the mRNAs, probably as a result of massive invasion of introns from endosimbionts, as well as the apparition of monocistronic mRNAs and both the 40S and the 60S ribosomal subunits, happened at this stage. Initiation of translation occurred perhaps in an IRES-dependent manner via the direct recruitment of the 40S ribosomal subunit by the mRNA. (C) The evolution in the cytoplasm of a proto-eIF4G (Pr-4G), along with the emergence of eIF3 and eIF4B, gradually improved the delivery of the 40S ribosomal subunit to the early mRNAs during evolution. The scanning process (orange arrow) evolved probably at this stage due to the activity of RNA helicases coming from different processes of metabolism that at the same time could participate in the unwinding of mRNA secondary structure during translation initiation. Among them, however, only an eIF4A-like helicase evolved functional interactions with eIF4G thus becoming later the canonical initiation factor eIF4A. (D) It is not know when eIF4E evolved during eukaryogenesis. However, an interaction of eIF4G with eIF4E, eIF4A and PABP evolved, which eventually led to the establishment of today's widespread cap-dependent mechanism to initiate translation.

5. Diversification of eukaryotes and further evolution of the translation apparatus

5.1. Functional divergence of initiation factors

After eukaryotes emerged, components of the translation machinery further evolved along the radiation into different phyla (Fig. 3). The continue appearance of different levels of organismal complexity led to the arousal of new phyla, developmental programs, behavioral patterns, and the invasion of novel ecological niches by eukaryotes. These events most probably were the causes of a further evolution, to different levels, of components and mechanisms of the translation apparatus in different taxa (*11, 12*). In the following, I will summarize the most studied examples of this.

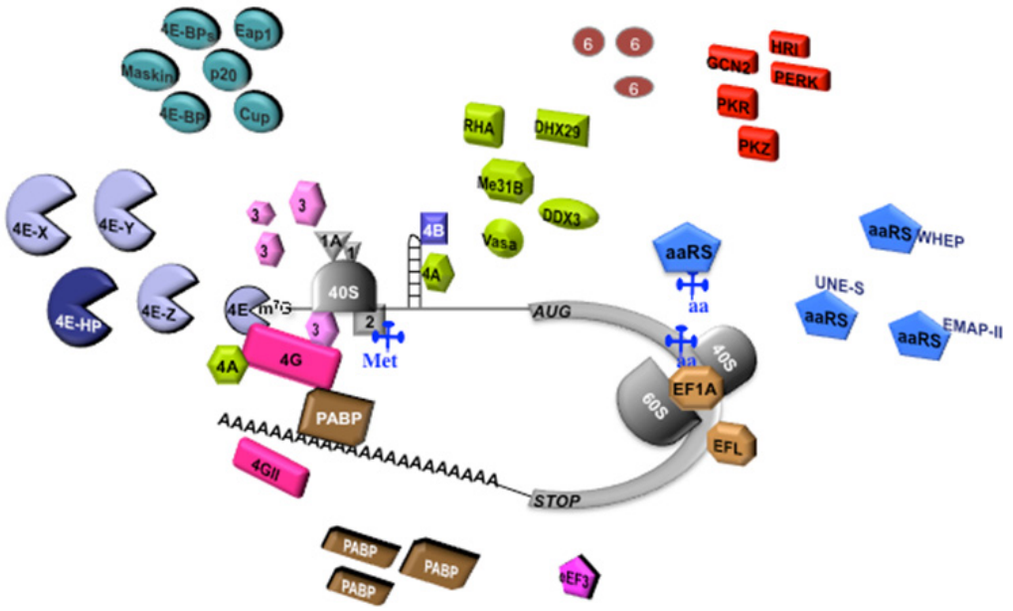

Figure 3. Diversity in the components of the translation apparatus across eukaryotes. The different components of the translation machinery with well-studied diversity in different phyla are shown in colors. Components with some diversity that is not discussed here are depicted in gray. Several cognates of eIF4E (purple) and eIF4G (red) have been found in plants, metazoan, protists and some fungi. In some cases, eIF4E cognates have evolved towards translational repressors (4E-HP, dark blue, is an example). Many 4E-binding proteins (including Maskin, 4E-BPs, Eap1, p20 and Cup, light blue) have been discovered in different species. The subunit composition of eIF3 (pink) ranges from 5 to 13 nonidentical polypeptides in different phyla. There is, however, a core of five homolog subunits shared by most eukaryotes. Some diversity has been found in eIF6. Several RNA helicases (light green) from diverse organisms are involved in the *Initiation* step. A family of five kinases (*HRI*, *PERK*, *GCN2*, *PKR* and *PKZ*, red) phosphorylate the alpha subunit of eIF2 to inhibit global translation under stress conditions. The presence of eIF2alpha kinases varies in different lineages. Different domains, such as *WHEP*, *EMAPII*, and *UNE-S*, have been added to different aminoacyl-tRNA synthetases (*aaRSs*, blue) in distinct phyla of multicellular species. For *Elongation* to happen, a number of protist, algae and fungi lack eEF1A (light brown) and instead possess the related factor elongation factor-like (EFL, dark brown). Ribosomes from all eukaryotes perform *Elongation* with eEF1A and eEF2. However, the yeast *S. cerevisiae* requires an additional essential factor, eEF3 (dark pink), for *Elongation* to proceed. Genes encoding eEF3 have been found exclusively in many species of fungi. Evidence supports the notion that eEF3 activity promotes ribosome recycling. Several cytoplasmic PABP (dark brown) cognates have been found in many phyla.

Most evidence for molecular and functional diversification among the translation components has been found in the eIF4 proteins. All major eukaryotic lineages possess several paralog genes for members of the eIF4 families. For some of them, differential expression patterns, and even variable biochemical properties among paralogs of the same organism, have been found (*12, 14, 61-66*). For eIF4E and eIF4G cognates, physiological specialization has been also found and, in some cases, eIF4E cognates have evolved towards translational repressors (*12, 14, 61-63, 65*). These findings support the hypothesis that in organisms with several paralogs, a ubiquitous set of eIF4 factors supports global translation initiation whereas other paralogs perform their activity in specific cellular processes (*61*). Whereas the need for distinct eIF4 proteins in different tissues may have been the driving force behind the evolution of various paralogs in multicellular organisms, in unicellular eukaryotes different paralogs may be differentially needed during distinct life stages (*67*).The multisubunit eIF3 is another example of factor that has undergone molecular diversification across eukaryotes, whose subunit composition ranges from 5 to 13 nonidentical polypeptides in different phyla (*68*). However, the functional relevance of these phenomena is not known.

5.2. Multiple helicases involved in the initiation step

eIF4A is the factor traditionally thought to perform RNA helicase activity to unwind the 5′-UTR secondary structure during the scanning. Recently, other RNA helicases from diverse organisms have also been found to be involved in different steps of translation initiation. Such is the case of the mammalian, *Drosophila* and yeast helicases DDX3 and Ded1, as well as the human helicases RHA and DHX29 (*69-71*). Evidence supports the idea that in *Drosophila*, the helicase Vasa is a translational activator of specific mRNAs involved in germline development (*5, 6*). In contrast, orthologs of the *Xenopus* helicase Xp54 in several organisms, including *Drosophila* Me31B, *Saccharomyces* Dhh1, human rck/p54, and *Caenorhabditis* CGH-1 have been found to repress translation of stored mRNAs and promote aggregation into germplasm-containing structures (*72*). In *Arabidopsis*, the eIF4F complex contains eIF4A in proliferating cells but different RNA helicases in quiescent cells (*73*). These findings show that evolutionary convergence has happened in different lineages to fulfill the need of RNA helicase activity during translation initiation.

5.3. Divergence in molecules involved in the elongation step

In contrast to the initiation step, the process of elongation is highly conserved among all forms of life. Strikingly, a recent genome-wide analysis revealed that a number of eukaryotic lineages lack eEF1A, a canonical factor that delivers aa-tRNAs to the A-site of ribosomes during the elongation step. Instead, they possess a related factor called elongation factor-like (EFL) protein that retains the residues critical for eEF1A (*74*). It was later found that EFL-encoding species are scattered widely across eukaryotes and that *eEF1A* and *EFL* genes display mutually exclusive phylogenetic distributions. Thus, it is assumed that eEF1A and EFL are functionally equivalent (*74-82*). It is thought that *eEF1A* is ancestral to all extant eukaryotes and that a

single duplication event in a specific lineage gave rise to *EFL*. *EFL* genes were then spread to other lineages via multiple independent lateral gene transfer events, where EFL took over the original eEF1A function resulting in secondary loss of the endogenous *eEF1A*. It is thought that both genes co-existed for some time before one or the other was lost. Indeed, the diatom *Thalassiosira* bears both *EFL* and *eEF1A* genes (*79*) and might be an example of this situation. It is also possible that there was a single gain of *EFL* early in evolution followed by differential loss of it (*74, 78, 79, 81, 82*). So far, *EFL* genes have been identified in widespread taxa, including diatoms, green and red algae, fungi, euglenozoans, foraminiferans, cryptophytes, goniomonads, katablepharid, chlorarachniophytes, oomycetes, dinoflagellates, choanozoans, centrohelids and haptophytes. Most of them are unicellular organisms. In contrast, most eukaryotes contain only *eEF1A* (*74-82*).

Key molecules for elongation are aaRSs, which catalyze the aminoacylation reaction whereby an amino acid is attached to the cognate tRNA (*21, 26, 27*). aaRS represent an intriguing and perhaps unique case of evolution among the components of the translation apparatus. Throughout evolution of multicellularity, different domains such as the tripeptide ELR (Glu-Leu-Arg), the oligonucleotide binding fold-containing EMAPII domain, the WHEP domain, the glutathione S-transferase (GST) domain and a specialized amino-terminal helix (N-helix), have been progressively added to different aaRSs in distinct phyla. The tripeptide ELR and the EMAPII domain were incorporated simultaneously to TyrRSs in metazoans starting from insects; the WHEP domain is present in TrpRS only in chordates; and a unique sequence motif, UNE-S, became fused to the C-terminal of SerRS of all vertebrates. In bilaterian the glutamylRS and prolylRS were linked via WHEP domains giving rise to a bifunctional glutamyl-prolylRS (*83, 84*).

It has been found that the function of the aaRSs was either increased or impaired by the addition of the new domains. Whereas the WHEP domain regulates interaction of TrpRS with its cognate receptor, with MetRS this domain plays a tRNA-sequestering function. The Leu-zipper motif in ArgRS is important for the formation of multi aaRSs complex (MSC), which enhances channeling of tRNA to the ribosome. Moreover, different aaRSs play diverse roles in cellular activities beyond translation, such as stress response, plant and animal embryogenesis, cell death, immune responses, transcriptional regulation, and RNA splicing (*83-85*). It has been found that the incorporation of domains to aaRSs correlates positively with the increase in organism's complexity. For example, the number of aaRSs carrying the GST domain increases from two in fungi to four in insects, to five in fish and six in humans (*83*). Thus, it has been proposed that the newly fused aaRSs domains triggered the appearance of new biological functions for these proteins in different lineages, and that the fusion of domains to aaRSs could have played an important part in expanding the complexity of newly emerging metazoan phyla (*83*).

5.4. Divergence in termination and recycling factors

Termination is governed by eRF1, which is a monophyletic protein that was inherited by eukaryotes from archaeal ancestors. eRF1 is universally present in eukaryotes and, with the

exception of some vascular plants and some ciliates, eukaryotes contain only one *eRF1* gene (*86-89*). Interestingly, unusually high rates of eRF1 evolution have been found in organisms with variant genetic codes (mostly protists and unicellular fungi), especially in the N-terminal domain, which is responsible for stop-codon recognition (*86, 87, 89-92*). eRF1 displays structural similarity to tRNA molecules and mimics its activity during binding of ribosomal A-site during recognition of a stop codon (*91-94*). Since mutations in eRF1 N-terminal domain switch from omnipotent to bipotent mode for stop-codon specificity (*94-98*), most likely the accelerated evolution of eRF1 in organisms with variations to the nuclear genetic code has been driven mainly to accommodate these variations (*89-99*).

Another striking case of evolutionary divergence was found in the fungi. Ribosomes from all eukaryotes perform elongation with eEF1A and eEF2. Surprisingly, it was found that the yeast *Saccharomyces cerevisiae* requires an additional essential factor, eEF3, for the elongation cycle to proceed (*100*). Genes encoding eEF3 were subsequently identified exclusively in other fungi, including *Candida*, *Pneumocystis*, *Neurospora*, *Aspergillus* and *Mucor* (*101-104*). eEF3 is an ATPase that interacts with both ribosomal subunits and that is required for the binding of aminoacyl-tRNA-eEF1A-GTP ternary complex to the ribosomal A-site by enhancing the rate of deacylated tRNA dissociation from the E-site (*105*). Recently, it was shown that post-termination complex, consisting of a ribosome, mRNA, and tRNA, is disassembled into single components by ATP and eEF3. Because the release of mRNA and deacylated tRNA and ribosome dissociation takes place simultaneously and no 40S—mRNA complexes remain, it is proposed that eEF3 activity promotes ribosome recycling (*106*). It remains unsolved what were the evolutionary forces that led to the emergence of eEF3 exclusively in fungi.

6. Concluding remarks

One of the enigmas in current Biology is how eukaryotic protein synthesis emerged. I have discussed that, in the absence of SD sequence in mRNAs and RPS1 in ribosomes, the evolution of translation machinery followed a gradual addition of scaffold proteins, namely eIF4G, eIF3 and eIF4B, which highly improved the efficiency and regulation of mRNA binding to the 40S ribosomal subunit (*14*). This, together with the incorporation of several RNA helicases, eIF4E and PABP, gradually improved the global efficiency, accuracy and possibilities of gene expression regulation at the level of translation initiation (*14*). Most likely the molecular diversification of the translation apparatus is among the basis that provided to early eukaryotes the scope to invade new ecological niches and overcome the different environmental and biological challenges this represented. Indeed, the evolution of the translation apparatus might have been both, cause and consequence of eukaryotic radiation.

Translation in eukaryotes is tightly coupled to other features and components of cellular metabolism. For example, translation control is coupled to RNA transport to ensure different developmental programs to occur (*107, 108*). The RNA transport machineries have also diverged in different phyla, and together with them some components of the translation

apparatus also diverged (*108*). Another fundamental aspect of RNA metabolism is the storage and degradation of mRNAs in different cytoplasmic bodies, such as P-bodies and P-granules, which contain translation factors (*109*). The diversity and conservation of these foci across phyla are a reflection of the general evolution that the translation machinery and its regulation have undergone during eukaryotes evolution.

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