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Structural insights into the evolution of late steps of translation initiation in the
three domains of life

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ABSTRACT

In eukaryotes and in archaea late steps of translation initiation involve the two initiation factors e/aIF5B and e/aIF1A. These two factors are also orthologous to the bacterial IF2 and IF1 proteins, respectively. Recent cryo-EM studies showed how e/aIF5B and e/aIF1A cooperate on the small ribosomal subunit to favor the binding of the large ribosomal subunit and the formation of a ribosome competent for elongation. In this review, pioneering studies and recent biochemical and structural results providing new insights into the role of a/eIF5B in archaea and eukaryotes will be presented. Recent structures will also be compared to orthologous bacterial initiation complexes to highlight domain-specific features and the evolution of initiation mechanisms.

INTRODUCTION

Translation of the genetic message into proteins is one of the fundamental cellular processes that appeared early on and was present in the Last Universal Common Ancestor (LUCA) [1-4]. Current phylogenetic analyses mainly based on genes coding for ribosomal proteins propose a two-domain tree of life with bacteria on one side and archaea and eukaryotes on the other. Eukaryotes could have emerged from within an archaeal branch [5-7]. Thus, studying and comparing translation mechanisms in different organisms helps to provide information about their evolutionary relationship.

While mechanisms of translation elongation are globally highly conserved in the three domains of life, initiation and termination show more pronounced domain specificities reflecting their more recent evolution. Translation initiation requires selection of the start codon on the mRNA leading to the formation of a ribosome competent for elongation. The process generally begins on the small ribosomal subunit (SSU) bound to an mRNA and a Met-initiator tRNA in the presence of initiation factors.

Archaeal mRNAs resemble the bacterial ones. They often contain Shine-Dalgarno sequences or have short or no 5' untranslated regions (leaderless mRNAs) and they lack a 5'-cap and a polyadenylated tail. However, other features of the translation apparatus bring archaea closer to eukaryotes. In particular, archaeal ribosome and translation initiation factors are of the eukaryotic type ([8-14] and Figure 1A). Archaeal translation initiation (TI) can be divided into two stages, start codon selection and joining of the large ribosomal subunit forming an elongation-competent ribosome ([11, 15] and Figure 1B). Start codon selection is achieved within a macromolecular complex made up of the SSU, the mRNA, aIF1, aIF1A and the ternary complex aIF2:GTP:Met-tRNA_i^{Met} ([15-19] and Figure 1B). The heterotrimeric protein aIF2 (α , β , γ subunits) and the small protein aIF1 contribute to the accuracy of this selection [17, 19-23]. After start codon selection and departures of aIF1 and aIF2-GDP, late steps of translation initiation leading to the formation of a ribosome competent for elongation take place. aIF5B replaces aIF2 on the Met-tRNA_i^{Met} and assists, in the presence of aIF1A, the joining of the large ribosomal subunit.

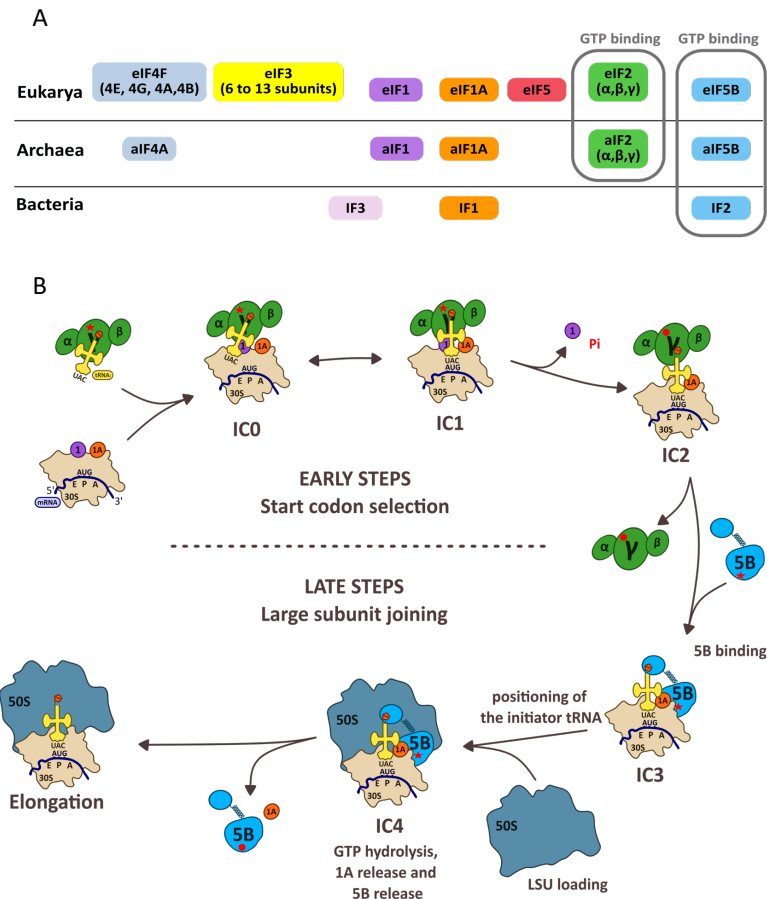


Figure 1: Schematic views of translation initiation

A- Translation initiation factors in the three domains of life.

eIF2B, the guanine exchange factor of eIF2, is not shown because it is not part of ribosomal initiation complexes. Archaea do not possess an eIF5 homolog and GTP hydrolysis on aIF2 is thought to be not assisted by a GTP hydrolysis accelerating protein [23]. aIF4A is found in many archaea. However its involvement in translation initiation remains to be proven [24]. Bacterial IF3 is a two-domain protein. Its C-terminal domain resembles e/aIF1 but the topology of its structure is different.

B- Steps of translation initiation in archaea. First steps are dedicated to start codon recognition. These steps involve aIF1, aIF1A and aIF2. GTP is represented as a red star whereas GDP is represented as a red circle. aIF1A binds the A site, aIF1 binds in front of the P site and aIF2 binds the initiator tRNA and the h44 helix. After start codon recognition, aIF1 is released from the SSU. aIF1 departure would trigger the release of the Pi group from aIF2 and, in turn, the release of aIF2 in its GDP-bound form. Cryo-EM structures illustrating initiation complexes IC0 (full initiation complex with initiator Met-tRNA_i^{Met} in P_{REMOTE} position), IC1 (full initiation complex with initiator Met-tRNA_i^{Met} in P_{IN} position, paired with the initiation codon) and IC2 (initiation complex after aIF1 departure) were determined [16, 17]. In IC2, aIF2 does not contact any longer the SSU but is not totally released from tRNA because a non-hydrolysable analogue of GTP was used [16]. After aIF2:GDP release, aIF5B is recruited on the SSU. Interactions of aIF5B with the SSU and with aIF1A stabilize the initiator tRNA in a position favorable to LSU joining. Assembly of the full ribosome would then trigger GTP hydrolysis on aIF5B, aIF1A release and final release of aIF5B bound to GDP. The exact order of these events remains to be studied in the archaeal system. In eukaryotes, studies of late steps of initiation in yeast and human showed that GTP hydrolysis on eIF5B accelerate eIF1A departure from the ribosome [25-27]. 80S:eIF5B structures determined so far do not contain eIF1A likely because of the occupation of its binding site by H69 helix of the LSU [28-30].

In eukaryotes, start codon selection is further regulated with the participation of other initiations factors, such as those of the eIF4F family involved in the mRNA 5'-cap binding, eIF3 involved in mRNA binding and eIF5 controlling GTP hydrolysis on eIF2 (Figure 1A). Since eIF1, eIF1A and eIF2 are homologous to their archaeal counterparts, selection of the start codon in eukaryotes and in archaea involve a common structural core [18]. In addition, late steps of translation initiation in eukaryotes are

also controlled by two initiation factors, eIF5B and eIF1A, that have homologous archaeal counterparts. Importantly, e/aIF5B and e/aIF1A are also orthologues of the bacterial proteins IF2 and IF1, respectively (Figure 1A). Late steps of translation initiation have therefore a universal character [31, 32]. In the past recent years, a wealth of genetic and biochemical data describing the late steps of translation initiation in eukaryotes were obtained. By contrast, available data for the archaeal case were much scarcer. Moreover, while structural data for binding of eIF5B to the 80S ribosome were available, structural data showing the binding of e/aIF5B to the small subunit of the ribosome were only recently obtained. In particular, how e/aIF5B cooperated with a/eIF1A during the late steps had remained an open question. In this review, pioneering studies and recent biochemical and structural results providing new insights into the role of a/eIF5B in archaea and eukaryotes will be presented. Recent structures will also be compared to orthologous bacterial initiation complexes to highlight domain-specific features and evolution of initiation mechanisms.

Identification of e/aIF5B and first studies of their functions

In the 90s, the *FUN12* gene (Fun for UNknown Function) was identified in *S. cerevisiae* chromosome I [33, 34]. Some years later, sequencing of *FUN12* identified a protein homologous to bacterial IF2 [35] in yeast. Then, the full genome of three archaeal species, *Methanocaldococcus jannaschii*, *Archaeoglobus fulgidus* and *Methanobacterium thermoautotrophicum* were published [36-38] identifying genes sharing homologies with the bacterial *infB* gene (encoding IF2), although more closely related to the eukaryotic *FUN12* gene. Based on this homology, the product of the *FUN12* gene was referred to as IF2 in some early studies [39-43]. This created a confusion due to the presence of initiation factor e/aIF2 in Eukarya and Archaea, unrelated to IF2 and Fun12 (Figure 1A). The ubiquitous character of Fun12 in eukaryotes and archaea raised the question of its function [9, 10].

Molecular genetics and biochemical studies showed that the product of *FUN12* was a critical yet nonessential protein in yeast, involved in translation initiation [39, 40]. Its role was shown to be complementary to that of eIF2, in that they perform distinct functions in TI process despite both being able to bind the Met-initiator tRNA (Met-tRNA_i^{Met}). Moreover, it was shown that GTP binding and hydrolysis were essential for the activity of Fun12 in translation. Human or archaeal (*M. jannaschii*) homologues were shown to be suitable substitutes in yeast *fun12Δ* cells, while this was not the case for the bacterial IF2 homologue [40]. This result reinforced the idea that archaea and eukaryotes have closely related mechanisms for TI. In the year 2000, the factor was renamed eIF5B considering that the mammalian protein was co-purified with eIF5, a GTPase activating protein, absent from archaea, that accelerates hydrolysis of GTP by eIF2 [44]. Recent studies on the interaction between eIF5 and eIF5B are discussed below. The role of eIF5B in binding Met-tRNA_i^{Met} and joining of the large ribosomal subunit was then shown [26, 44-47]. eIF5B-GTP, together with eIF1A, promotes ribosomal subunit joining. This is followed by GTP hydrolysis on eIF5B and release of eIF1A and eIF5B-GDP resulting in an 80S ribosome ready for elongation. GTPase activity of eIF5B is not required for subunit joining but for eIF5B release [26, 27, 43, 44, 46, 48]. These functional studies benefited from early biochemical results in mammals that had preceded identification of the *FUN12* gene [49-52].

Concomitantly, first insights into the function of the archaeal version of 5B were obtained [40-42, 47, 53, 54]. More recently, it was shown that *P. abyssi* aIF5B accelerated subunit joining

independently of GTP hydrolysis, as in eukaryotes. This result also suggested that GTP hydrolysis is only required for the release of the factor in the archaeal case [55].

In eukaryotes and archaea, e/aIF5B and e/aIF1A ensure the final checkpoint for the presence of the initiator tRNA on the SSU and facilitate the assembly with the large ribosomal subunit (LSU) [26, 39-41, 44, 45, 47, 55]. Subsequent GTP hydrolysis-dependent departure of eIF5B enables protein synthesis to begin [27, 43, 44, 48]. Finally, the joining step allows the positioning of the aminoacylated end of the initiator tRNA in the peptidyl transferase center. The two ribosomal subunits are in a rotated state (equivalent to the semi-rotated state in bacteria) allowing the binding of the first A site tRNA to start elongation.

e/aIF5B and e/aIF1A 3D structures

The first X-ray crystallographic structure of aIF5B was that from *Methanobacterium thermoautotrophicum* [42]. Nowadays, several crystallographic structures of e/aIF5B are available [42, 53-58]. These structures confirmed the structural homology between eIF5B and aIF5B, and highlighted some domain specificities (Figure 2 and 3). Compared to aIF5B, eukaryotic eIF5Bs contain an additional large N-terminal domain with little sequence conservation that was shown to be dispensable for yeast cell viability [59]. In human, in vitro assays showed that removal of the N-terminal domain of eIF5B reduced by 30% the methylpuromycin synthesis rate. The N-terminal domain of eIF5B could also influence the rate of GTP hydrolysis by eIF2 on the 48S initiation complex. However, N-terminal truncation did not affect the subunit joining activity of eIF5B [44]. Moreover, a recent cryo-EM study performed with native eIF5B isolated from rabbit reticulocytes failed to reveal the structure corresponding to the N-terminal domain [60]. In most of the studies, versions of eIF5B truncated of the eukaryotic specific N-terminal domain were used. Such versions closely resemble the archaeal factor.

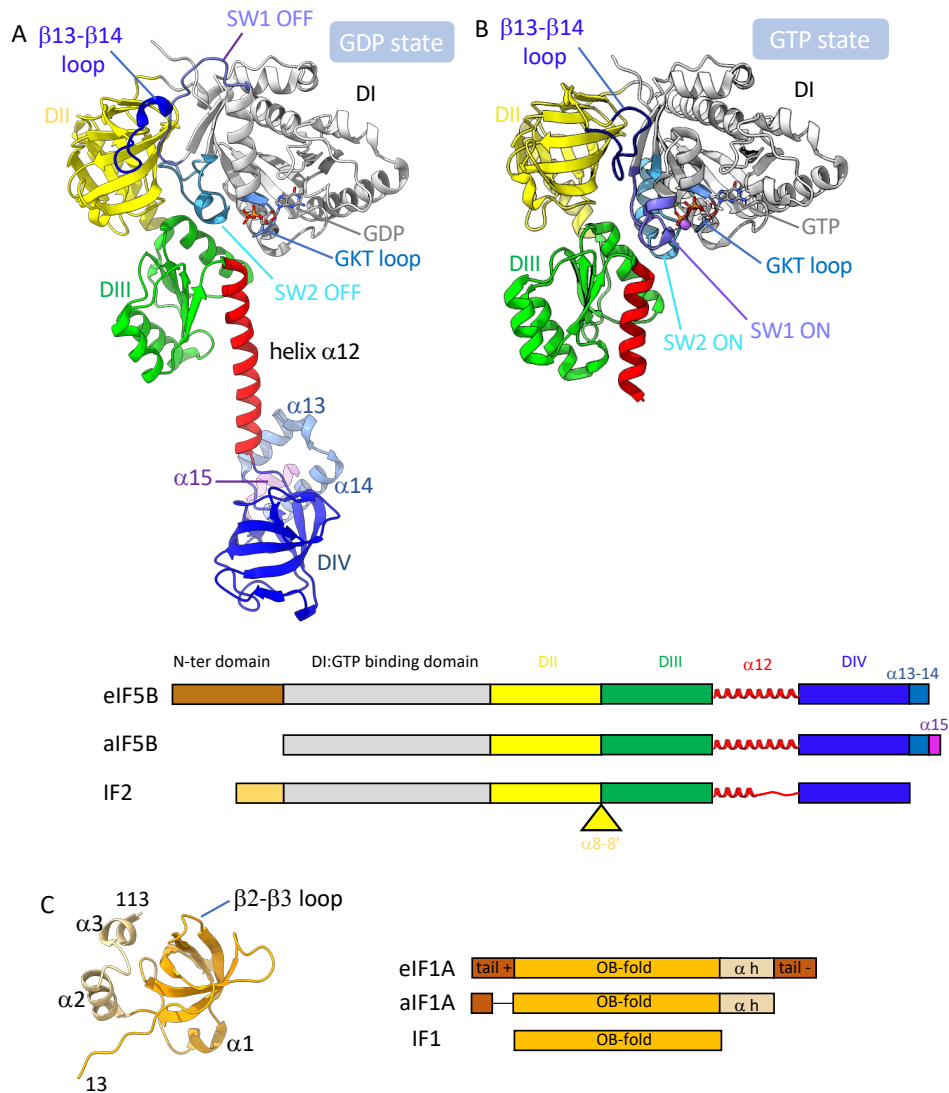


Figure 2: Crystallographic structures of aIF5B and aIF1A from *P. abyssi*

A-Cartoon representation of full-length aIF5B (PDB ID Code 7YYP). The aIF5B domains are colored as follows; domain I gray (1-229), domain II yellow (230-348), domain III green (350-438), $\alpha 12$ red, (439-464), domain IV blue (465-591) and the C-terminal archaeal-specific $\alpha 15$ helix magenta (591-598). The GDP is shown as sticks and main regions involved in the binding of the nucleotide, GKT loop, switch 1 (SW1), switch 2 (SW2) and the $\beta 13$ - $\beta 14$ loop, are colored in shades of blue.

B-Cartoon representation of aIF5B- ΔC (PDB ID Code 7YZN). Domains I of the structures A and B have been superimposed. The color code is the same as in view A. The factor is bound to GTP, resulting in “ON” conformations of SW1, SW2 and $\beta 13$ - $\beta 14$ loop. A comparison of the structural organization of e/aIF5B/IF2 in the three domains of life is shown below. DI, DII, DIII and DIV domains are represented with the same color code as in views A and B. Main specificities of each domains are shown. Briefly, the eukaryotic protein eIF5B has a long N-terminal domain with low conservation. This domain was shown to be dispensable for yeast viability [59]. The archaeal protein aIF5B has a supplementary $\alpha 15$ helix as compared to eIF5B. The bacterial protein has an N-ter domain of variable length depending on the phylum and a longer $\alpha 8$ helix linking domains II and III. In bacteria domain IV only contains a β -barrel. See also Figure 3. Because of their structural resemblance, e/aIF5B and IF2 are considered as universal initiation factors.

C- Cartoon representation of *P. abyssi* aIF1A (PDB ID Code 4MNO). The core of the protein is colored in orange and the two C-terminal α -helices are in pale orange. A comparison of the structural organization of e/aIF1A/IF1 in the three domains of life is shown alongside. The eukaryotic protein eIF1A has two long extensions at the N and C-terminal extremities. The C-terminal tail contains the conserved DIDD sequence. In contrast, the bacterial homologous protein IF1 corresponds only to the core domain colored in orange. Because of their structural resemblance, the IF1 and e/aIF1A proteins are considered as universal initiation factors.

Figure 2A and B were adapted from [55]. Figure 2C was adapted from [18]. Figures 2, 4, 5, 6 were drawn with ChimeraX [61].

X-ray structures of archaeal aIF5B have revealed a chalice-shaped protein containing four domains (as shown for *P. abyssi* aIF5B in Figure 2). Domain I binds the guanine nucleotide. It is built around a Rossmann-fold containing a seven stranded β -sheet and eight α -helices classically found in all translational GTPases. Domain II is a β -barrel homologous to domains II of e/aEF1A, e/aEF2 and the gamma subunit of e/aIF2. Domain III contains four beta strands sandwiched by three alpha helices. It is linked to domain II by $\alpha 8$. A long α -helix ($\alpha 12$) connects domain III and IV. The length of $\alpha 12$ and its amino acid composition are important for the function of e/aIF5B in Met-tRNA_i^{Met} binding and subunit joining [62] and Figure 3). Domain IV contains a β -barrel. It is responsible for the binding of the 3'-methionylated end of the Met-tRNA_i^{Met} [47]. Although structurally homologous, domain II and domain IV β -barrels do not present any sequence similarity. Two C-terminal helices ($\alpha 13$ and $\alpha 14$) follow the domain IV β -barrel in eIF5B, with an additional archaeal-specific $\alpha 15$ helix in aIF5B (Figures 2 and 3).

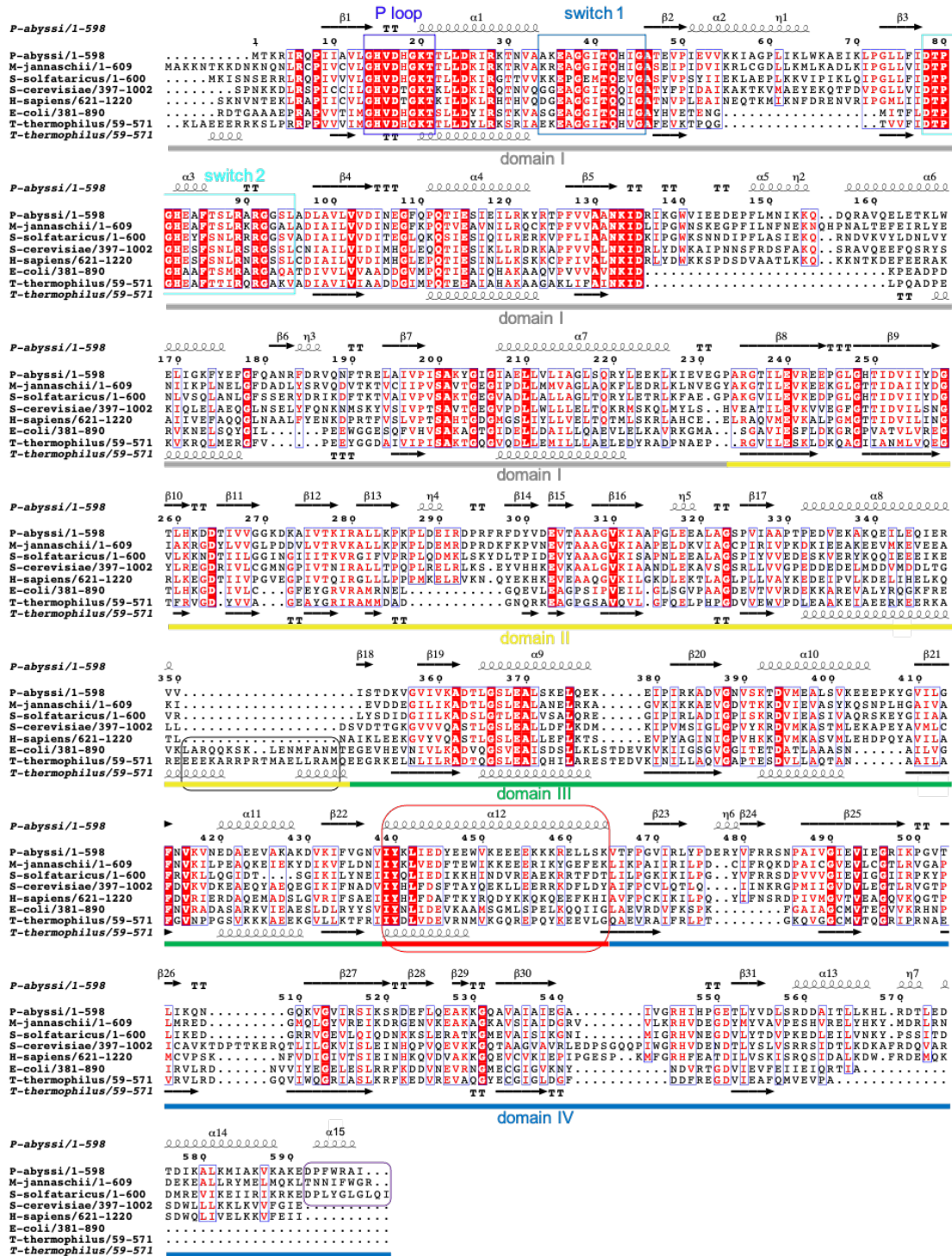


Figure 3: Sequence alignment of e/aIF5B/IF2.

The structures of *P. abyssi* aIF5B (PDB Code 7YYP) and *Thermus thermophilus* IF2 (PDB Code 5LMV) were used as references. The archaeal specific $\alpha 15$ helix is boxed in purple. $\alpha 12$ helix linking domains I, II, III to domain IV is boxed in red. Note that the length of the linking region is conserved in the 3 domains of life. The helical part is shorter in bacteria. The bacterial specific longer $\alpha 8$ region is boxed in black.

Binding of the nucleotide was studied using full-length and truncated versions of e/aIF5B.

Comparison of GTP and GDP bound states showed movements of the two switch regions (SW1 and

SW2) and of the β 13- β 14 loop of domain II [55, 57, 58] and Figure 2). The comparison also shows movements of domains II and III with respect to domain I [55]. As for all translational GTPases, GTP hydrolysis is activated by a conserved Histidine residue of switch 2. High-resolution structures of truncated versions of *Chaetomium thermophilum* eIF5B and *P. abyssi* aIF5B bound to GTP revealed the presence of two metal ions that may assist GTP hydrolysis by helping to neutralize the negative charges in the transition state [55, 57]. Finally, comparison of all available structures showed that domain IV can move freely with respect to domains I, II and III. These domain movements are important for the function of e/aIF5B on the ribosome, as discussed below [55].

aIF1A is a small protein (113 amino acid residues in *P. abyssi*), containing an OB-fold with 5 anti-parallel β -strands and two C-terminal α -helices [17]; Figure 2C). It binds to the A site on the SSU. Compared to archaea, eukaryotic eIF1A has unstructured N-terminal and C-terminal extensions that are important for its function [63]. In eukaryotes, eIF1A and eIF1 are necessary for mRNA scanning by the 48S pre-initiation complex (PIC, [64]). Both factors perform their tasks through a variety of interactions with other components of the initiation machinery [65]. eIF1A both promotes and inhibits scanning of the 48S PIC through the competing influences of its two unstructured N- and C-terminal extensions [63, 64, 66-68]. In cryo-EM structures of 48S PIC, the scanning inhibitor N-terminal tail of eIF1A stabilizes the codon:anticodon interaction [65, 69, 70].

After start codon selection and departures of e/aIF1 and e/aIF2.GDP, e/aIF1A remains bound to the A site on the SSU where it cooperates with e/aIF5B for late steps of the process. Interaction between eIF1A and eIF5B was early suggested from biochemical studies of initiation complexes purified from mammals [51]. In eukaryotes, eIF5B was shown to directly interact with the eukaryote-specific C-terminal tail of eIF1A through the well-conserved DIDDI sequence [56, 59, 71]. This interaction is required for optimal subunit joining [27, 46]. However, this interaction has not been yet observed on the ribosome. In archaea, aIF1A lacks the eukaryotic-specific C-terminal extension and the DIDDI sequence but aIF5B has a supplementary C-terminal helix [53]. Therefore, the possibility that the two proteins interact on the small ribosomal subunit remained to be explored until recently (see below).

Interestingly, translation initiation in mammals involve the interaction of eIF5B with another partner, namely eIF5. Indeed, consistent with early observations [44, 72], a recent study showed that

human eIF5 interacts with eIF5B on the SSU. This interaction involves a DIDDI motif found at the C-terminal extremity of eIF5 and domain IV of eIF5B. Strikingly, this motif is similar to that found in eIF1A. The binding affinity of eIF5 to eIF5B domain IV is 100-fold greater than that of eIF1A and the two proteins compete for the same binding site on eIF5B [73]. Whereas the C-terminus of eIF1A is conserved in most eukaryotes, with the notable exception of microsporidia, the DIDDI sequence of eIF5 is much less common and is only systematically found in Metazoa [73]. Thus, unlike human eIF5, *S. cerevisiae* eIF5 does not have a C-terminal tail and is not known to bind eIF5B. When present, the eIF5-CTT could participate in the recruitment of eIF5B on the 48S pre-initiation complex. Such an interaction would allow eIF5B to be pre-positioned to retrieve the initiator tRNA just after eIF2:GDP and eIF5 departures upon start codon selection. The presence of an eIF5-CTT would therefore be a selective advantage allowing for faster and more efficient translation initiation [73, 74].

Binding of eIF5B on the 80S ribosome.

First information on eIF5B binding to the 80S came from hydroxyl radical probing experiments [75, 76]. Then, a first cryo-EM structure of yeast eIF5B-GDPNP bound to the 80S ribosome was obtained in 2013 [28]. Since then, several structures of yeast complexes and of a mammalian 80S:mRNA-HCV-IRES:eIF5B initiation complex were published [28-30, 77, 78]. These structures show how domain IV of eIF5B is bound to the methionyl-CCA end of the initiator tRNA. eIF5B domain I interacts with the sarcin-ricin region of the LSU whereas eIF5B domains II and III are bound near the h5-h15 region and the h14-h5-uS12 region on the SSU (Figure 4A). In the presence of the GTP analogue GDPCP, the switch regions are ON. Interestingly, a tyrosine residue (Y837 in yeast) located at the N-terminal extremity of h12 and close to the catalytic histidine residue (H480 in yeast) could participate with the sarcin-ricin region of the LSU in triggering GTP hydrolysis on eIF5B. These two residues are strictly conserved in archaeal and eukaryotic 5B.

In another study, the cryo-EM structure of a yeast late initiation complex, prepared in the presence of GTP and rapidly frozen in order to trap intermediates, was determined [30]. eIF5B bound to the 80S ribosome was modelled in a GDP:Pi state, with SW1 not visible and SW2 in the ON conformation. Domain I, II and III were arranged as in the GDPCP bound structure [29] and the factor

was still bound to Met-tRNA_i^{Met} through its domain IV. The structure strongly suggests that GTP hydrolysis is not the rate limiting step for eIF5B dissociation from the 80S IC. The structure likely represents an intermediate 80S IC before eIF5B dissociation and transition to the elongation-competent state, but after GTP hydrolysis. Consistent with this idea, the configuration of the eIF5B-bound 80S structure is closer to an elongation-competent state. Notably, eIF1A was never observed in the eIF5B-bound 80S structures. Indeed, the positioning of the LSU helix 69 in the A site of the SSU is not compatible with the binding of the factor. The whole data indicate the following order of events: GTP hydrolysis on eIF5B would be followed by eIF1A departure before Pi release and finally eIF5B departure [26, 48].

These eIF5B-bound 80S structures supported by biochemical and genetic studies gave valuable information on late steps of TI. However, observation of e/aIF5B bound to the SSU after start codon recognition and release of e/aIF2 was still missing. In particular, the question of how e/aIF1A and e/aIF5B cooperate to facilitate the joining step was left unanswered.

e/aIF1A and e/aIF5B cooperate to favor ribosomal subunit joining

One difficulty in isolating an SSU:e/aIF5B complex is related to the labile binding of e/aIF5B to the small ribosomal subunit, as shown for the yeast system [26, 30]. However, very recently, two cryo-EM structures of mammalian initiation complexes containing eIF5B and eIF1A were determined [25, 60] as well as that of the corresponding archaeal complex [55]. After describing the archaeal and the eukaryotic e/aIF5B-bound SSU initiation complexes, similarities and differences featuring the two domains of life will be discussed.

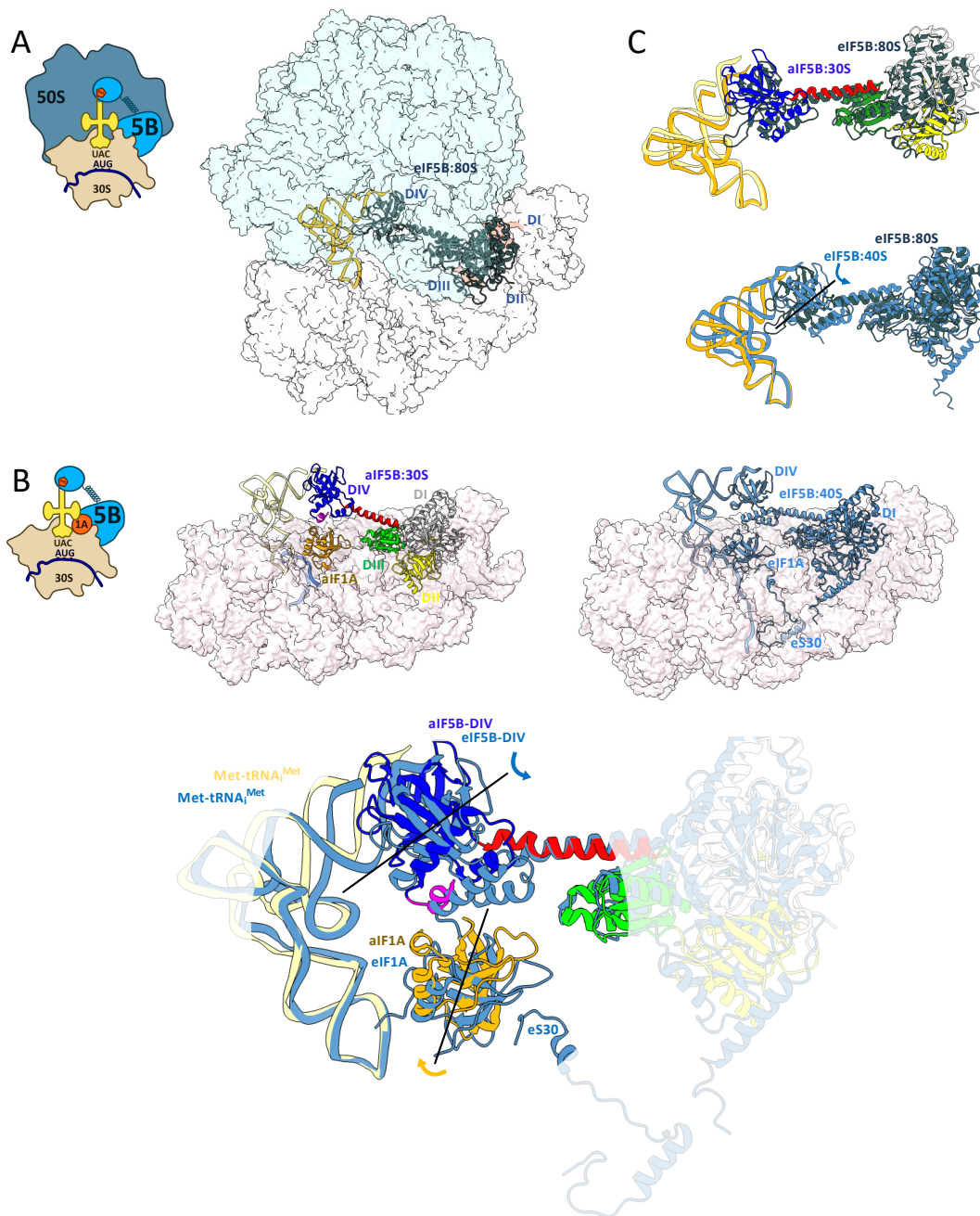


Figure 4: e/aIF5B in the late steps of translation initiation.

A. Binding of eIF5B to the ribosome. The cryo-EM structure of the yeast eIF5B-bound 80S is shown with the 60S and 40S as transparent molecular surfaces, eIF5B in blue and Met-tRNA^{Met} in orange (PDB ID Code 6W00; [30]). The pink patches indicate the h5-h15 region and the h14-h5-uS12 region on the SSU that contact eIF5B.

B. Binding of e/aIF5B to the SSU. Left: cryo-EM structure of the archaeal aIF5B-bound 30S structure with aIF5B and aIF1A shown as cartoons using the same color code as in Figure 2. The mRNA is shown in blue, (PDB ID Code 7ZAH; [55]). Right: cryo-EM structure of the rabbit eIF5B-bound 40S structure (PDB ID Code 7SYW; [60]). eIF5B, eIF1A, eS30 and Met-initiator tRNA are shown in blue and labeled. This structure is highly homologous to that of the human complex (PDB ID Code 7TQL; [25]). The superimposition of the two e/aIF5B:Met-tRNA^{Met}:e/aIF1A structures, obtained after alignment of the SSU rRNAs, is shown below. The differences between the two structures are highlighted (see text). Position of eIF1A is rotated by 18° and translated by 4.6 Å deeper in the A site, as compared to aIF1A. Position of eIF5B domain IV is rotated by 25° and translated by 8.8 Å as compared to that of aIF5B domain IV.

C. (Upper part) Superposition of *S. cerevisiae* eIF5B:Met-tRNA^{Met}-bound 80S structure on archaeal aIF1A:aIF5B:Met-tRNA^{Met}-bound 30S structure, obtained after alignment of the SSU rRNAs. aIF5B-domain IV is rotated by 6° and translated by 4.5 Å. (Lower part) Superposition of *S. cerevisiae* eIF5B:Met-tRNA^{Met}-bound 80S structure on mammalian eIF1A:eIF5B:Met-tRNA^{Met}-bound 40S structure, obtained after alignment of the SSU rRNAs. eIF5B-domain IV is rotated by about 32° and translated by 6 Å.

The cryo-EM structure of the archaeal IC (30S:mRNA:aIF1A:aIF5B:GDPNP:Met-tRNA_i^{Met}) was obtained using chemical crosslinking to deal with the dynamic binding of aIF5B to the SSU. A structure at an overall 2.7 Å resolution was obtained [55]. aIF5B is bound to GDPNP and the switch regions are ON. Domains II and III are bound to the 30S as expected from the yeast eIF5B-bound 80S structures [30] (Figure 4A and B). Domain I does not contact the 30S and it is pre-positioned to fit the GTPase activating center of the LSU after joining. Domain IV of aIF5B, interacts with the aminoacylated end of the tRNA and also contacts aIF1A (Figure 4B and Figure 5A). Interaction between domain IV of aIF5B and aIF1A is mediated by the archaeal specific $\alpha 15$ helix. This helix is packed onto a shallow groove delineated by the short C-terminal $\alpha 3$ helix of aIF1A on the one side and by the $\beta 2$ - $\beta 3$ loop on the other side (Figure 5A). The surface of interaction is about 330 Å². Comparison of structural classes isolated during cryo-EM data processing showed that interaction of aIF5B with the 30S and aIF1A is required to maintain the position of the initiator tRNA [55]. Moreover, comparison of the archaeal aIF5B-bound 30S structure to the yeast eIF5B-bound 80S structure shows that the initiator tRNA and aIF5B are already pre-positioned to allow efficient subunit joining (Figure 4C).

A human 40S:eIF1A:eIF5B complex was captured after a short-timed incubation of a 48S PIC complex with eIF5B in the presence of ATP and GTP under conditions defined by single-molecule kinetic studies [25]. On another hand, a 40S initiation complex containing eIF1A, eIF5B and the HCV IRES mRNA was assembled using ribosomes and initiation factors isolated from rabbit reticulocytes [60]. These structures were refined to global resolutions around 3.2 Å. Remarkably, the positionings of eIF1A, eIF5B and the initiator tRNA were very similar in the two mammalian structures (rmsd=0.96 Å for 512 atoms compared). eIF5B domains II and III contact the 40S as expected from the yeast eIF5B-bound 80S structures (Figure 4A-B). Domain I is bound to GTP and pre-positioned on the solvent side to reach the GTPase activating center of the LSU upon joining. Domain IV of eIF5B interacts with the methionylated end of the tRNA and with eIF1A. The two C-terminal helices of eIF5B ($\alpha 13$ and $\alpha 14$) contact the $\beta 2$ - $\beta 3$ loop and the C-terminal helix of eIF1A (Figure 4B and 5B). The surface of interaction is slightly smaller than in the archaeal structure (~150 Å² vs 330 Å² for the archaeal case). Surprisingly, the C-terminal tail of eIF1A carrying the DIDDI sequence previously shown to be important for the

interaction between the two factors [27, 46, 56, 59, 71, 79] was not visible in the cryo-EM maps. Possibly, binding of eIF1A DIDDI sequence to eIF5B has a dynamic character.

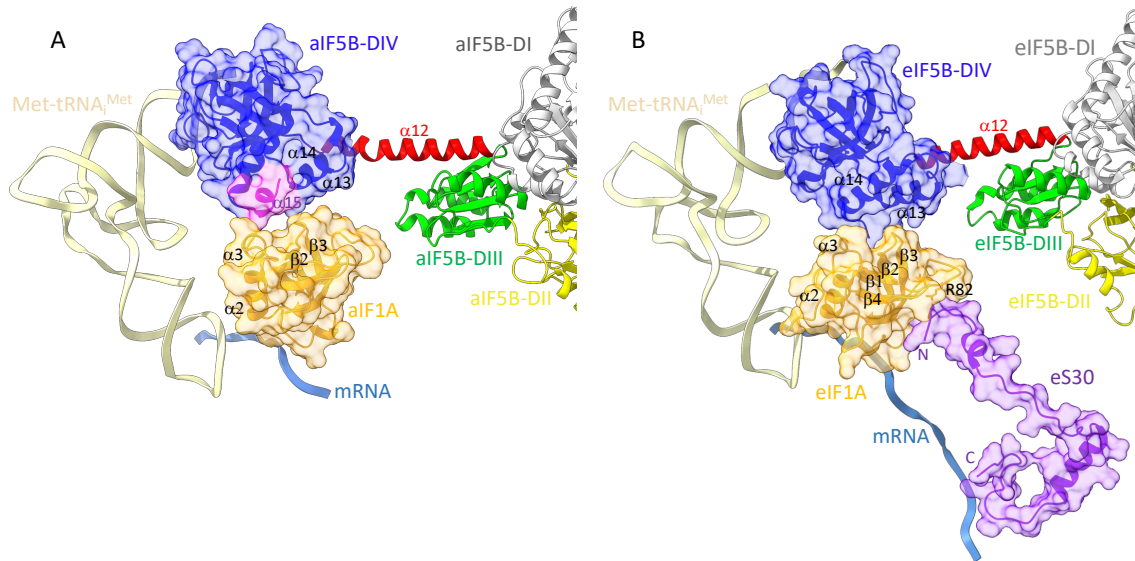


Figure 5: Interaction of e/aIF5B with e/aIF1A.

A-Closeup of the archaeal aIF5B-bound 30S structure (PDB ID Code 7ZAH:[55]). Interaction between domain IV of aIF5B and aIF1A is mediated by the archaeal specific $\alpha 15$ helix. This helix is packed onto a shallow groove delineated by the short C-terminal $\alpha 3$ helix of aIF1A on the one side and by the $\beta 2$ - $\beta 3$ loop on the other side. For clarity, only 9 nucleotides of the mRNA are shown.

B-Closeup of the mammalian eIF5B-bound 40S structure (PDB ID Code 7JYW:[60]). As compared to the archaeal case, the position of eIF5B-domainIV is more oriented towards the A site (see also Figure 4B). As a consequence helices 13 and 14 of eIF5B contact eIF1A. In the eukaryotic complex, eS30 interacts with eIF1A at the level of the eukaryotic signature sequence $^{81}\text{LR}_{82}$ (*H. sapiens* numbering) and with residues before $\beta 1$. Interestingly, eS30 is not present in *P. abyssi* and in most archaea although it is present in TACK and Asgard [11].

Even if the architectures of the archaeal and eukaryotic late SSU ICs are very similar (Figure 4B), superimposition of the structures highlights some differences in the positionings of e/aIF1A, of domain IV of e/aIF5B and of the Met-initiator tRNA (Figure 4B and 5). In particular, eIF1A is rotated and positioned more deeply into the A site, as compared to aIF1A. Position of domain IV of eIF5B is adjusted accordingly to maintain its interaction with eIF1A. Concomitantly, although the stem-loop anticodon regions of the initiator tRNAs are very well superimposed, the upper part of the initiator tRNA is more tilted towards the A site to maintain its interaction with eIF5B domain IV (Figure 4A). Finally, comparison of the eIF5B-bound 40S structures with the eIF5B-bound 80S structure highlights readjustments of the tRNA and domain IV of eIF5B occurring upon LSU assembly (Figure 4B).

eIF1A and aIF1A differ by the presence of long mobile eukaryotic-specific N and C-terminal tails (Figure 2C). In 48S complexes, the N-terminal tail of eIF1A stabilizes the codon:anticodon interaction in the P site [65, 69, 70]. In the mammalian eIF5B-bound 40S structures, the N-terminal tail

of eIF1A is not visible but its likely localization in the mRNA channel may influence the positioning of the core domain of eIF1A. On another hand eIF1A is stabilized by the N-terminal tail of eS30 (Figure 4A and 5B). Interestingly, eS30 is not present in *P. abyssi* and in most archaea [11]. Thus, the absence of eS30 may partly explain why the position of aIF1A is slightly different from that of eIF1A. To conclude, in eukaryotes and in archaea, e/aIF1A and e/aIF5B cooperate to maintain the initiator tRNA in an adequate position to facilitate joining with the large ribosomal subunit. However, some adjustments in the position of the initiator tRNA, e/aIF5B and e/aIF1A are visible in the two domains. These adjustments are likely to be linked to domain specificities.

Domain movements in e/aIF5B

Comparison of the available cryo-EM and X-ray crystallographic structures of e/aIF5B show that domains of e/aIF5B adopt a large variety of positions relative to domain I. A recent analysis shows that binding of aIF5B to the SSU causes a final adjustment of the positions of domains II and III with respect to domain I. This final adjustment would facilitate the binding of e/aIF5B to the GTPase center and facilitate subunit joining [55]. After GTP hydrolysis and release of the Pi group, movements of the switch regions in concert with the large movement of the β 13- β 14 loop in domain II (Figure 2) would trigger repositioning of domains II and III, contributing to e/aIF5B release from its SSU binding site. Moreover, transition of the switch 2 region to the OFF conformation would disrupt its interaction with helix 12. Because helix α 12 is linked to domain IV, this event would also contribute to destabilize the binding of domain IV to the initiator tRNA and to facilitate the departure of e/aIF5B from the LSU.

Comparison with the bacterial case

In bacteria, canonical translation initiation occurs on the SSU in the presence of an mRNA, a formyl-Met-tRNA_f^{Met} and three initiation factors, IF1, IF2, IF3. After start codon selection, the 50S subunit joins, triggering release of the initiation factors and final accommodation of the formyl-Met moiety of the initiator tRNA in the peptidyl-transferase center. Therefore, start codon selection and large subunit joining involve a same set of initiation factors and a single GTPase, IF2, responsible for the binding of the initiator f-Met-tRNA_f^{Met}. IF2 and IF1 are orthologous to e/aIF5B and e/aIF1A,

respectively (Figures 2 and 3). Cryo-EM studies have shown that GTP-bound IF2 adopts an extended conformation on the 30S subunit with domain IV of IF2 (domain C2, IF2 naming) interacting with the A₇₆-fMet moiety of the initiator tRNAs [80-84, 85, 86-88]. Interactions between IF1 and IF2 are also observed. However, these interactions occur differently from those in the eukaryotic and archaeal cases. Domain IV (or C2) of IF2 is shorter and does not contain the α 13 and α 14 C-terminal helices found in e/aIF5B (Figures 2, 3 and 6). Contacts between IF1 and IF2 do not involve IF2 domain IV (or C2) but are ensured by a bacterial-specific insertion in IF2- α 8 located between domain II and domain III (domain II and C1, IF2 naming) [80-84, 85, 87]. Like in eukaryotes and archaea, conformational changes within IF2 during the late steps of translation initiation facilitate the accommodation of fMet-tRNA_f^{Met} into the P site and IF2 dissociation from the initiator tRNA and from the ribosome [89, 90]. A recent cryo-EM study shows a compact form of IF2-GDP bound to the ribosome that could illustrate how domain rearrangements during GTP hydrolysis-related events would promote irreversible release from the initiator tRNA and IF2 dissociation [91].

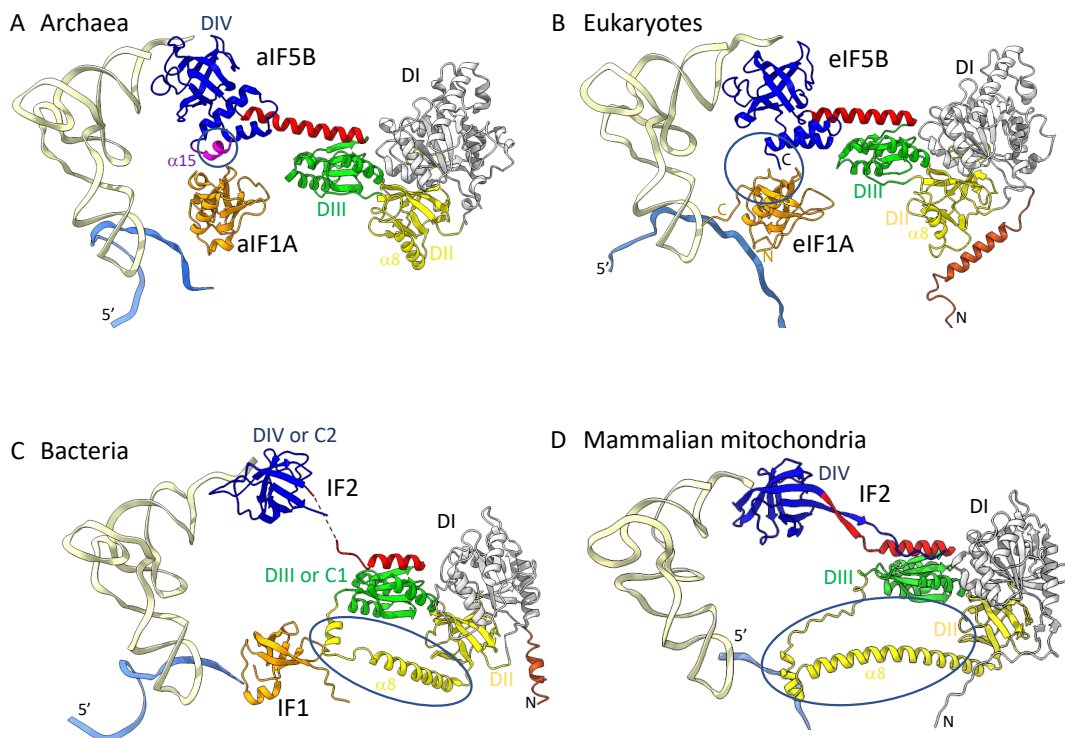


Figure 6: e/aIF5B/IF2 and e/aIF1/IF1 cooperate on the SSU to channel the initiator tRNA towards the LSU.

SSU rRNAs of all structures were superimposed for comparison.

A-The archaeal case is represented from the *P. abyssi* aIF5B:Met-tRNA_f^{Met}:aIF1A:30S complex (PDB ID Code 7ZAH; [55]).

B-The mammalian case is represented from the rabbit eIF5B:Met-tRNA_f^{Met}:eIF1A:40S complex (PDB ID Code 7SYW; [60]).

Only 30 residues (in brown) out of 620 in the N-domain were visible in the cryo-EM map.

C-The bacterial case is represented from the *T. thermophilus* 30S:IF1:IF2:f-Met-tRNA^{Met}:IF3 complex (PDB ID Code 5LMV); [84]. For the sake of clarity, IF3 is not represented.
D-The mammalian mitochondrial complex 30S:IF2:f-Met-tRNA^{Met} (porcine) is represented (PDB Code 6GAW; [92]). Domain specific features involved in the interaction between the two partners are encircled.

From an evolutionary point of view, it is also interesting to compare the late initiation complexes described above to those in mammalian mitochondria [92, 93]. In these organelles, IF1 is absent and IF2 has a very long insertion in the region of the $\alpha 8$ helix (Figure 6D). Part of this long insertion interacts with the A site thus playing a role similar to that of IF1 since it causes a supplementary contact of IF2 with the ribosome, blocks the decoding center and avoids mRNA slippage.

Concluding remarks

The occurrence of an interaction between e/aIF5B/IF2 and e/aIF1A/IF1 is universal, showing that there was an important evolutionary pressure that selected it. There are also domain-specific features that have evolved more lately. In all domains of life, the network of interactions involving the two initiation factors and the SSU helps to constrain the initiator tRNA in a conformation adequate for LSU joining, followed by the final accommodation of the acceptor end in the peptidyl transferase center. In this mechanism, the positioning of e/aIF1A/IF1 in the A site could also avoid mRNA slippage and thereby participate in the accuracy of translation initiation.

Therefore, one can imagine that ancestral initiation in the LUCA operated with the simplest versions of e/aIF5B/IF2 and e/aIF1A/IF1 to ensure start codon selection. Bacteria would then have evolved to formylate the Met-initiator tRNA while archaea and eukaryotes would have acquired e/aIF2. These two acquisitions have greatly improved specificity and efficiency of translation initiation. Later, in parallel with evolution of mRNA features, each domain of life would have developed its own strategies to regulate and further improve translation initiation mechanisms.

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