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Review

Eukaryotic and archaeal translation initiation factor 2: A heterotrimeric tRNA carrier

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ABSTRACT

Eukaryotic/archaeal translation initiation factor 2 (e/aIF2) is a heterotrimeric GTPase that plays a key role in selection of the correct start codon on messenger RNA. This review integrates structural and functional data to discuss the involvement of the three subunits in initiator tRNA binding. A possible role of the peripheral subunits in modulating the guanine nucleotide cycle on the core subunit is also addressed.

1. Introduction

Eukaryotic/archaeal initiation factor 2 (e/aIF2) has been characterized 30 years ago as a protein which forms a ternary complex with GTP and methionylated initiator tRNA, and subsequently binds to the small ribosomal subunit (e.g. [1–7]). eIF2 is composed of three subunits called α, β and γ [5,7], coded by *SUI2* [8,9], *SUI3* [10] and *GCD11* [11] genes, respectively. *SUI1* (coding for eIF1), *SUI2* and *SUI3* were first identified using a genetic reversion analysis of initiation codon mutations at the *HIS4* gene in yeast. This selection procedure was aimed at identifying specific interactions between pre-initiation complex and mRNA that mediate ribosomal recognition of a start codon [12]. Mutations in *GCD11* (GCD: general control derepressed) were first isolated because they alter translation efficiency at the transcription activator *GCN4* (GCN: general control non-inducible) AUG codon [11,13]. Later, a *gcd11* mutant was shown to suppress a mutant *his4* allele that lacks a functional AUG start codon [14]. Therefore, in addition to their role in delivering Met-tRNA$_i^{Met}$ to the ribosome, the three subunits of eIF2 also function in selecting the correct translational start site.

A 43S complex, comprising a ribosomal 40S subunit, eukaryotic initiation factors, 1, 1A, 3, 5 and eIF2:GTP: Met-tRNA$_i^{Met}$ binds to the 5′-capped end of mRNA with the help of eIF4s and scans downstream to the initiation codon to form a 48S complex. When the correct pairing between the initiation codon and the initiator tRNA anticodon is checked, GTP-bound to eIF2 is irreversibly hydrolyzed. eIF2–GDP then dissociates from the initiator tRNA and from the ribosome. Initiator tRNA lies in the P-site and after joining with the 60S ribosomal subunit catalyzed by eIF5B, the ribosome is committed to the elongation step. Therefore, irreversible GTP hydrolysis on eIF2 controls the accuracy of the translation initiation process, preventing initiation at non-AUG codons [15]. eIF1 and the GTPase activating protein (GAP) eIF5 participate in the control of this checking step [16–19]. After release from the ribosome, eIF2–GDP is further regenerated in eIF2–GTP through the action of a heteropentameric guanine nucleotide exchange factor, eIF2B. The exchange reaction is an important target for the control of translation. Indeed, inhibition of eIF2B activity prevents eIF2 recycling, thereby reducing rates of translation initiation and cell growth.

2. Eukaryotic and archaeal structural characteristics of e/aIF2

The genes coding for each subunit of eIF2 were shown to be essential for yeast cell viability [8,10,11]. These three subunits have orthologs in archaea, and the corresponding heterotrimeric factor was therefore named aIF2 [20,21]. However, archaea have no equivalent of the catalytic subunit of eIF2B (eIF2Bε and of eIF5). Therefore, GTP hydrolysis on aIF2 is likely to occur without GAP assistance, and the recycling of aIF2–GDP into aIF2–GTP is thought to be spontaneous.

2.1. The α subunit

Structural organization of α is conserved in eukaryotes and archaea, except that eukaryotic α subunits possess an acidic extension at the C-terminus of the protein (Fig. 2A). The structure of isolated domains of e/aIF2α and that of the entire protein were determined [22–26]. e/aIF2α is composed of three domains: an

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N-terminal β-barrell, followed by a helical domain, and by an α–β domain (Fig. 1A). Domains 1 and 2 form a rigid body linked to a mobile third domain. In eukaryotes, a serine residue (S51 in yeast) within a loop of domain 1 is the target of many types of kinases. As we will see below, phosphorylation of this residue is crucial for translational control. In archaea, the serine residue equivalent to S51 is not conserved. However, possible phosphorylation of a neighbor serine residue was proposed [27].

2.2. The β subunit

eIF2β and aIF2β differ in the presence of two additional domains in the eukaryotic version. At the N-terminus of the protein, a domain containing three lysine-rich boxes was shown to be involved in the binding to the C-terminal domains of two eIF2 partners, eIF5 and eIF2Bε [28,29]. Therefore, the absence of the N-terminal domain in archaea is likely to be related to the absence of eIF2Bε and eIF5 orthologues. The eukaryotic C-terminal extension is short (about 15 residues). So far, no role was assigned to this part of the protein. The 3D structure of aIF2β was solved by NMR and X-ray crystallography [30–33]. In the crystalline structures, β is bound to γ [32,33]. The conserved core of the β subunit is composed of three parts. An N-terminal α-helix (h1) is connected by a flexible linker to a central α–β domain, followed by a C-terminal zinc-binding domain. The N-terminal α-helix does not interact with the two other domains. The ZBD is packed onto the central α–β domain, with which it forms a rigid body (Fig. 1C).

2.3. The γ subunit, core of the heterotrimer

Two-hybrid interaction studies and GST pull down assays have shown that, in yeast, α and β are bound to the γ subunit but do not interact together [34,35]. Using purified archaeal versions of the three subunits, it was shown by in vitro assembly tests that γ is the core of the heterotrimeric protein, binding α and β which do not interact together [25,36,37]. Crystallographic studies of archaeal heterotrimeric proteins have confirmed this quaternary organization [33,38] (Fig. 2). Recent studies of human eIF2 have however reported interaction between α and β subunits [39,40].

eIF2γ and aIF2γ are homologous, except that the eukaryotic version contains an N-terminal domain that varies in length depending on the species (up to 90 residues). In *Saccharomyces cerevisiae*, this domain is not essential for function. However, a missense mutation in this domain has been reported to confer a slow growth phenotype [41]. In the rest of the protein, some sequence specificities of eukaryotic or archaeal IF2γ were noticed [37]. The sequence of eIF2γ contains all of the elements required for nucleotide binding. Therefore, it was proposed early that this subunit was sufficient for GDP and GTP binding. Moreover, sequence similarities between EF-Tu and eIF2γ have suggested that the γ subunit of eIF2 may also interact directly with the initiator tRNA [11,42].

2.4. Structure of aIF2γ, free or bound to guanine nucleotides

Structural homology between elongation factor Tu and eIF2γ was directly demonstrated by determination of the 3D structures of apo-aIF2γ from *Pyrococcus abyssi* (Pa-aIF2) [37], of apo-aIF2γ from *Methanococcus jannaschii* (Mj-aIF2) [43] and of apo-aIF2γ from *Sulfolobus solfataricus* (Ss-aIF2) [44].

aIF2γ shows three domains (Fig. 2B). Domain I (in yellow) contains the guanine nucleotide binding pocket delineated by the regions specifically encountered in all G-proteins (GKT loop, switch 1 and switch 2 regions, QNKIE and SALH sequences; Fig. 2). Switch 1 and switch 2 correspond to mobile regions. In all G-proteins, conformational changes of these two switch regions control the transition from an active GTP-bound state of the protein ("switch on") to an inactive GDP-bound state ("switch off") [45]. Domains II and III (in pale yellow and in orange, Fig. 2B) are β-barrels. These three domains are closely similar to those found in EF-Tu or eEF1A [46–49]. Superimposition of EF-Tu on aIF2γ has also allowed to evidence structural specificities of the initiation factor with regards to elongation factors (Fig. 2B, [37]). Hence, a zinc-binding domain

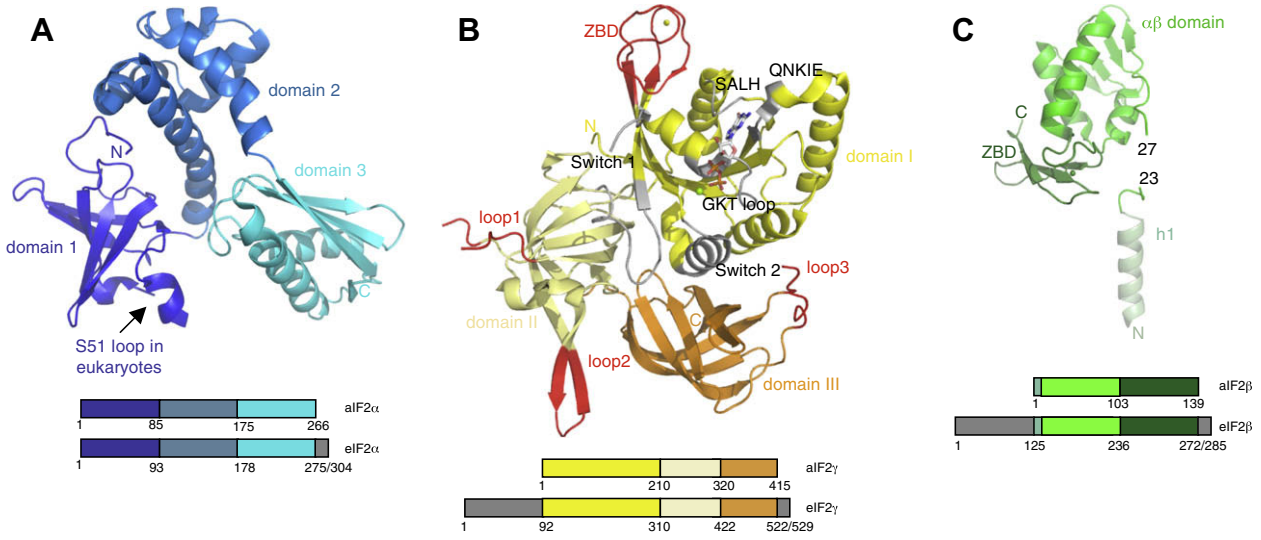

Fig. 1. Subunits of aIF2. (A) The α subunit. The three structural domains are colored as follows: domain 1 in dark blue, domain 2 in marine, and domain 3 in cyan. The loop carrying the S51 residue in eIF2α is indicated. The view is deduced from the structure of Ss-aIF2αγ (PDB ID 2AHO). (B) The γ subunit. The three structural domains are colored as follows: domain 1 in yellow, domain 2 in pale yellow, and domain 3 in orange. GDP is shown as sticks, Mg^{2+} as a green sphere, and Zn^{2+} as a yellow sphere. Regions involved in the binding of the nucleotide are colored in grey and labeled. Regions characteristic of the initiation factor are colored in red and labeled. The view is deduced from the structure of Pa-aIF2γ (PDB ID 1KK3). (C) The β subunit. The three structural domains are colored as follows: helix 1 in pale green, domain 2 in green, and domain 3 in dark green. Zn^{2+} is shown as a green sphere. Residues 23–27 are not visible.The view is deduced from the structure of Ss-aIF2α3βγ (PDB ID 2QMU). Below the cartoons are the schematic representations of e/aIF2 subunits. Colors of the boxes are related to the colors of the structural domains. For archaeal subunits, numbering is that of aIF2 from *S. solfataricus* and for eukaryotic subunits, numbering is that of eIF2 from *S. cerevisiae*. Domains specific of eukaryotic subunits are shown in grey.

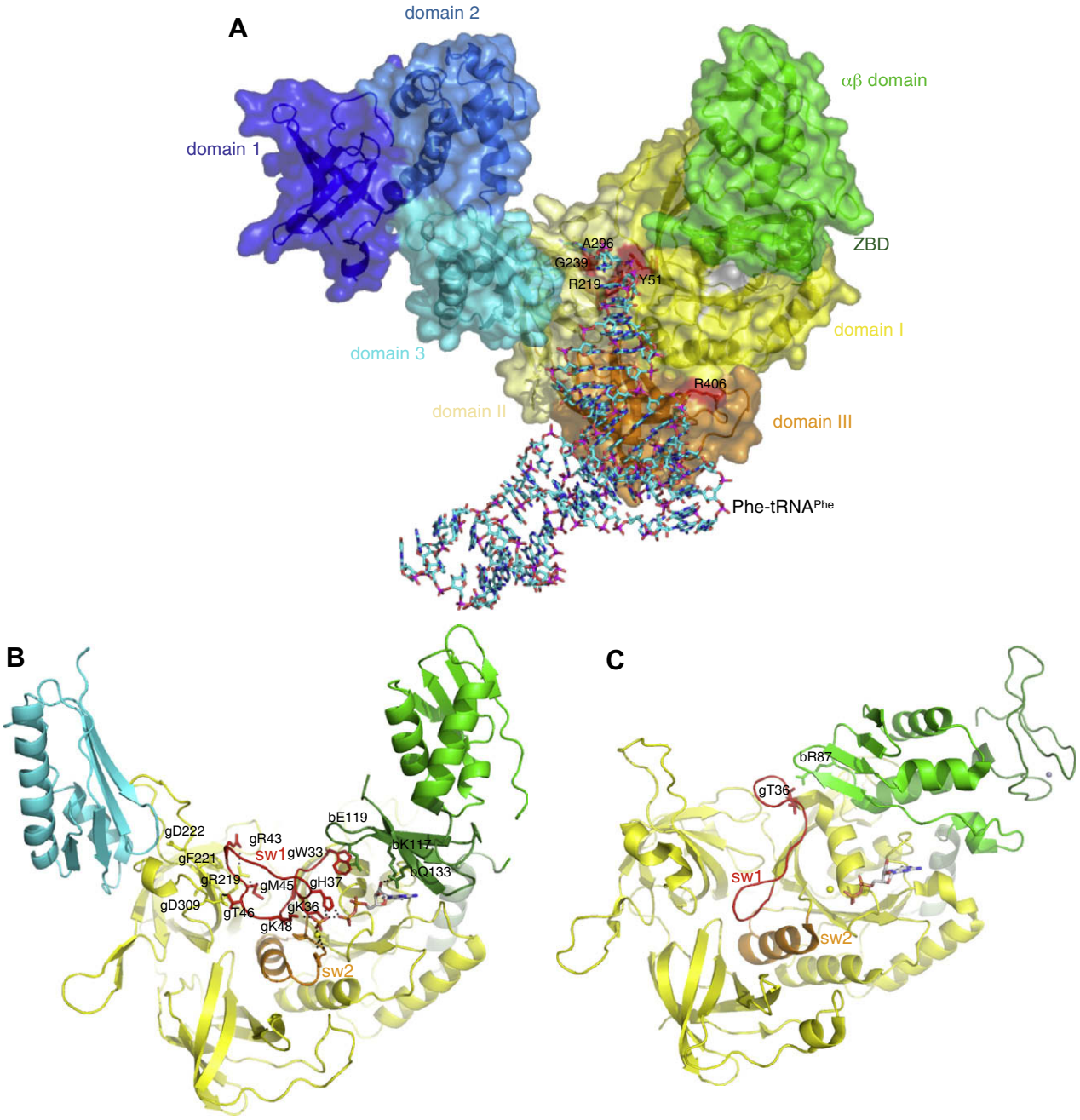

Fig. 2. (A) Docking of Phe-tRNA^{Phe} onto aIF2. A structure of the full aIF2αβγ heterotrimer is shown in the surface representation. This structure results from the positioning of domains 1 and 2 of aIF2α on the structure of Ss-aIF2α3βγ [33]. tRNA is drawn as sticks. The color code is the same as in Fig. 1. Red patches indicate residues important for tRNA binding. (B) Overall structure of Ss-aIF2α3βγ bound to GDP and Pi (PDB ID 2QMU) highlighting the switch 1-mediated communication between the three subunits. aIF2γ is colored in yellow, with switch 1 in red (31–49) and switch 2 in orange (93–113). The aIF2α3 domain (174–266) is in cyan. The aIF2β helix 1 (3–17) is in pale green. Residues 23–27 are not visible. The aIF2β central domain (28–103) is in green and the aIF2β ZBD (104–139) is in dark green. Relevant side chains are drawn. Switch 1 is colored in red and switch 2 is colored in orange. (C) Overall structure of Pf-aIF2βγ bound to GDP (PDB ID 2DCU). Color code is the same as in (B). Note that the conformation of the switch regions corresponds to "SW1 novel" and "SW2 on" in the case of Ss-aIF2α3βγ bound to GDP and Pi whereas both switch regions are "off" in the case of Pf-aIF2βγ bound to GDP-Mg^{2+}.

inserted within domain I, and protruding loops in domains II and III typify the initiation factor.

Crystal soaking experiments have allowed the determination of the 3D structures of Pa-aIF2γ complexed to GDP-Mg^{2+} or Gpp(NH)p-Mg^{2+} (a non-hydrolysable analogue of GTP) [37]. Moreover, the structure of GDP-Mg^{2+} bound to Ss-aIF2γ was obtained by co-crystallization [44]. In aIF2γ, the arrangement of the three domains is similar to that found in the active form of EF-Tu, bound to Gpp(NH)p-Mg^{2+} and aminoacylated tRNA [49]. The closed domain configuration is observed in all structures solved, thereby excluding the possibility that this conformation is due to lattice packing effects. Hence, this resemblance strongly argued in favor of the ability of isolated γ subunit to bind methionylated tRNA. However, only subtle conformational changes were observed when comparing the aIF2γ:GDP and GTP-bound forms to the apo form of the factor [37]. In particular the conformation of the two switch regions did not change but remained in the "off" state. Therefore, it appeared difficult to explain the GTP dependence of tRNA bind-

ing by an e/aIF2 trimer [1,4,42,50]. This raised the question of the possible roles of α and β subunits in tRNA binding.

3. tRNA binding by e/aIF2

By using archaeal aIF2 (Pa-aIF2 or Ss-aIF2), it was possible to easily purify individual subunits (α, β and γ) as well as heterodimers αγ, βγ and the heterotrimer αβγ. Protection-based assays were used to measure aminoacyl-tRNA binding to various forms of aIF2 [51]. Hence, it was shown that the isolated γ subunit of Pa-aIF2 is indeed able to bind methionylated initiator tRNA. Moreover, site-directed mutagenesis studies argue in favor of a binding mode of the tRNA molecule similar to that observed with the elongation factor [42,43,52]. The measured tRNA binding affinity for the isolated aIF2γ subunit is however highly reduced when compared to that obtained with the complete Pa-aIF2 heterotrimer (dissociation constants of 5 μM with γ alone and of 120nM with Pa-aIF2) [52]. Using Ss-aIF2γ alone, only a very weak protection was observed (K_d > 100 μM) whereas a K_d value of 1.5 nM is measured with complete Ss-aIF2. This difference in the behavior of the two archaeal versions of aIF2γ remains intriguing and may reflect the different conditions of growth of these two organisms [36,52]. Nevertheless, using Pa or Ss-aIF2 it was shown that the β subunit has only a small contribution whereas the α subunit provides the heterotrimer with almost its full tRNA binding affinity. Furthermore, the isolated C-domain of aIF2α, α3, responsible for binding to γ, is enough to retrieve the same binding affinity of tRNA as full aIF2 [25,52].

In eukaryotes, Met-tRNA$_i^{Met}$ K_d values for eIF2–GTP, determined by using nitrocellulose-binding assays, are in the 10 nM range [42,50,53]. The role of eIF2α and eIF2β subunits in tRNA binding remains controversial. Rabbit reticulocytes eIF2αγ heterodimers were shown to be unable to bind initiator tRNA therefore suggesting a crucial role of β in tRNA binding [54]. However, a number of earlier studies found little or no difference in Met-tRNA$_i^{Met}$ binding properties of mammalian eIF2 containing or lacking the β subunit [55–57]. In all cases, preparations lacking eIF2β were obtained as side-products during purification of eIF2, or were generated from purified eIF2 factor after proteolytic degradation of the β subunit. In the study of [54], the absence of β was checked using eIF2β antibodies from different sources. On another hand, isolation of βγ heterodimer during purification of rabbit reticulocyte eIF2 also suggested that α is not strictly required for Met-tRNA$_i^{Met}$ binding [58,59]. Finally, the construction of a yeast strain completely lacking eIF2α, allowed purification of eIF2βγ heterodimer. Therefore, it was shown that α contributes only slightly to tRNA binding affinity (no more than a factor of 5) [60]. β would then have an important role in tRNA binding.

In summary, a "eukaryotic behavior", with a major role for the β subunit in the binding of the tRNA and a minor role for the α subunit would be opposed to an "archaeal behavior" in which α has the major contribution.

The main nucleotidic identity element of eukaryotic and archaeal initiator tRNAs consist of an A1:U72 base pair in the acceptor stem, as opposed to a G1:C72 base pair found in most elongator tRNAs [50,52,61–63]. Moreover, the presence of a methionine group esterified to the tRNA is essential for its interaction with the initiation factor [50,52,64]. It was proposed that the A1:U72 base pair is critical for positioning the methionine moiety on the CCA end of the tRNA in its recognition pocket in eIF2 [50]. Other important characteristics of eukaryotic initiator tRNA correspond to the presence of a C3–G70 base pair within the acceptor stem, A54 and A60 in the TΨC loop (instead of T54 and pyrimidine 60 found in virtually all elongator tRNAs) and three consecutive GC base pairs within the anticodon stem [65]. The alteration of either one or both of the initiator-specific G:C base pairs at positions 29:41 and 31:39 in the anticodon stem of tRNA$_i$, or alteration of positions 54 and 60 in the T-loop had virtually no effect on the affinity of the resultant tRNA$_i$s for yeast eIF2:GTP [66].

4. The "on conformation of aIF2γ is observed in the structure of aIF2αγ heterodimer

The crystallographic structure of an Ss-aIF2αγ heterodimer bound to Gpp(NH)p-Mg^{2+}, able to bind Met-tRNA$_i^{Met}$ with almost full affinity, was refined at 3.0 Å resolution. The C-terminal domain of aIF2α (α3) interacts with domain II of aIF2γ. Two loops of α3 are buried in two pockets at the surface of γ domain II. One of these two pockets is bordered by the specific L1 loop (Figs. 1 and 2) [25]. Hence, the structure of the interface between the two subunits convincingly explains the behavior of mutants of α and γ subunits [43,52].

On another hand, in the γ subunit, the two switch regions involved in the binding of Gpp(NH)p-Mg^{2+} are in the "on" conformation, like in the structure of EF-Tu:Gpp(NH)p:Phe-tRNA^{Phe} [49]. Therefore, from the superimposition of aIF2γ on the active form of EF-Tu, a tight docking model of Met-tRNA$_i^{Met}$ binding to aIF2 was deduced. In particular, the altered position of switch 1 opens a channel between the G domain and domain II of the γ subunit to accommodate the methionyl group esterified to tRNA [25]. According to this model, the terminal A76 base is in a pocket formed by strands at the surface of domain II of aIF2γ. Y51, which belongs to switch 1, is stacked against the methionine group and residues R219, G239 and A296 contribute to the binding of the methionine and to that of the terminal adenosine (Fig. 2). These residues correspond to Y142, R319, G339 and G397 in yeast. Y142, R319 and G397 were indeed shown to be important for tRNA binding [14,42,43].

In order to bind Met-tRNA$_i^{Met}$ efficiently, eIF2 must be in the GTP-bound state [1,4,42,50]. Indeed, in yeast and in *S. solfataricus* the eIF2: Met-tRNA$_i^{Met}$ binding affinity is respectively 20 and 80 times larger in the presence of GTP than GDP [25,50]. In agreement with these results, in the Ss heterodimer, formation of a precise tRNA site is likely to result from the movement of the two switch regions also involved in the binding of Gpp(NH)p [25]. This convincingly explains the observed GTP dependence of Met-tRNA$_i^{Met}$ binding. Nevertheless, it should also be noted that the GTP dependence of Met-tRNA$_i^{Met}$ binding for e/aIF2 is less pronounced than that observed for EF-Tu. In this view, in the case of yeast eIF2, the GTP-bound state was proposed to allow positive interactions with the methionine group, whereas the binding site corresponding to the body of the tRNA was supposed to remain accessible in the two nucleotidic states [50]. This contrasts with EF-Tu, in which the GDP-bound state would not contain a binding site for the aminoacylated tRNA [67].

The EF-Tu based docking mode shows no direct contact between the α subunit and the tRNA molecule. Therefore, the participation of aIF2α in tRNA affinity is thought to be indirect. Indeed, a direct contact between α and the tRNA would require a large distortion of the αγ interface. Such a distortion seems unlikely, given the apparent rigidity of this interface. Rather, one possibility would be that the aIF2α subunit helps aIF2γ to maintain the switch in the "on" conformation. Several observations favor this idea. (i) The mutation (Y227A) of an aIF2α residue located at the interface with aIF2γ has a weak but significant negative effect on tRNA binding affinity. (ii) An of aIF2γ mutant, deleted of loop 1, becomes unable to bind aIF2α or tRNA. (iii) The only structure of aIF2γ showing the switch "on" conformation when Gpp(NH)p is bound is that of γ bound to α [25]. Moreover, it was observed that GTP binding affin-

ity is greater for aIF2 than for the γ subunit alone [36]. Finally, a recent thermodynamic study showed that the interactions between the α and γ subunits varied in the presence of Gpp(NH)p [68].

It should however be noted that an alternative docking model for the binding of the tRNA molecule on Ss-aIF2γ was proposed [44]. This model relies on the identification of two PPi sites within domain II of aIF2γ that would mimic the end of the acceptor stem of the tRNA. The acceptor stem of the initiator tRNA would be perpendicular to that of tRNA in the EF-Tu–Gpp(NH)p–Phe-tRNA^{Phe} complex. The elbow and T stem of Met-tRNA$_i^{Met}$ in this position would make extensive contact with the α subunit of aIF2. Up to now, no experimental data have been added in favor of such a model.

5. β Subunit lies on the side of the nucleotide binding pocket

Recently, three crystallographic structures in which the β subunit is bound to γ were obtained: that of aIF2βγ heterodimer from *P. furiosus* (Pf-aIF2βγ in the apo form or bound to GDP) [32], that of aIF2α3βγ from *S. solfataricus* obtained in the presence of GDP (Ss-aIF2α3βγ) [33] and that of the entire αβγ heterotrimer from *S. solfataricus* (Ss-aIF2αβγ) [38]. In the three structures, the N-terminal α-helix of β is wedged between two α helices of the G-domain of γ (Figs. 1 and 3). This interaction is sufficient to insure tight binding of β to γ, consistently with site-directed mutagenesis in yeast [34]. In Ss-aIF2αβγ, the rest of the β subunit has a highly flexible structure and does not contact eIF2γ [38]. However, comparison of the structures of Ss-aIF2α3βγ with that of Pf-aIF2βγ indicates two modes of binding of the β subunit onto the γ one (Fig. 2). In Pf-aIF2βγ, the central domain of β is packed onto γ with the ZBD exposed to the solvent and making no contact with γ. In the Ss-aIF2α3βγ structure, the central domain of β has no contact with γ whereas the ZBD is close to the nucleotide binding site on γ. In the two structures, the β subunit is in contact with γ through switch 1 (Fig. 2). Therefore, even if it cannot be excluded that these two modes of binding are induced by the crystallization conditions, it is worth considering the possibility that the two resulting structures of aIF2β reflect snapshots of the aIF2β conformations in aIF2 at different steps of the nucleotide cycle. An important contribution of β to the function of yeast eIF2 is indicated by analysis of mutations that allow translation initiation at non-AUG codons [34,69,70]. Functionally important mutations in β map to the vicinity of the zinc-binding region, within the C-terminus of the protein, or in the α1 helix [15,35]. Among these, two mutations increase the intrinsic GTPase activity of eIF2, independently of the presence of eIF5 or of that of the ribosome [15]. These mutations, L254P and S264Y, affect residues that correspond to aIF2β-S122 and aIF2β-A132. In the structure of Ss-aIF2α3βγ, these two residues are at the surface of β, close to the nucleotide binding site of γ [33]. This argues in favor of the idea that the conformation of β observed in Ss-aIF2α3βγ has functional significance.

In Ss-aIF2α3βγ structure, the ZBD directly contacts the switch 1 region. As a result, switch 1 adopts a novel conformation. Moreover, unexpectedly for a GDP bound state, switch 2 has the "on" conformation (Fig. 2). The stability of the switch 1 novel conformation is accounted for by a ligand, most probably a phosphate ion, bound near the nucleotide binding site. The structure suggests that this novel GDP-Pi bound state of aIF2 may be proficient for tRNA binding. Transient occurrence of a distinct phosphate binding site on eIF2γ has already been predicted in the case of eukaryotes. The prediction was gained based on a biochemical analysis showing that Pi release from eIF2, and not GTP hydrolysis itself, was the actual step triggered by recognition of an AUG start codon [71]. The novel nucleotide state identified in aIF2 is indicative of a similar mechanism in archaea.

Finally, in the two structures, aIF2β is bound to aIF2γ far away from the putative tRNA binding site and no direct contact between β and the tRNA is predictable. In the case of aIF2, β has only a minor role in tRNA binding affinity. However, the contribution of this subunit is thought to be important in eukaryotes (see above). In this view, it should be reminded that eIF2β presents two additional domains as compared to aIF2β. Notably, the presence of the three lysine boxes in the N-terminal part of the β subunit does not influence the GTP-dependent initiator Met-tRNA$_i^{Met}$ binding by the eIF2 complex [72]. Therefore, the way by which eIF2β influences tRNA binding on eIF2γ remains to be determined.

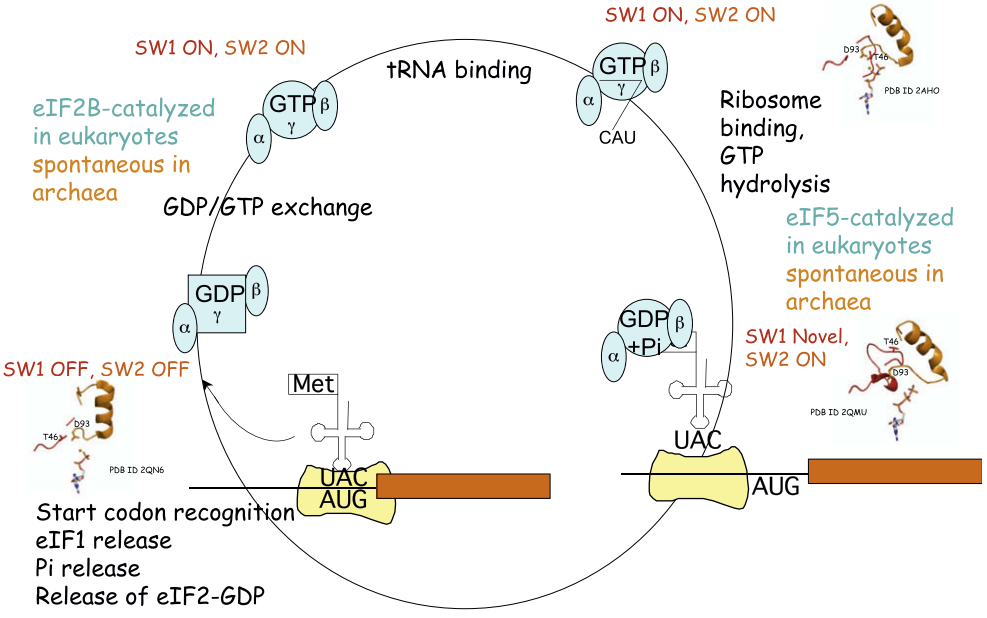

Fig. 3. Scheme of the nucleotide cycle on e/aIF2.

6. Role of eIF2 in translational control

One of the best characterized mechanisms of translational control in eukaryotic cells is associated with phosphorylation of Ser51 in the α subunit of eIF2. Following phosphorylation, the affinity of eIF2–GDP for eIF2B is much increased, and eIF2 is converted from a substrate to an inhibitor of the exchange factor. As a consequence, the pool of eIF2–GTP is rapidly depleted, and, on the whole, translation is inhibited in the cell. Because the intracellular concentration of eIF2B is small regarding that of eIF2, phosphorylation of a fraction of eIF2 is sufficient to inhibit protein synthesis.

The increased affinity of eIF2 for eIF2B has generally been interpreted to reflect very slow dissociation of the eIF2–eIF2B complex, resulting in the sequestering of eIF2B (e.g. [73]). Alternatively, it has been proposed that phosphorylation of eIF2α increases the rate of association of eIF2 with eIF2B, in such a way that phosphorylated eIF2 becomes a competitive, rather than an irreversible, inhibitor of the nucleotide exchange reaction [74]. This mechanism is further supported by in vivo overproduction experiments in yeast cells [75]. Notably, since Ser51 is likely to be in contact with eIF2B, the latter competitive mechanism allows for rapid reversion of translation inhibition upon production of an eIF2 phosphatase. Two such phosphatase activities, one constitutive (termed CReP) and the other inducible (PP1c-GADD34), have been evidenced in mammals [76].

Up to now, four types of kinases able to specifically phosphorylate Ser51 of eIF2α have been identified [77]. These proteins share a conserved kinase domain insuring high specificity for eIF2α [78]. These four kinases however respond to different stimuli. The most widespread one, GCN2, is controlled by uncharged tRNA that accumulates in case of shortage of any amino acid. The kinase activity of GCN2 is indeed triggered upon naked tRNA binding thanks in particular to a histidyl-tRNA synthetase-like domain [79]. PKR (for Protein Kinase RNA-dependent [80,81]), present in vertebrates, is induced by interferon and is activated upon double-stranded RNA binding. Such an activator frequently appears in the cell upon viral infection. Therefore, by inhibiting protein synthesis, the action of PKR participates in cell defense against viral propagation. The third kinase, PERK (for PKR-like Endoplasmic Reticulum Kinase [82], also called PEK for Pancreatic eIF2α Kinase [83]) is present in animals. PERK is activated in response to an ER stress, occurring in case of an imbalance in the lumen between unfolded proteins and chaperones. This allows coupling of the rate of protein synthesis in the cytoplasm to the folding capacity in the lumen [82]. Finally, the HRI (Heme Regulated Inhibitor) kinase [84] exists in vertebrates, and in a few other organisms such as *Schizosaccharomyces pombe* and *Bombyx mori*. HRI is activated upon heme deprivation in erythrocytes, as well as upon various oxidative stresses in other cell types.

Most interestingly, phosphorylation of eIF2α can also positively regulate translation of specific genes. This positive control was extensively studied in the case of *S. cerevisiae GCN4* (reviewed in [85]), a gene whose translation is induced upon the activation of GCN2. The GCN4 protein is a transcriptional activator of many genes encoding amino acid biosynthetic enzymes, aminoacyl-tRNA synthetases and pathway-specific activators [86]. In this way, phosphorylation of eIF2α triggers a global cellular response in yeast [87], reorienting the protein synthesis capacity toward production of charged tRNAs. The mechanism for *GCN4* translational control involves four short open-reading frames (uORF1-4; 2–3 sense codons each) upstream from that encoding GCN4 on the mRNA. Initiating 40S subunits scan the messenger RNA and translate uORF1. However, after translation termination, the 40S subunits can resume scanning with high frequency, thanks to specific features of uORF1 [88]. If the availability of eIF2–GTP-initiator tRNA ternary complex is high, such 40S subunits will rebind a ternary complex and translate one of the uORF2, uORF3 or uORF4. Because ribosomes do not resume scanning after having translated the latter ORFs, translation of the *GCN4* ORF is inhibited. On the contrary, in the presence of low amounts of ternary complex, after translation of uORF1, many 40S subunits will bind a ternary complex only after having bypassed the inhibitory uORF2-4, thereby reinitiating at the *GCN4* start codon.

Essentially the same mechanism has also been evidenced in higher eukaryotes in at least one case, that of the Activating Transcription Factor 4 (ATF4) [89,90]. This transcription factor may be considered as the metazoan counterpart of GCN4, and controls an "integrated stress response" including the expression of genes involved in amino acid metabolism and transport, as well as resistance to oxidative stress [91]. The function of ATF4 is however not limited to the stress response. A striking example is its clear involvement in the synaptic switch to the late phase of long-term potentialisation and long-term memory [92]. Indeed, ATF4 is a repressor of the cAMP response element binding protein-mediated gene expression, which is critical for long-term synaptic plasticity and memory [93].

More generally, an increasing number of important biological responses, such as the sensing of amino acid deficiency for dietary selection [94,95], are associated with eIF2α phosphorylation. This renders likely that beyond ATF4, other transcription factors may be activated by this phosphorylation, which clearly has broad biological consequences [96,97].

7. Conclusion and perspectives

Control of the nucleotide cycle of e/aIF2 is crucial for the accuracy of the initiation of translation. In eukaryotes, eIF5 and eIF2Bε, the catalytic subunit of eIF2B, modulate the nucleotide state of the heterotrimeric protein eIF2 (Fig. 3). Recent biochemical and structural data have given new ideas to explain how the control of the nucleotide cycle may occur.

Beside their ability to bind eIF2β, eIF5 and eIF2Bε are also able to contact the G-domain of eIF2γ [98,99]. Binding of eIF5 to γ involves its N-terminal part. This part comprises an α-β domain and a ZBD one homologous to those of aIF2β and probably adopting the same relative orientation [100]. In addition, eIF1, the other key factor involved in the nucleotide cycle of eIF2, also displays a structure similar to that of the α−β domain in e/aIF2β [101]. All these observations give credit to the idea that the three factors, eIF2β, eIF5 and eIF1, play concerted roles during the nucleotide cycle of eIF2 [33,100]. Indeed, because of their structural similarities, the factors may target the same region on the G domain of eIF2γ. Archaea possess an equivalent of eIF1. Thus, in the context of the above idea, one can imagine that, either in eukaryotes or in archaea, e/aIF1 release will be sufficient to trigger rearrangement of the full eIF2 structure leading to release of Pi and making codon selection irreversible.

e/aIF2 consists of a rigid central part, formed by the γ subunit, domain 3 of the α subunit and the h1 helix of the β subunit. Two mobile "wings" formed by domains 1 and 2 of the α subunit, and by domains α–β and ZDB of the β subunit, are appended to this central core. The mobility of the wings is probably crucial for the interaction of e/aIF2 with the ribosome, with the mRNA and/or with e/aIF2 partners. Results of UV cross-linking experiments and of assays of 48S complex formation done using α-deficient eIF2 indicate that eIF2α is involved in recognition of initiation codon context, probably by interacting with nucleotide [−3] (with respect to AUG codon) of the mRNA. The resulting stabilization of the 48S complex is dependent on the presence of eIF1 [102]. On another hand, eIF2β interacts directly or indirectly with eIF5 and eIF1.

These two factors are essential for the control of the nucleotide cycle. The network of interactions depicted in Fig. 2C indicates that the switch 1 region may use the α and β subunits as relays to exchange information with the ribosome, with mRNA, and with eIF1 and eIF5. In addition to biochemical and genetic studies, structural studies of translation initiation complexes are required to understand the central function played by e/aIF2 in faithfully deciphering the start codon on mRNA.

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