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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

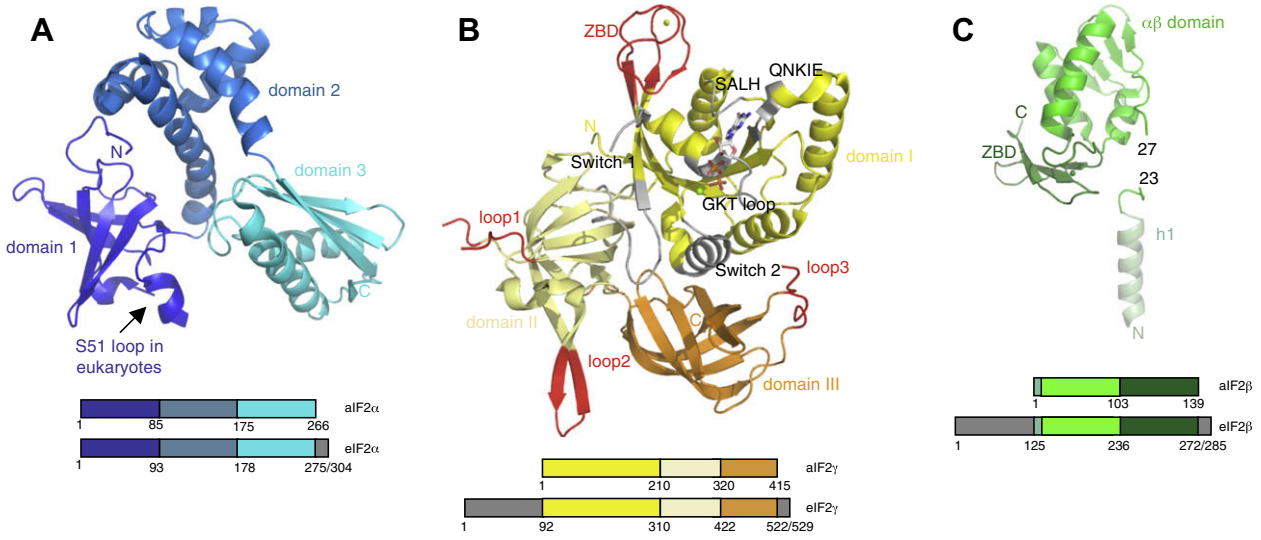

Fig. 1. Subunits of aIF2. (A) The α subunit. The three structural domains are colored as follows: domain 1 in dark blue, domain 2 in marine, and domain 3 in cyan. The loop carrying the S51 residue in eIF2α is indicated. The view is deduced from the structure of Ss-aIF2αγ (PDB ID 2AHO). (B) The γ subunit. 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.