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# Roles of yeast $eIF2\alpha$ and $eIF2\beta$ subunits in the binding of the initiator methionyl-tRNA

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## ABSTRACT

Heterotrimeric eukaryotic/archaeal translation initiation factor 2 (e/aIF2) binds initiator methionyl-tRNA and plays a key role in the selection of the start codon on messenger RNA. tRNA binding was extensively studied in the archaeal system. The  $\gamma$  subunit is able to bind tRNA, but the  $\alpha$  subunit is required to reach high affinity whereas the  $\beta$  subunit has only a minor role. In Saccharomyces cerevisiae however, the available data suggest an opposite scenario with  $\beta$  having the most important contribution to tRNA-binding affinity. In order to overcome difficulties with purification of the yeast  $elF2\gamma$ subunit, we designed chimeric eIF2 by assembling yeast  $\alpha$  and  $\beta$  subunits to archaeal  $\gamma$  subunit. We show that the  $\beta$  subunit of yeast has indeed an important role, with the eukaryote-specific N- and C-terminal domains being necessary to obtain full tRNA-binding affinity. The  $\alpha$  subunit apparently has a modest contribution. However, the positive effect of  $\alpha$  on tRNA binding can be progressively increased upon shortening the acidic C-terminal extension. These results, together with small angle X-ray scattering experiments, support the idea that in yeast eIF2, the tRNA molecule is bound by the  $\alpha$  subunit in a manner similar to that observed in the archaeal alF2-GDPNP-tRNA complex.

## INTRODUCTION

In eukaryotic and archaeal cells, the initiator tRNA carrier is the eukaryotic/archaeal translation initiation factor 2 (e/aIF2) heterotrimer. In its GTP-bound form, this factor specifically binds Met-tRNA<sub>i</sub><sup>Met</sup> and handles it in the translation initiation complex. After start codon recognition, the factor, in its GDP-bound form, loses affinity for Met-tRNA<sub>i</sub><sup>Met</sup> and eventually dissociates from the initiation complex. This leaves Met-tRNA<sub>i</sub><sup>Met</sup>

in the P-site of the small ribosomal subunit and allows the final steps of initiation to occur (1,2). In this process, specific binding of the initiator tRNA by e/aIF2 is crucial for accuracy. In both archaea and eukaryotes, the  $K_d$ values of the e/aIF2–GDPNP–Met-tRNA<sub>i</sub><sup>Met</sup> complexes are in the nanomolar range (3–8).

e/aIF2 results from the association of three subunits,  $\alpha$ ,  $\beta$  and  $\gamma$ . In archaea, the heterotrimer consists of a rigid central part, formed by the  $\gamma$  subunit, the C-terminal domain 3 of the  $\alpha$  subunit and the N-terminal helix of the  $\beta$  subunit. Two mobile parts formed by domains 1 and 2 of the  $\alpha$  subunit, and by the  $\alpha$ - $\beta$  and the zinc-binding domains of the  $\beta$  subunit are appended to the central core (Figure 1A) (6,9–12).

Until very recently, based on high structural resemblance of the  $\gamma$  subunit with the elongation factor EF1A as well as on site-directed mutagenesis studies, it was believed that the binding mode of the tRNA molecule on aIF2 was similar to that observed with the elongation factor (3,7,9,13,14). However, biochemical results and determination of the 5-A crystal structure of archaeal Sulfolubus solfataricus IF2 (Ss-aIF2) bound to initiator methionyl-tRNA have broken this model (15,16). In the 3D structure of the aIF2-GDPNP-tRNA complex, the tRNA is bound by the  $\alpha$  and  $\gamma$  subunits of aIF2 (Figure 1A). aIF2 approaches tRNA from the acceptor stem minor groove side, whereas EF1A approaches tRNA from the T-stem minor groove side. Despite this, thanks to a kinked conformation, the acceptor end of the tRNA fits in a channel on  $aIF2\gamma$ , which corresponds to the tRNA acceptor end-binding channel on EF1A. This model clearly explains why the isolated  $\gamma$  subunit of archaeal aIF2 is indeed able to bind initiator methionyltRNA but with a binding affinity highly reduced when compared with the tRNA-binding affinity for the complete aIF2 heterotrimer. Indeed, the  $\alpha$  subunit provides the heterotrimer with almost its full tRNAbinding affinity, whereas the  $\beta$  subunit only slightly contributes to tRNA binding (5–7,15).

In contrast with archaeal aIF2, the construction of a *Saccharomyces cerevisiae* strain completely lacking  $eIF2\alpha$ 

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**Figure 1.** Eukaryotic and archaeal e/aIF2. (A) Cartoon representation of Ss-aIF2 in complex with GDPNP and Met-tRNA<sub>f</sub><sup>Met</sup>. The cartoon was drawn from PDB ID 3V11 (15). The color code is as follows: G-domain of  $\gamma$  ( $\gamma$ DI, 1–210) in green, domain II ( $\gamma$ DII, 211–327) in yellow, domain III ( $\gamma$ DIII, 328–415) in orange, domain 1 of  $\alpha$  in dark blue ( $\alpha$ D1, 1–85), domain 2 of  $\alpha$  in blue ( $\alpha$ D2, 86–174), domain 3 of  $\alpha$  in cyan ( $\alpha$ D3, 175–266). The N-terminal  $\alpha$  helix of the  $\beta$  subunit (3–19) anchored to  $\gamma$ DI is colored in pink. Note that the position of the rest of the  $\beta$  subunit (residues 33–139, encircled with a dotted line) is only a tentative model, derived from SAXS data (15). The figure was drawn with PyMOL (http://www.pymol. org). (B) Schematic structural organizations of e/aIF2  $\alpha$  and  $\beta$  subunits. The colored boxes indicate the structural domains. Specific eukaryotic domains and extensions are colored in orange. Gray bars symbolize the K-boxes in the N-terminal domain of yeast eIF2 $\beta$ .

and allowing purification of an eIF2 $\beta\gamma$  heterodimer has shown that, in yeast,  $\alpha$  would only slightly contribute to tRNA-binding affinity (no more than a factor of 5) (17), whereas  $\beta$  would have an important effect (18). From these data, an 'eukaryotic behavior', with a major role for the  $\beta$  subunit in the binding of the tRNA and a minor role for the  $\alpha$  subunit would be opposed to an 'archaeal behavior' in which  $\alpha$  has the major contribution. Hence, the possibility that the structural involvement of the peripheral subunits in the eukaryotic ternary initiation complex differs from that in the archaeal one cannot be completely excluded. Notably, eIF2 from the primitive eukaryote *Encephalitozoon cuniculi* displays an intermediate behavior with  $\alpha$  and  $\beta$  subunits equally contributing to tRNA-binding affinity (19). Functional dissection of *S. cerevisiae* eIF2 *in vitro* was impaired by the inability to produce the isolated  $\gamma$  subunit. However, very recently, we reported that a chimeric protein formed by assembling the  $\gamma$  subunit of *S. solfataricus* aIF2 with the  $\alpha$  subunit of *S. cerevisiae* eIF2 was useful to study the role of the yeast eIF2 $\alpha$ subunit (15). Therefore, chimeric proteins appear as an attractive tool for studying the role of the yeast peripheral subunits of eIF2 in tRNA binding. In the present study, using chimeric e/aIF2, we show that the  $\beta$  subunit of yeast has indeed an important role in tRNA-binding affinity. The N- and C-terminal domains of yeast eIF2 $\beta$  are necessary to obtain full tRNA-binding affinity, the C-terminal extension of  $\beta$  having the most important role. The effect of the  $\beta$  subunit on tRNA-binding affinity may be either direct or indirect. The intact  $\alpha$  subunit apparently only slightly contributes to tRNA binding. However, shortening of the acidic C-terminal extension revealed a positive effect of the  $\alpha$  subunit on tRNA binding (15). Here, we show that the negative effect of the C-terminal extension is directly related to the size of the acidic C-terminal tail. Moreover, the small-angle X-ray scattering (SAXS) diffusion curve obtained with the Ch-eIF2 $\alpha\Delta C\gamma$ -GDPNPmet-tRNA<sub>f</sub><sup>Met</sup> complex agrees well with the theoretical curve computed from the crystallographic structure of Ss-aIF2 $\alpha\gamma$  bound to GDPND and met-tRNA<sub>f</sub><sup>Met</sup>. Therefore, altogether, the results are compatible with the idea that, in yeast eIF2, the tRNA molecule is bound in an orientation similar to that observed in the archaeal aIF2– GDPNP–tRNA complex.

## MATERIALS AND METHODS

# Cloning, expression and production of yeast eIF2 $\alpha$ and $\beta$ subunits and their variants

The genes encoding the  $\alpha$  and  $\beta$  subunits of eIF2 from *S. cerevisiae* were amplified by PCR from genomic DNA and cloned in pET-derivative vectors. The gene coding for the  $\alpha$  subunit was cloned between the NdeI and BamHI restriction sites of pET15b. The resulting plasmid called pET15bY- $\alpha$  led to the expression of an N-terminally tagged version of yeast  $\alpha$  subunit in *Escherichia coli*. The gene coding for the  $\beta$  subunit was cloned between the NdeI and SacII restriction sites of pET3a to give pET3aY- $\beta$ . This plasmid allows expression of an unmodified version of yeast eIF2 $\beta$ .

Deletions in the target genes were carried using the QuikChange Site-directed Mutagenesis method (Stratagen). The full sequence of all mutated genes was confirmed by resequencing. Deletion of the C-terminal extension of yeast eIF2a was achieved using pET15bY-a to give various truncated forms. pET15bY- $\alpha\Delta$ C274 produced a protein ending at residue T274 (15). pET15bY- $\alpha\Delta$ C283 produced a protein ending at residue L283, pET15bY- $\alpha\Delta$ C292 produced a protein ending at residue S292 and pET15bY- $\alpha\Delta$ C298 produced a protein ending at residue E298. The DNAs coding for yeast aD3 or yeast  $\alpha D_{3}\Delta C_{274}$  were obtained by the QuikChange Site-directed Mutagenesis method with the introduction of a start codon at position 178.

Deletion of the C-terminal domain of yeast eIF2ß was achieved using pET3aY- $\beta$  to give pET3aY- $\beta\Delta C$ . The construction allowed expression of a  $\beta$  subunit ending at residue I271. pET15bY-ß allowed expression of an N-terminally tagged version of yeast eIF2<sub>β</sub>. pET15bY- $\beta \Delta N$  was a derivative of pET15bY- $\beta$  producing a β subunit deleted of its N-terminal domain (residues 1-125) His-tagged at its N-terminus. The doubly deleted mutant  $eIF2\beta\Delta N\Delta C$ expressed was from pET15b $\beta\Delta N\Delta C$ , a derivative of pET15b $\beta$ Y- $\Delta N$ . The resulting protein corresponded to an N-terminally histidine-tagged version of yeast eIF2 $\beta$  comprised of the core domain of eIF2 $\beta$ , from residue E126 to I271.

Each subunit was overexpressed separately in *E. coli* BL21 Rosetta pLacI-Rare (Merck, Novagen). One-liter

cultures were in 2xTY containing 50  $\mu$ g/ml of ampicillin and 34  $\mu$ g/ml of chloramphenicol. Expression was induced after an overnight culture at 37°C (OD<sub>650</sub> ~ 2.5) by adding 1 mM of IPTG. After induction, the cultures were continued for 5–6 h at 18°C.

#### Purification of chimeric eIF2

Overexpression of Ss-aIF2y subunit was performed as described (6). Cultures of cells each overproducing one of the three subunits (250 ml for yeast  $\alpha$ , 500 ml for yeast  $\beta$  and 500 ml for S. solfataricus aIF2 $\gamma$ ) were harvested, mixed in 80 ml of buffer A (10 mM HEPES pH 7.5, 500 mM NaCl, 3 mM 2-mercaptoethanol, 0.1 mM PMSF and 0.1 mM benzamidine) and disrupted by sonication. After centrifugation, the supernatant was loaded onto a column (3 ml) containing Talon affinity resin (Clontech) equilibrated in the same buffer. The resin was first washed with 50 ml of buffer A and then with 50 ml of buffer A supplemented with 10 mM imidazole. The fractions containing the heterotrimer were finally recovered after elution with buffer A containing 125 mM imidazole. To remove the excess of the yeast  $\alpha$  subunit, the affinity column eluate was diluted to 300 mM NaCl and then loaded onto a 5 ml S-Hiload column ( $10 \text{ mm} \times 5 \text{ cm}$ ; GE Healthcare) equilibrated in buffer B (10 mM HEPES pH 7.5, 300 mM NaCl, 10 mM 2-mercaptoethanol, 0.1 mM PMSF and 0.1 mM benzamidine). gradient Α from 300 mM NaCl to 600 mM NaCl was used for elution (128 ml at a flow rate of 2 ml/min). After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the fractions containing the heterotrimer were pooled and dialyzed against buffer B. Finally, the protein was concentrated by using Centricon 30 concentrators. Either GDPNP- $Mg^{2+}$  (1 mM) or GDP- $Mg^{2+}$ (1 mM) was added before storage at 4°C of the recovered heterotrimer (Figure 2, lane 1). The same procedure was applied to purify the Ch-eIF2 $\alpha\Delta C\beta\gamma$ , Ch-eIF2 $\beta\Delta N\gamma$  and Ch-eIF2 $\beta$  $\Delta$ N $\Delta$ C $\gamma$  (Figure 2, lanes 4, 6 and 5).

## Purification of Ch-eIF2 $\beta\gamma$ and Ch-eIF2 $\beta\Delta C\gamma$

Cultures of cells each overproducing one of the two subunits (500 ml for yeast  $\beta$  or yeast  $\beta \Delta C$  and 500 ml for S. solfataricus aIF2 $\gamma$ ) were harvested, mixed in 40 ml of buffer B (10 mM HEPES pH 7.5, 300 mM NaCl, 10 mM 2-mercaptoethanol, 0.1 mM PMSF and 0.1 mM benzamidine) and disrupted by sonication. After centrifugation, the supernatant was loaded onto a 15 ml S-Hiload column ( $16 \text{ mm} \times 20 \text{ cm}$ ; GE Healthcare) equilibrated in buffer B. A gradient from 300 mM NaCl to 600 mM NaCl was used for elution (120 ml at a flow rate of 2 ml)min). After SDS–PAGE analysis, the fractions containing the heterodimer were pooled and dialyzed against buffer B. To remove remaining nucleic acids, the recovered protein was then loaded onto a 15ml Q-Hiload column  $(16 \text{ mm} \times 20 \text{ cm}; \text{ GE Healthcare})$  equilibrated in buffer B. The heterodimer was recovered in the flow-through fraction and then concentrated by using Centricon 30 concentrators. Either GDPNP-Mg<sup>2+</sup> (1 mM) or GDP-Mg<sup>2+</sup> (1 mM) was added before storage at 4°C of the recovered heterodimer (see Figure 2, lane 3 for purified Ch-eIF2 $\beta\gamma$ ).



**Figure 2.** SDS–PAGE analysis of purified Ch-eIF2 variants. The 12.5% SDS–PAGE were stained with Coomassie Blue. Lane 1, Ch-eIF2 heterotrimer. Lane 2, Ch-eIF2 $\alpha\gamma$  heterodimer. Lane 3, Ch-eIF2 $\beta\gamma$  heterodimer. Lane 4, Ch-eIF2( $\alpha\Delta$ C274) $\beta\gamma$ . Positions of tagged yeast eIF2 $\alpha$  (Y- $\alpha$ , 36.9 kDa), tagged yeast eIF2 $\alpha\Delta$ C274 (Y- $\alpha\Delta$ C, 33.4 kDa), yeast eIF2 $\beta$  (Y- $\beta$ , 31.4 kDa) and Ss-aIF2 $\gamma$  (Ss- $\gamma$ , 45.6 kDa) are indicated. Lane 5, Ch-eIF2 $\beta\Delta$ N $\Delta$ C $\gamma$  heterodimer. Lane 6, Ch-eIF2 $\beta\Delta$ N $\gamma$  heterodimer. Lane 7, Ch-eIF2 $\alpha\Delta$ C274 $\gamma$  heterodimer. Positions of yeast eIF2 $\beta\Delta$ N $\Delta$ C (Y- $\beta\Delta$ N $\Delta$ C), eIF2 $\beta\Delta$ N (Y- $\beta\Delta$ N) and eIF2 $\alpha\Delta$ C274 (Y- $\alpha\Delta$ C) are indicated. Lane 8, ChEncc-eIF2( $\alpha\Delta$ C274) $\gamma$  heterodimer, Lane 9, ChEncc-eIF2 $\alpha\gamma$  heterodimer. Positions of tagged yeast eIF2 $\alpha\Delta$ C274 (Y- $\alpha\Delta$ C, 33.4 kDa), tagged yeast eIF2 $\alpha$  (Y- $\alpha$ , 36.9 kDa) and *E. cuniculi* eIF2 $\gamma$  (Encc- $\gamma$ , 48.9 kDa) are indicated.

#### Purification of Ch-eIF2ay heterodimers and of its variants

The procedure used for the purification of Ch-eIF2 $\alpha\gamma$  heterodimer was described previously (15) (Figure 2, lane 2). To purify the Ch-eIF2 $\alpha\Delta$ C $\gamma$  heterodimer variants ( $\alpha\Delta$ C274,  $\alpha\Delta$ C283,  $\alpha\Delta$ C292 and  $\alpha\Delta$ C298), the S-Hiload step was replaced by a molecular sieve using a Superdex 200 HR 10/30 column (GE Healthcare). The purified proteins (Figure 2, lane 7) were stored in buffer B in the presence of either 1 mM GDPNP-Mg<sup>2+</sup> or 1 mM GDP-Mg<sup>2+</sup>.

The procedure used to purify Ch-eIF2 $\alpha$ D3 $\gamma$  and Ch-eIF2 $\alpha$ D3 $\Delta$ C $\gamma$  heterodimers was the same as that used for Ch-eIF2 $\alpha$  $\Delta$ C $\gamma$  heterodimer variants, except that after the Talon column, size-exclusion chromatography on Superdex 75 HR 10/30 (GE Healthcare) was used to polish the preparation.

Ch-eIF2 $\alpha\Delta C\gamma$  and Ch-eIF2 $\alpha$ D3 $\Delta C\gamma$  protein used to perform SAXS studies were purified as follows.  $\alpha\Delta C$ and  $\alpha$ D3 $\Delta C$  were first purified using Talon affinity resin. The His-tag extensions of the recovered proteins were then removed during overnight dialysis in a buffer containing 10 mM HEPES pH 7.5, 200 mM NaCl, 3 mM 2-mercaptoethanol, 10 mM CaCl<sub>2</sub> and Thrombin (0.25 U/mg of substrate protein) at 4°C. The  $\gamma$  subunit of *S. solfataricus* was purified as described (6).  $\alpha\Delta C$ and/or  $\alpha$ D3 $\Delta C$  was assembled with Ss-aIF2 $\gamma$  and the heterodimers were finally purified using a last step of molecular sieving using a Superdex 200 HR 10/30 column (GE Healthcare) in buffer C (10 mM HEPES pH 7.5, 200 mM NaCl and 3 mM 2-mercaptoethanol).

# Purification of Ch-Encc-eIF2 $\alpha\gamma$ and of Ch-Encc-eIF2 $\alpha\Delta C\gamma$

BL21 Rosetta pLacI-Rare containing the pET28b+ $\gamma$ tc-Encc plasmid was used to overexpress the  $\gamma$  subunit from *E. cuniculi* (19). The resulting protein carried a 6-histidine tag at its C-terminus. One-liter cultures were in 2xTY containing 25 µg/ml of kanamycin and 34 µg/ml of chloramphenicol. Expression was induced by adding 1 mM of IPTG when OD<sub>650</sub> reached 0.8. After induction, the cultures were continued for 8–12h at 18°C.

Cultures of cells each overproducing one of the two subunits (250 ml for yeast  $\alpha$ , 500 ml for *E. cuniculi* eIF2 $\gamma$ ) were harvested, mixed in 40 ml of buffer A and

disrupted by sonication. After centrifugation, the supernatant was loaded onto a column (3 ml) containing Talon affinity resin (Clontech) equilibrated in the same buffer. The resin was first washed with 50 ml of buffer A and then with 50 ml of buffer A supplemented with 10 mM imidazole. The fractions containing the heterodimer were finally recovered after elution with buffer A containing 125 mM imidazole. To remove the excess of the yeast  $\alpha$ subunit, the affinity column eluate was diluted to 250 mM NaCl and then loaded onto a 5ml Q-Sepharose HP column (10 mm × 5 cm; GE Healthcare) equilibrated in buffer C (10 mM HEPES pH 7.5, 250 mM NaCl, 10 mM 2-mercaptoethanol, 0.1 mM PMSF and 0.1 mM benzamidine). The heterodimer flowed through the column whereas the excess yeast  $\alpha$  subunit was retained. After SDS-PAGE analysis, the fractions containing the heterodimer were pooled and dialyzed against buffer B. Finally, the protein was concentrated by using Centricon 30 concentrators. Either GDPNP- $Mg^{2+}$  (1 mM) or GDP-Mg<sup>2+</sup> (1 mM) was added before storage at  $4^{\circ}C$  of the recovered heterodimers.

The same procedure was used to purify ChEncceIF2 $\alpha\Delta C\gamma$  except that after the Talon column, the protein was loaded onto a Superdex 75 column (HR10/ 30, GE Healthcare) equilibrated in buffer B. The eluted heterodimer was loaded onto a 5ml S-Sepharose HP column  $(10 \text{ mm} \times 5 \text{ cm}, \text{ GE Healthcare})$  equilibrated in buffer D (10mM HEPES pH 7.5, 200mM NaCl, 10mM 0.1 mM PMSF 2-mercaptoethanol, and 0.1 mM benzamidine). A gradient from 200 to 800 mM NaCl was used for elution (80 ml at a flow rate of 2 ml/min). The fractions containing the heterodimer were pooled and dialyzed against buffer B. Finally, the protein was concentrated by using Centricon 30 concentrators. Either GDPNP-Mg<sup>2+</sup> (1 mM) or GDP-Mg<sup>2+</sup> (1 mM) was added before storage at 4°C of the recovered heterodimer (Figure 2, lanes 8 and 9).

#### **Protection assay**

The protocol used was derived from (20).  $tRNA_f^{Met}$  was produced in *E. coli* from synthetic genes and purified as described (21,22). Endogenous  $tRNA_i^{Met}$  purified from *S. cerevisiae* was a generous gift of Dr Gérard Keith (Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France). Full aminoacylation with [ $^{35}$ S]-methionine (~10 000 dpm/pmol; Perkin Elmer) was achieved using homogeneous *E. coli* M547 MetRS (23). tRNA<sub>f</sub><sup>Met</sup>UAC, a derivative of tRNA<sub>f</sub><sup>Met</sup> carrying a UAC (Val) anticodon was produced as described (21,22). Valylation with [ $^{14}$ C]Val (563 dpm/pmol) was performed with homogeneous *E. coli* valyl-tRNA synthetase as described (7). Aminoacyl-tRNAs were precipitated with ethanol in the presence of 0.3 M NaAc pH 5.5 and stored at  $-20^{\circ}$ C in 100% EtOH in small aliquots. Before use, aminoacylated tRNAs were redissolved in water and full aminoacylation was systematically controlled through measurement of radioactivity after precipitation in 5% trichloroacetic acid (TCA).

Protection by chimeric eIF2 (Ch-eIF2) variants of methionyl-tRNA<sub>f</sub><sup>Met</sup> against spontaneous hydrolysis was assaved as follows. Reaction mixtures (150 ul) contained 20 mM HEPES-NaOH pH 8.0, 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA (bovine serum albumin; ROCHE), 5% glycerol, 0.1% triton X-100, 1mM GDPNP and 2nM of E. coli methionyl-tRNA<sub>f</sub><sup>Met</sup>. The concentrations of the proteins were determined from A280 measurements using extinction coefficients computed from the amino acid sequences. Concentrations of Ch-eIF2 variants were varied from 1 nM to 30  $\mu$ M, using a range depending on the  $K_d$  value to be measured. The mixtures were incubated at 30°C. To determine the rate constants of deacylation, 20 µl aliquots were withdrawn at various times (usually, six aliquots from 5 to 60 min) and precipitated in 5% TCA in the presence of 80 µg of yeast RNA as carrier. In all cases, the deacylation curve as a function of time could be fitted with a single exponential. For each  $K_d$  measurement, a set of 8-10 experiments corresponding to 8-10 different protein concentrations was performed. The rate constants measured at variable protein concentrations were then fitted to simple binding curves (20) from which the dissociation constant of the studied protein-tRNA complexes and their associated standard errors could be deduced using the MC-Fit program (24). Each experiment was independently repeated at least twice, without significant variation of the results.

## Small-angle X-ray scattering

SAXS experiments were conducted on the SWING beamline at the SOLEIL synchrotron as described (15). For these experiments, the His-tags of eIF2 $\alpha$ 3 $\Delta$ C and of eIF2 $\alpha$ 3 $\Delta$ C were removed before assembly with Ss-aIF2 $\gamma$ , as described above. A concentrated sample of Ch-eIF2( $\alpha$ 3 $\Delta$ C) $\gamma$  (~4 nmol in 40 µl) was injected onto a size-exclusion column (agilent Bio column SEC3; 300 Å) using an Agilent HPLC system and eluted directly into the SAXS flow-through capillary cell at a flow rate of 0.4 ml/min.

To collect data on Ch-eIF2( $\alpha\Delta C$ ) $\gamma$ -GDPNP-Met-tRNA<sub>f</sub><sup>Met</sup> complex, the protein was mixed with a 1.15-fold molar excess of Met-tRNA<sub>f</sub><sup>Met</sup> and injected as a concentrated sample (~20 nmol in 250 µl) onto a size-exclusion column (Superdex 200 HR10/30, GE Healthcare), using an Agilent HPLC system and eluted

directly into the SAXS flow-through capillary cell at a flow rate of 0.4 ml/min. The same procedure was used to collect data on Ss-aIF2–GDPNP–Met-tRNA<sub>f</sub><sup>Met</sup>, Ss-aIF2–GDPNP–Met-tRNA<sub>f</sub><sup>Met</sup>(A1–U72) and Ss-aIF2–GDPNP–Ss-Met-tRNA<sub>i</sub><sup>Met</sup> complexes. tRNA<sub>f</sub><sup>Met</sup>(A1–U72) and Ss-tRNA<sub>i</sub><sup>Met</sup> were purified and aminoacylated as described (7,25).

For all experiments, the elution buffer consisted of 10 mM MOPS (pH 6.7), 200 mM NaCl and 5 mM MgCl<sub>2</sub>. SAXS data were collected continuously, with a frame duration of 1.5 s and a dead time between frames of 1.0 s. Selected frames corresponding to the main elution peak were averaged (26). A large number of frames were collected during the first minutes of the elution, and these were averaged to account for buffer scattering, which was subsequently subtracted from the signal during elution of the protein or protein-tRNA complex. Data reduction to absolute units, frame averaging and subtraction were done using FOXTROT (26). All subsequent data processing, analysis and modeling steps were carried out with PRIMUS and other programs of the ATSAS suite (27). Scattered intensity curves were calculated from the atomic coordinates of the crystallographic structure, using CRYSOL with 50 harmonics (28). This program was also used to fit the calculated curve to the experimental one, by adjusting the excluded volume, the averaged atomic radius and the contrast of the hydration layer surrounding the particle in solution.

## RESULTS

## Production and assembly of the Ch-eIF2 heterotrimer

The chimeric heterotrimer (Ch-eIF2) was formed by assembling the archaeal  $\gamma$  subunit of *S. solfataricus* aIF2 with the  $\alpha$  and  $\beta$  subunits of *S. cerevisiae* eIF2. To facilitate purification of Ch-eIF2, we used an N-terminally tagged version of yeast eIF2 $\alpha$  and native versions of yeast eIF2 $\beta$  and Ss-aIF2 $\gamma$ . The three subunits were produced independently in *E. coli*. The first step of purification of the assembled Ch-eIF2 heterotrimer was a metal affinity chromatography. This step showed that the archaeal  $\gamma$  subunit was able to interact with both yeast  $\alpha$  and  $\beta$  subunits. To remove the excess of the yeast  $\alpha$  subunit, we then used anion exchange chromatography. This purification step allowed to recover a correct stoechiometry between the three subunits, as judged by SDS–PAGE analysis (Figure 2, lane 1). Binding of tRNA<sub>i</sub><sup>Met</sup> onto e/aIF2 leads to protection

Binding of tRNA<sup>i</sup><sup>Met</sup> onto e/aIF2 leads to protection of aminoacylated tRNA against spontaneous deacylation. Thus, dissociation constants of Met-tRNA<sup>i</sup><sup>Met</sup> from an e/ aIF2–GDPNP–Met-tRNA<sup>i<sup>Met</sup></sup> complex can be estimated by following deacylation rates in the presence of various eIF2 concentrations at a fixed tRNA concentration (7,20). The sequence of the *E. coli* initiator tRNA<sup>Met</sup> has a high homology with the one from the archaeal initiator tRNA (Figure 3). In particular, the acceptor stems of both tRNAs are identical, with the exception that the A1–U72 base pair in the archaeal tRNA is replaced by C1–A72 in the bacterial one. Moreover, it was previously shown that *E. coli* Met-tRNA<sup>Met</sup> was as good a ligand of



Figure 3. Cloverleaf representations of initiator Met-tRNAs from *S. solfataricus, E. coli* and *S. cerevisiae* cytoplasm. Nucleotides denoted with gray boxes are universally conserved in initiator tRNAs. Post-transcriptional modifications are indicated in the cases of *E. coli* and *S. cerevisiae*.

archaeal aIF2 as the cognate initiator Ss-tRNA<sub>i</sub><sup>Met</sup> produced in *E. coli* (15). To further validate at a structural level, the use of *E. coli* initiator Met-tRNA<sub>f</sub><sup>Met</sup> as a model ligand, we compared the SAXS curve of Ss-aIF2– GDPNP–Met-tRNA<sub>f</sub><sup>Met</sup> with the corresponding ones with either Met-tRNA<sub>f</sub><sup>Met</sup>A1–U72 variant or MettRNA<sub>i</sub><sup>Met</sup> from *S. solfataricus*. The three SAXS curves were nicely superimposable (Supplementary Figure S1), showing that the three complexes were highly similar.

*Escherichia coli* Met-tRNA<sub>f</sub><sup>Met</sup> initiator tRNA was used as a model ligand to study tRNA binding by Ch-eIF2. Ch-eIF2 bound efficiently initiator methionyl-tRNA in the presence of GDPNP-Mg<sup>2+</sup> with a  $K_d$  value of  $87 \pm 10 \text{ nM}$  (Table 1, row 1). Moreover, in the presence of GDP instead of GDPNP, the  $K_d$  value was increased to  $700 \pm 300 \,\mathrm{nM}$ . Therefore, GDP lowered the binding affinity of the initiator methionyl-tRNA by one order of magnitude, in keeping with the nucleotide effects observed with yeast eIF2 [factor of 20 (4)] or Ss-aIF2 [factor of 100, (6)]. Furthermore, we verified that Ch-eIF2 could still authentic yeast Met-tRNA<sub>i</sub><sup>Met</sup> interact with  $(K_{\rm d} = 27 \pm 5 \,\mu\text{M}; \text{ Table 1, row 1; Figure 3)}$ . The binding affinity of both initiator tRNAs for the chimeric factor in the presence of GDPNP were reasonably high, when compared with the affinity for eIF2 from yeast  $[K_d = 9 \text{ nM}, (4)]$  or for Ss-aIF2  $[K_d = 1.5 \text{ nM}, (6)]$ . The observed discrepancies between the  $K_d$  values measured with yeast eIF2, Ss-aIF2 or Ch-eIF2 could be in part due to differences in the assay conditions. Indeed, in the case of yeast eIF2 the temperature used in the assay was 26°C and in the case of Ss-aIF2, the temperature used in the assay was 51°C. Here, to avoid thermal effects on yeast  $\alpha$  and  $\beta$  proteins, we performed the measurements at 30°C, a temperature sub-optimal for the archaeal  $\gamma$  subunit of Ch-eIF2 (5). Finally, it is known that archaeal and eukaryotic eIF2 strongly recognize the methionine moiety on initiator tRNAs (4,7,29,30). Therefore, as an additional control to validate the use of Ch-eIF2 as a tool, we verified that the chimeric heterotrimer had the same property. We used Val-tRNA<sub>f</sub><sup>Met</sup> UAC in a standard protection assay. Instead of protection against deacylation, we observed a surprising 2-fold increase of the rate of deacylation in the presence of 7.4  $\mu$ M Ch-eIF2. Possibly, low-affinity unspecific binding of the Val-tRNA<sub>f</sub><sup>Met</sup> UAC on the factor may favor hydrolysis of the esterified amino acid. Nevertheless, this experiment allowed to conclude that the presence of the valyl group strongly affected specific aminoacyl-tRNA binding.

# The yeast $\beta$ subunit strongly influences tRNA binding on Ch-eIF2

At 30°C, only weak, though significant, GDPNPdependent binding of initiator tRNA to Ss-aIF2y occurs (Table 1, row 2). Therefore, the large difference between the  $K_d$  value measured with the Ss- $\gamma$  subunit alone and that measured for the full Ch-eIF2 trimer showed that the  $\alpha$  and/or the  $\beta$  subunits participate in tRNA binding (Table 1, rows 1 and 2). In order to examine the contribution of each peripheral subunit, Ch-eIF $2\alpha\gamma$  and Ch-eIF2 $\beta\gamma$  heterodimers were produced and purified (Figure 2, lanes 2 and 3). Using Ch-eIF2 $\beta\gamma$ , the  $K_d$  value of Met-tRNA<sub>f</sub><sup>Met</sup> was 55 ± 10 nM (Table 1, row 3) whereas using Ch-eIF2 $\alpha\gamma$ , the  $K_d$  value of Met-tRNA<sub>f</sub><sup>Met</sup> was 9200 ± 2000 nM (Table 1, row 7). Hence, the  $\beta$  subunit of yeast eIF2 improved the binding affinity of Met-tRNA<sub>f</sub><sup>Met</sup> by three orders of magnitude (Table 1, rows 2 and 3, Supplementary Figure S2A). The yeast  $\alpha$  subunit also participated in the binding affinity of Met-tRNA<sub>f</sub><sup>Met</sup>. Its contribution was however two orders of magnitude less important than that of the  $\beta$  subunit (Table 1, rows 2 and 7, Supplementary Figure S2A). Notably, in the absence of the  $\alpha$  subunit, the affinity of Ch-eIF2 $\beta\gamma$  for Met-tRNA<sub>f</sub><sup>Met</sup> was similar to that

		Υ-α	Υ-β	Ss-γ	$K_{\rm d} ({\rm nM})$ Met-tRNA <sub>f</sub> <sup>Met</sup> (GDPNP) <sup>a</sup>	$K_{\rm d} ({\rm nM})$ Met-tRNA <sub>f</sub> <sup>Met</sup> (GDP) <sup>a</sup>	$K_{\rm d}$ (nM) Met-tRNA <sub>i</sub> <sup>Met</sup> (GDPNP) <sup>b</sup>
1	Ch-eIF2	wt	wt	wt	$87 \pm 10$	$700 \pm 300$	27 ± 5
2	Ss-aIF2y	_	_	wt	$55000\pm12000$	n.d.	$27000\pm10000$
3	Ch-eIF2βγ		wt	wt	$55 \pm 20$	$180 \pm 50$	$34 \pm 6$
4	Ch-eIF2( $\beta \Delta N$ ) $\gamma$	_	βΔΝ	wt	$231 \pm 30$	$557 \pm 125$	n.d.
5	Ch-eIF2( $\beta \Delta C$ ) $\gamma$	_	βΔC	wt	$532 \pm 140$	$1670 \pm 960$	n.d.
6	Ch-eIF2( $\beta \Delta N \Delta C$ ) $\gamma$	_	βδνδς	wt	$3500 \pm 1400$	>9500	n.d.
7	Ch-eIF2ay	wt	_	wt	$9200 \pm 2000$	>120000	$4200 \pm 2200$
8	Ch-eIF2( $\alpha\Delta$ C274) $\gamma$	$\alpha \Delta C$	_	wt	$73 \pm 10$	$740 \pm 70$	$36 \pm 8$
9	Ch-eIF2( $\alpha \Delta C274$ ) $\beta \gamma$	$\alpha \Delta C$	wt	wt	$3 \pm 1$	$26 \pm 4$	n.d.
10	Ch-eIF2( $\alpha \Delta C283$ ) $\gamma$	$\alpha \Delta C$	_	wt	$950 \pm 180$	n.d.	n.d.
11	Ch-eIF2( $\alpha \Delta C292$ ) $\gamma$	$\alpha \Delta C$	_	wt	$2600 \pm 600$	n.d.	n.d.
12	Ch-eIF2( $\alpha \Delta C298$ ) $\gamma$	$\alpha \Delta C$	_	wt	$4200 \pm 200$	n.d.	n.d.
13	Ch-eIF2( $\alpha$ 3) $\gamma$	α3	_	wt	>30 000	n.d.	n.d.
14	Ch-eIF2( $\alpha$ 3 $\Delta$ C274) $\gamma$	$\alpha 3\Delta C$	-	wt	$2900\pm500$	>44 000	n.d.

Table 1. tRNA binding by Ch-eIF2

Dissociation constants of Met-tRNA<sup>Met</sup> from its complexes with the indicated versions of Ch-eIF2 were determined from protection experiments as <sup>a</sup>Measured with *E. coli* Met-tRNA<sub>f</sub><sup>Met</sup> as a ligand. n.d., not determined.

measured with the complete chimeric heterotrimer (compare rows 1 and 3, Table 1 and Supplementary Figure S2A). The same effects of the  $\alpha$  and  $\beta$  subunits were observed using authentic yeast Met-tRNA<sub>i</sub><sup>Met</sup> as a substrate (Table 1, rows 2, 3 and 7). This indicated that the association of the  $\beta$  subunit to  $\gamma$  was enough to retrieve almost the same binding affinity for tRNA as full Ch-eIF2. Ch-eIF2 $\beta\gamma$  remained specific of the methionine moiety of the tRNA ligand. Indeed, the presence of  $10 \,\mu M$ Ch-eIF2 $\beta\gamma$  only decreased the deacylation rate of Val-tRNA<sub>f</sub><sup>Met</sup> UAC by 20%, consistent with a dissociation constant of the order of several tens of micromolar. Notably, the behavior of the yeast peripheral subunits was opposite to that observed with archaeal  $\alpha$  and  $\beta$  subunits (Supplementary Figure S2A).

# N- and C-terminal extensions of yeast $eIF2\beta$ are necessary for Met-tRNA $_{f}^{Met}$ binding

The yeast  $\beta$  subunit is made of a conserved structural core corresponding to the archaeal version of the protein to which are appended eukaryote-specific domains, namely, an N-terminal domain and a short C-terminal extension (Figure 1B). To evaluate the roles of the yeast-eIF2 $\beta$  extensions in tRNA binding, three truncated versions of the subunit were produced. In eIF2 $\beta\Delta N$ , the first 125 residues were removed. In eIF2 $\beta\Delta C$ , residues 272–285 were removed. The two deletions were also performed simultaneously giving eIF2 $\beta \Delta N \Delta C$ .

The three heterodimers Ch-eIF2 $\beta \Delta N\gamma$ , Ch-eIF2 $\beta \Delta C\gamma$ and Ch-eIF2 $\beta$  $\Delta$ N $\Delta$ C $\gamma$  were purified and Met-tRNA<sub>f</sub><sup>Met</sup>binding affinities were measured. As shown in Table 1, the N-terminal domain contributed by a factor of 4.2 to tRNA-binding affinity (rows 3 and 4) and the C-terminal extension of eIF2ß contributed by a factor of 9.7 (rows 3 and 5). These results showed that both eukaryotic extensions participate in the binding of the tRNA molecule with the contribution of the short basic C-terminal extension being twice as much as that of the

N-terminal domain. Moreover, with the double truncation (Ch-eIF2 $\beta \Delta N \Delta C \gamma$ ), the dissociation constant fell down by a factor of 63 when compared with unmodified Ch-eIF2 $\beta\gamma$  (rows 3 and 6). Therefore, the positive effects of both extensions on the binding of the initiator tRNA were cumulative (Supplementary Figure S2B). Finally, using Ch-eIF2 $\beta$ \DeltaN $\Delta$ C $\gamma$ , a  $K_d$ -value for Met- tRNA<sub>f</sub><sup>Met</sup> binding of  $3500 \pm 1400 \,\text{nM}$  was measured. The affinity of the tRNA remained 15-fold higher than that with Ss-aIF2y alone. Hence, the conserved core of yeast eIF2β still contains some features allowing it to participate in tRNA-binding affinity (Supplementary Figure S2B).

#### The acidic C-terminal extension of yeast eIF2a interferes with tRNA binding

The yeast  $\alpha$  subunit of eIF2 comprises a conserved structural core corresponding to the archaeal version of the protein, with an additional highly acidic (pI = 3.14) C-terminal tail, containing 16 Asp or Glu out of 30 residues. This acidic C-terminal extension is characteristic of eukaryotic eIF2 $\alpha$  (Figures 1B and 4). Previously, the C-terminal region corresponding to residues 275-304 was removed through site-directed mutagenesis of the yeast eIF2 $\alpha$  gene (15). With the heterodimer Ch-eIF2 $\alpha\Delta$ C274 $\gamma$ , a  $K_{d}$ -value for the initiator methionyl-tRNA of  $73 \pm 10$  nM was measured (15). Therefore, removal of the C-terminal tail increased the binding affinity by more than two orders of magnitude (rows 7 and 8 in Table 1 and Supplementary Figure S2C). The same effect was observed using authentic yeast Met-tRNA<sub>i</sub><sup>Met</sup> as a substrate ( $K_d = 36 \pm 8 \text{ nM}$ ; Table 1). Hence, the C-terminal extension of the yeast  $\alpha$ subunit is strongly unfavorable to the binding of the tRNA on Ch-eIF $2\alpha\gamma$ . Again, we verified that Ch-eIF2 $\alpha\Delta$ C274 $\gamma$  remained specific of the methionine moiety of the tRNA ligand. Indeed, the presence of  $10\,\mu\text{M}$  Ch-eIF2 $\alpha\Delta$ C274 $\gamma$  only decreased the deacylation

rate of Val-tRNA<sub>f</sub><sup>Met</sup> UAC by 37%, consistent with a dissociation constant in the  $10 \,\mu$ M range.

In order to delineate the region responsible for the negative effect on tRNA binding, three deletions of various lengths in the C-terminal extension were performed. The three shortened proteins were named  $\alpha \Delta C283$ ,  $\alpha \Delta C292$  and  $\alpha \Delta C298$  (Figure 4). The corresponding chimeric heterodimers Ch-eIF2 $\alpha\Delta C\gamma$  were purified and their tRNA-binding affinities were measured. As shown in Table 1 (rows 8 and 10–12), the tRNA-binding affinity increased when the size of the acidic C-terminal extension decreased, the highest tRNA-binding affinity being obtained upon deletion of the complete acidic C-terminal extension (Table 1 and Supplementary Figure S2C). In the context of the heterotrimer, complete removal of the acidic tail of  $\alpha$  $(\alpha \Delta C274)$  increased the affinity by a factor of 30 (rows 1) and 9) leading to a heterotrimer even more efficient in tRNA binding than Ch-eIF2. These results strongly suggested that the apparent weak effect of yeast  $eIF2\alpha$  on tRNA-binding results from a positive effect of the core part of the subunit, compensated by a negative effect of the acidic residues in its appended C-terminal tail.

To confirm the effect of the C-terminal tail of yeast eIF2 $\alpha$ , we examined it using another eIF2 $\gamma$  subunit. We therefore produced Ch-eIF2 using the central eukaryotic eIF2γ subunit from E. cuniculi (Encc-eIF2). Two chimeric proteins were produced and purified (Figure 2, lanes 8 and 9). ChEncc-eIF2 $\alpha\gamma$  corresponds to the  $\alpha$  subunit of S. cerevisiae bound to the  $\gamma$  subunit of E. cuniculi and ChEncc-eIF2 $\alpha\Delta$ 274C $\gamma$  corresponds to the  $\alpha$  subunit of S. cerevisiae truncated of its C-terminal tail bound to the  $\gamma$  subunit of *E. cuniculi*. Dissociation constants of Met-tRNAf<sup>Met</sup> from these proteins were determined using the same procedure as that used for yeast Ch-eIF2. Encc-eIF2 $\gamma$  bound Met-tRNA with a  $K_d$  value of  $1700 \pm 300 \,\text{nM}$  (19). Association of yeast eIF2 $\alpha$  to Encc-eIF2 $\gamma$  only increased the affinity of Met-tRNA by a factor of 5.6 (Table 2, rows 1 and 2) whereas the increase of affinity was by a factor of 105 when  $eIF2\alpha\Delta C$  was used in place of  $eIF2\alpha$  (Table 2, rows 1 and 3).

# Domains 1 and 2 of $eIF2\alpha$ contribute to tRNA-binding affinity

In the archaeal system, the  $\alpha D3$  domain is sufficient to confer on aIF2 $\gamma$  its full tRNA-binding affinity (6,7). Nevertheless, the 3D structure of aIF2 bound to the initiator tRNA revealed contacts of the  $\alpha D12$  domains with the tRNA molecule (15). To explain the apparent discrepancy between biochemical data and the observed contacts of  $\alpha D12$  with the tRNA in the structure, it was proposed that the gain in enthalpy resulting from the contacts of the  $\alpha D12$  domains with the tRNA just compensates for the entropic cost of the immobilization of  $\alpha D12$ . We anticipated that these contributions may quantitatively vary depending on the  $\alpha$  subunit studied.

We produced the  $\alpha D3$  domain of yeast eIF2 $\alpha$ . Then, tRNA-binding affinity of Ch-eIF2( $\alpha D3$ ) $\gamma$  heterodimer was measured. Interestingly, the tRNA-binding affinity for Ch-eIF2( $\alpha D3$ ) $\gamma$  heterodimer was markedly lowered compared with that measured with the entire yeast  $\alpha$  subunit bound to archaeal  $\gamma$ . Only slight protection of Met-tRNA was observed at a concentration of Ch-eIF2( $\alpha$ D3) $\gamma$  heterodimer of 30  $\mu$ M (compare rows 7 and 13 in Table 1). This result strongly suggested that domains 1 and 2 of yeast eIF2 $\alpha$  contribute to tRNA-binding affinity. Consistently, although deletion of the C-terminal acidic tail from  $\alpha$ D3 increased the binding affinity of the tRNA for the chimeric heterodimer by a factor of at least 10 (compare rows 13 and 14 in Table 1), the affinity remained much lower than that measured in the presence of the  $\alpha$ D12 domains, with the Ch-eIF2 $\alpha\Delta$ C274 $\gamma$  heterodimer (compare rows 14 and 8 in Table 1).

#### Structural studies in solution using SAXS

The region of contact between  $e/aIF2\alpha$  and  $e/aIF2\gamma$ mainly involves two loops of the C-terminal domain of  $\alpha$  ( $\alpha$ D3) as well as an elongated loop and a  $\beta$ -strand of  $\gamma$ [Figure 1A (6)]. Moreover, the structure of the human  $eIF2\alpha$  C-terminal domain is highly similar to the structure of archaeal  $aIF2\alpha$  (6,31,32). Therefore, besides the C-terminal extension, the structure of  $\alpha$ D3 is conserved between eukaryotes and archaea.

In a first step, we checked the structural similarity between the Ch-eIF2( $\alpha$ 3 $\Delta$ C) $\gamma$  protein and the archaeal Ss-aIF2 $\alpha$ D3 $\gamma$  protein. This was achieved by measuring the X-ray scattering curve of Ch-eIF2( $\alpha$ 3 $\Delta$ C) $\gamma$ . This experimental curve was then compared with the theoretical X-ray scattering curve computed from the crystallographic structure of the archaeal  $\alpha$ D3 $\gamma$  protein (from PDB ID 2AHO). The two curves showed a very good agreement (Supplementary Figure S3A,  $\chi = 1.9$ ). This result strongly argued in favor of a binding mode of yeast eIF2 $\alpha$ 3 $\Delta$ C protein onto Ss-aIF2 $\gamma$  similar to that observed for the archaeal aIF2 $\alpha$ D3 domain (6).

Finally, in order to evaluate the binding mode of the tRNA molecule by the yeast  $eIF2\alpha\Delta C$  subunit, the scattering curve of Ch- $eIF2\alpha\Delta C\gamma$  bound to Met-tRNA<sub>f</sub><sup>Met</sup> was measured after purification of the complex (see 'Materials and Methods' section). The SAXS diffusion curve obtained with the Ch- $eIF2\alpha\Delta C\gamma$ -GDPNP–Met-tRNA<sub>f</sub><sup>Met</sup> complex agreed well with the theoretical curve computed from the crystallographic structure of Ss- $aIF2\alpha\gamma$  bound to Met-tRNA<sub>f</sub><sup>Met</sup> (from PDB ID 3V11, Supplementary Figure S3B,  $\chi = 2.8$ ). This strongly argued in favor of a binding mode of the tRNA by yeast  $eIF2\alpha$  similar to the binding mode observed for the archaeal Ss- $aIF2\alpha$  subunit.

## DISCUSSION

In this study, we have constructed Ch-eIF2 formed by assembling yeast eIF2 $\alpha$  and  $\beta$  subunits to archaeal Ss-aIF2 $\gamma$  core subunit. In such chimeric factors, the peripheral  $\alpha$  and  $\beta$  subunits are likely to adopt a binding mode to the central  $\gamma$  subunit identical to that observed for archaeal aIF2 [Figure 1A (6,10–12)]. Indeed, the binding of the  $\beta$  subunit to the  $\gamma$  one involves an  $\alpha$ -helix of e/aIF2 $\beta$  wedged between two  $\alpha$ -helices of the G-domain

S. mansoni/1-328	TPKAVK I RADFE VS CFTYDG I DAVKSALRSGL ELNSDSL P I R I NL I APPL YVL TTQTMDRAAGLE QL NEVL DV I KKSIESQ-GGSFK I QQAARV VSDTDDADL QRQMDE LEKANRE VSGDE DDE DE DDGDE E DDDE AS NDGDQNGLK
C. elegans/1-342	TPQAVK IRADIEVS CF DYDGIDAVKAALIAGKNCS NGTFPIK INLIAAPHFVVTTQTL DREGGIEAVNS IL DT IKNAIEGF-KGKFT IKEEARIVTD IDDEKKKGDGDEDEEESEDEDEDDGLMAPKGLDQQVDAEEAS RDNRKKAGDDEDS DEEDD-
P. falciparum/1-329	TP QALKLRGRI DVWCF GYE GI DAVKEALKKGKE I SNDKVS I NIKLIAPP QYVI VTS CHDKDLGMAKI QEAMKVI SDKI KEYKGGDF KQQGE I LVI GGDE E KRLEELLDKHDGI SS DDDDYNTS DE DDE NSSEE DE NTSE DE EE D
B. bovis/1-332	TP QALKLERCMI DVWCFGP DG I EAVKS AL MLAKANEEHN I QVKL I APP QYE I MTTCHDKDKGMDI I TTALEE I SNKI RSYAGGEFKQRGDI I I VGDE DE RHLYS LLE GQESSESEE DE GMGRVDES MLPP DAVEP DADS D
T. amulata/1-334	VP QALKLKCST DVWCF GP GTNAVKNST GKAKL-LDPQTSTRLTAPP QYETLTSCF DKEAGLALMNQTLEVTEKNTKSFTGGDF KQKCDVVVTCD-DEKHLEDLLELHETTDEEDEGDE-EEEEED GMGRVDESMLPP NLSNEMEEEEESDE
P. chabaudi/1-325	IPQALKLEGEIDVWCFCYEGIDAVKEALKKKKE ISNNEVIINIKLIAPPQYVVISCHDKELCMCKIQEANKVISDEIKEYKGCDFKQQGEILVIGCDDEKKLEELDKHOCLSSDDEVSSDEDDDSSDEDDDSSDEDDD
C. hominis/1-335	SPQQLKLRARTHVCCCCVDCTDAVRHATTACQE - IVTKDDVE
D. melanogaster/1-341	VSPTIVKTIKADIE USCTOTE GLDAVKASLI KOLELSTEELPTKINLTAPPLTVMITISTIKKI DUCKALEVATEHTIKADIE CSCTOTE GLDAVKASLI KOLELSTEELPTKINLTAPPLTVMITISTIKKI DUCKALEVATEHTIKADIE CSCTOTE GLDAVKASLI KOLELSTEELPTKINLTAPPLTVMITISTIKKI DUCKALEVATEHTIKADIE CSCTOTE GLDAVKASLI KOLELSTEELPTKINLTAPPLTVMITISTIKKI DUCKALEVATEHTIKADIE CSCTOTE GLDAVKASLI KOLELSTEELP
B. mori/1-332	IS QUAY IRADIC ACTOR TO DAVKARCHAGE ALST YOMP TATICITY FEW DICKAL QUALDA LONDON OF TEAS-GUY NEQUARY VY LATDEARL ARQWERA LENACY MOD SHOULD STORE OF DECOMPOSITION OF YOUR ASSOCIATION
C. quipquafarciatur/1-241	
R malavi/1-220	TROAM I BADIEV CES YOC DAWENALIE CO
O lucimarinus/1-322	TPOPLK (RADVE) TCFOYDCVI H KSANRAAF USTEDCK VKMSI VASPLYVI STOTSEREEGISI VDKAISVI OFVITSK - CCKLI LKESARVVSERDDKI   AFOITNI NKVERGSDTDDDTKCEVEI
O. satiya/1-339	TPOPLK IRADVE MKCFOFDGVI HIKOAMRKAFAAGNDNCPVKIKI VAPPI VVI TTOTI DKDOG I SVI TDA I KACTAFIFKH-KGKI I VKFAPRAVSFREDKI FNEHIFTI NEONAFVDGDADS-FFFDTCMGDI DI TNSCVNAD
A. thaliana/1-344	TP OP MK I RADIELKCF OF DGVLHIKEAMKKAE AVGT DDCPVKIKLVAPPLYVLTTHTHYKEKGIVTLNKAIEACITAIEEH-KGKLVVKEGARAVSERDDKLLAEHMAKLRMDNEEMSCDEGSEDEEEDTGMGEVDIDGG-SGIIE
P. patens/1-333	TPQPLK I RAD I E LKCF OF DGVL H I KDAMRKAEAAGT E CPVK I KLVAPPLYV I TTQTL DKDL G I TVL ENA I KACTKE I E KH-KGKLLVKE PPRAVS E RE E RFLAET I KNLHDANE E VDC DE DS DQE DQGMGS V DVE RGGLS E
B. fuckeliana/1-310	TPQPTKVRADVEVTCF GYE G I DAVKTALRTAE ARNTADNQ VKVKLVSPPL YVLTSTCL EKS VG I STL EAA I VD I RKN I EAA-GGGL I VKME PKAVTE NDDAEL QAL MEKRERENAE VS GDES MS ES DE N-QVE VM
A. clavatus/1-308	TPAPTKVRAD IE VTCF GYE GIDAVKTALRTAE ANNTPENQ I KVKLVAPPLYVLTS QCLDKTMGIKLLE EA IE KIQVNIKEA-GGGCIVKMAPKAVTE HDDAAL QELMDKRE RENME VS GDE SMS ES DE G-VPE
C. albicans/1-300	TPQAINIRADVDVSCFSYEGIDAIKEALKAAESVSTSQNQVKAKLVAAPLYFITVQALDKNEGVKLLEKAIEKITESIEAS-GGNCKVSMPPKAVSANEDAELERMLQRREAEDKDSSDEEDDA
C. neoformans/1-300	TPKPVKVRAD I EVKCF SYAG I DA I KRALTAGE AVSTPDVP I KVRL VAPPLYVNSTTSTDKNAA I ELMEKAVEV I GETVRKD-KGDI TI KMKPKVVS ETE DAELKALME OF EAANMDQAGDDESSEEDE
S. sclerotiorum/1-313	TPQPTKVRADVEVTCFGYEGIDAVKTALRTAE ARNTADNQ VKVKLVSPPLYVLTSTCLEKSVGISTLEAA IVDIRKNIEAA-GGGLIVKMEPKAVTENDDAELQALMEKRERENAEVSGDESMSESDEN-QVEVM
5. pombe/1-306	TPQPIKIRADVEVTCFCYEGINAIKAALKAAEDVHTEEVPIKVKLVAPPLYVLLTNALDKSLGLKKLEEAIGAIEKSITAS-NGTCTVKMKPKAVSETDELELADLMKKFEKENAEISCDEEDDQSGSE
A. gossypii/1-302	TPQAIKIRADVEVSCFSYEGIEAIKEALKAAEATSTEQMQVKVKLVAAPLYVITTQALDKNQGIEALNHAISKIEEYIAKY-QGVCNVTMPPKAVTATEDAELQALLESKELENRSDSEEEDSDYE
Y. lipolytica/1-313	TP NPVKVRAD I DI TCFAYE CI EA I KKALAACE TLNSE LVP VKVKLVAAPLFVLTSTCL DKQKA I DTLCEA I EK I RES I EAS - GCSLTVKWE PKAVTETDDAE LAKLMEKTE QONKLVDCDDDDADE EAGDFE
D. hansenii/1-300	IP QATKTRADVEVSCFSYEGTDATKKALTAAEETSTEGMAVKAKLVAAPLYVTSVQALDKNQCTALLEKATEKVSESTENS-GGTCKVTNSPKAVTATEDAELQALLEKKEAEDKESSDEEEED
P. nodorum/1-312	IPOPTIXVRSDTEVTCFCYFCTDAVKEALKTAFAKNTPENQVKVKLVSPPLYVLTSQCDKTVGTACECYSTKDTKAF-CGDCQXTMMAPKAVTENDDAELAALMEKKEKENQUSGDDESTSDEGVVEADS
E. nidulans/ 1-308	IPHETIX VRADI EVICE OF DUT DAVINALENTAL AENT PENQTAVIC VAPPETVE ISOLO IN THE EATER ETTER ASS-GUGCEV VRADAVA ENDALACUEL WRATE REINE VGC DUS QUE SUB
N vertensis/1-313	TP OAK IRADE EVENTY SCHOLDARTS ALKARE - AVSTERING - VARCUARE LETAGUS UKANDI ALCETALEKTED FOR VARVANDARTAKT AS DARLENALDARE UKANDU DU PODE - DU POD
O myleiss/1-315	TPOAVK I RADI EVACYCY FCI DAVK DA I RAGI SCSTEAMP I KINI I APPRYVMTTTI ERTECI SVI NOAMAA I KERI EK-RCVENI OMEAKVYTDTDETEL AROL ERI FRENAFVDCDDEDCEMEAKAED
S. frugiperda/1-331	TS OAVK I RAD I E CACY CY E GI DAVRAAL KAGL ALST VOL P I KINLIAPPLY WITTS TPEKADGLKAL ODA I DKIKET I DAA-GGY EN OMAPKY VTAT DE AE LAROMERAE AE NAE VAGDS AE E DE DOGMGDY RMDDE POONGAS DE DDD
H. sapiens/1-315	TP OAVK I RAD I E VACYGY E G I DAVKE AL RAGL NCSTE NMP I K I NL I APP RY WITTTLE RTE GLSVLS OAMAV I KE KIE E K-RGVF NY OME P KVYTDTDE TE LAROMER LE RE NAE VDCDDDAE E MEAKAE D
D. rerio/1-315	TP OAVK I RAD I E VACY GYE G I DAVKEAL RAGL NCST E AMP I K I NL I APP RYVMTTTTL E RTE GL S VL NOA MAA I K E R I E E K-RGV F NV OME P K VVT DT DE TE L OROL E R LE RE NAE V DG DD DA E E ME AKT E D
E. cuniculi/1-283	QAS TYKVRAD I DVVCS S R GG I VS I KKVF S DAL R L DPR L EF CL I KTP TYS V TRVS S NK DKAY EVVREACNVL I S NI QKE - GGT AT V I S QP KLYGE KS RYNL L NF EE NEESS SE
E. cuniculi/1-283 T. thermophila/1-320	QASTYXWRADIDVVCSSRGCIVSIKKVFSDAL - RLDPR- LEFCLIKTPTYSVTRVSSNDBAYEVVERACKVISISNIQKE GGTATVISQPKLYGEKSRYNLINE EENEESSSE NOTYM RIKABPELRCHEYEGIDAKIAALLAGE - AKSRNICG - VKEFLIGSPYVAGCIVTODNIG IE VUEALKAVEEVISK- KGSYKKITEFN KIKORAQDNLEBKAKEEVEDDDEDSEVECMGDDDEDEDEDEDE
E. cuniculi/1-283 T. thermophila/1-320 S. cerevisiae/1-304	QASTYXWRADIDVVCSSRGCIVS IKKWFSDALRLDPRLEFCLIKTPTYSVTRVSSNDDAYEVVPEACVUSIISIN QE-CGTATVISOPKLYCEKSRSVNLINEEENEESSE NTOPMRIKADFELRCHEYEGIDAIKAALLAGEAKSRNKCDVKFEIIGSPVAGCIVTODKNLGIEVLHEALKAVEEVIISK-KGSYKLETEPKIYGDKAQDNLEALEKAEEEDEDDEDSEVECMCDDDEEDEEND
E. cuniculi/1-283 T. thermophila/1-320 S. cerevisiae/1-304	QASTYKYRADI DVVCSSRGCI VS I KKVFS BAL KLDPR LEFCLI KTPTYS VTRVSSN.BLAY EVVERCAVLI I SIN QKE_GCTATVI SQPKLYCEKSRYNLLINE EENEESSSE. NYGY MRI KABDE ELNCHNEGE LIGH KAALLAGE KASRNCCF VKFKLVAAPLYVLTTQAL DKQKG I EQLESA I EK I TEVI TKY-GGVCNI TMP FKAVTATEDAELQALLESKEL DNR DSEDDEDESDE DE - TPQAVKI RADVEVSCFSYEG I DA I KDALKSAE DNSTEQNQ - VKVKLVAAPLYVLTTQAL DKQKG I EQLESA I EK I TEVI TKY-GGVCNI TMP FKAVTATEDAELQALLESKEL DNR DSEDDEDESDE DE -
E. cuniculi/1-283 T. thermophila/1-320 S. cerevisiae/1-304	QAS TYX KNAD I DVVCSSRGCI VS I KK VF SDAL RLDPRLEFCLI KTPTYS VTRVS SN KDRAY EVVERACKUL I SNI QKE-CGTATVI SQPKLI V GEKSRYNLL IN EENEESS SE TYTOP MIR KADE ELICHENE GI BAI KAALLAGE AKS RNICCHVER I I OSPYVACI VTOPKNI CIE OV LEALLAVE VEI I SNI QKE-KGYKLETEN KEI KAELEGE DODEDDEDDEDDEDDEDDEDDEDDEDDEDDEDDEDDEDDE
E. cuniculi/1-283 T. thermophila/1-320 S. cerevisiae/1-304	QASTYKVRADI DVVCSSRCGI VS I KKVF SDAL EL DPR
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304	QASTYKVRADI DVVCSSRGCI VS I KKVFSBAL ELDPRLEFCLI KTPTYSVTRVSSN KDBAY EVVERACKUS I SIN QKE_GCTATVI SQPKLY CEKSRSVNLL INE EENEESSSE. NYTOP MRI KABJE ELRCHEYEG I BAI KAALLAGE AKSRNCCE VKSELI VTDOKNA OL EVVERALKAVE EVI I SAN KASTKATE TEN EKSTKE TET FIN UT KY-GGVCN I TMP KAVTA JEDAEL QALLESKEL DNS DSEDDEDDS DE DE
E. cunicul(1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259	QASTYXYRADI DVVCSSRCGI VS I KKVF SDAL EL DPR
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266	QASTYXWRADI DVVCSSRCGI VS I KKVF SDAL ELDPREFCLI KTPTYS VTRVSSNLDBAY EVYERACKVI [S NI QKE_GCTATI SQPKLY CEKSSYNLL IN EENEESSSE TYTOY MI KAB DELENCHEYEG I BAL KAALLGA AKSNKCÖ VKSEN (STOTODING, GEVELKALKAVEEVI I SNI QKE_GCTATI SQPKLY CEKSSYNLL IN EENEESSSE TY QAVK I RADVEVSCFSYEG I DA I KDALLKSA DMSTEQMQ VKVKLVAAPLYVLTT QALDKOKG I EQLESA I EKI TEVI TYY-GGVCNI TMP FKAVTAT EDALLQALLESKEL DNS DSEDDEDES DE
E. cunicult/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263	QASTYXVRADI DVVCSSRCGI VS I KKVFS BAL ELEPCLI KTPTYSVTRVSSNEDA ** EVVEACAVLI I SIN QKE_GCTATVI SQPKLYCEKSKYNLL INFEENEESSSE. NOTYM I KABP ELRCHEYEG I BALKAALLGE AKS RIXCE VER I I GSPYXGCI VTODING I EV LEALLAVE VI I SN. KCSY KK. TEFK I KKAELE DEDEDEDE SE VE CONCODDEEDEENEN TPQAVKI RADVEVSCFSYEG I DA I KDALKSAE DINSTEONG VKVKLVAAPLYVLTT QALDKOKGI EQLESA I EK I TEVI TKY-GGVCNI TMPPKAVTATEDAEL QALLESKED. DINS DS EDDEDEDES DE elf2udC274 elf2udC283 elf2udC292 elf2udC298 RLPSVEI RGI ME I TNSRPGCVEVI RDTLSAAAEGN
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263 P. abyssi/1-275	QAS TYXKRAD I DVVCSSRCGI VS I KKVF SDAL EL DPR
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263 P. abyssi/1-275 M. marianigi/1-259	QASTYXYRADI DVVCSSRCGI VS I KKVFS BAL RL DPR LE FC LI KTPTYS VTRVS SN DBAY EVYE EACHVI SI NI QKE -GCTATI SQ PKLY CEKSKYNL I.NE EENEESS SE NTOY MI KAB PLENCHEYE GI BA I KAALLAG AKS RNCCH VKE I I GS PYYACI VTDENS GI EV LEAL KAVE YE I I SS NY KAEL KKSTKK TETEN KKAEL KEEDEDEDE DS EVE CONCODDEEDEENEN TP QAVK I RADVEVSCFSYEG I DA I KDALKSAE DHS TEQMQ VKVKLVAAPLYVLTT QALDKQKGI EQLESA I EK I TEVI TKY-GGVCNI TMP PKAVTAT EDAEL QALLESKE DIRS DS EDDEDEDE DS EVE CONCODDEEDEENEN GIF200C274 eIF200C293 eIF200C294 eIF200C293 eIF2
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263 P. abyssi/1-275 M. maringir/1-259 A. fulgidus/1-267	QASTYXYRADI DVVCSSRCGI VS I KKVF SDAL EL DPR
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263 P. abyssi/1-275 M. marisnigri/1-259 A. fulgious/1-267 M. thermophila/1-257	QASTYXYRADI DVVCSSRCGI VS I KKVF SDAL RLDPR LEFC LI KTPTYS VTRVS SN.BLAY EVYEACAVL I SN QKE_CGTATVI SQPKLY CEKSAYNL LINE EENEESS SE. TYDY MI KAD PLENCHEYE GI BALKAALLGE AKS RNLCE VER FILI SGPYRGUTTODING, GI EVUEALLAYEVE I I SN KK-KGSYKK TEFK LIK KASKALGE LIKE KASELEDEDEDE SE VE CONCIDIDEEDEEDED GIF200C274 GIF200C292 GIF200C292 GIF200C292 GIF200C293 GIF200C2
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashi/1-266 M. woltae/1-263 P. abyst/1-275 M. martingi/1-259 A. fulgidus/1-267 M. thermophila/1-257 M. stadtmanae/1-260	QASTYKYRADI DVVCSSRCGI VS I KKVF SDAL EL DPREL FC LI KTPTYSYTRYS SN DDAY EVYE CACYU I SI NO KE-GCTATYI SO PKLY CEXSRYNLL IN EENEESS SE TYPOWRI KAD EL RCHENE GE I DA I KAALL CAC
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symblosum/1-259 M. jannachil/1-266 M. voltae/1-263 M. matingli/1-257 M. thermophila/1-257 M. startungli/1-265	QASTYXWRADI DVVCSSRCGI VS I KKVF SDAL RLDPR LEFC LI KTPTYSVTRVS SN.BLAY EVVE ACAVLY I SN QKE_CGTATVI SQPKLY CEKSSYNL LINE EENEESSE. TYO WI KABD ELINCHEYE GI BALKAALLGE AKS RNLCE VEVE I I SSPYRACI VTODING I EV LEAL KAVE EVI I SN N KKE. KCSY KKI TEFVI HY KORKAONLE KKAELEEDEDEDE SE VE CONCODDEEDEEDED TYO WI KABD EVSCFSYEG I DA I KDALKSAE DINS TEOMO VKVKLVAAPLYVLT GALD KOK GI EOLESA I EK I TEVI TKY-GGVCNI TMPP KAVTAT EDALE GALLES KEL DINS DS EDDEDEDE SD EVE CONCODDEEDEEDED GIF20LC274 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LC238 eIF20LCE VEVKITYLGAPTYR I SI TAPDY KTAEKELKPLVE GI ORSAEKN-RGTFKFTREESKKTREE ELTNIKEVE COVI EMISTAPD GI KOLKKALTTAL KANPYED VEVKITYLGAPYRYVI I APDYKSGE EVFKKVCEKAAVT I KLL-GCEGTYYRESKK- ELSNIKVDOL LITSTTEPD GIKVKIVKI KKAI KANARVED VEVKITYLGAPYRYVI I APDYKSGE EVFKKVCEKAKEK KKAKKKKKKKKKKKKKKKKKKKKKEN VEVKITYLGAPYRY I SI TAPDYKATE VLES VAELI I EX I KRV KGRA COG SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKITYL DYKAERKE KKASAAAVCVVERA CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKITYL DYKAERKE KKASAAAVCVVERA CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKITYL DYKAERKE KKASAAAVCVVERA CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKITYL DYKAERKE KKASAAAVCVVERA CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKI HYV EDAPYR I DI TAPDYKATE KALKSAAAA KKASAAAA I KYKE CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKI DYV HAD DYKAERKA KKASAAAVCVVERA CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKU CYGPYPYR I KYL AD DYKAERA ALKSAAAA I KYKE ALKSAAAA I KYKE CGC SKT I RKOKGAO KYRKIVKGYFELKSSAB GI ER I KKALLBAKRAL VNG VEVKU CYGPYPYR I KYL AD DYKKARA KKASAAAA I KYKE CGC SKF I RKAA STPEVIG GI TYVD JUSYKSNOVEI I I EAL KABARDAG EEEE VEVKU CYGPYPYR I KYL AD DYKKARA KKAAAAAR KYKE I RKAAFAARCE EI VERA CGC SKSF I RKE D
E. cunicul/1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashii/1-266 M. voltae/1-263 P. abysi/1-275 M. maringiy/1-257 M. thermophila/1-257 M. stadtmanae/1-260 M. smithi/1-265 M. shandmanae/1-260	QASTYXYRADI DVVCSSRCGI VS I KKVFS BAL RL DPR LEFCLIKTPTYSYTRVS SN DBAY EVYREACYNLISIN QE-CGTATYI SQ PKLYCEKSYNLL NE EENESSSE. TYD WRI KAD FLENCHEYGE I BAL KAALLAGE KKS RNKCE VKYRLY AAPLYVLTTQNS N. BUAY EVYREACYNLISIN QE-CGTATYI SQ PKLYCEKSYNLL NE EKABE EEDBOEDS BE VE COMCODDE EDE BNN TYD WRI KAD VEVSCFSYEG I DA I KDALLKSA E DNS TEOMQ VKYRLY AAPLYVLTTGAL DKQKGI E QLESA I EKITEVI TKY-GCVCNI TMP FKAYTAT EDALL QALLES KELDRY DS EDDE DE SD ED EI F202C274 EIF202C292 EIF202C292 EIF202C292 EIF202C292 EIF202C292 EIF202C99 RLPSVE I RG I ME I TNSRP DCVEV I RDTLS AAAECN
E-cunicult/283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashi/1-366 M. voltaer/-263 P. abys/si/1-267 M. marting/i/1-259 M. stachmana-1-320 M. stachmana-1-320 M. stachmana-1-320 M. shadhu/1-263 M. shadhu/1-263 M. shadhu/1-263	QASTYXWRADI DVVCSSRCGI VS I KKVF SDAL RL DPR
E-cunically1-283 T. thermophila/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. jannashi/1-266 M. voltae/1-283 P. abyssi/1-225 M. thermophila/1-257 M. thermophila/1-257 M. thatdhors/1-267 M. thatdhors/1-267 M. thatdhors/1-267 M. thatdhors/1-267 M. thatdhors/1-267 M. thatdhors/1-267	QASTYKYRADI DVVCSSRCGI VS I KKVFSBAL RLDPR LEPCLIKTPTYSVTRVSSNBAY EVVEACAVLISIN QE-CGTATVISOPKIL YCEKSYNIL INF EENEESSSE TYOM KIRAD ELKRENEYE GI BALKAALLAGE KASRNCCD VKVELVAAPLYVLTTQKSSRUP (VLEALLAVEY I ISK. KGSTKK TEFK KIKKAK KASTKK TEFK KIKKAKKAK TYOM KIRAD EVSCFSYEGI DA I KDALLASA E DIASTEONG VKVELVAAPLYVLTTGALDKOG I EQLESA I EKITEVI TKY-CGVCNI TMPTKAVTAT EDAEL QALLESKED DRS DE EDDE DE SVEOKODDDE EDEEND EIFZuGZC274 EIFZuGZC283 EIFZuGZC283 EIFZuGZC292 EIFZuGZC293 EIFZuGZC293 EIFZuGZC293 EIFZuGZC293 EIFZUGZC294 E
E-cunically 1-283 Thermophilus 1-320 S. cerevisiae1-304 C. symbiosum/1-259 M.janusabil-266 M. autostabil-263 M. autostabil-263 M. stadenta-263 M. bandent-263 M. bandent-26	QASTYXWRADI DVVCSSRCGI VS I KKVF SDAL RL DPR
E cunically 1-283 Thermophilu 1-320 S. cenvisike1-394 C. symbiosum/1-259 M. janashi 1-266 M. janashi 1-265 M. shorthof 1-267 M. shorthof 1-267 M. sandhof 1-267 M. sandhof 1-268 M. sandhof 1-267 M. sandhof 1-268 M. sandhof 1-267 M. sa	QASTYKYRADI DVVCSSRCGI VS I KKVF SDAL RL DPR LE PC LI KTPTYSVTRVS SN DDAY EVYEACAVL I SIN QAE-CGTATVI SQPKLY CEKSAYNI. INF EENEESS SE. TYO MI KAD PLENCHEYE GI DAI KAALLGA
E cunically 1-283 Thermophilus 1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. janusabi/1-366 M. voltae/1-263 P. abys/s1/-265 M. tode/s1-263 M. tode/s	QAS TYXYRADI DVVCSSRCGI VS I KKVF SDAL KL DPR
E cunically 1-283 Thermophilu 3-320 S. cenvisiae1-304 C. symbiosum/1-259 M. jantashi1-366 M. jantashi1-366 M. jantashi1-265 M. sandari 2-267 M. sandari 2-267 M. sandari 1-263 M. sand	QAS TYXYRADI DVVCSSRCGI VS I KKVF SBAL RL DPRLEF CL IKTPTYSYTRYS SN DBAY EVYEACAVL ISIN QAE-CGTATYI SQ PKLYCEKSYNLL IN EENEESS SE. TYO MY IKABY ELRCHENE'G EDA KAALLACA
E cunically 1-283 Thermophilus 1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. janusabi/1-366 M. voltae/1-263 P. abys/s1/-265 M. tode/1-263 M. tode/	QAS TYXYRADI DVVCSSRCGI VS I KKVF SDAL RL DPR
E cunically 1-283 T. thermophila, 1-320 S. cenvisiaer1-304 C. symbiosum/1-259 M. janashi/1-266 M. sandaright-275 M. thermophila/1-267 M. stadbmane-1-260 M. sandaright-276 M. s	QASTYKYRADI DVVCSSRCGI VS I KKVF BAL RL DPR LE FC LI KTPTYSVTRVS SN DBAY EVYE ACAVL SI JN QKE -CGTATI SQ PKLYGEKSYNL I. NE EENESSSE TYO MKI KAD VELKRENEY GE JA I KAALLAGE AKS RNKCD VYE KI JCS PYYAGCI TYODKN GE VL HAELKASE VE JN KKS KK RTEN KK KKS KK RTEN KKS KKS KK RTEN KKS KKS KK RTEN KKS KKS KKS KKS KKS KKS KKS KKS KKS KK
E cunically 1-283 Thermophilus 1-320 S. cerevisiaer1-304 C. symbiosum/1-259 M. janusabil-364 W. voltaer1-263 P. abys/s1-265 M. maring/n1-265 M. bander1-263 M. bander	QAS TYXYRADI DVVCSSRCGI VS I KKVF BAL RL DPR LE FC LI KTPTYS VTRVS SN DBAY EVYREACYU I SI N QKECGTAT VI SQ PKLY CEKSSYNL I.NE EENEESS SE MTOY MI KAB VELKRENEY GE I KAALLAGE AKS RNLCE VKYRLY AAPLYVLT TAU DKQK GI EQLESA I EK I TEVI TYCGAVON I TMP PKAYTAT EDALE QALLES KEL DRS DB EDDEDEDS DE VE CONCODDEEDEEND 
E cunically 1-283 T. thermophilay 1-320 S. cenvisiaer1-304 C. symbiosum/1-259 M. janashi/1-266 M. sandaright-275 M. thermophila/1-267 M. stadbmane-1-260 M. sandaright-276 M. sandaright-277 A. penkint/-277 S. maniangl-1-267	QASTYKYRADI DVVCSSRCGI VS I KKVFS BAL RL DPR LE FC LI KTPTYS VTRVS SN DBAY EVYE ACAVL SI JNI QAE -CGTATI SQ PKLY GENSKYNL LINE EENEESSE. TY OW KI KAD LENCHENCE (LA KAALLAGE DKS TEOMQ VKVL VAAPLYVL TTQNS ON GUE ACAVEY I I SN. KCS YK KT TEYN KI KASK KAE EENE BOEDE DS EVE CONCODDE EENEN TY QAVKI KADVEVSCTS YEG I DA I KDALLKS AE DKS TEOMQ VKVL VAAPLYVL TTGAL DKQG I EQ LESA I EK I TEVI TKY - CGVCNI TMP FKAVTAT EDAEL QALLES KEL DNR DS EDDE DDE DS EVE CONCODDE EENEN GEF20AC274 EF20AC238 EF720AC238 EF7
E cunicult/283 Tuhemophilu/3320 S. cerevisiae/1-304 C. symbiosum/1-259 M.jannashi/1-366 M.voltae/1-263 P.abysi/1-263 M.stadia/1-263 M. stadia/1-263 M. stadia/1-263 M. stadia/1-263 M. stadia/1-263 M. banden/1-263 M. banden/1-263 M. banden/1-263 M. banden/1-263 M. banden/1-264 M. banden/1-264 M. banden/1-264 M. banden/1-265 M. banden/1-265 S. stadaulu/1-265 S. stadaulu/1-263	QAS TYXYRADI DVVCSSRCGI VS I KKVF BAL RL DPR
E cunically 1-283 Thermophilu 3-320 S. cenvisiser 1-304 C. symbiosum/1-259 M. janzabil-265 M. janzabil-265 M. sandtarju-275 M. standmark-260 M. sandtarju-276 M. sandtarju-276 S. sand	QAS TYXYRADI DVVCSSRCGI VS I KKVF BAL RL DPR LE FC LI KTPTYS VTRVS SN DBAY EVYE ACAVL SI I SI Q KE_CGTATVI SQ PKLYCEKS SYNLL INE EENEESSE. TY Q VKI KABV ELKRENEY EG I DA I KAALLGE DKS TE QNQ VKVK V AAPLYVL TT QAL DKQG I EQ LESA I EK I TEVI TKY-CGVCN I TMP FXAVTAT EDAEL QALLES KEL DRS DS EDDE DES DE VE CONCODDE EDEEND 
E cunically 1-283 T. thermophilu/1-320 S. cerevisiae/1-304 C. symbiosum/1-259 M. janusabi/1-366 M. voltae/1-263 P. abys/s1/-265 M. toda/s1-263 M. toda/s1-263 S. sadda/s1-263 S.	QAS TYXWRADI DVVCSSRCGI VS I KKVF BAL RL DPR LE PC LI KTPTYS VTRVS SN DBAY EVVE ACAVLY I SN QKE -CGTAT VI SQ PKLY GEXSRYNL LINE EENEESSE. TY QVK I RADVE VS CFS YEG I DA I KAALLAGE DIAS TEOMQ VXVKL VAAPLYVLT TQALDAGK GI EQLESA I EK I TEV I TKY -GGVCNI TMP PKAYTAT EDAELQALLES KEL DIRS DB EDDEDEDS DE YEOKODDDEEDEEND 
E cunically 1-283 T. thermophilay 1-320 S. cenvisiaer1-304 C. symbiosum/1-259 M. janashi/1-266 M. sandaright-275 M. sharefully-1275 M. sharefully-1275 M. sharefully-1276 M. sandaright-276 M. sandaright-277 M. sharefully-1276 M. sandaright-278 M. sandaright-278 S. stadiaditt-287 S. Stadiatt-287 S. Stadiatt-286 S. Stad	QAS TYXWRADI DVVCSSRCGI VS I KKVF BAL RL DPR LE FC LI KTP TYX TRVS NDBAY EVYE ACAVL ISIN QAE -CGTAT VIS QP KLY GEXSRYNL LINE EXAEESES SE. TY QAVKI RADVEVSCFSYEG I DA I KAALLGE DIAS TEOMQVXVL VAAPLYVL TT QALD DKQG I EQLESA I EK I TEVI TKY-CGVCNI TMP FXAVTAT EDAEL QALLES KEL DRY. DS EDDE DES DE VEOKODD DE EDEEND 

Figure 4. Alignment of  $e/aIF2\alpha$  domain 3 sequences. Representative eukaryotic (upper block) and archaeal (lower block) sequences are shown. Aspartate and glutamate residues are boxed in gray. Secondary structures are drawn below the Ss-aIF2 $\alpha$  sequence. The positions of the last residue in C-terminal truncated versions of eIF2 $\alpha$  are indicated.

Table 2. Effects of the C-terminal acidic tail of yeast  $\alpha$  subunit on tRNA binding in the context of Encephalitozoon cuniculi  $\gamma$  subunit

		Υ-α	Encc-γ	$K_{\rm d}$ (nM) Met-tRNA <sub>f</sub> <sup>Met</sup> (GDPNP) <sup>a</sup>	$K_{\rm d}$ (nM) Met-tRNA <sub>f</sub> <sup>Met</sup> (GDP) <sup>a</sup>
1	Encc-eIF2 $\gamma^{b}$	_	wt	$1700 \pm 300$	>24 000
2	ChEncc-eIF2ay	Wt	wt	$300 \pm 50$	>16000
3	ChEncc-eIF2( $\alpha\Delta C$ ) $\gamma$	$\alpha \Delta C$	wt	$16 \pm 1$	$3380\pm550$

Dissociation constants were determined from protection experiments as described in 'Materials and Methods' section.

<sup>a</sup>Measured with *E. coli* Met-tRNA<sub>f</sub><sup>Met</sup> as a ligand.

<sup>b</sup>Data from (19).

of  $\gamma$  (10–12). The rest of the  $\beta$  subunit is mobile with respect to  $\gamma$  (10–12). Since the residues of the anchoring helix contacting the  $\gamma$  subunit are well conserved in all e/ aIF2 $\beta$  species, it is most likely that yeast eIF2 $\beta$  is caught by Ss-aIF2 $\gamma$  in a similar manner. This idea is consistent with site-directed mutagenesis studies in the yeast system (33). The binding of yeast eIF2 $\alpha$  to the archaeal  $\gamma$  subunit was probed by SAXS (Supplementary Figure S3A). The results clearly argued in favor of a binding mode of the yeast eIF2 $\alpha$  subunit to the archaeal  $\gamma$  identical to that observed for the archaeal  $\alpha$  subunit.

Consistently, the chimeric protein, Ch-eIF2, is indeed able to bind yeast initiator methionyl-tRNA with a  $K_d$ value (27 nM) similar to those measured with authentic versions of eIF2 (4,6,7,19). Moreover, we verified that the chimeric factor remained specific for the methionine moiety of its aminoacyl-tRNA ligand. These results validated the use of such chimeric proteins to study the influence of the  $\alpha$  and  $\beta$  peripheral subunits on the tRNAbinding affinity. The role of each peripheral subunit in tRNA binding was studied in the context of heterodimers (Ch-eIF2 $\alpha\gamma$  and Ch-eIF2 $\beta\gamma$ ). We observe that yeast eIF2 $\beta$ is essential to retrieve full tRNA-binding affinity, whereas yeast eIF2 $\alpha$  only weakly contributes to tRNA-binding affinity. These results are in keeping with the observations made using the yeast system (17,18) and shows that binding of yeast  $\alpha$  and  $\beta$  subunits to archaeal  $\gamma$  subunit confers on Ch-eIF2  $\alpha$  eukaryotic behavior. The same effects of the yeast  $\alpha$  and  $\beta$  subunits on tRNA binding were observed when *E. coli* Met-tRNA<sub>f</sub><sup>Met</sup> was used as the ligand instead of authentic *S. cerevisiae* MettRNA<sub>i</sub><sup>Met</sup>. The authentic tRNA was preferred by a factor of 2–3, whatever the combination of subunits (Table 1). This preference was also observed with the aIF2 $\gamma$  subunit alone, consistent with the idea that the stimulatory role of the A1–U72 pair of the eukaryotic initiator tRNA is mainly exerted through binding by the  $\gamma$  subunit (4,15,30,34). The *E. coli* initiator tRNA therefore appeared as a good substitute to further dissect the structure–function relationships of the yeast eIF2 $\alpha$  and eIF2 $\beta$  subunits. Hence, the bacterial tRNA was used in the rest of the study since it can be obtained with a higher purity.

The two eukaryotic-specific extensions of eIF2ß positively contribute to tRNA affinity. Removal of the two extensions makes a truncated eIF2ß structurally homologous to its archaeal counterpart. However, in contrast to archaeal aIF2 $\beta$ , the core-restricted version of eIF2 $\beta$ retains a significant ability to increase tRNA-binding affinity (by a factor of at least 28). Therefore, the eIF2ß core domain contains specific features involved in tRNAbinding affinity. Overall, each of the three parts of the  $\beta$ subunit contributes by one order of magnitude to tRNAbinding affinity. Furthermore, these contributions are additive, thereby suggesting that the two extensions independently cooperate to enhance tRNA affinity. Whether these contributions result from direct contacts of the  $\beta$ subunit with tRNA or from indirect effects, for instance on the conformation of the  $\gamma$  subunit, remains to be elucidated.

Interestingly, the near absence of effect of yeast  $eIF2\alpha$ on tRNA affinity is only apparent. Indeed, a positive effect of the  $\alpha$  subunit is compensated by a negative effect due to the appended eukaryotic acidic tail. The extent of the negative effect of the C-terminal tail increases with the size of the acidic peptide. The  $\alpha \Delta C274$  version of yeast eIF2a contributes by at least three orders of magnitude to tRNA-binding affinity. Within this subunit, domain 3 has apparently a minor role whereas domains 1 and 2 of eIF2 $\alpha$  bring the major contribution to tRNA affinity. Nevertheless, SAXS experiments argue in favor of a binding mode for the tRNA molecule by Ch-eIF2 $\alpha\Delta C\gamma$ identical to that observed within the authentic archaeal complex. The precise interactions of the domains of  $e/aIF2\alpha$  with the tRNA molecule may explain the differences observed in the  $K_d$  measurements. In addition, at this stage, we cannot exclude that a part of the effects of the  $\alpha$  subunit on tRNA binding may be indirect, through modulating the conformation of the  $\gamma$  subunit, for example at the level of the switch regions.

Finally, the present study, together with those of eIF2 from *E. cuniculi* (19) and archaeal aIF2 (6,7,14,35), shows that the roles of the peripheral subunits of e/aIF2 in tRNA binding vary from one organism to another. Despite this, the overall structure of the ternary initiation complex in solution is likely to remain similar in all organisms. The observed differences in the contributions of the peripheral subunits to tRNA binding might reflect species-specific adjustments in the mechanisms of handling of the initiator tRNA by e/aIF2 within the ribosomal initiation complex. For example, one may propose that the negative effect of the C-terminal tail of yeast  $eIF2\alpha$  is cancelled on the small ribosomal subunit, thanks to an interaction with another partner of the ribosomal initiation complex. In agreement with this idea, the C-terminal tail of human  $eIF2\alpha$  was shown to be mobile in solution (31). More generally, contacts of the  $\alpha$  and the  $\beta$  subunits with other components of the initiation complex may contribute to modulate the affinity of e/aIF2 for the initiator tRNA along the translation initiation process (16). Such modulations would, in turn, contribute to start codon recognition, in agreement with the known importance of  $eIF2\alpha$ and  $eIF2\beta$  for translation start specificity (36,37).

#### SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Figures 1–3.

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