Conserved sequences in the β subunit of archaeal and eukaryal translation initiation factor 2 (eIF2), absent from eIF5, mediate interaction with eIF2 γ

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The eukaryotic translation initiation factor 2 (eIF2) binds the methionyl-initiator tRNA in a GTP-dependent mode. This complex associates with the 40 S ribosomal particle, which then, with the aid of other factors, binds to the 5' end of the mRNA and migrates to the first AUG codon, where eIF5 promotes GTP hydrolysis, followed by the formation of the 80 S ribosome. Here we provide a comparative sequence analysis of the β subunit of eIF2 and its archaeal counterpart (aIF2 β). aIF2 β differs from eIF2 β in not possessing an N-terminal extension implicated in binding RNA, eIF5 and eIF2B. The remaining sequences are highly conserved, and are shared with eIF5. Previously isolated mutations in the yeast eIF2 β , which allow initiation of translation

at UUG codons due to the uncovering of an intrinsic GTPase activity in eIF2, involve residues that are conserved in aIF2 β , but not in eIF5. We show that the sequence of eIF2 β homologous to aIF2 β is sufficient for binding eIF2 γ , the only subunit with which it interacts, and comprises, at the most, 78 residues. eIF5 does not interact with eIF2 γ , despite its similarity with eIF2 β , probably because of a gap in homology in this region. These observations have implications for the evolution of the mechanism of translation initiation.

Key words: aIF2, eIF5, eIF2, translation initiation, two-hybrid assays.

INTRODUCTION

The protein synthesis machinery is highly conserved throughout evolution, with its basic components found in both bacteria and eukaryotes, functioning in analogous steps in these two types of cells. However, the process of initiation of translation has differed considerably in the two types of organisms, perhaps to accommodate an increasing need for control at this stage of gene expression in eukaryotes. The differences are found both in the number of factors required for the formation of the translating ribosomal particle and also in the mode of recognition of the translation start site on the mRNA. Recently, with the complete sequencing of several Archaea genomes, another level of complexity has emerged, since these organisms display a hybrid system between bacteria and eukaryotes for the initiation of protein synthesis. Archaea have polycistronic mRNAs, which are uncapped, and lack long polyadenylated tails. The coding sequences on these mRNAs are, in most cases, preceded by typical Shine–Dalgarno elements. On the other hand, Archaea have counterparts of several eukaryotic translation initiation factors, except those known to be involved in 'cap' recognition and binding. Sequences highly homologous to eIF1A, the three subunits of eIF2, yIF2, two of the five subunits of eIF2B and eIF4A, have been identified in all archaeal genomes sequenced to date [1–6].

eIF2 is a key factor in the translation initiation process in eukaryotes (reviewed in [7]). It binds GTP and methionyl-initiator tRNA (Met-tRNA_i^{Met}). This ternary complex then associates with the 40 S ribosomal subunit. With the aid of a number of factors, which stabilize this interaction (eIF1, eIF1A and eIF3), recognize the cap structure and undo hairpin structures on the message (eIF4F, eIF4B), the 40 S eIF2–

GTP–Met-tRNA_i^{Met} complex binds to the 5' end of mRNA, and migrates along the message until the first AUG codon is found. At this point, eIF5 promotes the hydrolysis of GTP on the complex, with the concomitant release of all initiation factors. With the Met-tRNA_i^{Met} in the P site, formation of the 80 S translating ribosomes then occurs. For another round of initiation, the GDP molecule bound to the released eIF2 factor must be exchanged for GTP, in a step catalysed by eIF2B.

The α subunit of eIF2 is a major site of regulation of overall protein synthesis in eukaryotes. Its phosphorylation on serine-51 by protein kinases, such as the double-stranded RNA-dependent kinase and the haem-regulated inhibitor kinase in mammalian cells, and Gcn2 in yeast, results in the inactivation of eIF2 by blocking the exchange of GDP to GTP catalysed by eIF2B (reviewed in [8,9]). The γ subunit contains GTP-binding motifs, as well as sequences that have been shown for bacterial elongation factor (EF-Tu) to be involved in tRNA binding [10–13]. The β subunit contains two RNA-binding regions [14–16]. In the N-terminal half, three runs of lysine residues mediate interaction with mRNA *in vitro*; the C-portion of the protein, which encompasses a C₂–C₂ motif, also has RNA-binding properties. Both eIF2 β and eIF2 γ can be cross-linked to GTP and to Met-tRNA_i^{Met}, suggesting that the two subunits may be involved in the binding of these moieties [17–19].

The recognition of the initiator AUG codon during the scanning process is an intrinsic property of the Met-tRNA_i^{Met}, as shown in the yeast model [20]. However, other participants of this process also include the three subunits of eIF2, and eIF5. Genetic analysis in *Saccharomyces cerevisiae* identified mutations in these factors that allow the Met-tRNA_i^{Met} to recognize UUG codons for protein synthesis initiation (called suppressor mutants, for their capacity to suppress initiator codon mutations

Abbreviations used: aIF2, archaeal translation initiation factor 2; EF-Tu, bacterial elongation factor; eIF2, eukaryotic translation initiation factor 2; GAD, Gal4 activation domain; GBD, Gal4 DNA-binding domain; GST, glutathione S-transferase; Met-tRNA_i^{Met}, methionyl-initiator tRNA.

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in the *HIS4* locus in yeast) [14,21,22]. The biochemical basis for this abnormal initiation event seems to be a higher GTPase activity in the mutant eIF5 when associated with the initiation complex, and the uncovering of an intrinsic GTPase activity in the mutant eIF2, independent of eIF5 and of the ribosomes. Both of these defects probably result in the premature release of the translation initiation factors upon encountering an UUG codon [22].

Archaea has counterparts of all three subunits of eIF2. Because eIF2 is a central player in the determination of the start codon in eukaryotes and, in Archaea this process seems to have mechanistic divergences, a comparative analysis of the eIF2 and archaeal translation initiation factor 2 (aIF2) sequences should aid in the understanding of the participation of this factor in the translation initiation process.

In the present study, we describe a detailed comparison of this factor, mainly regarding the β subunit, which differs in a significant manner from the Archaea orthologues. Also included in this comparison is eIF5, which although sharing a high degree of sequence similarity with eIF2 β , has a completely different role in translation initiation. This analysis, together with the large body of mutational data available for the β subunit of eIF2, suggested important functional features of this protein, which were tested experimentally in yeast.

MATERIALS AND METHODS

Strains and genetic methods

Standard yeast genetic techniques and media were used as described elsewhere [23]. Yeast strain 167-3C (*MAT α* , *ura3-52*, *leu2-3,-112*, *his4^{UG}-306*, *ino1-13*) has been described previously [24]. The yeast strain used in the two-hybrid assays was PJ69-4A (*MAT α* , *trp1-901*, *leu2-3,-112*, *ura3-52*, *his3-200*, *gal4 Δ* , *gal80 Δ* , *GAL2-ADE2*, *LYS2::GAL1-HIS3*, *met2::GAL7-lacZ*) [25]. For the two-hybrid interaction studies, 1 mM 3-aminotriazole was added to synthetic dextrose media supplemented with the appropriate nutritional requirements.

Plasmid constructions

Fusions of the wild-type eIF2 subunits to the Gal4 DNA-binding (GBD) and activation (GAD) domains were made as follows: the *SUI3* gene, coding for eIF2 β in *S. cerevisiae*, containing a *Bam*HI site at the initiator AUG codon, was transferred as a *Bam*HI fragment from plasmid pBE149, a pGEX2 fusion described previously [16], to plasmids pGAD-C1 or pGBD-C1 [25]. The *SUI2* gene, encoding eIF2 α , was cloned as an *Nde*I (blunt ended with Klenow enzyme)–*Bam*HI fragment from plasmid pJLA603 [26] into pGBT9 and pGAD424 [27], which are identical to the above set of two-hybrid vectors, except for the restriction sites used for the fusions, digested with *Sma*I and *Bam*HI. The *GCD11* gene, encoding eIF2 γ , was obtained as an *Acc*I (blunt ended with Klenow enzyme)–*Pst*I fragment and cloned into pGBT9 and pGAD424 plasmids digested with *Sma*I and *Pst*I.

Deleted forms of eIF2 β fused to GAD were obtained by transferring to pGAD-C1 *Bam*HI fragments from plasmids pBE163, pBE192, pBE217, pBE242, which are glutathione S-transferase (GST) fusion constructions, described previously in [16]. The deletion removing amino acid residues from 235 to the C-terminus was constructed by inserting a *Bam*HI–*Xho*I fragment from pBE163 into pGAD-C1 digested with *Bam*HI and *Sal*I. Fusions of sequences encoding amino acid residues 1–186 and 187–285 were obtained by using a *Bgl*II site located between the sequences encoding amino acids 186 and 187, transferring a *Bam*HI–*Bgl*II and a *Bgl*II–*Bam*HI fragments respectively,

from plasmid pBE149 to pGAD-C1 digested with *Bam*HI. Fusion of amino acid residues 128–205 to GAD was obtained by inserting a 250 bp *Bam*HI–*Hinc*II fragment from pBE192 into pGAD-C1 digested with *Sal*I, made blunt ended by the Klenow polymerase, followed by digestion with *Bam*HI.

The fusion of amino acid residues 128–205 of eIF2 β with residues 128–205 of eIF2 β with GST was obtained by inserting the 250 bp *Bam*HI–*Hinc*II fragment from pBE192 into plasmid pGEX2T [28] digested with *Bam*HI–*Sma*I, originating plasmid pBE324.

The eIF5 coding sequence (*TIF5*) [29] was isolated by PCR from total genomic DNA, with primers 5'-CGGGATCCTCTATTAATATTTGTAGAGATAATC-3' and 5'-CGGTGCGACCTATTCGTCGTCTTCTTCATCAT-3', and cloned into pBluescript-polyT. The sequenced insert was transferred as a *Bam*HI fragment into *Bam*HI-digested pGAD-C1 or pGBD-C1. The fusion of the N-terminal portion of eIF5 to the Gal4 sequences was obtained by isolating a 642 bp *Bam*HI–*Eco*RV fragment from the cloned *TIF5* gene, and ligating it to pGAD-C1 or pGBD-C1 digested with *Sal*I, made blunt ended with the Klenow polymerase, and digested with *Bam*HI.

The high copy plasmid pBE185 carries the sequences coding for eIF2 γ [10], cloned as a *Hind*III fragment in YEp351 [30].

GST-pull down assay

The GST protein and GST–eIF2 β , GST–eIF2 $\beta^{\Delta 96/179}$ and GST–eIF2 $\beta^{128-205}$ fusion proteins were purified from *E. coli* cells containing plasmids pGEX2T, pBE149, pBE217 and pBE324, respectively, as described previously [16]. Yeast whole cell extract from strain 167-3C carrying plasmid pBE185, overexpressing eIF2 γ , was prepared from cells grown to saturation in synthetic dextrose medium supplemented with uracil, histidine and inositol. Cells were broken by agitation with glass beads in 10 mM Tris/HCl (pH 7.5), 100 mM NaCl, 30 mM MgCl₂, and 1 mM PMSF. For the GST-pull down assays, approx. 5 μ g of the purified fusion proteins, estimated from samples separated by SDS/PAGE after Coomassie Blue staining, were incubated for 30 min at 4 °C with 100 μ g of yeast whole cell extract, in buffer containing 20 mM Tris/HCl (pH 7.5), 100 mM KCl, 5 mM MgCl₂, 1 mM EDTA, 7 mM β -mercaptoethanol, 1 mM PMSF, 2 μ g/ml aprotinin, and 0.05% Igepal CA-630, in a final volume of 200 μ l. GSH–Sepharose beads (Pharmacia) were added (20 μ l) and after 15 min of incubation at 4 °C, the beads were washed three times with 0.5 ml PBS, resuspended in 20 μ l of 2 $\times$ Laemmli loading buffer [31], and subjected to SDS/10% PAGE. The purified GST protein (approx. 15 μ g), used as control in an identical pull-down assay, was added to the yeast whole cell extract in order to approach the amount of the degradation product present in some of the fusion protein preparations. The proteins were blotted to a Hybond-C membrane (Amersham) and probed with rabbit serum raised against a GST–eIF2 γ fusion protein [16]. After incubation with Protein A–horseradish peroxidase (1:2000; Sigma), the immune complexes were detected using the ECL* detection kit (Amersham).

RESULTS

Comparative analysis of eIF2 from Archaea and Eukarya

All sequenced Archaea genomes revealed the presence of orthologues of the three subunits of eIF2. The γ subunit is the most conserved between Eukarya and Archaea (47% identity), as expected from the strong homology of eukaryotic eIF2 γ with bacterial EF-Tu. The motifs implicated in the interaction with guanine nucleotides, as inferred from EF-Tu and yeast

Figure 1 Sequence alignment of the conserved sequences of the β subunit of eIF2 from Eukarya and Archaea and of eIF5

Residues that are identical or conserved in at least eight sequences are highlighted in black. * and ◆ denote the residues in *S. cerevisiae* eIF2 β and eIF5 sequences, respectively, which are altered in the suppressor alleles. The accession codes and references for these sequences are: *S. cerevisiae* eIF2 β , 320774 [14]; *Homo sapiens* eIF2 β , P20042 [15]; *Drosophila melanogaster* eIF2 β , P41375 [32]; *Triticum aestivum* eIF2 β , 024473 [33]; *Caenorhabditis elegans* eIF2 β , Q21230 [34]; *Methanococcus jannaschii* aIF2 β , Q57562 [1]; *P. horikoshii* aIF2 β , 3257011 [4]; *Archaeoglobus fulgidus* aIF2 β , 027958 [2]; *Methanobacterium thermoautotrophicum* aIF2 β , 027797 [3]; *S. cerevisiae* eIF5, P38431 [29]; *H. sapiens* eIF5, 1229140 [35]; *C. elegans* eIF5, Q22918 [34]; *Zea mays* eIF5, 4160402. The alignment was obtained using the Clustal algorithm in the MegAlign program of the DNASTAR package for Macintosh.

eIF2 γ , are present in the Archaea sequences [12,13]. For the α subunit, the identity between Eukarya and Archaea is in the range of 23%.

The most striking feature in this comparison resides in the β subunit. The β subunit of Archaea consists of only the C-terminal two-thirds of the eukaryal sequence, as shown in Figure 1. The region that is common to eIF2 β and aIF2 β is also found in the N-terminal region of eIF5, as shown in the alignment presented in Figure 1. Considering only the conserved sequences present in the three groups of proteins, equivalent to the complete archaeal aIF2 β , the average pairwise amino acid identity indexes, derived from the alignment shown in Figure 1, are 27% between eIF2 β and aIF2 β , 20% between eIF5 and aIF2 β , and 19% between eIF5 and eIF2 β . Thus it is likely that the archaeal sequence is more related to eIF2 β than to eIF5, suggesting that eIF5 has appeared later in evolution to accommodate a functional need of eukaryotic translation initiation, along with eIF2 β acquiring an N-terminal extension.

The N-terminal extension of eukaryal eIF2 shows little sequence conservation except for the presence of two or three lysine runs, of which at least one is required for protein function, as determined in yeast. These basic residues have been implicated in binding RNA, eIF5 and eIF2B ϵ [16,36]. The absence of this feature in the archaeal sequences may therefore be related to the mode of initiation of translation in these organisms. In eIF5, the C-terminal extension contains motifs that have been shown to be required for this factor to bind to the N-terminal region of eIF2 β [36].

The conserved sequences in aIF2 β , eIF2 β and eIF5 involve residues that have been subjected to extensive mutational analysis in the yeast eIF2 β [37]. These residues map in or near the C2-C2 motif (marked with arrows in Figure 1). The cysteine residues in this motif are essential for eIF2 β function. The elimination of only one of them, alteration of their spacing, or the elimination of the complete motif, results in inactive proteins. The residues in

the yeast eIF2 β sequence (marked with asterisks in Figure 1) are the sites of missense mutations that lead to translation initiation at non-AUG codons [14,37]. These mutations, resulting in non-conservative substitutions, are: R248T or R248G, R253I or R253S, L254P, S264Y or S264F, S267P, V268G or V268F. In three cases, where the same residues were altered in two independent mutants, the inserted residues are functionally not related. These results therefore suggest that these alterations result in loss of function. For both S264Y and L254P, it was shown biochemically that the mutant eIF2 complexes display an intrinsic GTPase activity, independent of eIF5 and of the ribosomes [22]. The residues that were found mutated in the suppressor forms of yeast eIF2 β are identical or conserved in all eukaryotic eIF2 β and in archaeal aIF2 β subunits, except for the positions relative to yeast S264 and S267 in all Archaea, and L254 in *Pyrococcus horikoshii*. In Archaea, position 267 carries a proline residue in all four sequences, as does the suppressor mutant in yeast; in addition, Archaea shares a conserved alanine residue with eIF5 (position 261 relative to yeast eIF2 β) in this region. These observations suggest that aIF2 might have a higher intrinsic GTPase activity. Regarding eIF5, no conservation is found in any of the positions involving suppressor mutations, except that corresponding to yeast valine-268. The residue that is mutated in yeast eIF5 (G31R) leading to initiation at UUG codons due to a premature GTPase activity on the pre-initiation complex, is identical in all eIF5 sequences and, interestingly, aligns with a gap in both eIF2 β and aIF2 β . The comparison of the C2-C2 region of the β subunit of both archaea and eukaryotes with that of eIF5 suggests that the latter has diverged considerably in function from an ancestral aIF2 β -like molecule.

Interaction of eIF2 β and eIF2 γ

The comparative analysis described above indicated that the eukaryotic sequence that is homologous to the archaeal β

in a protein unable to interact with eIF2 γ , indicating that this interaction requires sequences present in the central portion of eIF2 β . As expected, the lack of the lysine runs did not affect the interaction. In order to determine whether sequences in this central portion were sufficient to mediate the interaction with eIF2 γ , a protein was constructed containing only residues 128–205 fused to GAD. This protein associated with eIF2 γ to the same extent as the wild-type, indicating that this central region of eIF2 β mediates the association of this subunit with eIF2 γ . All deleted forms of eIF2 β described above were also tested for interaction with eIF2 γ in the opposite orientation of the two-hybrid system, with identical results as depicted in Figure 2(B) (results not shown).

To biochemically ascertain that the central portion of eIF2 β is responsible for the interaction with eIF2 γ , as suggested from the two-hybrid assays, a GST-pull down experiment was performed. A GST fusion protein carrying residues 128–205 of eIF2 β (GST-eIF2 β ^{128–205}), and a GST-eIF2 β fusion lacking residues 96–179 (GST-eIF2 β ^{Δ 96/179}) were purified from bacteria and incubated with whole cell extracts, prepared from a yeast strain overexpressing the eIF2 γ subunit from plasmid pBE185, and with GSH-Sepharose beads. As controls, the GST and the GST-eIF2 β proteins were used in parallel assays. eIF2 γ which associated with the beads through interaction with the GST fusion proteins was detected by Western blots, as shown in Figure 2(C). While the fusion protein lacking the central residues of eIF2 β was unable to associate with the γ subunit, the GST-eIF2 β ^{128–205} protein bound eIF2 γ at levels comparable with the complete eIF2 β . These results are in agreement with the two-hybrid data, giving further support for the role of the central 78 residues of eIF2 β , at the most, in being required and sufficient to maintain the interaction with the γ subunit. In this portion of the eIF2 β sequence, two groups of hydrophobic residues are spaced such that, in an α -helical simulation, their lateral chains are exposed on one surface, whereas the intervening residues are polar, an arrangement similar to 'leucine zipper' motifs. This arrangement of hydrophobic residues is maintained in all eukaryotic eIF2 β proteins and in the Archaea homologues. This region might, therefore, be largely responsible for the interaction with eIF2 γ through hydrophobic contacts. Interestingly, this same sequence was found to be irrelevant for the interaction of eIF2 β with RNA [16].

Interaction of eIF5 with eIF2

eIF5 shows a large gap in homology relative to eIF2 β in the sequences that were shown here to be involved in the interaction with eIF2 γ . From the sequence comparison shown in Figure 1, the region of strong sequence conservation between these two proteins begins at position 158, relative to the yeast eIF2 β . If the region comprising residues 128–180 is required for maintaining the interaction of eIF2 β with eIF2 γ , as our results suggest, then eIF5 may not interact with eIF2 γ . Previous *in vitro* data indicated that eIF5 interacts only with the β subunit of eIF2 [39]. However, because those results were obtained with proteins immobilized on filters after denaturation and renaturation, they might not reflect the actual situation in the cells. To evaluate this interaction *in vivo*, we performed two-hybrid analysis of eIF2 with eIF5, testing all pairwise combinations in both GAD and GBD fusions. Only the β subunit was capable of interacting with eIF5 *in vivo* (Figure 2D). This interaction was very weak, not allowing the growth of cells in the presence of 3-aminotriazole. It was possible that the N-terminal region of eIF5 would only be accessible for interaction with eIF2 γ under circumstances during the translation initiation process which would cause a conformational

modification in its structure, such as after interaction with other initiation factors or the ribosome. Therefore a construct bearing only the N-terminal portion of eIF5, containing the eIF2 β -homology motif, was also tested in the two-hybrid assays against eIF2 γ . Again, this was unable to interact with eIF2 γ in either fusion orientation (results not shown). These results, together with the available *in vitro* binding data, strongly indicate that eIF5 does not associate stably with eIF2 γ . This could be explained by the fact that the N-terminal region of eIF5 only differs in a significant manner from eIF2 β and aIF2 β for the large gap in the alignment in the region implicated in the interaction with eIF2 γ .

DISCUSSION

As expected from the Archaea sequences of the β subunit, we show in this paper that the N-terminal extension of eIF2 β , comprising the three lysine runs, is not required for maintaining the interaction with eIF2 γ , the only subunit that seems to bind to eIF2 β . We have previously shown that the lysine runs of eIF2 β are not required for the GTP-dependent binding of the Met-tRNA^{Met} by eIF2, nor for the binding of the ternary complex to the 40 S ribosomal subunit [16]. Therefore it seems that the sequence corresponding to aIF2 β is sufficient for the formation of the 43 S pre-initiation complex in eukaryotes. Thus it must be in the subsequent steps in translation initiation that this N-terminal portion of eIF2 β is required. We have shown that this region can mediate *in vitro* binding of eIF2 to mRNA, through the lysine repeats, in a charge-dependent mode [16]. Given that this extension is a characteristic feature of eukaryotic eIF2 β , and that Archaea and Eukarya differ in their modes of recognition of the AUG codon, this comparative analysis provides a rather intriguing hypothesis that the N-terminal extension of eIF2 β , through its lysine runs, might function as an anchor of the pre-initiation complex on to the mRNA, much as the Shine-Dalgarno sequence does in Archaea and Eubacteria. In addition to this idea, given that eIF5 is not found in Archaea, it is possible that the N-terminal region of eIF2 β has also evolved to accommodate the interaction with eIF5, as shown by recent data demonstrating that this eIF2 β domain is required for the binding of eIF5 and of the catalytic subunit of eIF2B [36]. The interactions of eIF5 and eIF2B with eIF2 β through this region are mutually exclusive, which is consistent with their functioning in different steps of translation initiation. As with these factors, the interaction of the N-terminal region with mRNA might also be mutually exclusive with the binding of eIF5.

eIF2 β can be thus divided in three domains: the N-terminal region, responsible for a eukaryotic specific step in translation initiation, involving the binding of eIF5 and eIF2B and of mRNA; a small central region, involved in binding the γ subunit, as determined in this present paper; and the remaining C-terminal region of the protein, that includes the sites of the mutations that alter translation initiation specificity, which may be involved in the control of GTP hydrolysis, and also in the binding of RNA.

In the genetic screen for suppressors of initiator codon mutations in yeast, mutations in the β subunit of eIF2 were the most frequently isolated suppressors [24]. This is consistent with the idea that these suppressors seem to represent loss-of-function mutations, as our comparative sequence analysis suggested. Considering that they result in the uncovering of an intrinsic GTPase activity on eIF2, these observations taken together seem to suggest that the β subunit of eIF2 may have a major role in inhibiting the hydrolysis of GTP in the pre-initiation complex. The suppressor mutations would then result in a defect in this inhibitory role, and their observed co-dominant phenotypes can

Figure 3 Schematic model for the function of eIF2 β

See text for details. * indicates the GTP molecule; + indicates the Met-tRNA^{Met}.

thus be explained by the fact that the interacting domain with eIF2 γ , as defined in the present study, is intact in these mutants.

From the sequence comparison of eIF2 γ with EF-Tu, and ligand-binding data obtained for eIF2, it seems that eIF2 γ contains the complete motifs for binding GTP and tRNA [13] and probably for the GTP hydrolysis induced upon ribosomal binding and codon/anticodon recognition, as defined for EF-Tu. The complex EF-Tu-GTP-tRNA binds to the elongating ribosomal particle, and GTP hydrolysis can thus occur immediately when a proper codon-anticodon interaction takes place. For eIF2, however, this activity must be tightly controlled in the 40 S eIF2-GTP-Met-tRNA^{Met} complex, to allow for a long period of association with the 40 S subunit and with the mRNA during the scanning process, until the pre-initiation complex is positioned at the initiator AUG codon. Therefore a mechanism is likely to exist in this complex for controlling the hydrolysis of GTP in the 40 S-associated eIF2, which may involve the β subunit, as this present study suggests. The function of eIF5, in promoting the GTP hydrolysis on the pre-initiation complex, according to this model, would then be to displace eIF2 β from its binding to eIF2 γ , thus allowing GTP hydrolysis to occur (as shown in the scheme depicted in Figure 3). eIF5, through its N-terminal region, containing the C₂-C₂ motif, may replace eIF2 β by binding to the same site as the C₂-C₂ motif of eIF2 β in the pre-initiation complex, and through its C-terminal extension to eIF2 β . The C₂-C₂ region of eIF5 shows important features that differ from eIF2 β , namely the fact that five of the six residues mutated in eIF2 β suppressors are not conserved in and among eIF5 sequences. This suggests that these eIF5 residues, as opposed to the same positions in the β subunit, do not bind specifically to any translational component, therefore suggesting that its role is to simply displace the C₂-C₂ region of eIF2 β from a very specific contact in the complex.

The sequencing of archaeal genomes did not identify any homologue of eIF5. The archaeal aIF2 β has a small gap in homology in the region mapped here to bind to the γ subunit, perhaps lowering its affinity for aIF2 γ . In addition, they contain

a residue in the C₂-C₂ region that results in higher GTPase activity in eIF2, and share an alanine residue with eIF5, suggesting that aIF2 might be more prone to GTP hydrolysis than its eukaryotic counterpart. Therefore no eIF5-like molecule would be required for activating the hydrolysis of GTP in these organisms. Archaea might cope with a higher intrinsic GTPase activity in aIF2 probably because the pre-initiation complex would bind directly on to the initiator region, through the Shine-Dalgarno sequence, without the involvement of a long period of message association for the scanning process to reach the AUG codon. Certainly, the functional study of archaeal aIF2, with the determination of the rate of spontaneous GTP hydrolysis, will be very informative regarding the role played by the β subunit in this complex.

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REFERENCES

- Bult, C. J., White, O., Olsen, G. J., Zhou, L., Fleischmann, R. D., Sutton, G. G., Blake, J. A., FitzGerald, L. M., Clayton, R. A., Gocayne, J. D. et al. (1996) *Science* **273**, 1058–1073.
- Klenk, H.-P., Clayton, R. A., Tomb, J.-F., White, O., Nelson, K. E., Ketchum, K. A., Dodson, R. J., Gwin, M., Hickey, E. K., Peterson, J. D. et al. (1997) *Nature (London)* **390**, 364–370.
- Smith, D. R., Doucette-Stamm, L. A., Deloughery, C., Lee, H., Dubois, J., Aldredge, T., Bashirzadeh, R., Blakely, D., Cook, R., Gilbert, K. et al. (1997) *J. Bacteriol.* **179**, 7135–7155.
- Kawarabayashi, Y., Sawada, M., Horikawa, H., Haikawa, Y., Hino, Y., Yamamoto, S., Sekine, M., Baba, S., Kosugi, H., Hosoyama, A. et al. (1998) *DNA Res.* **5**, 55–76.
- Kyrpides, N. C. and Woese, C. R. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 224–228.
- Kyrpides, N. C. and Woese, C. R. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3726–3730.
- Merrick, W. C. and Hershey, J. W. B. (1996) in *Translational Control*, (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp 31–69, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Clemens, M. J. (1996) in *Translational Control*, (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds.), pp 139–172, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hinnebusch, A. G. (1996) in *Translational Control*, (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds.), pp 199–244, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hannig, E. M., Cigan, A. M., Freeman, B. A. and Kinzy, T. G. (1993) *Mol. Cell. Biol.* **13**, 506–520.
- Dorris, D. R., Erickson, F. L. and Hannig, E. M. (1995) *EMBO J.* **14**, 2239–2249.
- Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B. F. C. and Nyborg, J. (1995) *Science* **270**, 1464–1472.
- Erickson, F. L. and Hannig, E. M. (1996) *EMBO J.* **15**, 6311–6320.
- Donahue, T. F., Cigan, A. M., Pabich, E. K. and Castilho-Valavicius, B. (1988) *Cell* **54**, 621–632.
- Pathak, V. K., Nielsen, P. J., Trachsel, H. and Hershey, J. W. B. (1988) *Cell* **54**, 633–639.
- Laurino, J. P., Thompson, G. M., Pacheco, E. and Castilho, B. A. (1999) *Mol. Cell. Biol.* **19**, 173–181.
- Anthony, D., Jr, Kinzy, T. and Merrick, W. C. (1990) *Arch. Biochem. Biophys.* **281**, 157–162.
- Bommer, J. A., Kraft, R., Kurzchalia, T. V., Price, N. T. and Proud, C. G. (1991) *Biochim. Biophys. Acta* **1079**, 308–315.
- Gaspar, N. J., Kinzy, T. G., Scherer, B. J., Humbelin, M., Hershey, J. W. B. and Merrick, W. C. (1994) *J. Biol. Chem.* **269**, 3415–3422.
- Cigan, A. M., Feng, L. and Donahue, T. F. (1988) *Science* **242**, 93–97.
- Cigan, A. M., Pabich, E. K., Feng, L. and Donahue, T. F. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2784–2788.
- Huang, H., Yoon, H., Hannig, E. M. and Donahue, T. F. (1997) *Genes Dev.* **11**, 2396–2413.
- Guthrie, C. and Fink, G. R. (1991) *Guide to Yeast Genetics and Molecular Biology*, Academic Press, New York.

-
- 24 Castilho-Valavicius, B., Yoon, J. and Donahue, T. F. (1990) *Genetics* **124**, 483–495
25 James, P., Halladay, J. and Craig, E. (1996) *Genetics* **144**, 1425–1436
26 Schauder, B., Blocker, H., Frank, R. and McCarthy, J. (1987) *Gene* **52**, 279–283
27 Chien, C. T., Bartel, P. L., Sternglanz, R. and Fields, S. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9578–9582
28 Smith, D. B. and Johnson, K. (1988) *Gene* **67**, 31–40
29 Chakravarti, D. and Maitra, U. (1993) *J. Biol. Chem.* **268**, 10524–10533
30 Hill, J. E., Myers, A. M., Koerner, T. J. and Tzagoloff, A. (1986) *Yeast* **2**, 163–167
31 Laemmli, U.K. (1970) *Nature (London)* **227**, 680–685
32 Ye, X. and Cavener, D. R. (1994) *Gene* **142**, 271–274
33 Metz, A. M. and Browning, K. S. (1997) *Arch. Biochem. Biophys.* **342**, 187–189
34 Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J. et al. (1994) *Nature (London)* **368**, 32–38
35 Si, K., Das, K. and Maitra, U. (1996) *J. Biol. Chem.* **271**, 16934–16938
36 Asano, K., Krishnamoorthy, T., Phan, L., Pavitt, G. D. and Hinnebusch, A. G. (1999) *EMBO J.* **18**, 1673–1688
37 Castilho-Valavicius, B., Thompson, G. M. and Donahue, T. F. (1992) *Gene Expression* **2**, 297–309
38 Fields, S. and Song, O.-K. (1989) *Nature (London)* **340**, 245–246
39 Das, S., Maiti, T., Das, K. and Maitra, U. (1997) *J. Biol. Chem.* **272**, 31712–31718
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