Universal Translation Initiation Factor IF2/eIF5B

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Translation factors are thought to accelerate the rate of protein synthesis and/or increase the fidelity of the process. In addition, the positive contributions of translation factors to cellular protein synthesis provide a means to regulate this process in response to cellular or environmental cues. Along with the requirement for factors to facilitate translation elongation, a distinct set of factors have been identified that promote assembly of a functional ribosome•mRNA•initiator Met-tRNA$_i^{Met}$ complex in which the anticodon of the Met-tRNA$_i^{Met}$ and the AUG codon of the mRNA base-pair within the ribosomal P site. Whereas three translation initiation factors (IF) have been identified in prokaryotes, translation initiation in eukaryotes requires at least 12 independent factors (Fig. 1) (for review, see Hershey and Merrick 2000). In addition, GTP is an essential requirement for translation in both prokaryotes and eukaryotes. Although biochemical and genetic analyses have provided insights into the roles of the translation initiation factors, the precise molecular function for most of these factors has not been resolved.

The recent deciphering of the genomes of a large number of organisms has revealed that a subset of translation initiation factors has been conserved among prokaryotes, archaea, and eukaryotes. As indicated in Figure 1, the factors IF1/eIF1A and IF2/eIF5B are conserved in all three kingdoms. The factor EF-P/eIF5A has also been conserved; however, whereas eIF5A stimulates model assays of translation initiation, in vivo studies have not confirmed a role for the protein in translation (Kang and Hershey 1994). The homology of the factors IF1 and eIF1A has been revealed by both amino acid sequence conservation (Kyrpides and Woese 1998) and similarity of factors' three-dimensional structures (Sette et al. 1997; Battiste et al. 2000). The conservation between IF2 and eIF5B is discussed in detail below. In addition to these universally conserved factors, it is interesting to note that factors eIF1 and eIF2 have been conserved between archaea and eukaryotes. Genetic studies in yeast have implicated both of these factors in AUG start codon recognition by scanning 40S ribosomal complexes (for review, see Donahue 2000). It is intriguing to speculate that eIF2 may functionally substitute for the mRNA Shine-Dalgarno sequence/16S rRNA interaction that promotes AUG start codon recognition in bacterial cells. As the structures of eIF1 (Fletcher et al. 1999) and the prokaryotic factor IF3 (Biou et al. 1995) show some resemblance, and a role of IF3 is to ensure AUG start-site specificity, it is also intriguing to speculate that the function of IF3 may have been assumed by eIF1 during evolution. Finally, the function of many of the eukaryotic-specific translation factors, notably in the eIF4 family and eIF3, is to facilitate binding of the 40S ribosomal complexes to the 5′-capped end of the eukaryotic mRNAs.

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CONSERVATION OF TRANSLATION FACTOR IF2/eIF5B

Analyses of the amino acid sequences of IF2 and eIF5B from prokaryotes and eukaryotes reveal that the protein can be subdivided into three regions (Fig. 2): an amino-terminal region with an abundance of charged residues, a highly conserved consensus GTP-binding domain, and a carboxy-terminal region that is fairly well-conserved. The eIF5B from archaea is highly similar to the prokaryotic and eukaryotic factors; however, it lacks the amino-terminal region (Fig. 2). Interestingly, the amino-terminal region of yeast eIF5B can be removed with no effect on yeast cell growth or on the ability of eIF5B to promote translation in vitro (Choi et al. 2000). The amino-terminal region of bacterial IF2 has been reported to bind ribosomes (Moreno et al. 1999), and like eIF5B, this region of IF2 is nonessential in vivo; however, deletion of the amino-terminal region of IF2 severely cripples bacterial cell growth (Laalami et al. 1991).

In the yeast *Saccharomyces cerevisiae*, eIF5B is encoded by the gene *FUN12*. Deletion of the *FUN12* gene results in a severe slow-growth phenotype, and polyribosome profiles from isogenic wild-type and *fun12*Δ strains reveal a translation initiation defect in the strains lacking eIF5B (Choi et al. 1998). This translation defect is also observed in vitro. When programmed with a luciferase reporter mRNA, whole-cell translation extracts prepared from wild-type strains produce ~15- to 25-fold more luciferase than extracts prepared from strains lacking eIF5B (Fig. 3) (Choi et al. 1998). Importantly, addition of recombinant GST-yeast-eIF5B to extracts prepared from strains lacking eIF5B fully restored translational activity (Fig. 3). This in vitro result demonstrates that eIF5B directly stimulates translation and indicates that eIF5B is a translation initiation factor.

Human and archaeal eIF5B fully or partially, respectively, substituted for yeast eIF5B and restored high-level

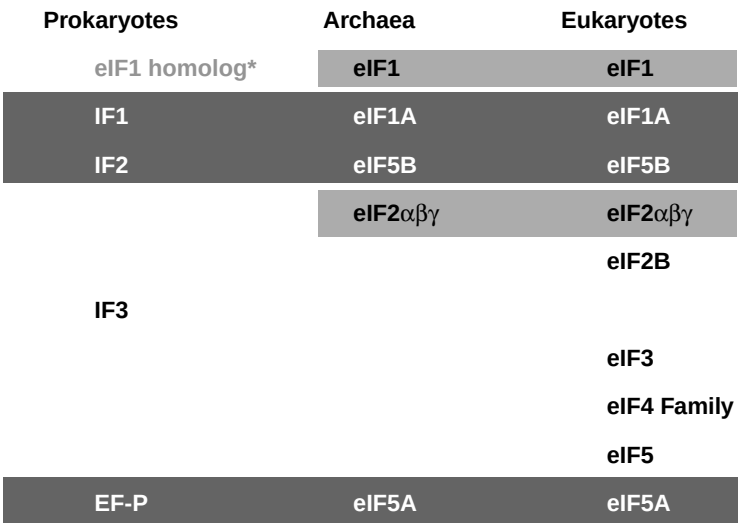

Figure 1. A core set of translation initiation factors is conserved through evolution. The factors in archaea have been identified on the basis of amino acid sequence similarity to the factors in prokaryotes and eukaryotes. As indicated, an ortholog of eukaryotic translation factor eIF1 has been identified in some, but not all, of the prokaryotic genomes that have been sequenced.

translational activity in extracts prepared from the eIF5B-deficient yeast strain (Fig. 3) (Lee et al. 1999). Consistent with their ability to restore translational activity in vitro, human and archaeal eIF5B substituted for yeast eIF5B in vivo and partially suppressed the slow-growth phenotype of strains lacking eIF5B (Lee et al. 1999). These in vivo and in vitro results demonstrate that the function of eIF5B has been conserved between yeast and mammals, and likely reflects a conservation in eIF5B function among all archaea and eukaryotes. To date, in vivo cross-complementation studies between archaeal or yeast eIF5B and *Escherichia coli* IF2 have been unsuccessful (J.H. Lee et al., unpubl.), suggesting that IF2 and eIF5B make critical contacts with other components of the translational machinery or ribosomes that are specific for bacterial versus archaeal/eukaryotic organisms. This lack of complementation between IF2 and eIF5B may reflect the fact that we have been unable to detect binding of eIF5B to initiator Met-tRNA$_i^{Met}$ (T.V. Pestova et al., unpubl.), whereas IF2 has been reported to specifically bind fMet-tRNA$_f^{Met}$ (see Wu and RajBhandary 1997; Guenneugues et al. 2000; Szkaradkiewicz et al. 2000). To summarize, archaeal and eukaryotic eIF5B appear to be functional homologs, and their function may be slightly diverged from that of bacterial IF2.

REQUIREMENT FOR AN ADDITIONAL MOLECULE OF GTP IN EUKARYOTIC VERSUS PROKARYOTIC TRANSLATION INITIATION

Role for eIF5B in Ribosomal Subunit Joining

As described elsewhere in this volume, Tatyana Pestova in a biochemical study identified eIF5B as a fac-

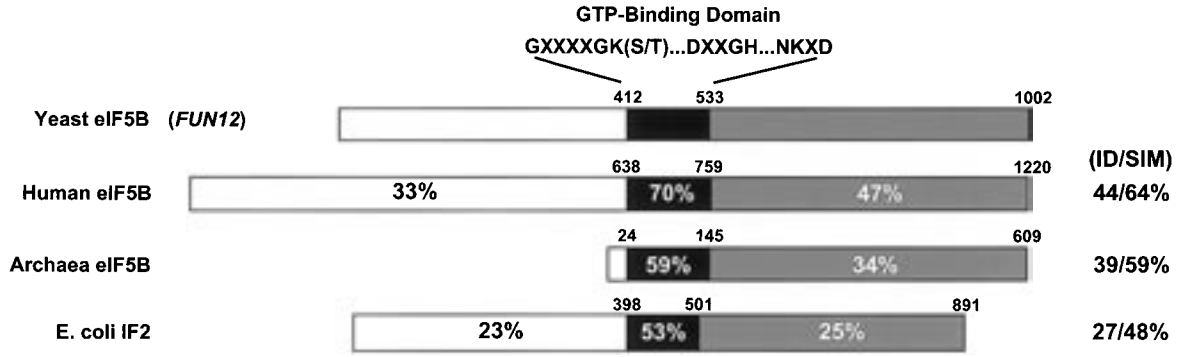

Figure 2. Conservation of IF2/eIF5B in prokaryotes, archaea, and eukaryotes. The schematics representing the full-length IF2/eIF5B from the yeast *S. cerevisiae* (encoded by the gene *FUN12*), humans, the archaea *Methanococcus jannaschii*, and *E. coli* are aligned through their highly conserved GTP-binding domains (containing the indicated consensus sequence motifs). Numbers above the schematics indicate the amino acid residue. Numbers within the schematics of the human, archaea, and *E. coli* factors are the percentages of amino acid sequence identities in the amino-terminal, GTP-binding, and carboxy-terminal domains relative to yeast eIF5B. At the right is indicated the percentage of amino acid sequence identities (ID) and similarities (SIM) of the full-length proteins compared to yeast eIF5B.

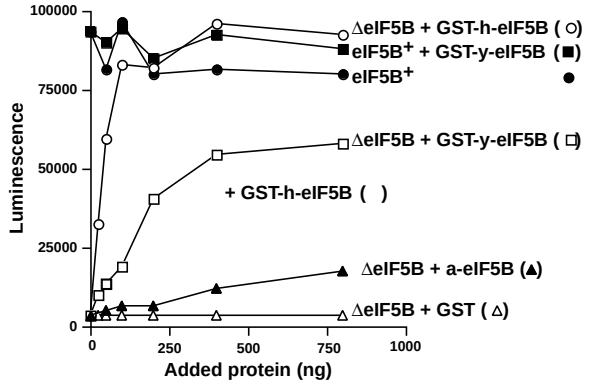

Figure 3. Restoration of translational activity in extracts from *fun12*Δ yeast strains lacking eIF5B by addition of recombinant yeast, human, or archaeal (*M. jannaschii*) eIF5B. In vitro translation extracts were prepared from isogenic wild-type (eIF5B$^+$) and eIF5B-deletion (ΔeIF5B) yeast strains. The extracts were supplemented with the indicated amounts of recombinant proteins and programmed with an in-vitro-transcribed luciferase reporter mRNA. Translational activity was determined after 15 minutes' incubation by measuring luminescence. (GST-y-eIF5B) Yeast eIF5B fusion protein; (GST-h-eIF5B) human eIF5B fusion protein; (a-eIF5B) *M. jannaschii* eIF5B (no GST).

tor required for the ribosomal subunit joining step of protein synthesis. Following scanning of the small ribosomal complex containing Met-tRNA$_i^{Met}$ and most likely the factors eIF2, eIF3, eIF5, eIF1, and eIF1A from the 5′ end of the mRNA to the AUG start codon, eIF5 is thought to promote GTP hydrolysis by eIF2, resulting in release of the factors from the ribosome (for review, see Hershey and Merrick 2000). Pestova et al. (2000) found that an additional factor, eIF5B, was required for 60S subunit joining. Recombinant human eIF5B substituted for the native protein and stimulated 60S subunit joining as well as methionyl-puromycin synthesis, a model assay for first peptide-bond formation. Consistent with the presence of the GTP-binding domain in eIF5B, Pestova et al. (2000) found that eIF5B bound GTP and that the factor hydrolyzed GTP in a ribosome-dependent reaction. Finally, eIF5B acted catalytically to promote subunit joining, and blocking the GTPase activity of eIF5B using the nonhydrolyzable GTP analog GDPNP permitted subunit joining but prevented the release of eIF5B from the 80S ribosomes following subunit joining (Pestova et al. 2000). Interestingly, the requirement for eIF5B for subunit joining is consistent with the observation that bacterial IF2 promotes subunit association (Godefroy-Colburn et al. 1975) and that the GTPase activity of IF2 is triggered upon subunit association (Kolakofsky et al. 1968; Tomsic et al. 2000).

Kinetic Studies Reveal GTP Requirement in a Late Step of Translation Initiation

Two recent kinetic studies have provided new insights into the roles of GTP and IF2 in translation initiation. Lorsch and Herschlag (1999) studying translation initiation in a mammalian in vitro system obtained evidence for a new GTP-dependent step late in the translation initiation pathway. This GTP requirement followed subunit joining and resulted in a 30-fold activation of the 80S complex, generating what they referred to as the 80S* complex (Lorsch and Herschlag 1999). This GTP-dependent conversion of 80S to 80S* was attributed to a soluble factor, as opposed to a ribosomal constituent. Finally, conversion of 80S to 80S* was inhibited by inclusion of GMP-PNP, resulting in formation of dead 80S complexes. These attributes are strikingly similar to the properties reported by Pestova et al. (2000) for eIF5B, and it will be very interesting to see whether recombinant human eIF5B can promote the conversion of 80S to 80S* in this system.

In a related study using a reconstituted bacterial translation initiation system, Tomsic et al. (2000) reported that GTP hydrolysis by IF2 occurred with fast kinetics following subunit joining; however, P_i release from the IF2•ribosomal complex was slow and rate-limiting for subsequent binding of the first elongating aminoacyl tRNA in complex with EF1A. It was proposed that a conformational rearrangement of IF2 or the ribosome is required subsequent to subunit joining to allow for IF2 release and first peptide-bond formation (Rodnina et al. 2000). This model is strikingly similar to the 80S to 80S* conversion proposed by Lorsch and Herschlag (1999). Surprisingly, the results of the kinetic study on bacterial translation suggest that GTP is not required for subunit joining and that GTP hydrolysis by IF2 is not linked to release of IF2 from the 70S ribosome. However, a point mutation in the IF2 GTP-binding domain that blocks GTP hydrolysis causes a dominant-lethal phenotype in vivo and results in retention of IF2 on the 70S ribosome following subunit joining (Luchin et al. 1999). Interestingly, this latter result is consistent with results of Pestova et al. which indicated that GTP hydrolysis by eIF5B is required for release of the factor following 80S complex formation (Pestova et al. 2000). The source of the discrepancy between the results of the kinetic and mutational studies on IF2 is unclear at present, and additional work is necessary to resolve the role of GTP hydrolysis by IF2 in translation initiation.

Model for the Roles of GTP in Translation Initiation

The discovery of eIF5B together with the previous identification of eIF2 and the work of Lorsch and Herschlag (1999), indicates that there are at least two GTP-dependent steps in eukaryotic translation initiation. In contrast, IF2 is the only GTPase required for prokaryotic translation initiation. A model to account for the additional GTP requirement in eukaryotic translation initiation in comparison to prokaryotic translation is presented in Figure 4. In prokaryotes, IF2 facilitates fMet-tRNA$_f^{Met}$ binding to the 30S ribosomal subunit, and in addition, IF2 promotes subunit joining. Hydrolysis of GTP by IF2 occurs rapidly following subunit joining and may be necessary for IF2 release from the 70S ribosome following subunit joining. In eukaryotes, the IF2 homolog eIF5B is

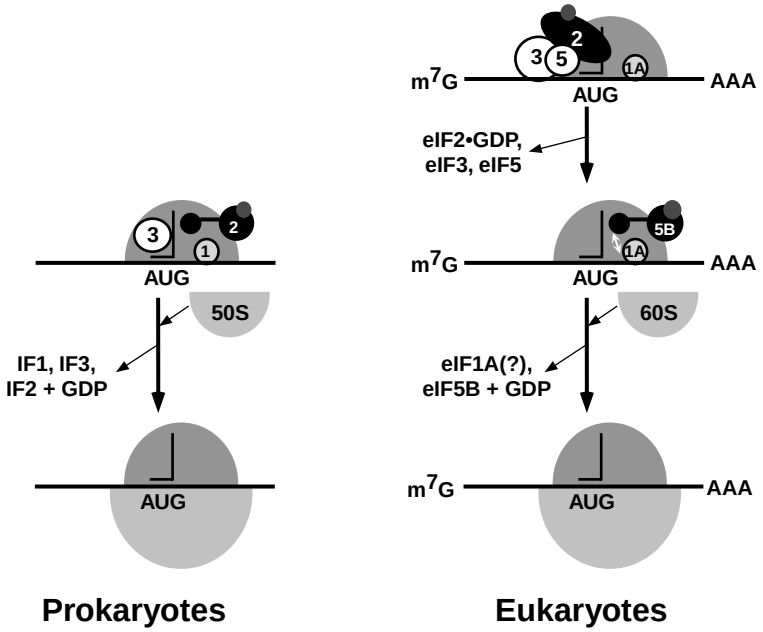

Figure 4. Model depicting two GTP-dependent steps in eukaryotic translation initiation versus only a single GTP requirement in prokaryotic translation initiation. (Left) In prokaryotic translation initiation, IF2 is the lone GTPase. The preinitiation complex with bound initiator fMet-tRNAfMet and factors localizes to the AUG start codon via interactions with the Shine-Dalgarno sequence on the mRNA. Following binding of the large 50S ribosomal subunit, hydrolysis of GTP by IF2 is coupled to release of the factors from the 70S ribosome. (Right) In eukaryotic translation initiation, the preinitiation complex containing factors eIF2, eIF3, eIF5, eIF1 (not depicted), and eIF1A, and initiator Met-tRNAiMet scans on the mRNA to the AUG codon. Proper base-pairing between the anticodon of the Met-tRNAiMet and the AUG start codon triggers eIF5-dependent GTP hydrolysis by eIF2 and release of the factors and deposition of Met-tRNAiMet in the ribosomal P site. The factor eIF5B then binds and promotes binding of the large 60S ribosomal subunit. Assembly of the 80S ribosome triggers the GTPase activity of eIF5B, leading to release of the remaining initiation factors from the ribosome.

required for efficient subunit joining, and GTP hydrolysis by eIF5B is required for release of the factor from the 80S ribosome following subunit joining. Accordingly, IF2 and eIF5B are performing complementary roles to promote subunit joining in prokaryotes and eukaryotes, respectively.

The presence of a second GTPase, eIF2, is restricted to archaea and eukaryotes (Fig. 1). The factor eIF2 binds the Met-$tRNA_i^{Met}$ to the ribosome in eukaryotes (for review, see Hershey and Merrick 2000). Thus, together eIF2 and eIF5B perform the functions attributed solely to IF2 in bacteria. The eIF2 forms a ternary complex with GTP and Met-$tRNA_i^{Met}$, and this ternary complex then binds to a 40S ribosomal subunit together with the translation factors eIF1, eIF1A, eIF3, and eIF5 to form a 43S preinitiation complex. This complex associates with the mRNA and scans to the AUG start codon. Recognition of the AUG start codon is accomplished by base-pairing with the anticodon loop of the $tRNA_i^{Met}$. This base-pairing is thought to trigger eIF5 to activate GTP hydrolysis by eIF2. In this way, eIF5, together with eIF2 and the Met-$tRNA_i^{Met}$, stringently ensures the fidelity of the initiation process (Huang et al. 1997; Donahue 2000). In prokaryotes, the selection of the AUG start codon is specified by interactions between the ribosome-binding site (Shine-Dalgarno sequence) on the mRNA and the 3′ end of the 16S rRNA in the 30S subunit. Thus, the novel GTP-dependent step in eukaryotic translation initiation is a fidelity checkpoint that replaces the simple base-pairing interaction used by prokaryotes to specify the translational start site. In addition, it should be noted that introduction of eIF2 in eukaryotic translation initiation provides a new opportunity to regulate protein synthesis. Like EF1A, eIF2 requires a guanine nucleotide exchange factor (eIF2B) to regenerate the active eIF2•GTP complex following each round of translation initiation. Phosphorylation of eIF2 on Ser-51 of its α subunit by specific stress-responsive kinases converts eIF2 from a substrate to a competitive inhibitor of eIF2B (Dever 1999). The phosphorylation of eIF2 is a common mechanism employed by eukaryotic cells to regulate both general and gene-specific mRNA translation (Dever 1999).

Finally, a question that arises concerns the function of eIF2 in archaea. Several studies have reported the presence of Shine-Dalgarno-like sequences, complementary to the 3′ end of 16S rRNA, near the AUG start codons of open reading frames in various archaea. These findings suggest that archaea use a prokaryotic-like mechanism to locate translational start sites and, as such, archaea should not require eIF2. However, a recent report suggests that in the archaeon *Sulfolobus solfataricus* the first open reading frame in a number of operons lacks Shine-Dalgarno ribosome-binding sequences, whereas subsequent open reading frames in these operons are preceded by a Shine-Dalgarno sequence (Tolstrup et al. 2000). Thus, it can be proposed that archaea employ a eukaryotic scanning-type mechanism involving eIF2 to locate the first open reading frame on a polycistronic mRNA, whereas

the subsequent open reading frames are translated via a prokaryotic-like mechanism which may or may not require eIF2 to bind the Met-tRNA$_i^{Met}$ to the ribosome.

STRUCTURAL ANALYSIS OF IF2/eIF5B

Recently, we determined the structure of eIF5B from the archaeon *Methanobacterium thermoautotrophicum* (Roll-Mecak et al. 2000). The protein consists of four domains and resembles a molecular chalice. The GTP-binding domain and domains II and III form the cup of the chalice, and they are connected via a long α helix (the stem of the chalice) to domain IV, which forms the base of the chalice (Fig. 5A). The GTP-binding domain resembles the GTP-binding domains of the translation factors EF1A and EF2, as well as that of Ras and the heterotrimeric GTP-binding proteins. Domains II and IV are antiparallel β barrels, whereas domain III is a novel α/β/α-sandwich.

Domain IV of eIF5B, as expected, resembles the carboxyl terminus of IF2 from *Bacillus stearothermophilus* (Meunier et al. 2000). This carboxy-terminal domain of IF2 is responsible for binding fMet-tRNA$_f^{Met}$, and mutagenesis studies and spectroscopic methods have implicated several residues of IF2 in fMet-tRNA$_f^{Met}$ binding (Misselwitz et al. 1999; Guenneugues et al. 2000). Interestingly, these residues are not conserved in archaeal and eukaryotic eIF5B, consistent with the inability to detect specific binding of eIF5B to Met-tRNA$_i^{Met}$. In contrast to the binding of aminoacyl tRNA to EF1A, the binding of fMet-tRNA$_f^{Met}$ to IF2 appears to only require fMet and the 3′ end of the tRNA. Competitive binding studies revealed that fMet-AMP can effectively compete the binding of full-length fMet-tRNA$_f^{Met}$ to IF2 (Szkaradkiewicz et al. 2000). In addition, deacylation protection and spectroscopic titration experiments demonstrated that fMet linked to the last six residues of the acceptor end of tRNA$_f^{Met}$ interacted with similar affinity as the full-length fMet-tRNA$_f^{Met}$ to IF2 (Guenneugues et al. 2000). Thus, it can be proposed that a primary function of the fMet-tRNA$_f^{Met}$ binding property of prokaryotic IF2 is to ensure that translation initiates with a formylated amino acid.

Comparison of the active and inactive structures of IF2/eIF5B revealed a concerted movement of domains II, III, and IV triggered by small conformational changes in the active site of the G domain induced by Mg^{++}/GTP binding (Fig. 5B) (Roll-Mecak et al. 2000). eIF5B seems to employ an articulated lever mechanism to amplify the small conformational changes in the active site over a distance of 90 Å to the carboxy-terminal domain IV. We presume that these conformational changes induced by Mg^{++}/GTP binding allow the proper interaction of the factor with the ribosome.

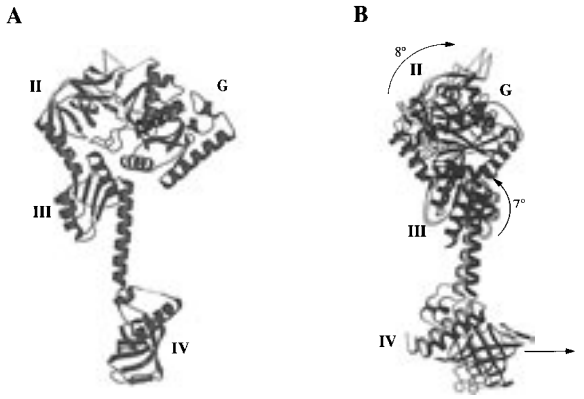

Figure 5. Three-dimensional structure of IF2/eIF5B. (*A*) Ribbon diagram showing the nucleotide-binding face of IF2/eIF5B from the archaeon *M. thermoautotrophicum* in complex with the nonhydrolyzable GTP analog GDPNP (Roll-Mecak et al. 2000). The locations of the GTP-binding domain (G domain) and domains II–IV are indicated. (*B*) Domain movements in IF2/eIF5B induced by Mg^{++}/GTP binding. Ribbon diagrams of IF2/eIF5B•GDPNP (*black*) and IF2/eIF5B•GDP (*gray*) superpositioned based on conserved elements in their GTP-binding domains (Roll-Mecak et al. 2000). The view of eIF5B is rotated 90° about a vertical axis relative to the image in *A*. The arrows denote the relative domain movements induced by GTP binding to eIF5B. (Reprinted, with permission, from Roll-Mecak et al. 2000 [copyright Elsevier Science].)

Similarity of IF2/eIF5B to Translation Elongation GTPases Suggests a Model for eIF5B Function in Translation Initiation

As mentioned above, the GTP-binding domain of eIF5B is structurally similar to the GTP-binding domains of translation factors EF1A and EF2. However, the structural similarity between eIF5B, EF1A, and EF2 extends to the domain II β barrel (see Fig. 6) (Roll-Mecak et al. 2000). Not only are the first two domains of eIF5B•GTP, EF2•GDP, and EF1A•GTP structurally similar, they also display the same relative orientation, suggesting that these domains form a common ribosome-binding platform. However, domains III and IV in IF2/eIF5B are not structurally similar to the corresponding domains in EF2. Comparison of the structures of EF1A•GTP•Phe-tRNA^{Phe} and EF2•GDP revealed that domains III–V of EF2 appear to mimic the shape of the tRNA moiety of the EF1A ternary complex, with domain III acting as the acceptor stem, domain V as the T stem, and domain IV as the anticodon helix (Nyborg et al. 1996). The surface charge distribution on domain IV of EF2 is similar to the anticodon helix of the tRNA, and electron microscopic studies have shown that this domain inserts into the ribosomal A site (Agrawal et al. 1998; Stark et al. 2000), presumably to promote ribosomal translocation during translation elongation. When the GTP-binding domains of eIF5B and EF1A are superimposed, domain IV of eIF5B is positioned at roughly 90° relative to the anticodon helix of the tRNA in the EF1A ternary complex (Fig. 6). Thus, as opposed to domain IV of EF2, which inserts into the decoding center on the small subunit, we predict that domain IV of IF2/eIF5B traverses across the top of the A site where it can be in close proximity to the 3′ end of the P-site tRNA, as discussed below.

A model that takes into account the structure of eIF5B and its resemblance to the elongation factors EF1A and

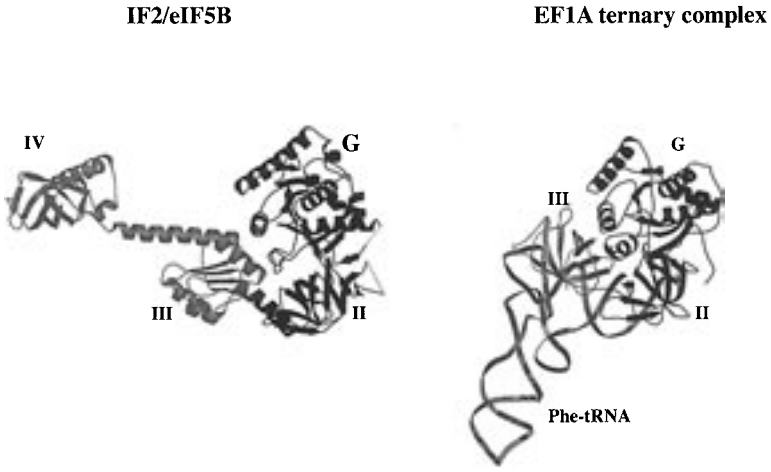

Figure 6. Comparison of the structures of the translational GTPases IF2/eIF5B•GDP and EF1A•GDPNP•Phe-tRNA^{Phe}. Ribbon diagrams of IF2/eIF5B•GDP (*left*, Roll-Mecak et al. 2000) and EF1A•GDPNP•Phe-tRNA^{Phe} (*right*, Nissen et al. 1995) oriented based on the alignment of the conserved P loop and α-helix H1 in the GTP-binding domains of the factors. The conserved GTP-binding (G domain) and β-barrel (II) domains of the factors are depicted in black; domains III and IV of IF2/eIF5B, domain III of EF1A, and Phe-tRNA^{Phe} are depicted in gray. The dorsal face of the factors is shown from the perspective of the small 40S ribosomal subunit. (Adapted and reprinted, with permission, from Roll-Mecak et al. 2000 [copyright Elsevier Science].)

EF2 is presented in Figure 7. Prior to describing the model, it is important to note that domain IV of yeast eIF5B directly interacts with the translation factor eIF1A. As mentioned earlier, eIF1A structurally resembles IF1 and, together with IF2/eIF5B, these proteins are the only universally conserved translation initiation factors (Fig. 1). Protein–protein interaction assays revealed that the carboxyl terminus of eIF5B directly interacts with eIF1A, and the two proteins form a complex independent of the ribosome (Choi et al. 2000). Similarly, the factors IF1 and IF2 have been reported to be cross-linked when bound to the ribosome (Boileau et al. 1983). Thus, these homologous factors likely physically interact on the ribosome to promote translation initiation in all organisms. Previously, the IF1-binding site on the ribosome was mapped to the A site (Moazed et al. 1995), and more recently, the X-ray structure of IF1 bound to the 30S subunit confirmed that the factor binds to the base of the A site adjacent to the mRNA (Carter et al. 2001). In our proposed model for eukaryotic translation initiation (Fig. 7), the 40S ribosomal subunit scans to the AUG codon where eIF2 hydrolyzes its GTP and many of the factors are released. The resulting complex contains Met-tRNA$_i^{Met}$ base-paired to the AUG codon of the mRNA in the P site and eIF1A in the A site of the 40S subunit. We propose that this complex is unstable in the absence of eIF5B, and that this instability at least partly accounts for the slow-growth phenotype of yeast strains lacking eIF5B. Inter-

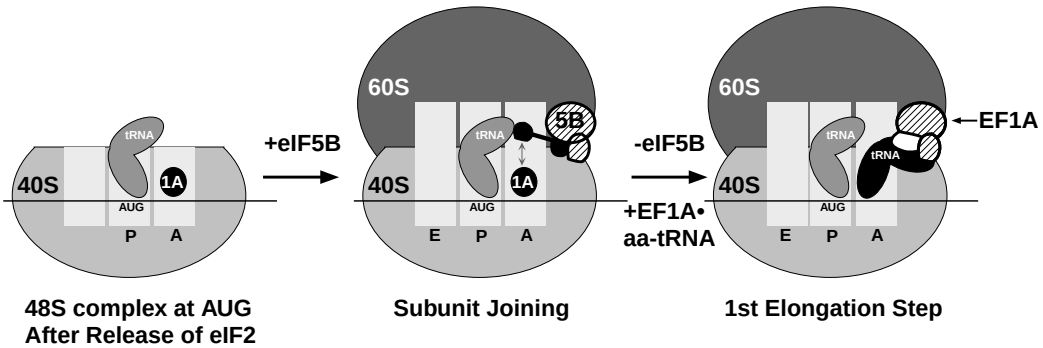

Figure 7. Structure-based model for eIF5B function in promoting the final steps of translation initiation. (*Left*) Following scanning of the 40S ribosomal complex to the AUG start codon, GTP hydrolysis by eIF2 is coupled to release of factors from the ribosome. The Met-tRNA$_i^{Met}$ remains bound in the P site, and we propose that eIF1A remains in the A site. (*Middle*) eIF5B binds to the complex mediated by interactions between domain IV and eIF1A. Domain IV of eIF5B is also in a position to interact with the aminoacyl end of the initiator Met-tRNA$_i^{Met}$ or perhaps with ribosomal constituents of the P site. The binding of eIF5B to the 40S complex facilitates 60S subunit binding. Hydrolysis of GTP by eIF5B following subunit joining is presumably accompanied by conformational changes that could reposition Met-tRNA$_i^{Met}$ in the P site and/or trigger release of eIF1A and eIF5B. (*Right*) The release of eIF5B and eIF1A presents a vacant A site on the 80S ribosome that is filled by the first elongating tRNA as part of the ternary complex EF1A•GTP•aminoacyl-tRNA.

estingly, this slow-growth phenotype can be partially rescued by increasing the dosage of $tRNA_i^{Met}$ genes in the cell (Choi et al. 1998). We propose that the increased abundance of Met-$tRNA_i^{Met}$ in these strains alters the equilibrium of the binding reaction, resulting in more Met-$tRNA_i^{Met}$ bound to the ribosome, and thus increasing the efficiency of protein synthesis.

According to our model, the eIF1A in the A site serves as a docking point for eIF5B. Domain IV of eIF5B will also be in position to interact with the aminoacyl end of Met-$tRNA_i^{Met}$ bound in the ribosomal P site (Fig. 7). This latter interaction may be more significant in bacterial translation, where domain IV of IF2 specifically recognizes fMet as described above. The binding of eIF5B to the 40S subunit facilitates joining of the large ribosomal subunit. Upon subunit joining, eIF5B hydrolyzes its GTP and presumably undergoes a conformational change that could dislodge eIF1A, weaken the binding of eIF5B to the ribosome, and/or alter the conformation of the ribosome or the Met-$tRNA_i^{Met}$ in the P site to facilitate the transition to translation elongation. (These changes may reflect the conversion of 80S to 80S* as described above.) Finally, the domain movements of eIF5B may result in formation of a functional A site such that following release of eIF5B, a molecular imprint is left for binding the EF1A ternary complex in the first step of translation elongation.

CONCLUDING REMARKS

Two translation initiation factors have been conserved through evolution. These factors, IF1/eIF1A and IF2/eIF5B, physically and functionally interact to promote assembly of a translationally competent ribosome with Met-$tRNA_i^{Met}$ in the P site base-paired with the AUG start codon of the mRNA. Interestingly, the third prokaryotic translation initiation factor IF3 and the eukaryotic factor eIF1 appear to have somewhat overlapping roles to ensure fidelity in the initiation process and specify initiation at an AUG codon. The identification of eIF5B reveals that there are at least two GTP-dependent steps in eukaryotic translation initiation as opposed to the single GTP requirement in prokaryotes. In eukaryotes and archaea, the GTPases eIF2 and eIF5B together perform the functions attributed to IF2 in prokaryotes. Whereas eIF5B is required for subunit joining, the factor eIF2 is needed for AUG start-codon selection and appears to functionally substitute for the ribosome-binding site (Shine-Dalgarno sequence) in prokaryotic mRNAs.

Key questions to address concerning the function of eIF5B in translation initiation include: (1) Which translation factors are released from the 40S subunit upon GTP hydrolysis by eIF2? (2) When does eIF5B bind to the ribosome and what factors are present on the 40S subunit when eIF5B binds? (3) What ribosomal constituents are required to stimulate the GTPase activity of eIF5B? (4) What is the conformational transition of eIF5B upon binding to the ribosome and after GTP hydrolysis? In addition, based on the critical role of eIF5B to promote subunit joining, it is surprising that eIF5B is not essential for viability in *S. cerevisiae*. In *Drosophila melanogaster,* eIF5B appears to be required for proper development and viability of the organism (Carrera et al. 2000); however, it remains to be determined whether eIF5B is required for cellular viability in *Drosophila* or other higher eukaryotes. Finally, electron microscopic and/or X-ray crystallographic images of IF2/eIF5B bound to the ribosome will provide important insights to further reveal the function of this universally conserved translation initiation factor.

ACKNOWLEDGMENTS

We thank Tatyana Pestova and Alan Hinnebusch for insights and useful discussions, and members of our labs for advice during the course of our studies. S.K.B. is an investigator in the Howard Hughes Medical Institute. A.R.-M. was supported by a National Science Foundation graduate fellowship and a Burroughs-Welcome Fund Interfaces training grant to The Rockefeller University.

REFERENCES

Agrawal R., Penczek P., Grassucci R., and Frank J. 1998. Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: The mechanism of translocation. *Proc. Natl. Acad. Sci.* **95:** 6134.

Battiste J.B., Pestova T.V., Hellen C.U.T., and Wagner G. 2000. The eIF1A solution structure reveals a large RNA-binding surface important for scanning function. *Mol. Cell* **5:** 109.

Biou V., Shu F., and Ramakrishnan V. 1995. X-ray crystallography shows that translational initiation factor IF3 consists of two compact α/β domains linked by an α-helix. *EMBO J.* **14:** 4056.

Boileau G., Butler P., Hershey J.W., and Traut R.R. 1983. Direct cross-links between initiation factors 1, 2, and 3 and ribosomal proteins promoted by 2-iminothiolane. *Biochemistry* **22:** 3162.

Carrera P., Johnstone O., Nakamura A., Casanova J., Jackle H., and Lasko P. 2000. VASA mediates translation through interaction with a *Drosophila* yIF2 homolog. *Mol. Cell* **5:** 181.

Carter A., Clemons W., Brodersen D., Morgan-Warren R., Hartsch T., Wimberly B., and Ramakrishnan V. 2001. Crystal structure of an initiation factor bound to the 30S ribosomal subunit. *Science* **291:** 498.

Choi S.K., Lee J.H., Zoll W.L., Merrick W.C., and Dever T.E. 1998. Promotion of Met-$tRNA_i^{Met}$ binding to ribosomes by yIF2, a bacterial IF2 homolog in yeast. *Science* **280:** 1757.

Choi S.K., Olsen D., Roll-Mecak A., Martung A., Remo K., Burley S., Hinnebusch A., and Dever T. 2000. Physical and functional interaction between eukaryotic orthologs of prokaryotic translation initiation factors IF1 and IF2. *Mol. Cell. Biol.* **20:** 7183.

Dever T.E. 1999. Translation initiation: Adept at adapting. *Trends Biochem. Sci.* **24:** 398.

Donahue T. 2000. Genetic approaches to translation initiation in *Saccharomyces cerevisiae*. In *Translational control of gene expression* (ed. N. Sonenberg et al.), p. 487. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Fletcher C.M., Pestova T.V., Hellen C.U.T., and Wagner G. 1999. Structure and interactions of the translation initiation factor eIF1. *EMBO J.* **18:** 2631.

Godefroy-Colburn T., Wolfe A.D., Dondon J., Grunberg-Manago M., Dessen P., and Pantaloni D. 1975. Light-scattering studies showing the effect of initiation factors on the reversible dissociation of *Escherichia coli* ribosomes. *J. Mol. Biol.* **94:** 461.

Guenneugues M., Caserta E., Brandi L., Spurio R., Meunier S., Pon C., Boelens R., and Gualerzi C. 2000. Mapping the fMet-$tRNA_f^{Met}$ binding site of initiation factor IF2. *EMBO J.* **19:** 5233.

Hershey J.W.B. and Merrick W.C. 2000. Pathway and mechanism of initiation of protein synthesis. In *Translational control of gene expression* (ed. N. Sonenberg et al.), p. 33. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Huang H., Yoon H., Hannig E.M., and Donahue T.F. 1997. GTP hydrolysis controls stringent selection of the AUG start codon during translation initiation in *Saccharomyces cerevisiae*. *Genes Dev.* **11:** 2396.

Kang H.A. and Hershey J.W. 1994. Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **269:** 3934.

Kolakofsky D., Dewey K.F., Hershey J.W., and Thach R.E. 1968. Guanosine 5′-triphosphatase activity of initiation factor f2. *Proc. Natl. Acad. Sci.* **61:** 1066.

Kyrpides N.C. and Woese C.R. 1998. Universally conserved translation initiation factors. *Proc. Natl. Acad. Sci.* **95:** 224.

Laalami S., Putzer H., Plumbridge J.A., and Grunberg-Manago M. 1991. A severely truncated form of translation initiation factor 2 supports growth of *Escherichia coli*. *J. Mol. Biol.* **220:** 335.

Lee J.H., Choi S.K., Roll-Mecak A., Burley S.K., and Dever T.E. 1999. Universal conservation in translation initiation revealed by human and archaeal homologs of bacterial translation initiation factor IF2. *Proc. Natl. Acad. Sci.* **96:** 4342.

Lorsch J.R. and Herschlag D. 1999. Kinetic dissection of fundamental processes of eukaryotic translation initiation in vitro. *EMBO J.* **18:** 6705.

Luchin S., Putzer H., Hershey J.W., Cenatiempo Y., Grunberg-Manago M., and Laalami S. 1999. In vitro study of two dominant inhibitory GTPase mutants of *Escherichia coli* translation initiation factor IF2. Direct evidence that GTP hydrolysis is necessary for factor recycling. *J. Biol. Chem.* **274:** 6074.

Meunier S., Spurio R., Czisch M., Wechselberger R., Guenneugues M., Gualerzi C., and Boelens R. 2000. Structure of the fMet-tRNA^{fMet}-binding domain of *B. stearothermophilus* initiation factor IF2. *EMBO J.* **19:** 1918.

Misselwitz R., Welfle K., Krafft C., Welfle H., Brandi L., Caserta E., and Gualerzi C. 1999. The fMet-tRNA binding domain of translational initiation factor IF2: Role and environment of its two Cys residues. *FEBS Lett.* **459:** 332.

Moazed D., Samaha R.R., Gualerzi C., and Noller H.F. 1995. Specific protection of 16 S rRNA by translational initiation factors. *J. Mol. Biol.* **248:** 207.

Moreno J.M., Drskjotersen L., Kristensen J.E., Mortensen K.K., and Sperling-Petersen H.U. 1999. Characterization of the domains of *E. coli* initiation factor IF2 responsible for recognition of the ribosome. *FEBS Lett* **455:** 130.

Nissen P., Kjeldgaard M., Thirup S., Polekhina G., Reshetnikova L., Clark B.F., and Nyborg J. 1995. Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. *Science* **270:** 1464.

Nyborg J., Nissen P., Kjeldgaard M., Thirup S., Polekhina G., and Clark B.F.C. 1996. Structure of the ternary complex of EF-Tu: Macromolecular mimicry in translation. *Trends Biochem. Sci.* **21:** 81.

Pestova T.V., Lomakin I.B., Lee J.H., Choi S.K., Dever T.E., and Hellen C.U.T. 2000. The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature* **403:** 332.

Rodnina M., Stark H., Savelsbergh A., Wieden H.-J., Mohr D., Matassova N., Peske F., Daviter T., Gualerzi C., and Wintermeyer W. 2000. GTPase mechanisms and functions of translation factors on the ribosome. *Biol. Chem.* **381:** 377.

Roll-Mecak A., Cao C., Dever T.E., and Burley S.K. 2000. X-ray structures of the universal translation initiation factor IF2/eIF5B: Conformational changes on GDP and GTP binding. *Cell* **103:** 781.

Sette M., van Tilborg P., Spurio R., Kaptein R., Paci M., Gualerzi C.O., and Boelens R. 1997. The structure of the translational initiation factor IF1 from *E. coli* contains an oligomer-binding motif. *EMBO J.* **16:** 1436.

Stark H., Rodnina M., Wieden H.-J., van Heel M., and Wintermeyer W. 2000. Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. *Cell* **100:** 301.

Szkaradkiewicz K., Zuleeg T., Limmer S., and Sprinzl M. 2000. Interaction of fMet-tRNA^{fMet} and fMet-AMP with the C-terminal domain of *Thermus thermophilus* translation initiation factor 2. *Eur. J. Biochem.* **267:** 4290.

Tolstrup N., Sensen C., Garrett R., and Clausen I. 2000. Two different and highly organized mechanisms of translation initiation in the archaeon *Sulfolobus solfataricus*. *Extremophiles* **4:** 175.

Tomsic J., Vitali L., Daviter T., Savelsbergh A., Spurio R., Striebeck P., Wintermeyer W., Rodnina M., and Gualerzi C. 2000. Late events of translation initiation in bacteria: A kinetic analysis. *EMBO J.* **19:** 2127.

Wu X.-Q. and RajBhandary U. 1997. Effect of the amino acid attached to *Escherichia coli* initiator tRNA on its affinity for the initiation factor IF2 and on the IF2 dependence of its binding to the ribosome. *J. Biol. Chem.* **272:** 1891.