Minimum Requirements for the Function of Eukaryotic Translation Initiation Factor 2

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ABSTRACT

Eukaryotic translation initiation factor 2 (eIF2) is a G protein heterotrimer required for GTP-dependent delivery of initiator tRNA to the ribosome. eIF2B, the nucleotide exchange factor for eIF2, is a heteropentamer that, in yeast, is encoded by four essential genes and one nonessential gene. We found that increased levels of wild-type eIF2, in the presence of sufficient levels of initiator tRNA, overcome the requirement for eIF2B in vivo. Consistent with bypassing eIF2B, these conditions also suppress the lethal effect of overexpressing the mammalian tumor suppressor PKR, an eIF2α kinase. The effects described are further enhanced in the presence of a mutation in the G protein (γ) subunit of eIF2, ged11-K250R, which mimics the function of eIF2B in vivo. Interestingly, the same conditions that bypass eIF2B also overcome the requirement for the normally essential eIF2α structural gene (SUI2). Our results suggest that the eIF2γ complex is capable of carrying out the essential function(s) of eIF2 in the absence of eIF2α and eIF2B and are consistent with the idea that the latter function primarily to regulate the level of eIF2-GTP-Met-tRNA Met ternary complexes in vivo.

In the current model for eukaryotic translation initiation (Hinnebusch and Liebman 1991; Merrick 1992; Pain 1996), initiator methionyl-tRNA is delivered to the 40S ribosomal subunit in the form of a eukaryotic translation initiation factor 2 (eIF2)-GTP-Met-tRNA Met ternary complex. The resulting 43S complex, which also includes eIF3 and eIF1A, binds at or near the 5′ end of capped eukaryotic messenger RNAs in a process that appears to involve interactions between eIF3 and proteins bound at the mRNA 5′ cap (Sachs et al. 1997). Once bound, the ribosome traverses the mRNA in a 5′-to-3′ direction and locates the AUG codon representing the translational start site. Recognition of the start site is accompanied by GTP hydrolysis, which releases Met-tRNA Met to the ribosomal peptidyl site and converts eIF2 to an eIF2-GDP binary complex. The eIF2-GDP complex must be converted to the GTP form to rebind Met-tRNA Met and to participate in another cycle of translation initiation. Mammalian eIF2-GDP is extremely stable in vitro in the presence of physiological concentrations of magnesium and requires the guanine nucleotide exchange factor eIF2B to promote rapid conversion of eIF2-GDP to eIF2-GTP. Although the latter complex is less stable, GTP binding is stabilized upon binding Met-tRNA Met.

In the yeast Saccharomyces cerevisiae, subunits of the eIF2 heterotrimer are encoded by the single-copy essential genes SUI2 (α), SUI3 (β), and GCD11 (γ). The primary structures of the eIF2 subunits are conserved between yeast and mammals (Ernst et al. 1987; Donahue et al. 1988; Pathak et al. 1988; Cigan et al. 1989; Erickson et al. 1997). The γ-subunit of eIF2 is a member of the GTP-binding (G) protein superfamily and is highly similar to eubacterial elongation factor EF1A (formerly EF-Tu; Hannig et al. 1993; Gaspar et al. 1994). Functional similarity between EF1A and eIF2γ proteins is further suggested by genetic and biochemical data that indicate the γ-subunit plays a significant role in binding nucleotide and tRNA ligands (Erickson and Hannig 1996). The guanine nucleotide exchange factor for eIF2, eIF2B, is a heteropentamer that, in yeast, is encoded by four essential genes (GCD1, GCD2, GCD6, and GCD7) and one nonessential gene (GNC3) that are also conserved in mammals (Bushman et al. 1993a; Cigan et al. 1993; Koorn 1995; Price et al. 1996a,b). The exchange reaction is regulated indirectly via phosphorylation of the α-subunit of eIF2, which converts eIF2 into a competitive inhibitor of the exchange reaction (Rowlands et al. 1988; Dever et al. 1995; Kimball et al. 1998). In vivo, this leads to reduced global rates of translation initiation and, in some cases, gene-specific enhancement of translation (Hershey 1991; Hinnebusch 1993; Rhoads 1993). A mammalian eIF2α kinase, PKR, has been proposed to function as a tumor suppressor and underlies the importance of regulating the exchange reaction for normal homeostasis in higher eukaryotic organisms (Koromilas et al. 1992; Meurs et al. 1993; Barber et al. 1995a,b; Donze et al. 1995).
We previously described a mutation, *gcd11-K250R*, that conferred phenotypes consistent with reduced eIF2 function, i.e., reduced growth rates and increased expression of *GCN4* (Erickson and Hannig 1996). This mutation alters the lysine residue within the NKXD nucleotide-binding motif of eIF2γ that is conserved in G proteins (Dever et al. 1987; Bourne et al. 1991). Both phenotypes were suppressed by increased dosage of the yeast initiator tRNA gene (*IMT*). In *vivo*, *gcd11-K250R* led to increased dissociation rates for both eIF2-GDP and eIF2-GTP, while the binding of Met-tRNA\textsubscript{Met} to eIF2-GTP complexes stabilized GTP binding for both the wild-type and γ\textsubscript{K250R} forms of eIF2 (Erickson and Hannig 1996). Together, these data suggested that the γ\textsubscript{K250R} alteration might also promote more rapid formation of ternary complexes and thereby reduce the requirement for eIF2B in *vivo*. In this article, we confirm these predictions. Furthermore, sufficient levels of wild-type eIF2 also reduce the requirement for eIF2B in *vivo*, though much less efficiently than in the presence of *gcd11-K250R*. This suggests that increasing the rate of dissociation of eIF2-GDP complexes is an essential function provided by eIF2B. Interestingly, the α-subunit of eIF2, which appears to play an important role in eIF2/eIF2B interactions (Vazquez de Aldana and Hinnenbusch 1994; Pavitt et al. 1997, 1998; Nika et al. 2001), is no longer required for viability under conditions that lead to bypass of eIF2B. Our combined data suggest that the eIF2βγ complex is capable of carrying out all essential eIF2B functions, including essential interactions with additional components of the eukaryotic translational machinery. We propose that eIF2B and the α-subunit of eIF2 comprise an elaborate regulatory system for modulating levels of ternary complex that, although not essential *per se* for growth, plays a critical role in maintaining homeostasis and viability in wild-type cells.

**MATERIALS AND METHODS**

Plasmids: pSB32/ (LEU2), YCp50/ (URA3; Rose and Broach 1991), and pRS316/ (URA3; Sikorski and Hieter 1989) are yeast-*Escherichia coli* shuttle vectors containing yeast centromere sequences for maintenance in low copy number in yeast. YEp24/ (URA3), YEp13/ (LEU2; Rose and Broach 1991), and YEpplac112/ (TRPI; Gietz and Sugino 1988) are yeast-*E. coli* shuttle vectors maintained in high copy in yeast due to the presence of the 2\textmu m origin of replication. Construction of YEp13/ (SU2/SU3/GCDD1\textsubscript{HIDP} (Ep847), YEp13/SU2/SU3/gcd11\textsubscript{HIDP}-K50R (Ep922), and YEpplac112/IMT (Ep1013) were described previously (Erickson and Hannig 1996). Unless otherwise noted, the GCDD1 alleles used in this study contain a C-terminal octyl-histidine tag. The tag does not appear to alter the function of wild-type eIF2 in *vivo* or affect ligand binding in *vivo* (Erickson and Hannig 1996). The galactose-inducible PKR construct (in YCp50) was a generous gift from T. Dever (National Institutes of Health, Bethesda, MD).

Ep1087 is a pSB32/ GCDD6/GCDD7 plasmid. A 3.3-kb SpeI fragment from pJB6 (Bushman et al. 1993a) containing GCDD6 was converted to a HindIII fragment following treatment with Klenow enzyme and ligation with HindIII linkers. This fragment was inserted into HindIII-cleaved pSB32 to create Ep1033. The GCDD7 fragment was obtained from pJB100 (Bushman et al. 1993a) by first converting the EcoRI site to an *Eag*I site using oligonucleotide linkers as described above. A 2.1-kb *Nof* fragment containing GCDD7 was removed from the modified pJB100, ligated to *Eag*I-cleaved YEp24 to create Ep673, and then subcloned as a 2.1-kb *Eag*I fragment into *Eag*I-cleaved Ep1033. The resulting plasmid, Ep1037, was cleaved with *AadI* (in vector sequences), flush-ended, and converted to an *Adh* site using oligonucleotide linkers to create Ep1066.

The pSB32/ GCDD1/GCDD2/GCDD6/GCDD7 plasmid Ep127 was constructed by inserting an *Adh* fragment containing GCDD1 and GCDD2 into *Adh*-cleaved Ep1066. The GCDD1 fragment was obtained as a 2.4-kb *BamHI* fragment from YCp50/Sc4014 (Hill and Struhl 1988) that was initially subcloned into *BamHI*-cleaved pBluescript (Promega, Madison, WI) to create Ep1082. The GCDD2 fragment was obtained from pY26 (Dever et al. 1995) as a 2.6-kb *Eag*I fragment following conversion of the *Clal* site to an *Eag*I site as above. This fragment was subcloned into *Eag*I-cleaved Ep1082. Vector Xhol and SphI sites were sequentially converted to *Adh* sites by linker tailing to create Ep1120. A 5.0-kb *Adh* fragment from Ep1120 containing GCDD1 and GCDD2 was then subcloned into *Adh*-cleaved Ep1066 to create Ep1127.

Additional plasmids containing the four essential eIF2B subunit genes, with or without GCDD1\textsubscript{HIDP}, were constructed as follows. Ep1174/ (pBluescript with Xhol and SphI sites altered to *Adh*) was cleaved with *BamHI* and *HindIII* and ligated with a 2.1-kb *BamHI/HindIII* fragment containing GCDD1\textsubscript{HIDP}. The resulting plasmid (Ep1246) was digested with *BamHI* and ligated with the 2.4-kb *BamHI* GCDD1 fragment from YCp50/Sc4014. The resulting plasmid was cleaved with *Eag*I and ligated with the 2.6-kb *Eag*I GCDD2 fragment to create Ep1247. A 7.1-kb *Adh* fragment, containing GCDD1, GCDD2, and GCDD1\textsubscript{HIDP}, was ligated to *Adh*-cleaved Ep1067 and Ep1066 to create Ep1250/ (pRS316/ GCDD1/GCDD2/GCDD6/GCDD7/GCDD1\textsubscript{HIDP} and Ep1262/ (pSB32/ GCDD1/GCDD2/GCDD6/GCDD7/GCDD1\textsubscript{HIDP}), respectively. The pRS316/ GCDD1/GCDD2/GCDD6/GCDD7 plasmid Ep1125 was constructed by first inserting the 2.1-kb *Eag*I GCDD7 fragment from Ep673 into *Eag*I-cleaveed pJB5 to create Ep1042. The 5.1-kb GCDD1/GCDD2 *Adh* fragment from Ep1120 was then inserted at the *Adh* site in Ep1042, which had been modified using *Adh* oligonucleotide linkers, to create Ep1125.

Plasmids used in the *Snf2* suppression experiments contained the 2.1-kb *HindIII/SnaBI* GCDD11 fragment (where the *SnaBI* site was altered to a *BamHI* site by linker tailing; Hannig et al. 1993) and/or a 2.5-kbp *BamHI* fragment from pD14-6 containing *SU2* (a gift from T. Dever, National Institutes of Health). Construction of YEp15/GCDD1\textsubscript{HIDP} (Ep832) and YEp13/ gcdd11-K250R\textsubscript{HIDP} (Ep921) were described previously (Erickson and Hannig 1996). YEp15/ GCDD1\textsubscript{HIDP}/SU2 (Ep1064) and YEp13/ gcdd11-K250R\textsubscript{HIDP}/SU2 (Ep1065) were constructed by removing the 2.5-kbp *BamHI* SU2 fragment from Ep847 and Ep992, respectively.

**Strain construction**: The parent strain for EY878 (MATa *leu2-3, 112 *trpl-D3 ura3-52 gcd1:hisG gcd2:hisG gcd6:hisG gcd7:hisG gen3::hisG <Ep1125>) is EY809 (MATa *leu2-3, 112 *trpl-D3 ura3-52 gcd1:hisG gcd2:hisG <Ep1042 [pRS316/URA3] / GCDD6/GCDD7>). The gcd7::hisG allele (from pJB110; Bushman et al. 1993a) removes 66% (residues 79–325) of the 381-amino-acid GCDD7 open reading frame (ORF). The gcd6::hisG allele was from pJB96 (Bushman et al. 1993a), and removes 87% (residues 93–713) of the 713-amino-acid GCDD6 ORF. The remainder of the eIF2B subunit genes were deleted in EY809 after replacing the pRS316-based plasmid with the low-copy
LEU2 plasmid Ep1127 that contains GCD1, GCD2, GCD6, and GCD7. The gedΔ::hisG-URA3-his3Gallele (Ep1191) was derived from Ep175 (a URA3 disruption version of Ep174; HANNIG and HINNEBUSCH 1988) by replacing the disrupting URA3 fragment with the isgG-URA3-his3G cassette from pNYK51 (ALANI et al. 1987). This removes 52% (residues 1–299) of the 578-amino-acid GCD1 ORF. The gedΔ::hisG-URA3-his3G allele (Ep1145) removes 95% (residues 26–632 on a PvuII/ EcoRI restriction fragment) of the 651-amino-acid GCD2 ORF (PADDON et al. 1989). The gedΔ::hisG-URA3-his3G allele was derived from Ep308 (HANNIG et al. 1990) by replacing the disrupting LEU2 fragment with hisG-URA3-his3G as above to create Ep545. This construct removes the entire 305-amino-acid GCN3 ORF. DNA fragments used for gene disruptions were obtained following digestion of the corresponding plasmids with BamHI (GCD1), NcoI (GCD2), or BglII/PvuII (GCN3) and purification by agarose gel electrophoresis. Gene disruptions were confirmed by Southern blot analysis of Ura⁺ trans-formants, using appropriate probes to distinguish chromosomal and plasmid-borne (in Ep1127) alleles (data not shown). Chromosomal disruptions were plated on YEPD plates with 5-fluoroorotic acid (5-FOA) medium to select for recombination between the direct hisG repeats, an event that evicts the URA3 gene, leaving behind a single copy of the hisG repeat. GCD2, GCD1, and GCN3 were disrupted sequentially in the EY809 background. The URA3 plasmid Ep1125 was then used to replace Ep1127 to create EY878. The absence of each essential eIF2B subunit gene in EY878 was also demonstrated genetically by the inability of plasmids containing only three of the four essential eIF2B subunit genes, in all possible combinations, to complement in the absence of Ep1125.

Disruption of GCD11 in EY878 utilized a derivative of EY878 in which Ep1626 (LEU2) replaced Ep1125 (URA3); GCD11 was then disrupted using the gedΔ::hisG-URA3-his3G allele from Ep523 as described (DORRIS et al. 1995), followed by growth on 5-FOA. The URA3 plasmid Ep1250 was used to replace the LEU2 plasmid Ep1262 to create EY923.

EY740 (MATa leu2-3, -112 ura3-52 trp1Δ63 gedΔ::hisG GAL2’<Ep293; Ycp50/CD11>) was obtained as a meiotic segregant from a cross between a H1515 (MATa leu2-3, -112 ura3-52) strain and EY878 (MATa leu2-3, -112 ura3-52 gedΔ::hisG GAL2’<Ep293>). The gedΔ::his3Gallele lacks the entire GCD11 open reading frame (HANNIG et al. 1999). EY885 (MATa leu2-3, -112 ura3-52 trp1Δ63 sui2Δ gedΔ::hisG GAL2’<Ep1130; Ycp50/CD11/SUI2>) was obtained from a cross between EY740 and EY779 (MATa leu2-2, -112 ura3-52 trp1Δ63 sui2Δ <pSB32/SUI2>). The sui2Δ allele removes the N-terminal two-thirds of the SUI2 ORF (DEVER et al. 1992). EY779 is a derivative of H1816 (VAZQUEZ DE ALDANA and HINNEBUSCH 1994).

**Growth rate determination:** Doubling times at 30°C were determined for log-phase cultures grown in minimal (SD) media supplemented as necessary (SHERMAN et al. 1986).

**eIF2B assay:** Reactions (30 μl) contained 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM MgCl₂, 0.1 mM Na₂EDTA, 1 mM dithiothreitol, 5% glycerol, 1 mg/ml creatine kinase (as carrier), 10 μM GDP, 100 μM GTP, and 500 nM [3H]Met-tRNAiMet (65 kcpm/pmol). eIF2B preparations (3 μg, >80% pure; ERICKSON and HANNIG 1996) were prebound to GDP for 10 min at 25°C in the absence or presence of 1 μg yeast eIF2B (NIKA et al. 2001) and then transferred to a 10°C water bath for an additional 5 min. Under these conditions, both wild-type eIF2 and eIF2K250R are maximally bound to GDP (ERICSON and HANNIG 1996). GDP and [3H]Met-tRNAiMet were then added, and 5-μl aliquots were withdrawn and filtered through nitrocellulose as described previously (ERICSON and HANNIG 1996).

**RESULTS**

**Yeast eIF2 exhibits an intrinsic nucleotide exchange activity:** We measured nucleotide exchange activity by testing the ability of GDP-bound yeast eIF2 to form eIF2-GTP-Met-tRNAiMet ternary complexes in vitro (Figure 1). In mammalian systems, this reaction requires eIF2B to dissociate the eIF2-GDP complex (reviewed in MERRICK 1992). In contrast to the mammalian system, wild-type yeast eIF2-GDP readily formed ternary complexes in the absence of eIF2B, with 40% of maximal complex formation seen after 16 min. We attribute this effect to an appreciable intrinsic GDP off-rate for yeast eIF2 that is not seen for the mammalian factor (AHMAD et al. 1985; ERICKSON and HANNIG 1996). Addition of catalytic amounts of yeast eIF2B further increased ternary complex formation by wild-type eIF2 to ~64% of maximum over the same time period. In the absence of eIF2B, eIF2K250R displayed an apparent rate of ternary complex formation (at early time points) that was greater than that for wild-type eIF2 alone and similar to the eIF2B-promoted reaction. The possibility that the eIF2K250R preparation was contaminated with eIF2B is unlikely, since the addition of eIF2B to the eIF2K250R reaction did not further enhance ternary complex formation (i.e., reaction rates at early time points are similar).

The ability of the γK250R alteration to mimic eIF2B activity in vitro suggested that gedΔ::K250R strains might demonstrate a reduced requirement for eIF2B function in vivo.
All strains grew well on dextrose media (Figure 2). In gcd11-K250R plasmid containing a galactose-inducible PKR construct, tide off-rate seen for wild-type eIF2αGDP complexes. The chromosomal gcd11 allele alone. Co-overexpression of the three eIF2 subunit genes (Figure 3; data not shown). Western blot was spotted onto minimal media plates containing either 2% dextrose (or the empty vector). Transformants were then plated on 5-FOA. The resulting strain is viable because it contains a low-copy URA3 plasmid harboring the four essential eIF2B subunit genes. To test the requirement for eIF2B in PKR-mediated growth inhibition is suppressed by increased eIF2 gene dosage: We reasoned that yeast cells less dependent upon eIF2B might show decreased sensitivity to overexpression of the mammalian eIF2α kinase PKR, which confers a severe slow-growth phenotype in wild-type yeast (Dever et al. 1993). This phenotype appears to result from an increase in phosphorylated eIF2α (at residue serine 51), which converts eIF2 to a competitive inhibitor of the exchange reaction (Rowlands et al. 1988; Chong et al. 1992; Dever et al. 1995). Because eIF2B is typically present at reduced levels relative to eIF2 (Price and Proud 1994), the resulting functional sequestration of eIF2B leads to decreased levels of ternary complex and thus reduced growth rates. We constructed yeast strains harboring a high-copy plasmid containing gcd11 or gcd11-K250R in combination with SU2 and SU3, or a low-copy plasmid containing the GCD11 allele alone. Co-overexpression of the three eIF2 subunit genes has shown an increased level of this initiation factor complex at least fivefold in vivo (Hannig et al. 1993; Dever et al. 1995; Erickson and Hannig 1996). The chromosomal GCD11 allele was deleted in these strains, which, in addition, harbored a plasmid containing a galactose-inducible PKR construct, as well as a high-copy IMT plasmid (or the empty vector). All strains grew well on dextrose media (Figure 2). In the absence of the high-copy IMT plasmid, all strains failed to grow upon induction of PKR expression on galactose media (data not shown). However, in the presence of increased IMT gene dosage, gcd11-K250R suppressed the PKR-mediated growth defect in the presence of elevated levels of eIF2α and eIF2β (Figure 2), suggesting that suppression is mediated by the eIF2 complex. Under these same conditions wild-type eIF2 was a much weaker suppressor, as would be predicted on the basis of its slower intrinsic off-rate for GDP. eIF2αγβδ, which demonstrates a wild-type GDP off-rate in vitro (Erickson and Hannig 1996), suppresses at a level similar to wild-type eIF2. The effects of overexpression of eIF2 are likely not due to inhibition of phosphorylation of the α-subunit by PKR, as these conditions have been shown to increase the level of phosphorylated eIF2 in the cell (Dever et al. 1995). Our results suggest that elevated levels of eIF2 and initiator tRNA reduce the requirement for eIF2B at a level proportional to the intrinsic nucleotide off-rate for yeast eIF2.

**Bypass of the essential function of eIF2B:** If the only essential function provided by eIF2B is to promote the rapid dissociation of eIF2-GDP complexes, we reasoned that gcd11-K250R might suppress a deletion of some or all of the four essential eIF2B subunit genes. To test this hypothesis, we constructed a yeast strain lacking the chromosomal GCD1, 2, -6, and -7 genes that encode the essential eIF2B subunits (Cigan et al. 1991, 1993), as well as the nonessential eIF2B subunit gene GCN3 (Hannig and Hinnenbusch 1988). The resulting strain (EY878) is viable because it contains a low-copy URA3 plasmid harboring the four essential eIF2B subunit genes. To test the requirement for eIF2B in gcd11-K250R strains, we constructed LEU2 plasmids containing either gcd11-K250R or GCD11 in combination with SU2 and SU3. These plasmids were introduced into EY878 that also harbored either a high-copy IMT plasmid or the empty vector. Transformants were then plated on 5-FOA medium to examine the ability of the eIF2 plasmids to suppress the complete loss of essential eIF2B genes. 5-FOA selects for Ura- cells that have lost the URA3 plasmid (Boeke et al. 1987), which, in the case of EY878, contains the only copies of the essential eIF2B subunit genes. As shown in Figure 3, the gcd11-K250R/SU2/ SU3 combination was an effective suppressor in the eIF2B quintuple-deletion strain. Suppression was dependent upon increased IMT gene dosage and, in addition, required high-copy expression of all three eIF2 subunit genes (Figure 3; data not shown). Western blot analysis confirmed the absence of detectable eIF2B subunits in the suppressed strain (data not shown). Overexpression of wild-type eIF2 also suppressed the complete absence of eIF2B in an IMT-dependent manner, albeit at a reduced level, consistent with the intrinsic nucleotide off-rate seen for wild-type eIF2-GDP complexes. Enhanced dissociation of eIF2-GDP resulting from the gcd11-K250R mutation (Figure 1) is consistent with
more efficient bypass of the nucleotide exchange function of eIF2B. Our results lend strong support to the notion that the rapid dissociation of eIF2-GDP complexes is an essential eIF2B function.

**Bypass of the essential function of SUI2 (eIF2α):** In cells that no longer require eIF2B, it is possible that certain eIF2 subunit(s) with which eIF2B interacts are not required. We chose the α-subunit of eIF2 to test this idea, on the basis of previous genetic evidence that suggested a direct interaction between the α-subunit of eIF2 and eIF2B (Vazquez de Aldana et al. 1993, Pavitt et al. 1997, 1998). We constructed a Δgcd11 Δsui2 strain (EY835) that harbored a URA3/GCD11/SUI2 plasmid and used the plasmid shuffle technique described above to examine the ability of GCD11 and gcd11-K250R constructs (LEU2) to suppress the Δsui2 mutation by conferring viability in the absence of the resident URA3 plasmid. To demonstrate the absence of chromosomal SUI2 and GCD11 in this strain, a low-copy LEU2 plasmid containing both SUI2 and GCD11, but not plasmids harboring either gene alone, supported the viability of EY835 in the absence of the URA3/GCD11/SUI2 plasmid (Figure 4). High-copy plasmids containing gcd11-K250R, either alone or in combination with SUI3 (eIF2B), suppressed the Δsui2 mutation, increasing doubling times 1.5- to 2.2-fold compared with controls (Figure 5, bottom panel). Suppression by gcd11-K250R was independent of the presence of a multi-copy IMT plasmid, although suppression was more efficient with the IMT plasmid (20–40% decrease in doubling times). This result suggests that the two-subunit form (βγ) of eIF2 is functional in this strain, but does not rule out the additional possibility that the γ-subunit alone is functional. A low-copy plasmid containing gcd11-K250R also suppressed Δsui2, albeit less efficiently (data not shown). Overexpression of wild-type GCD11 weakly suppressed Δsui2 (5-fold increase in doubling time) and suppression required co-overexpression of SUI3 and IMT. Our results suggest that the contribution of the α-subunit to eIF2 function is not essential for ligand binding or the interaction of eIF2 with additional components of the translational apparatus.

**Comparative requirements for bypass of essential eIF2B and eIF2α functions:** Examination of results presented in Figures 3 and 4 reveals a difference in the requirements for suppression in the eIF2B deletion strains.
strain compared with the Δsui2 strain. In the former instance, suppression in all cases required increased IMT gene dosage, whereas suppression of Δsui2 by gcd11-K250R is independent of, though enhanced by, the presence of additional copies of IMT. A trivial explanation for this difference may be related to the presence of the chromosomal GCD11 allele in EY878 used in the eIF2B bypass experiments (Figure 3). In this case, the presence of wild-type eIF2 complexes may compete with eIF2γK250R and thereby reduce the efficiency of suppression in these strains. To test this idea, we created a Δgcd11 strain (EY923) isogenic with EY878 and repeated the eIF2B bypass experiments. The results, shown in Figure 6, are essentially identical to those shown in Figure 3, i.e., bypass of the essential function of eIF2B requires overexpression of both eIF2 and initiator tRNA and is independent of the presence of a chromosomal GCD11 allele in the host strain. Again, eIF2γK250R is a more efficient suppressor, resulting in a 1.4-fold increase in doubling time (vs. the control) compared with a 3-fold increase for wild-type eIF2 (Figure 5, top).

DISCUSSION

Previous biochemical studies using mammalian factors indicated that eIF2 and eIF2B play critical roles in the initiation of eukaryotic protein synthesis (reviewed in Merrick 1992; Price and Proud 1994; Pain 1996). Genetic analyses in yeast provided evidence that both factors are required for growth and viability (Hinnebusch 1997). In yeast, each of the three conserved eIF2 subunits and four of the five conserved eIF2B subunits are encoded by essential genes. We previously described a mutation in the gene encoding the γ-subunit of eIF2, gcd11-K250R, that increased the intrinsic rate of dissociation of guanine nucleotides from binary complexes in vitro (Erickson and Hannig 1996). Although eIF2γK250R showed increased dissociation for both GDP and GTP in vitro, GTP binding by both eIF2γK250R and wild-type eIF2 could be stabilized by forming ternary complexes with charged initiator tRNA. These results were consistent with in vivo experiments that demonstrated increased IMT gene dosage suppressed both the slow growth and increased expression of GCN4 (i.e., the Gcd2 phenotype) in gcd11-K250R strains. The latter are indicative of at least partial restoration of eIF2 function (Hin-
not require, additional copies of IMT. These combined results suggested to us that gcd11-K250R strains might exhibit a reduced dependence upon eIF2B, resulting in partial or complete bypass of the requirement for eIF2B dependent upon (or enhanced by) increased IMT gene dosage. The data presented here demonstrate that overexpression of either the wild-type or \( \gamma_{\text{K250R}} \) form of eIF2 suppresses deletion of the four essential eIF2B subunit genes and that bypass of essential eIF2B function(s) requires co-overexpression of initiator tRNA. Consistent with a reduced requirement for eIF2B function, strains grown under bypass conditions but containing all eIF2B subunit genes show a reduced sensitivity to the eIF2\( \alpha \) kinases PKR (Figure 2) and Gcn2p (Dever et al. 1995). The efficiency of suppression of both the PKR-induced growth phenotype and the absence of eIF2B correlated directly with the rate of dissociation of guanine nucleotides determined previously with purified eIF2 preparations; i.e., gcd11-K250R strains were more efficient than strains harboring the wild-type GCD11 allele. Our data imply that enhancing the rate of nucleotide dissociation from eIF2 is an essential function of eIF2B.

Kinzey and Woolford (1995) demonstrated previously that additional copies of the TEF2 gene, encoding elongation factor eEF1A (previously eEF1\( \alpha \)), were sufficient to bypass the requirement for its export factor, eEF1B, when provided on a low-copy-number plasmid. The requirement for elevated levels of both wild-type eIF2 and initiator tRNA in bypassing eIF2B function suggests that eIF2 and/or initiator tRNA are normally maintained at limiting levels such that eIF2B is essential for promoting levels of ternary complex required in rapidly growing cells. Such a mechanism would also allow for rapid and effective changes in the level of ternary complexes by modulating eIF2B activity in response to various stimuli and, as such, may play an important role in regulating cell growth. Our results make the prediction that cells harboring mutations analogous to gcd11-K250R may be less sensitive to growth regulation mediated through protein kinases that phosphorylate the \( \alpha \)-subunit of eIF2 (Samuel 1993; Wek 1994; Shi et al. 1998; Sood et al. 2000a,b). Additional strategies developed to circumvent this regulatory mechanism, such as specific alterations in eIF2\( \alpha \) that render it refractory to phosphorylation (Donze et al. 1995) or dominant negative mutations in PKR (Koromilas et al. 1992; Meurs et al. 1995; Barber et al. 1995a), have been shown to promote tumor formation in mammals, implying that PKR may function as a tumor suppressor (Lengyel 1993).

Conditions required to suppress a \( \Delta \text{sui2} \) mutation differed somewhat from those required to suppress the deletion of essential eIF2B subunit genes. In the latter case, increased IMT gene dosage was absolutely required, whereas suppression of \( \Delta \text{sui2} \) in gcd11-K250R strains was more efficient in the presence of, but did not require, additional copies of IMT. The difference in gene dosage requirements for IMT raises the possibility that eIF2B provides a function, in addition to nucleotide exchange, that is substituted (in the eIF2B bypass experiments) by elevated levels of initiator tRNA. It is possible that catalyzed nucleotide exchange proceeds through an eIF2-GTP-eIF2B intermediate that facilitates the interaction of eIF2 with initiator tRNA, perhaps by increasing the on-rate for tRNA relative to eIF2-GTP binary complexes. Manchester and Stasiowski (1990) proposed a similar model based upon theoretical considerations of association and dissociation rate constants under physiological conditions and the reaction rates for protein synthesis initiation. If this is indeed the preferred pathway for ternary complex formation \( \text{in vivo} \), increasing the level of initiator tRNA may overcome the requirement for this eIF2B function via mass action. However, this model does not fully explain the requirement for increased IMT gene dosage in gcd11-K250R strains in the absence of eIF2B. In the presence of eIF2B, the viability of gcd11-K250R \( \Delta \text{sui2} \) strains does not require additional copies of IMT, despite the fact that these conditions would be expected to bypass the eIF2B nucleotide exchange function. In fact, co-overexpression of gcd11-K250R, SUI3, and IMT is sufficient to bypass eIF2B \( \text{in vivo} \) (Nika and E. M. Hannig, unpublished observations). Furthermore, we demonstrated recently that eIF2\( \alpha \) is required to promote efficient interaction between eIF2 and eIF2B \( \text{in vitro} \) (Nika et al. 2001). These combined observations suggest that eIF2B may contribute to the formation of eIF2\( \gamma_{\text{K250R}} \) ternary complexes in a manner that does not appear to require catalyzed nucleotide exchange and that is independent of (or less dependent upon) direct eIF2-eIF2B interaction. An alternative means through which eIF2B may facilitate ternary complex formation is by increasing local concentrations of tRNA, perhaps through a channeling type of mechanism. Such a mechanism may be direct or indirect, would not require direct interaction between eIF2 and eIF2B, and may be facilitated by a ribosomal localization of at least a portion of the eIF2B pool (Matts et al. 1988; Gigan et al. 1991; Chakrabarti and Matta 1992; Ramaiyah et al. 1992; Bushman et al. 1993b; Mueller et al. 1998). However, we cannot rule out completely the involvement of at least some form of an eIF2-eIF2B intermediate. In this respect, it is interesting to note recent data demonstrating genetic as well as physical interaction between GCD11 and LOS1 (Hellmuth et al. 1998; Grosshans et al. 2000). LOS1 encodes a member of the \( \beta \)-importin family that plays a nonessential role in transport of tRNA across the yeast nuclear membrane. However, it is unclear whether this interaction is important in the specific transport and/or localization of initiator tRNA. On the other hand, eEF1A (the functional homolog of prokaryotic EF1A) does appear to be required for efficient nuclear export of certain noninitiator tRNAs and has been suggested to func-
Our combined data predict that eIF2βγ carries out all eIF2 functions required for translation initiation, including interactions with ribosomes and other translation factors, start site recognition, nucleotide exchange (in the presence or absence of eIF2B), and formation of ternary complexes. This suggests a model in which eIF2βγ comprises the eIF2 functional core, whereas the α-subunit of eIF2 and the eIF2B heteropentamer form a regulatory core that modulates the level of eIF2 function by regulating nucleotide exchange and formation of ternary complexes in vivo. The availability of yeast strains lacking normally essential subunits of eIF2 and eIF2B should provide valuable tools for dissecting the functions of individual polypeptides in these mult-subunit complexes. Such functions could include roles in catalysis, as well as regulatory functions involved in cellular responses to stress or other environmental stimuli (Welsh and Proud 1992; Engelberg et al. 1994; Kimball and Jefferson 1994; Brostrom et al. 1996; Gallie et al. 1997; Qu et al. 1997; Scheper et al. 1997).

In this regard, results of previous studies have indicated a role for Gcd6p in catalysis, whereas Gcd2p, Gcd7p, and Gcn3p appear to form a regulatory subcomplex (Yang and Hinnebusch 1996; Fabian et al. 1997; Pavitt et al. 1997, 1998; Gomez and Pavitt 2000). Biochemical analysis of individual eIF2B polypeptides and subcomplexes that are devoid of contaminating subunits, purified from strains using the genetic backgrounds developed in this article, in addition to further genetic analyses of these strains, will allow these questions to be addressed more directly.

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LITERATURE CITED


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