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Molecular cloning and characterization of cDNA encoding the α subunit of the rat protein synthesis initiation factor eIF-2B

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ABSTRACT **Eukaryotic initiation factor 2B (eIF-2B) is an essential component of the pathway of peptide-chain initiation in mammalian cells, yet little is known about its molecular structure and regulation. To investigate the structure, regulation, and interactions of the individual subunits of eIF-2B, we have begun to clone, characterize, and express the corresponding cDNAs. We report here the cloning and characterization of a 1510-bp cDNA encoding the α subunit of eIF-2B from a rat brain cDNA library. The cDNA contains an open reading frame of 918 bp encoding a polypeptide of 305 aa with a predicted molecular mass of 33.7 kDa. This cDNA recognizes a single RNA species ≈1.6 kb in length on Northern blots of RNA from rat liver. The predicted amino acid sequence contains regions identical to the sequences of peptides derived from bovine liver eIF-2B α subunit. Expression of this cDNA *in vitro* yields a peptide which comigrates with natural eIF-2Bα in SDS/polyacrylamide gels. The predicted amino acid sequence exhibits 42% identity to that deduced for the *Saccharomyces cerevisiae* GCN3 protein, the smallest subunit of yeast eIF-2B. In addition, expression of the rat cDNA in yeast functionally complements a *gcn3* deletion for the inability to induce histidine biosynthetic genes under the control of GCN4. These results strongly support the hypothesis that mammalian eIF-2α and GCN3 are homologues. Southern blots indicate that the eIF-2Bα cDNA also recognizes genomic DNA fragments from several other species, suggesting significant homology between the rat eIF-2Bα gene and that from other species.**

Protein synthesis is a highly regulated cellular process. An important site of regulation is peptide chain initiation, the first step in the translation of mRNA into protein. Peptide chain initiation is mediated by a group of proteins termed eukaryotic initiation factors (eIFs) (reviewed in ref. 1). Modulation of the activity of the initiation factors, especially by phosphorylation, has been well documented (2, 3).

A large body of work has established an important role for one of the initiation factors, eIF-2B, in the regulation of protein synthesis under a variety of conditions in which peptide chain initiation is inhibited (reviewed in ref. 2). In each of these conditions, inhibition of peptide chain initiation is correlated with a reduction in eIF-2B activity. eIF-2B is a heteropentameric protein complex that mediates guanine nucleotide exchange on eIF-2 to promote GDP release and rebinding of GTP. Inhibition of eIF-2B activity is often associated with an enhanced phosphorylation of the α subunit of eIF-2, a heterotrimeric protein that mediates the binding of initiator methionyl-tRNA (Met-tRNA$_i$) to the 40S ribosomal subunit as a ternary complex, eIF-2·GTP·Met-tRNA$_i$.

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Phosphorylation of eIF-2α does not directly inhibit formation of a ternary complex, as this reaction proceeds efficiently *in vitro* with the phosphorylated factor (4). Instead, eIF-2 phosphorylated on its α subunit [eIF-2α(*P*)] has at least a 100-fold greater affinity for eIF-2B than does the unphosphorylated factor, which may impede eIF-2B activity by sequestration of eIF-2B in an inactive eIF-2α(*P*)·GDP·eIF-2B complex (5).

The eIF-2B complex has been purified from several mammalian sources (2) and in each case it is composed of α, β, γ, δ, and ε subunits. The guanine nucleotide exchange mechanism of eIF-2B remains a subject of controversy, even though initial studies were reported a decade ago (6). It is unlikely that all five subunits are required for the guanine nucleotide exchange activity of eIF-2B, as other factors which perform similar functions (such as eEF-1 and the Ras GDP-dissociation factor superfamily) are composed of no more than three subunits (7, 8). No definitive evidence is available concerning the functions of the individual subunits; however, photoaffinity labeling studies have shown that several of the subunits (β, γ, and δ) possess nucleotide binding sites (9). The activity of eIF-2B has also been shown to be regulated *in vitro* by the $NADP^+$/NADPH ratio (10, 11) and by phosphorylation of the ε subunit with casein kinase II (10).

Each of the subunits of a putative eIF-2B complex in *Saccharomyces cerevisiae* has been cloned (12), and the predicted molecular masses of the individual components of this complex are similar to those observed for mammalian eIF-2B subunits (2, 13). In contrast, only a single full-length mammalian cDNA (for the rabbit reticulocyte δ subunit) has been reported (14). The 34-kDa yeast protein GCN3 (corresponding in size to the mammalian α subunit) is not an essential component of the eIF-2B complex in yeast (in that it is not required for normal cell growth), yet specific mutations within GCN3 lead to an inhibition of general translation initiation (15). Other work suggests that GCN3 may be required for the interaction of the yeast eIF-2B complex with the α subunit of eIF-2 (yeast SUI2) and may mediate the inhibition of the GTP exchange reaction associated with phosphorylation of eIF-2α (16).

To investigate the regulation of mammalian eIF-2B, the interactions among its subunits, and its potential interaction(s) with eIF-2, we have isolated the individual subunits from bovine and rat liver and have begun to clone and express the corresponding cDNAs. Here we report the cloning of a full-length cDNA for rat eIF-2Bα and show that this subunit and yeast GCN3 are functionally equivalent.¶

Abbreviations: eIF, eukaryotic initiation factor; 3-AT, 3-amino-1,2,4-triazole.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. U05821).

MATERIALS AND METHODS

Materials. The rat brain cDNA library in λZAPII was purchased from Stratagene (catalogue no. 936515). All reagents for protein digestion, polymerase chain reaction (PCR) amplification, and restriction endonuclease digestion were purchased from Promega. Oligonucleotide primers were synthesized on an automated DNA synthesizer (Applied Biosystems, model 380A). ^{32}P- and ^{35}S-labeled compounds were purchased from Amersham.

Determination of the Amino Acid Sequences of eIF-2Bα Fragments. Purified eIF-2B complex was isolated from bovine liver (17). The α subunit was isolated by SDS/PAGE, blotted onto a poly(vinylidene difluoride) membrane (Bio-Rad), and subjected to microsequencing. No sequence was obtained, suggesting that the N terminus of the protein is blocked. Another aliquot of the α subunit was isolated by SDS/PAGE and then coelectrophoresed into a second gel along with an aliquot of protease (trypsin, *Staphylococcus aureus* V8 protease, or Lys-C endoproteinase), and partial digestion was allowed to occur in the stacking gel (18). After electrophoresis, the peptide fragments were electrophoretically transferred to a poly(vinylidene difluoride) membrane, and slices of the membrane containing the individual peptide fragments were used in microsequencing reactions.

Reverse Transcription (RT)–PCR Amplification of eIF-2Bα cDNA. Degenerate oligonucleotide primers were designed corresponding to peptide sequences derived from bovine eIF-2Bα (Fig. 1). First-strand cDNA was synthesized and then amplified with primers A (sense; 5′-GGHAARAARATG-GCHAARGC-3′; H = A, C, or T; R = A or G) and B (antisense; 5′-ACRAAYTTRAARSYYTCIGC-3′; Y = C or T; S = G or C; I = deoxyinosine) as described (19). An aliquot of this reaction mixture was reamplified with 20 pmol each of primers A (sense) and C ("nested" antisense; 5′-ACIACRT-ARAAYTTRTTYTTIGC-3′). Reaction products were purified by electrophoresis in a 0.8% agarose gel and then subcloned and sequenced as described (19). One of the inserts consisted of a 210-bp open reading frame that contained amino acid sequence identical to that derived from bovine liver eIF-2Bα.

Screening the Rat Brain cDNA Library. The 210-bp eIF-2Bα cDNA was isolated and radiolabeled to a specific activity of $>1 \times 10^9$ cpm/μg with [α-^{32}P]dCTP by use of random primers (MultiPrime kit; Amersham). This probe was purified and used to screen a rat brain cDNA library in λZAPII as described (19). The recombinant pBluescript SK(−) plasmids were excised *in vivo* from the λZAPII phages, used to transform *Escherichia coli* XL1-Blue, isolated from overnight cultures, and characterized by multiple restriction digests.

Sequencing of eIF-2Bα cDNA. Unique clones were sequenced by using gene-specific primers and vector-specific (T3 and T7) primers. A 1.5-kb cDNA was identified (pBrα$_{1.5}$) which had a 17-nt stretch of poly(dA) at one end and the 210-bp eIF-2Bα internal sequence. This insert was sequenced completely across both strands by primer "walking." Sequence alignment and analysis were performed with the DNASTAR computer program (DNASTAR, Madison, WI).

Southern Blot Analysis. Human, rabbit, rat, mouse, and *Drosophila melanogaster* genomic DNA were digested at 37°C for 16 hr with *Bam*HI restriction endonuclease. Digestion products were transferred to a Magna nylon membrane (Micron Separations, Westboro, MA) and hybridized at 55°C to a radiolabeled pBrα$_{1.5}$ cDNA as described (19).

Northern Blot Analysis. Total RNA and poly(A)$^+$ RNA were isolated from rat liver and analyzed by Northern blot

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CTCGACACGCTGTCAGGCGGTGCTTTGCTGCTGGCGGGGTTGTGGTCCGCGTCGCGAGGAAGCCATGGAGGACGGTGAGT 80
                                                          M  E  D  G  E

TAATCAAGTACTTTAAGTCCCAGATGAAAGGAGACCCTAACATGGCCTCGGCGGTGGCTGCCATCCAGACCTTGCTGGAA 160
L  I  K  Y  F  K  S  Q  M  K  G  D  P  N  M  A  S  A  V  A  A  I  Q  T  L  L  E

TTCTTGAAGAGAGATAAAGGAGAGACAATCCAGGGCCTGAGAGCCCATCTCACCAAAGCCATCGAAACCCTCTGCGCTGT 240
 F  L  K  R  D  K  G  E  T  I  Q  G  L  R  A  H  L  T  K  A  I  E  T  L  C  A  V

GGACTCCTCGGTGGCCGTGTCCTCGGGCGGGGAACTCTTCCTTCGCTTCATCAGCCTCACCTCCCTGGAGTACTCCGACT 320
  D  S  S  V  A  V  S  S  G  G  E  L  F  L  R  F  I  S  L  T  S  L  E  Y  S  D

ACTCCAAATGTAAAAAGATCATGATCGAGAGAGGAGAGCTTTTCCTGAGCAGAATATCCCTGTCAAGAACTAAGATCGCC 400
Y  S  K  C  K  K  I  M  I  E  R  G  E  L  F  L  S  R  I  S  L  S  R  T  K  I  A

AGTCTATGCCATGCTTTTATCAAAGACGGGGCGAGAATCTTGACTCACGCCTACTCCAGAGTTGTCCTGAGGGTCCTGGA 480
 S  L  C  H  A  F  I  K  D  G  A  R  I  L  T  H  A  Y  S  R  V  V  L  R  V  L  E

                                                                              A
AGAAGCCGTGGCAGCCAAGAAACGTTTCAGTGTGTATATCACGGAGTCACAGCCTGATTTATCTGGTAAGAAAATGGCCA 560
  E  A  V  A  A  K  K  R  F  S  V  Y  I  T  E  S  Q  P  D  L  S  G  K  K  M  A

AAGCCCTCTGCCACCTCAATGTCCCTGTCACTGTGGTGCTGGACGCTGCTGTTGGCTATATCATGGAGAAAGTAGACCTT 640
K  A  L  C  H  L  N  V  P  V  T  V  V  L  D  A  A  V  G  Y  I  M  E  K  V  D  L

GTCATCGTTGGTGCTGAAGGAGTGGTAGAGAATGGAGGAATTATTAACAAGATCGGAACCAACCAGATGGCCGTGTGTGC 720
 V  I  V  G  A  E  G  V  V  E  N  G  G  I  I  N  K  I  G  T  N  Q  M  A  V  C  A

          C                                  B
CAAAGCCCAGAACAAGCCCTTCTATGTGGTTGCAGAAAGTTTCAAGTTTGTACGGCTCTTTCCACTCAACCAGCAAGATG 800
  K  A  Q  N  K  P  F  Y  V  V  A  E  S  F  K  F  V  R  L  F  P  L  N  Q  Q  D

TCCCAGATAAGTTTAAGTACAAGGCAGACACCCTGAAGTCTGTGCAGGCTGGACAGGACCTCAAAGAGGAACACCCATGG 880
V  P  D  K  F  K  Y  K  A  D  T  L  K  S  V  Q  A  G  Q  D  L  K  E  E  H  P  W

GTCGACTACACCTCCCCATCCCTCATCACACTGCTGTTTACGGACTTAGGTGTGTTGACACCATCCGCTGTAAGCCACGA 960
 V  D  Y  T  S  P  S  L  I  T  L  L  F  T  D  L  G  V  L  T  P  S  A  V  S  H  E

GCTCATCAAGCTGTACCTGTGAGCAGTGCCCCTCCTGCCAACAGTCAGGGAGCACATCTTGTGCAGGGTGAGCAGCCTTT 1040
  L  I  K  L  Y  L

CCACTCCAGCCAGCCAGGGGCCAACAATGGGACTTTCAGACCTGTATGCCAAGGCTCATTTCAGAGGTCCCTGAAGAATC 1120
CTTTACTCTTGCTGTCACATCTGAAGCCTGGTCCCAGTTCTAGAACTCAAGGTGAAGCAACCTTGTGGTTTTTAGAGACT 1200
CAGTCTCCGGAAGAACAAGTTAATTACAGACTGCAAAGCTGAGGAGAGATGGCACAGGAGCAGGGACCCCCCAGCCGTGC 1280
TGCATCACTGAGAGATGCGGTGACATCGGCTGAGTACACACCTTCAGTGCCTCACAAGACAGCAGACACTGTAGCTGACG 1360
GCATCTGGCTCCATGCCCTCAGCAGGATCCAGCCAGATAGAAAAAACACCAATGCAGGAGCCATTCACATGGCACTGTCA 1440
CAGAATGTTGTCTGTAAAAGATGCGAGTTTACTCTAAACATGTTTAAAACCTGAAAAAAAAAAAAAAAAA 1510
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FIG. 1. Nucleotide and deduced amino acid sequence of the cDNA for eIF-2Bα. The nucleotide sequence shown is of a cDNA of eIF-2Bα isolated from a rat brain cDNA library. An open reading frame of 305 aa is shown with single-letter code for the predicted amino acids. Putative translation initiation consensus nucleotide sequences are underlined. The boxed amino acid residues correspond to peptide sequence derived from purified preparations of eIF-2Bα from bovine liver. Positions of oligonucleotides used for PCR amplifications and their orientation with respect to the sense strand are indicated by arrows, and are identified by a single-letter designation (A–C) above the arrow.

hybridization (19) with the radiolabeled pBr$\alpha_{1.5}$ *Bam*HI fragment.

***In Vitro* Translation of eIF-2Bα cDNA.** The pBr$\alpha_{1.5}$ cDNA was transcribed and translated *in vitro* with the T3 TnT coupled reticulocyte lysate system (Promega). Nonrecombinant pBluescript SK(−) was used as a control. One picomole of each purified plasmid was used in each reaction. The radiolabel used was [^{35}S]methionine. The reaction mixtures were incubated at 30°C for 120 min and an aliquot (5 μl) of the mixture was fractionated by SDS/PAGE. The gel was stained, soaked in En^{3}Hance (NEN/DuPont) for 30 min, dried, and exposed to x-ray film at −80°C for 16 hr.

Analysis of eIF-2Bα Function in Yeast. The *Bam*HI–*Xba* I fragment comprising nt 1–1159 of the pBr$\alpha_{1.5}$ cDNA insert was subcloned into the yeast expression vector pEMBLyex4 in the sense orientation by standard techniques (20). The resulting plasmid, pαYEX4, was transformed into yeast strains H2525 (*MAT***a** *ino1 leu2-3 leu2-112 ura3-52 gcn3Δ HIS4–lacZ*) and H2524 (*MAT***a** *ino1 leu2-3 leu2-112 ura3-52 gcn2Δ HIS4–lacZ*). These strains were derived from EY448 (21) and H1149 (22), respectively, by replacing *LEU2* disruptions of *GCN3* or *GCN2* with unmarked deletions by using plasmid pNKY85 (23). The *gcn3Δ* H2525 and *gcn2Δ* H2524 strains were also transformed with each of the plasmids pEMBLyex4 (empty vector), mp116 (12), and p585 (24). Strains containing each plasmid were grown to confluence on SG medium (25) supplemented with all 20 amino acids (each at ≈0.1 mg/ml) and replica plated to SG medium supplemented with 30 mM 3-amino-1,2,4-triazole (3-AT), 40 mM leucine, and all other amino acids (at ≈0.1 mg/ml) except histidine. Plates were incubated at 30°C for 1 day, replica plated a second time to the same SG medium containing 3-AT, and incubated for an additional 2 days at 30°C.

RESULTS AND DISCUSSION

eIF-2Bα was cleaved with trypsin, *S. aureus* V8 protease, or Lys-C endoproteinase, and the N-terminal amino acid sequences of six of the resulting peptides were determined by automated microsequencing. Degenerate oligonucleotides were designed from these sequences and were used for PCR amplification of a rat eIF-2Bα cDNA. Nucleotide usage at degenerate sites within the oligonucleotides was based on an analysis of codon preference in 74 rat liver-derived cDNA sequences listed in GenBank release 75. The approximate positions of the oligonucleotides within the eIF-2Bα mRNA were suggested by the partial homologies of the corresponding peptides to the predicted amino acid sequence of GCN3, the yeast equivalent of mammalian eIF-2Bα (12). First-strand cDNA was synthesized from rat liver total RNA by using random hexamers and Superscript reverse transcriptase (Life Technologies). The first-strand cDNA was used as a template for PCR amplification using primers A and B (Fig. 1), and an aliquot of this reaction mixture was reamplified with primers A and C. Three products from this reaction (210, 400, and 450 bp) were isolated by electrophoresis in an agarose gel, subcloned into the pCRII vector, and sequenced with T7 and SP6 primers. The 210-bp cDNA fragment contained sequence which encoded additional eIF-2Bα peptide sequence (i.e., sequence not used to design the primers). The other PCR-generated cDNAs showed no significant homology to any of the eIF-2Bα peptides or to GCN3.

To isolate a cDNA corresponding to the entire coding region of eIF2Bα, the 210-bp fragment was radiolabeled by random priming and used to screen a rat brain λZAPII cDNA library. From 3.0×10^5 recombinant phages screened, 7 positive phage clones were purified and the inserts were excised in pBluescript SK(−) vector. These clones were analyzed by multiple restriction digests and partial sequencing and were found to contain overlapping cDNA fragments. The largest of these clones (pBr$\alpha_{1.5}$) contained a 1.5-kb insert which was sequenced completely across both strands. The nucleotide and deduced amino acid sequences of this cDNA are shown in Fig. 1. The eIF-2Bα cDNA is 1510 bp long with an open reading frame encoding a protein of 305 aa with a predicted molecular mass of 33.7 kDa. The initial ATG codon of this open reading frame is contained within a translation initiation consensus site (26); this is also true for the third ATG codon, located 57 bp (i.e., 19 aa) downstream. The TGA termination codon is located at nt 980. The cDNA contains a 3′-terminal poly(dA) region 17 bp in length but has no classical polyadenylylation signal (27). The deduced amino acid sequence of this cDNA contains all of the amino acid sequences of the peptides derived by proteolytic digestion of bovine eIF-2Bα.

The size of the rat eIF-2Bα mRNA was determined by Northern blot analysis of rat liver total and poly(A)$^+$ RNA. A single band was observed at a position of ≈1575 nt (Fig. 2) after stringent washing. A 5′-RACE (rapid amplification of cDNA ends) procedure (28) was performed with several nested antisense nondegenerate oligonucleotide primers in an attempt to obtain further upstream sequence. This yielded no additional sequence other than that already contained in the cDNA (data not shown). Taken together, these results suggest that nearly all of the 5′ untranslated mRNA sequence is contained within the eIF-2Bα cDNA.

The pBr$\alpha_{1.5}$ plasmid was translated *in vitro* with a coupled transcription/translation system (Promega). An aliquot of the ^{35}S-labeled translation reaction mixture was size-fractionated by SDS/PAGE and visualized by autoradiography. A predominant peptide product was observed with an apparent molecular mass of 34 kDa that comigrated with eIF-2Bα purified from rat liver (Fig. 3). This peptide was not observed in reaction mixtures with the nonrecombinant pBluescript vector (data not shown).

Southern blots of *Bam*HI digests of genomic DNA from several species were prepared and were probed with a radiolabeled eIF-2Bα cDNA (Fig. 4). Under stringent hybridization and washing conditions, specific fragments from human, rabbit, rat, and mouse genomic DNA were recognized by this probe. The simple band patterns are consistent with eIF-2Bα transcription from a single gene. The crossreactivity of the cDNA probe suggests that there is significant homology of the eIF-2Bα gene among these species. No bands were observed in the lane containing *D. melanogaster* genomic DNA, which is consistent with the lack of observable eIF-2B activity in this

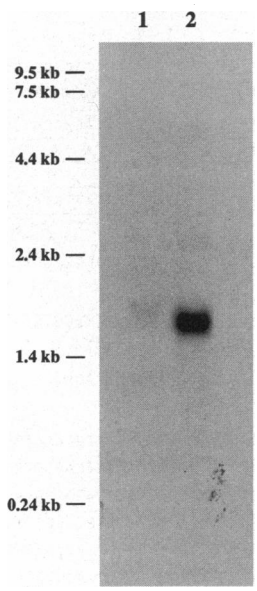

FIG. 2. Northern blot analysis for eIF-2Bα mRNA. Rat liver total RNA (15 μg, lane 1) and poly(A)$^+$ RNA (2 μg, lane 2) were electrophoresed in a denaturing agarose/formaldehyde gel, transferred to a nylon membrane, and probed with radiolabeled eIF-2Bα cDNA fragments comprising nt 1–1416. Positions of standard RNA size markers are indicated on the left.

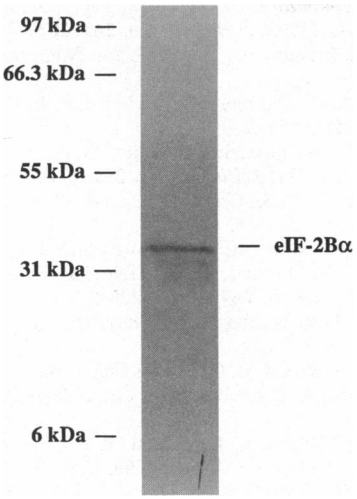

FIG. 3. Expression of the eIF-2Bα protein. The eIF-2Bα cDNA contained within the pBluescript plasmid was transcribed and translated *in vitro* with the T3 TnT polymerase/reticulocyte lysate system (Promega). Molecular mass standards and rat liver eIF-2B were electrophoresed in parallel lanes; positions of the markers are indicated on the left, and the position of eIF-2Bα is indicated on the right.

organism (29) but may simply be due to a low level of homology between the two species.

The deduced amino acid sequence of the rat eIF-2Bα cDNA displays 42% identity to that of *S. cerevisiae* GCN3 and 40% identity to a hypothetical open reading frame from a *Caenorhabditis elegans* genomic cosmid clone (30). The three proteins display 27% identity overall, with the greatest variability occurring at the N termini of the proteins (Fig. 5).

In wild-type yeast cells, amino acid deprivation leads to the phosphorylation of eIF-2α (SUI2) by the protein kinase GCN2. In addition to reducing the efficiency of general translation initiation, this elicits increased translation of GCN4 mRNA (31). GCN4 protein is a transcriptional activator of many amino acid biosynthetic genes, including those in the histidine pathway. Induction of GCN4 translation and the attendant increased transcription of histidine biosynthetic genes under GCN4 control is required for growth in the presence of 3-AT, an inhibitor of histidine biosynthesis. Thus, inactivation of the eIF-2α kinase GCN2 makes yeast cells

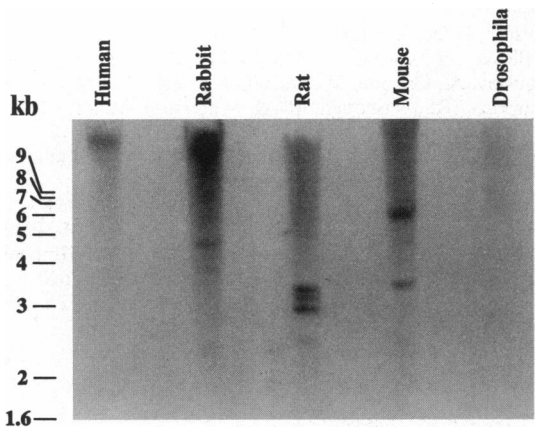

FIG. 4. Southern blot analysis of genomic DNA. Genomic DNAs from the species indicated (5 μg of each) were digested with *Bam*HI restriction endonuclease, electrophoresed in an agarose gel, and transferred to and immobilized on a nylon membrane. The blot was probed with radiolabeled eIF-2Bα cDNA fragments comprising nt 1–1416 and exposed to film for 120 hr. Positions of standard DNA markers (1-kb ladder, GIBCO) are indicated on the left.

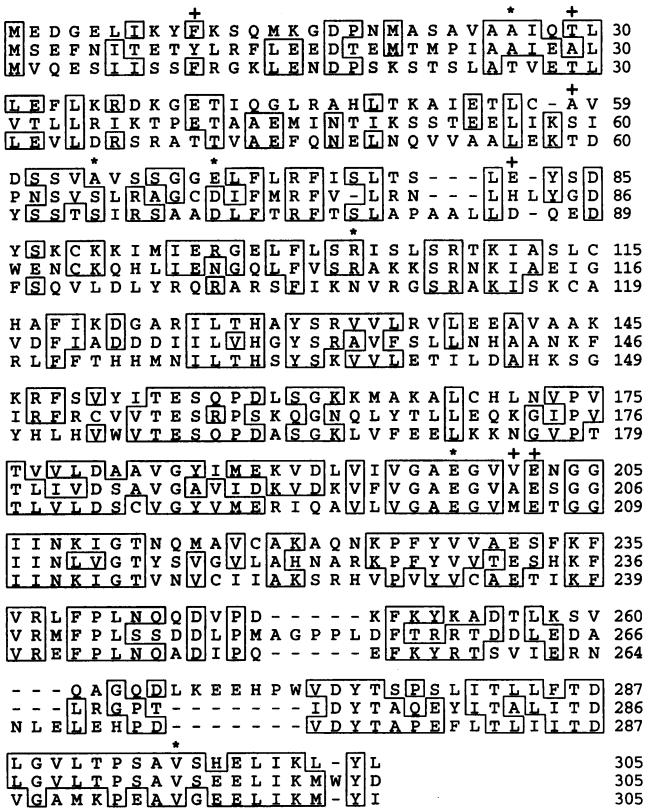

FIG. 5. Alignment of the deduced amino acid sequences of eIF-2Bα cDNAs. The top sequence is the predicted amino acid sequence of rat eIF-2Bα, the middle sequence is that of *S. cerevisiae* GCN3, and the lower sequence is that of the hypothetical open reading frame in the *C. elegans* genomic cosmid clone ZK1098. Identical residues are boxed. Residues mutated in yeast (23) are indicated by stars (more severe mutations, causing slow-growth phenotype) and plus signs (less severe mutations, causing induction of GCN4 only). Residues are numbered on the right. Alignment was constructed with the program MEGALIGN (DNASTAR).

sensitive to 3-AT. Deletion of *GCN3*, encoding yeast eIF-2Bα, also gives this phenotype (ref. 21; reviewed in ref. 32). This last observation, plus the fact that inactivation of *GCN3* allows yeast cells to survive levels of eIF-2α phosphorylation that are lethal in *GCN3*$^+$ cells (33), led to the idea that GCN3 mediates the inhibitory effect of phosphorylated eIF-2 on the recycling function of eIF-2B. To determine whether rat liver eIF-2Bα is functionally homologous to GCN3, we asked whether expression of the rat protein in a yeast mutant lacking GCN3 would restore transcriptional activation of genes in the histidine pathway under the control of GCN4.

To answer this question, the rat cDNA was inserted 3′ of a galactose-inducible promoter on a high-copy-number autonomously replicating plasmid. The resulting construct, pαYEX4, was introduced into strains containing a chromosomal deletion of either *GCN3* or *GCN2*. Introduction of pαYEX4 into the *gcn3*Δ strain conferred increased resistance to 3-AT relative to vector alone, at a level somewhat lower than that given by plasmid mp116 bearing *GCN3* (Fig. 6). The 3-AT-resistant phenotype of the pαYEX4 transformants was observed when cells were grown with galactose, but not glucose, as carbon source (data not shown). These results suggest that the rat eIF-2Bα protein can substitute for GCN3 in the yeast eIF-2B complex and restore the induction of GCN4 and its target genes in the histidine pathway. Introduction of pαYEX4 into the *gcn2*Δ strain did not confer 3-AT resistance relative to vector alone (Fig. 6), indicating that rat eIF-2Bα, like GCN3, is dependent on phosphorylation of eIF-2α by

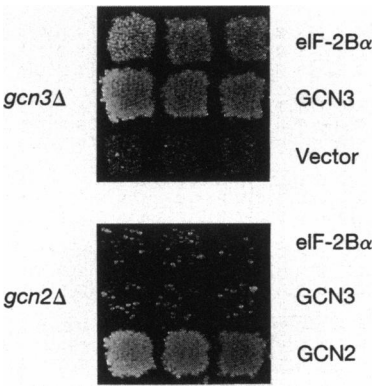

FIG. 6. Expression of rat eIF-2Bα complements a *gcn3Δ* mutation for growth of yeast cells on histidine starvation medium. Transformants of *gcn3Δ* strain H2525 (*Upper*) or *gcn2Δ* strain H2524 (*Lower*) bearing plasmid pαYEX4 (eIF-2Bα), mp116 (GCN3), pEMBLyex4 (vector), or p585 were grown to confluence and replica plated. Plates were incubated at 30°C for 1 day, replica plated a second time, and incubated for an additional 2 days at 30°C. The 3-AT-resistant papillae appearing in the sectors containing pαYEX4 or pEMBLyex4 transformants of the *gcn2Δ* strain probably contain spontaneous mutations in one of the genes encoding other subunits of eIF-2 or eIF-2B in yeast (34).

GCN2 for its stimulatory effects on histidine biosynthesis. Thus, rat eIF-2Bα is functionally substituting for GCN3 rather than bypassing the requirement for eIF-2α phosphorylation—e.g., by interfering with eIF-2 or eIF-2B function.

Hannig *et al.* (21) have characterized 12 point mutations in yeast eIF-2Bα which appear to affect eIF-2B function. These mutations fall into two phenotypic groups: (*i*) relatively severe mutations which lead to an induction of GCN4 under non-starvation conditions and also a retardation of cell growth rate and (*ii*) apparently less severe mutations which also cause the induction of GCN4 but do not affect growth (21). The residues identified by this mutational analysis as being important for yeast eIF-2B function are generally conserved among rat, yeast, and *C. elegans* or show conservative substitution (Fig. 5), and the degree of conservation of these residues appears to correlate with the severity of the effect of their mutation.

Mutations in yeast eIF-2Bα which cause the slow-growth phenotype appear to alter the interaction of eIF-2Bα with eIF-2 and with the other subunits of eIF-2B. The conservation of these residues in the rat protein and the functional equivalence of rat and yeast eIF-2Bα demonstrated here suggest that similar interactions may occur in mammalian cells. Expression of wild-type and mutant eIF-2Bα proteins in yeast and mammalian cells will allow us to begin investigating the mechanisms and consequences of eIF-2B/eIF-2 interactions in both normal and perturbed physiological conditions.

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