Structure of the Catalytic Fragment of Translation Initiation Factor 2B and Identification of a Critically Important Catalytic Residue*§

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Eukaryotic initiation factor (eIF) 2B catalyzes the nucleotide activation of eIF2 to its active GTP-bound state. The exchange activity has been mapped to the C terminus of the eIF2B2 subunit. We have determined the crystal structure of residues 544–704 from yeast eIF2B2 at 2.3-Å resolution, and this fragment is an all-helical protein built around the conserved aromatic acidic (AA) boxes also found in eIF4G and eIF5. The eight helices are organized in a manner similar to HEAT repeats. The molecule is highly asymmetric with respect to surface charge and conservation. One area in the N terminus is proposed to be directly involved in catalysis. In agreement with this hypothesis, mutation of glutamate 569 is shown to be lethal. An acidic belt and a second area in the C terminus containing residues from the AA boxes are important for binding to eIF2. Two mutations causing the fatal human genetic disease leukoencephalopathy with vanishing white matter are buried and appear to disrupt the structural integrity of the catalytic domain rather than interfering directly with catalysis or binding of eIF2.

The initiation phase of protein synthesis in eukaryotic cells is a complex series of highly regulated interactions between ribosomal subunits, mRNA, aminoacylated initiator methionyl-tRNA (Met-tRNA^{Met}), and eukaryotic translation initiation factors (eIFs).1 They all function to correctly position Met-tRNA^{Met} to the 40 S ribosomal subunit as part of an eIF2-GTP-Met-tRNA^{Met} ternary complex (TC). When TC is bound to both the 40 S ribosomal subunit and the initiator AUG codon of an mRNA, GTPase activating protein (GAP) eIF5 stimulates GTP hydrolysis releasing an eIF2-GDP binary complex. eIF2B acts as a nucleotide exchange factor (GEF) and promotes release of GDP from eIF2 and formation of an eIF2-GTP complex. Only eIF2-GTP can form TC, so by controlling eIF2B function, cells can control TC levels and protein synthesis initiation (1). eIF2B activity is controlled both indirectly by phosphorylation of eIF2, and directly by phosphorylation of eIF2B.

Four protein kinases can phosphorylate the eIF2α subunit (eIF2α) at Ser51. Each kinase reacts to different cellular stress conditions. GCN2 responds to amino acid starvation (2); PEK/PERK counters damage caused by unfolded proteins in the endoplasmic reticulum; PKR is activated by double stranded RNA in response to viral infection and HRI is regulated by heme levels in reticulocytes (1). Phosphorylation of eIF2α (eIF2α(P)) reduces the activity of eIF2B by formation of a non-productive eIF2α-eIF2B complex (3). As eIF2 is more abundant than eIF2B, a small fraction of eIF2α(P) can have a large effect on eIF2B activity and therefore significantly reduce TC levels. The reduction in TC levels by eIF2α(P) has opposing effects; overall protein synthesis is lowered, but the translation of stress responsive genes is enhanced.

The activity of mammalian eIF2B can also be controlled directly in response to insulin signaling, which causes glycogen synthase kinase 3 inactivation and thereby contributes to activation of eIF2B (4). This permits increased eIF2B activity and protein synthesis in response to growth-promoting signals. It was recently demonstrated that mutations in eIF2B cause the fatal human genetically inherited brain disorder known as childhood ataxia with central nervous system hypomyelination or vanishing white matter leukoencephalopathy (5, 6).

eIF2 and eIF2B are proteins with three and five non-identical subunits, respectively. Whereas eIF2α appears to be largely required for regulation by phosphorylation, the β and γ subunits have a central role in GTPase function. eIF2γ contains the GDP/GTP binding domain and also binds Met-tRNA^{Met} (7), whereas eIF2α is required to bind both eIF5 and eIF2B (8).

Functions for each of the five subunits of eIF2B have also been assigned from molecular genetic and biochemical studies of the yeast factor. The eIF2B α, β, and δ subunits share extensive sequence similarity and these three subunits form a regulatory subcomplex that mediates the inhibition of eIF2B function in response to eIF2α(P) (9). This regulatory complex binds eIF2α(P) with higher affinity than non-phosphorylated eIF2.

In contrast, the γ and ε subunits of eIF2B are required for the catalytic function of nucleotide exchange. These subunits share extensive similarity over the entire length of eIF2Bγ, eIF2Bε subunits from rat, yeast, and Drosophila are capable of nucleotide exchange in vitro (3, 10, 11). We recently demon-
strated that the C-terminal ~200 amino acids of eIF2B contain the minimal eIF2B catalytic domain (12). This minimal fragment was proposed to contain two functional regions. The C-terminal 115 residues share sequence homology with the C termini of eIF5 and mammalian eIF4G, and has been defined as the W2 domain (two invariant tryptophans), and this domain also includes the shorter eIF5C domain. Within this region there are two AA boxes (rich in aromatic and acidic residues) (8), which are important for mediating protein-protein interactions. Multiple alanine substitutions in the AA boxes of eIF2B disrupt binding to eIF2β and equivalent substitutions in eIF5 have similar effects on eIF2β binding (8). The C terminus of mammalian eIF4G is proposed to interact with the protein kinase Mnk1, which phosphorylates eIF4E on serine 209 and has a regulatory role in translation (13).

The second region within this catalytic domain is proposed to function as the catalytic center of the enzyme. This region encompasses residues 518–583 of the yeast protein and is well conserved in all eIF2β proteins (12). In addition, mutation of either Thr552 to Ile (T552I) or Ser776 to Asn (S776N) directly impairs catalytic function without detectably impairing binding to eIF2β (14). Thus, although the eIF2β C terminus is only a small fragment of the entire ~275–295-kDa eIF2 complex, it contains the major functional regions required for nucleotide exchange and provides a structural model for the related C termini of eIF4G and eIF5. In this study we present the x-ray structure of the eIF2β catalytic domain to 2.3 Å and genetic evidence that Glu569 plays a major role in the function of eIF2B.

EXPERIMENTAL PROCEDURES

Protein Preparation,Crystallization, and Structure Determination—DNA encoding residues 524–712 was amplified by PCR using the pAV1693 plasmid as template (12). The PCR product was cleaved with Eco311 creating overhangs compatible with the NcoI and BamHI cloning sites of the pET24d vector into which it was ligated, resulting in a construct having the internal eIF2β Bev360 as start methionine. This construct was expressed in Escherichia coli BL21(DE3) Rosetta cells grown in a defined medium containing selenomethionine. The protein was purified to homogeneity by anion exchange chromatography. The columns were equilibrated in buffer A (20 mM Tris-Cl, pH 7.4, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA) with 150 mM NaCl and eluted with a gradient to 500 mM NaCl in buffer A. Finally, the fragment was purified by gel filtration using a Superdex 75 (Amersham Biosciences) column equilibrated in buffer A with 100 mM NaCl and concentrated to 5–8 mg/ml prior to crystallization. Crystals were obtained at 5 °C by vapor diffusion of drops with equal volumes of protein and reservoir solution containing 0.2 M ammonium acetate, 23–25% PEG 2000 monomethyl ether, 100 mM Na citrate, pH 5.75. The crystals belong to the tetragonal space group I422 (a = 106.34 Å, c = 91.71 Å) with one molecule per asymmetric unit. Crystals were flash-frozen in liquid nitrogen directly from the mother liquor supplemented with a few microliters of reservoir solution prior to data collection. Anomalous data collected from a selenomethionine-substituted crystal were collected at 100 K at the EMBL BW7A (Table I). The data were processed and scaled with MOSFLM and SCALA (15), and multiple wavelength anomalous dispersion (MAD) phases were calculated with CNS (16). After density modification a starting model containing 43% of the data from three wavelengths collected from a selenomethionine-substituted crystal (Fig. 1) or in the final 2F₀ − F₁ map. Hence, the first 20 residues and the last 8 residues are disordered in the crystal. This may explain the Rmin value of 27.2% (Table I), which is slightly higher than expected. The residues in the N terminus may require residues upstream in eIF2β or other residues in eIF2B to become ordered, whereas those in the C terminus may become ordered during complex formation with eIF2β (see below). Alternatively, phosphorylation of two conserved serines in the C terminus, as demonstrated for mammalian eIF2β, could have an effect on the structure of this region (4).

The structured fragment of eIF2B containing residues Asp544–Asp704 (eIF2βC) has overall dimensions of 47 × 43 × 32 Å. The protein is all-helical (Fig. 2) with α-helices I–VII arranged in pairs with an angle between them of 129°. One such helical hairpin then packs against the next pair with an angle of 35°–38°. This results in a left-handed superhelix with an axis approximately perpendicular to the axes of the individual helices (Fig. 3). There are also two 310 helices, the first in residues 619–624 is located right after helix IV, and the second with residues 684–686 prior to helix VIII. The protein has the overall appearance of a hairpin (Fig. 3, top) with helices III–VI forming the center, whereas helices I and II and VII–VIII are located on either side. Helices I, III, V, and VII form one surface of the molecule (the A-face), whereas the remaining helices are on the opposite side (the B-face). The three edges of the molecule can be named according to the contributing helices (Fig. 3). The N-edge is made from residues in the helices I and II, whereas helices VII and VIII contribute to the C-edge. Finally, the top edge contains residues from all helices.

The structure can be divided into a structural core containing helices III–VIII with structural homology to other proteins onto which N-terminal helices I and II are attached. This core is organized around the two AA boxes (Figs. 2 and 3), which is shared with eIF5 and mammalian eIF4G. For both eIF2βC and eIF5 the AA boxes are required for interaction with the common binding partner, the N-terminal half of the β-subunit from eIF2 (8). In contrast to the exchange activity of eIF2B, eIF5 acts as a GAP by stimulating hydrolysis of GTP bound to eIF2γ in the preinitiation complex (25). Hence, it is very likely that both eIF5 and eIF2β are in contact with the nucleotide binding pocket of eIF2γ although no such interaction has been reported.

Structural Homology of eIF2βC to Other Proteins—The structure of eIF2βC is the first structure of a protein containing the AA boxes (see below). A search for homologous proteins with DALI (26) reveals a similar arrangement of helices in several other proteins. The best Z-score, 12.0, is obtained by the 80-kDα subunit of the human nuclear cap binding complex (27), where all eight helices in eIF2βC and eight helices within residues 497–651 in domain 3 of the large subunit of the
complex can be superimposed with a root mean square deviation (r.m.s.d.) of 2.0 Å for 122 Cα atoms (Fig. 4). The second best match with a Z-score of 7.4 is obtained with a central fragment of initiation factor 4GII (MIF4G), and helices III–VIII of eIF2B can be matched with the six N-terminal helices of MIF4G with an r.m.s.d. of 2.1 Å over 77 Cα atoms (Fig. 4) (28).

Importantly, this fragment does not contain the AA boxes, which are found at the C terminus of mammalian eIF4G.

Because eIF2B acts as a GEF and eIF5 as a GAP, structural homology to other GEFs or GAPs is of interest. The only close structural homologues of eIF2B detected in a DALI search are the Ras GEF (SOS) and GAP (p120GAP). Helices III–VIII of eIF2B superimpose with six helices within residues 605–739 of SOS with a Z-score of 4.7 (78 Cα atoms with r.m.s.d. of 3.0 Å).

However, the matching fragment of SOS is not part of the catalytic site of SOS, but rather involved in binding of a second Ras molecule, which has been characterized very recently (29). After superimposition of eIF2BeC onto the complex between SOS and two molecules of Ras, a loop region of eIF2B containing non-conserved residues (Gln 603–Asp 609) comes close to both switch I and II of the second Ras molecule. Thus, the Gln603-609 loop could be implicated in binding to eIF2 and displacing GDP. An important role seems unlikely, however, as sequence is not well conserved among eIF2Be proteins in this region. The first six helices of eIF2BeC can also be superimposed on six helices within residues 732–900 of p120GAP, the GAP of Ras (DALI score 6.1, r.m.s.d. of 2.0 Å for 66 Cα atoms), but in contrast to the three other cases, there is a very large insert between the second and the third matching helical hairpin of p120GAP. Nevertheless, this similarity superimposes the

**Table I**

Statistics for data collection, phase determination and the final refinement for the eIF2BeC

| Dataset   | λ     | Resolution | Completeness | Mean I(σI) | Rsym | Wilson B-factor |
|-----------|-------|------------|--------------|------------|------|----------------|
| Peak      | 0.9821| 20–2.4 (20.53–2.4) | 99.2 (99.9) | 27.6 (6.1) | 5.3 (27.4) | 42 |
| Inflection| 0.9824| 20–2.4 (20.53–2.4) | 99.2 (99.9) | 27.8 (6.2) | 5.2 (26.4) | 43 |
| Remote    | 0.9121| 20–2.3 (20.42–2.3) | 99.2 (99.2) | 28.7 (6.5) | 5.0 (27.8) | 40 |

| Resolution | λ     | Sites | FOM |
|------------|-------|------|-----|
| MAD phasing| 20–2.4 | 5    | 0.73|

| Resolution | R/Rfree | Reflections | Atoms | r.m.s.d. bonds/angles | Ramachandran |
|------------|---------|-------------|-------|-----------------------|--------------|
| 20–2.3     | 24.3/27.2 | 20051/2111 | 1342/91 | 0.008/1.3 | 99.3/0.7/0 |

**Fig. 1.** Electron density of helix VIII from the experimental map obtained from MAD phases after density modification. Helix VIII of one molecule of eIF2BeC (gray carbons) packs with a symmetry related helix VIII (gold carbons). The contacts are dominated by the stacking of Trp699 with the corresponding residue of the neighbor molecule. The electron density map is contoured at 1.7 σ and plotted with the map_cover option in the program O (18) using a radius of 1 Å.
FIG. 2. Structure-based sequence alignment of the C-terminal part of eIF2B from 12 species, yeast eIF5, and human eIF4GII. The α-helical secondary structure derived from the structure of yeast eIF2B is shown as cylinders. Yeast eIF5 and human eIF4GII were aligned with yeast eIF2B. The numbering above the alignment is according to yeast eIF2B. Green asterisks denote mutations having no apparent effect on yeast viability, black denotes mutations reducing the catalytic activity of eIF2B, and red is lethal. Boxes indicate Trp618 and Met640 corresponding to human Trp628 and Glu650 that when mutated both cause the childhood ataxia with central nervous system hypomyelination disease. Green circles denote residues in AA box 1 and gray denote those in AA box 2. Blue arrowheads indicate the beginning and end (residues 591–706 in yeast eIF2B) of the W2 domain of the LOAD data base at NCBI (PSSM code 3659), black arrowheads (residues 619–706) indicate the eIF5C domain of the SMART data base (PSSM code 466), and red arrowheads (residues 634–711) indicate the W2 domain of the Pfam data base (PSSM code 2545). Species abbreviations: N.c., Neurospora crassa; P.a., Podospora anserina; S.p., Schizosaccharomyces pombe; C.e., Caenorhabditis elegans; A.t., Arabidopsis thaliana; C.a., Candida albicans (partial sequence); O.c., Oryctolagus cuniculus; M.m., Mus musculus; R.n., Rattus norvegicus; D.m., Drosophila melanogaster; H.s., Homo sapiens; S.c., Saccharomyces cerevisiae.
The Surface Properties of eIF2B—The surface properties of the fragment with respect to the distribution of charges and exposed conserved residues of putative functional importance are highly asymmetric (Fig. 3, middle and right column, respectively). The overall charge of the fragment is quite acidic with an isoelectric point of 4.3, and the surface electrostatic potential is dominated by a very large acidic belt, which starts at the N-edge, continues over the A-face over the top edge of the molecule, and ends at the B-face at the C terminus of helix VIII. A minor positively charged (basic) patch is located on the B-face and top edge around which the acidic belt is “tied.” The remaining charges are more or less randomly distributed (Fig. 3). The majority of the residues constituting the acidic belt and basic patch of eIF2Be are conserved. The acidic belt includes residues Asp564, Glu569, Glu548, Glu583, Asp634, Asp666, Glu670, Asp704, and Asp711, which are all highly conserved with respect to charge (the order of the listed residues following the direction of the belt as described above). Three of these residues, Glu670, Asp671, and Asp704, are conserved AA box residues (see below). The basic patch includes Arg574, Lys623, and Arg624.

As with the electrostatic potential, the exposed conserved residues are primarily found at the B-face, N-edge, and top edge (Fig. 3), and are roughly organized in two patches. The first of these contains residues from helices I and II and associated loops, whereas the second contains residues from helices VII–VIII and their associated loops. In this second area, residues from the AA boxes are dominating and the strictly conserved Tyr663, Glu670, Trp690, and Leu700 together with the highly conserved Ala703 form an exposed “handle” at the C-edge (Fig. 3).

The AA Boxes—The AA boxes contain conserved aliphatic, aromatic and acidic residues (Fig. 2). The first box contains 12 conserved residues within amino acids Leu655–Trp670, which...
are all located within helices VI and VII (Fig. 5). The second box has eight conserved residues within amino acids Trp<sup>696</sup>–Glu<sup>706</sup>, of which the two last, Glu<sup>705</sup> and Glu<sup>706</sup>, are disordered in our structure. This AA box is located at the C terminus of helix VIII. The first box is important for the structural integrity of the helix III–VIII core. Residues Ala<sup>658</sup> in helix VI and Ile<sup>667</sup> in the following loop contact helix IV and the following 3<sub>10</sub> helix, whereas Leu<sup>655</sup> and Leu<sup>659</sup> in helix VI together with the strictly conserved Trp<sup>676</sup> in helix VII pack with helix V. The indole ring of the Trp<sup>676</sup> also engages in a hydrogen bond with the side chain of Asn<sup>637</sup>, and furthermore, packs with side chains of Met<sup>636</sup>, Lys<sup>675</sup>, and Trp<sup>677</sup>.

The strictly conserved Tyr<sup>663</sup> is at the center of a cluster of five residues from the AA boxes linking helices VI–VIII (Fig. 5). It engages in van der Waal interactions with Glu<sup>670</sup>, Ile<sup>673</sup>, Trp<sup>676</sup>, and Leu<sup>700</sup>, which, except for the tryptophan, are strictly conserved in eIF2B<sub>e</sub>. Furthermore, the hydroxy group of Tyr<sup>663</sup> forms a hydrogen bond with the side chain carboxyl group of Glu<sup>670</sup>. On one side this cluster is flanked by Tyr<sup>674</sup>,...
which fixes the end of helix VIII through a hydrogen bond from its hydroxy group to the side chain of Asp704, the last residue in our structure. To the other side Phe656 flanks the cluster.

The N-terminal Helices—Although the two N-terminal helices are firmly associated with the six helical core of eIF2Bc, residues 544–576 show significantly higher temperature factors (46.1 Å²) compared with amino acids 577–704 (34.8 Å²). This is not because of very high mobility of a few disordered residues, but rather a general trend for all atoms. Hence, these two helices are by average more mobile than the rest of the structure. This could be of functional importance for the exchange reaction, as these two helices are likely to be directly involved in catalysis, but may also be caused by missing residues from either eIF2Be itself or the other subunits of eIF2B. One indication in favor of their functional importance is that these two helices and the loop to helix III contain three strictly conserved residues. Strictly conserved residues are otherwise only found in the AA boxes. A direct function of residues from the two helices in catalysis is also supported by genetic and biochemical data (12) (see also below). The interface between helices I–II and the rest of the molecule is not extensive in agreement with the elevated temperature factors. Central contacts are formed by the packing of Met557 and Leu693 with Arg566 and Trp518 from helices III and IV, respectively. Furthermore, there are important polar interactions; Asp569 forms a salt bridge with Arg624, and a water molecule bridges the side chains of Glu569 and Arg526. At the end of helix II Asn571 and Arg574 interact with Tyr581 in helix III.

Mutation of Glutamate 569 Is Critical for in Vivo Function—To identify residues important for catalysis of nucleotide exchange we introduced single alanines in place of selected conserved residues within helices I and II of eIF2BeC. As we had previously identified two residues within this region of yeast eIF2BeC, Thr552 and Ser778, that when mutated significantly impaired eIF2B function in yeast (14) we suspected that other changes here might significantly reduce eIF2B activity.

We selected conserved residues with side chains implicated in catalysis of nucleotide exchange in other GEFs. Charged residues were selected as these are important for function in many other GEFs. Leucine 938 is important for the exchange reaction in SOS. The RAS-SOS co-crystal structure reveals that it disrupts magnesium binding to the RAS nucleotide binding pocket (36). Thus, two leucine residues were selected. Finally, Asn728 was chosen as it is universally conserved. Each residue was changed to Ala. The mutations were introduced into the gCD6 gene on a low copy plasmid and were shuffled into a gcd6a strain. Surprisingly, of seven mutations analyzed, six exhibited no obvious growth defect (Fig. 6A). Only one mutation, E569A, had a significant phenotype. It was lethal. This suggested that nucleotide exchange was severely reduced in this strain. The essential function of eIF2B can be overcome in yeast by overexpressing four genes encoding TC factors: the three eIF2 subunits and one of the tRNAiMet genes (12, 33). The resulting strain (GP4115) is severely slow growing. We asked whether a plasmid bearing gcd6-E569A or any of our other previously described reduced activity mutants could improve the growth rate of our mutant strain where deletion of gcd6 is rescued by high copy TC. We found that wild type GCD6 and reduced activity mutants F250L, T552I, and S576N fully rescued the growth rate of this strain (Fig. 6B). The previously described mutation N249K was partially functional. This suggested that nucleotide exchange was severely reduced in this strain. The essential function of eIF2B can be overcome in yeast by overexpressing four genes encoding TC factors: the three eIF2 subunits and one of the tRNAiMet genes (12, 33). The resulting strain (GP4115) is severely slow growing. We asked whether a plasmid bearing gcd6-E569A or any of our other previously described reduced activity mutants could improve the growth rate of our mutant strain where deletion of gcd6 is rescued by high copy TC. We found that wild type GCD6 and reduced activity mutants F250L, T552I, and S576N fully rescued the slow growth phenotype of this strain (Fig. 6B). The previously described mutation N249K was partially functional in this assay. N249K, like E569A is lethal in an otherwise normal strain, but does retain some eIF2B activity under ideal growth conditions.
The Bipolar Properties of eIF2BeC—The catalytic activity of eIF2B has been mapped to residues 518–712 by genetic analysis, in vitro exchange, and pull-down assays (14). Furthermore, in vitro studies showed that deletion of residues 518–580 results in loss of exchange activity, whereas binding to eIF2 is preserved (12). These results are in excellent agreement with the structure presented here, because helices I and II are within 518–580. Hence, they are not required for binding eIF2 but very likely to be involved in catalysis, whereas helices III–VIII are sufficient for eIF2 binding but not for catalysis. One conserved surface patch at helices I and II seems well suited for participation in catalysis as this patch contains Glu569 (Figs. 3 and 7), and mutation of this residue to alanine eliminates eIF2B function in vivo (Fig. 6). The glutamate is located in the center of the patch at the N-edge/top edge, and flanks a negatively charged depression between helices I and II, which also contains Thr552. This threonine is engaged in hydrogen bonding to Glu548, hereby fixing this residue. Mutation of Thr552 and another residue close to this area, Ser576, has previously been shown to reduce exchange activity (Table II) (14).

Consistent with our findings that Glu569 is important for eIF2B catalytic function, structures of complexes between G-proteins and their GEFs have previously emphasized the importance of glutamates or aspartates in the reaction mechanism. In the Arf1-Sec7 complex, the Glu97 side chain of Sec7 overlaps with the binding site for the Mg2+ and γ-phosphate. It also forms a salt bridge with the conserved P-loop lysine (34), and mutation of this glutamate reduces exchange activity by orders of magnitude (35). In the Tiam1-Rac1 complex Glu1047 from Tiam1 interacts extensively with switch I of Rac1, and places an Ile from Rac close to the GDP ribose binding site (36). In the Ras-Sos complex, Glu492 from SOS forms a hydrogen bond with Ser147 from the P-loop of Ras, and this prevents binding of both phosphates and Mg2+ to Ras (37). In the EF-Tu-EF-Ts complex, Asp80 from EF-Ts displaces EF-Tu switch II, thereby disrupting the EF-Tu Mg2+ binding site (38). As shown by these examples, the GEF Asp/Glu do not recognize equivalent parts of the nucleotide binding pocket of their target G-protein, but can contribute to exchange by interaction with switch I, switch II, or the P-loop of the G-protein.

In vitro experiments showed that residues 580–712 are sufficient for binding to eIF2 (12), and this result can now be rationalized, as these residues contain helices III–VIII, the core of the molecule. The eIF2β subunit contains three lysine-rich boxes, which mediates binding of eIF2 to both eIF2B and the GAP eIF5 (8, 39). These positively charged stretches of eIF2β are likely to interact with the acidic belt observed in eIF2BeC. The AA box motifs of eIF4G have also been implicated in protein-protein interactions. eIF4G has been shown to bind to the eIF4E kinase, Mnk1. The N-terminal 23 residues of Mnk1 necessary for this interaction also contain a lysine-rich region very similar to the lysine-rich boxes in eIF2β (13).

Comparison of eIF2BeC with eIF5—The catalytic fragment of eIF2B shares a number of characteristics with the eIF2 GAP, eIF5. The AA boxes are important for interaction of both proteins with the common substrate eIF2β (8). Double mutations E346A,E347A and E384A,E385A of rat eIF5, corresponding to yeast eIF2Be Glu670-Asp671 and Glu705,Glu706 (Fig. 2), caused severe defects in eIF5 binding to eIF2B (Table II) (40). The hexamutant E345A,E346A,E347A,E384A,E385A,E386A showed strongly decreased binding to eIF2β (40). These residues can now be mapped to equivalents in yeast eIF2BeC, except for residues Glu705 and Glu706 for which we have no electron density. They are located in the acidic belt at the C-edge/top edge of eIF2BeC (Figs. 3 and 5). Thus, it is clear that this part of eIF2BeC (and eIF5) is critical for binding to eIF2β. However, one major functional difference between eIF2BeC and eIF5 is that Arg15 essential for GAP activity of eIF5 (41, 42) is located at the N terminus of the protein quite distant in the primary structure from C-terminal residues 241–405 in eIF5. The Exchange Factors of Translation—eIF2γ shares extensive structural and functional similarity to translation elongation factors eEF1A and prokaryotic EF-Tu. All three are G-proteins that also bind aminoacylated tRNAs and interact with the ribosome. In addition, all three factors require a nucleotide exchange factor: eIF2B, eIF1B, and EF-Ts, respectively. Despite these obvious similarities all three exchange factor catalytic domains do not share structural similarities. eIF2BeC is an all-helical protein, whereas the eEF1B catalytic fragment is organized with a central β-sheet surrounded by two helices (43) and EF-Ts offers a third structural solution (38). The common properties of the three homologous G-proteins do apparently not impose any restraints on the structure of their exchange factors.

Partial Structural Basis for a Genetic Disease—Childhood ataxia with central nervous system hypomyelination also called leukoencephalopathy with vanishing white matter, is a rare recessive fatal genetic disease. The disease is caused by mutations in human eIF2B. The tryptophan interacts tightly with human lysine is equivalent to the aliphatic side chain of the

| Residue | Function/Effect | Ref. |
|---------|-----------------|-----|
| W618 (human W628R) | Disease (CACH) | 6 |
| M640 (human E650K) | Disease (CACH) | 6 |
| 12 AA box (Leu659, Ala659, Leu662, Tyr663, Ile667, Ile668, Glu670, Asp671, Ile673, Tyr674, Trp676) | No binding to eIF2 | 8 |
| 7 AA box (Trp696, Val697, Thr699, Leu701, Asp704, Glu705, Glu706) | No binding to eIF2 | 8 |
| T652I | Reduced exchange activity | 14 |
| E659A | Lethal | This work |
| S676N | Reduced exchange activity | 14 |

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yeast methionine. Preliminary modeling shows that an arginine can roughly fill the space occupied by Trp in the yeast structure. An arginine in this position might be detrimental, as repulsion could occur with the human Lys, but it is also perfectly located for making a hydrogen bond with the backbone between yeast His and Asp.

For the second mutation, human E650K, the equivalent yeast Met is located between the Leu from AA box 1 and also in the vicinity of Trp. Modeling indicates that a lysine can be accommodated here, but such a lysine in human eIF2B might be attracted into a salt bridge with human Asp, and thereby decrease the stability of the hydrophobic core around human Met and Trp. Hence, the yeast structure of eIF2B shows that both the pathogenic mutations found in the C terminus of human eIF2B are buried, so these residues are not directly involved in catalysis or in the interface to eIF2 or other parts of eIF2B. This suggests that their mutation cause the phenotype by disturbing the structural integrity of the domain.

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REFERENCES

1. Dever, T. E. (2002) Cell 108, 545–556
2. Hinnebusch, A. G. (2000) in Translational Control of Gene Expression (Sonenberg, N., Hershey, J. W. B., and Matthews, M. B., eds) pp. 185–243, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Pavtit, G. D., Ramaiah, K. V., Kimball, S. R., and Hinnebusch, A. G. (1998) Genes Dev. 12, 514–526
4. Wang, X., Paulin, F. E., Campbell, L. E., Gomez, E., O'Brien, K., Morrice, N., and Proud, C. G. (2001) EMBO J. 20, 4349–4359
5. van der Knaap, M. S., Leegwater, P. A., Konst, A. A., Visser, A., Naidu, S., Oudejans, C. B., Schutgens, R. B., and Pronk, J. C. (2002) Ann. Neurol. 51, 264–270
6. Leegwater, P. A., Vermeulen, G., Konst, A. A., Naidu, S., Mulders, J., Visser, A., Kersbergen, P., Mobach, D., Fonds, D., van Berkel, C. G., Lemmers, R. J., Frants, R. R., Oudejans, C. B., Schutgens, R. B., Pronk, J. C., and van der Knaap, M. S. (2001) Nat. Genet. 28, 383–388
7. Erickson, F. L., and Hannig, E. M. (1996) EMBO J. 15, 6311–6320
8. Asano, K., Krishnamoorthy, T., Phan, L., Pavitt, G. D., and Hinnebusch, A. G. (1999) EMBO J. 18, 1673–1688
9. Yang, W., and Hinnebusch, A. G. (1996) Mol. Cell. Biol. 16, 6603–6616
10. Fabian, J. R., Kimball, S. R., Heinzinger, N. K., and Jefferson, L. S. (1997) J. Biol. Chem. 272, 12359–12365
11. Williams, D. D., Pavitt, G. D., and Proud, C. G. (2001) J. Biol. Chem. 276, 3733–3742
12. Gomez, E., Mohammad, S. S., and Pavitt, G. D. (2002) EMBO J. 21, 5292–5301
13. Waskiewicz, A. J., Johnson, J. C., Penn, B., Mahalingam, M., Kimball, S. R., and Cooper, J. A. (1999) Mol. Cell. Biol. 19, 1871–1880
14. Gomez, E., and Pavitt, G. D. (2000) Mol. Cell. Biol. 20, 3965–3976
15. Leslie, A. G. W. (1992) Joint CCP4 + EMBT-EAMCB Newsletter on Protein Crystallography, Vol. 26
16. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, S. J., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
17. Petrizzi, A., Morris, R., and Lamon, V. S. (1999) Nat. Struct. Biol. 6, 458–463
18. Jones, T. A., Cowan, S., Zou, J.-Y., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
19. Koradi, R., Billeter, M., and Wuthrich, K. (1996) J. Mol. Graph. 14, 29–32, 51–55
20. Gietz, R. D., Schiestl, R. H., Willems, A. R., and Woods, R. A. (1995) Yeast 11, 355–360
21. Bocke, J. D., Trueheart, J., Natsoulis, G., and Fink, G. R. (1987) Methods Enzymol. 154, 164–175
22. Bushman, J. L., Asuru, A. I., Matts, R. L., and Hinnebusch, A. G. (1993) Mol. Cell. Biol. 13, 1920–1932
23. Dever, T. E., Yang, W., Astrum, S., Bystron, A. S., and Hinnebusch, A. G. (1995) Mol. Cell. Biol. 15, 6351–6363
24. Hendrickson, W. A. (1991) Science 254, 51–58
25. Chakrabarti, A., and Maitra, U. (1991) J. Mol. Biol. 226, 110–119
26. Mazza, C., Ohno, M., Segref, A., Mattaj, I. W., and Cusack, S. (2001) Mol. Cell. 8, 383–396
27. Marcotrigiano, J., Lomakin, I. B., Sanberg, N., Pestova, T. V., Hellén, C. U., and Burley, S. K. (2001) Mol. Cell. 7, 193–203
28. Margaritis, S. M., Sondermann, H., Hall, B. E., Nagar, B., Hoelz, A., Pirruccello, M., Bar-Sagi, D., and Kuriyan, J. (2003) Cell 112, 685–695
29. Scheffzek, K., Ahmadian, M. R., Kabsch, W., Wiesmüller, L., Lauterwein, A., Schmitz, F., and Wittinghofer, A. (1997) Science 277, 333–338
30. Andrade, M. A., Petosa, C., O'Donnogue, S. I., Muller, C. W., and Bork, P. (2001) J. Mol. Biol. 309, 1–18
31. Andrade, M. A., and Bork, P. (1995) Nat. Genet. 11, 115–116
32. Erickson, F. L., Nika, J., Epel, S., and Hannig, E. M. (2001) Genetics 158, 123–132
33. Goldberg, J. (1998) Cell 95, 237–248
34. Her بد, Dufour, S., Robineau, S., Chardin, P., Paris, S., Chaubre, M., Cherfils, J., and Antonny, B. (1998) EMBO J. 17, 3651–3659
35. Worthylake, D. K., Rossman, K. L., and Sondek, J. (2000) Nature 408, 682–688
36. Boriack-Sjodin, P. A., Margaritis, S. M., Bar-Sagi, D., and Kuriyan, J. (1998) Nature 394, 337–343
37. Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S., and Leberman, R. (1996) Nature 379, 511–518
38. Das, S., Maiti, T., Das, K., and Maitra, U. (1997) J. Biol. Chem. 272, 31712–31718
39. Das, S., and Maitra, U. (2000) Mol. Cell. Biol. 20, 3942–3950
40. Pavitt, F. E., Campbell, L. E., O'Brien, K., Loughlin, J., and Proud, C. G. (2001) Curr. Biol. 11, 55–59
41. Das, S., Ghosh, R., and Maitra, U. (2001) J. Biol. Chem. 276, 6720–6726
42. Andersen, G. R., Pedersen, L., Valente, L., Chatterjee, L., Kinsey, T. G., Kjeldgaard, M., and Nyborg, J. (2000) Mol. Cell 6, 1281–1286
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