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eIF2B, the guanine nucleotide-exchange factor for eukaryotic initiation factor 2

Sequence conservation between the α, β and δ subunits of eIF2B from mammals and yeast

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The guanine nucleotide-exchange factor eIF2B mediates the exchange of GDP bound to translation initiation factor eIF2 for GTP. This exchange process is a key regulatory step for the control of translation initiation in eukaryotic organisms. To improve our understanding of the structure, function and regulation of eIF2B, we have obtained and sequenced cDNA species encoding all of its five subunits. Here we report the sequences of eIF2Bβ and δ from rat. This paper focuses on sequence similarities between the α, β and δ subunits of mammalian eIF2B. Earlier work showed that the amino acid sequences of the corresponding subunits of eIF2B in the yeast *Saccharomyces cerevisiae* (GCN3, GCD7 and GCD2) exhibit considerable similarity. We demonstrate that this is also true for the mammalian subunits. Moreover, alignment of the eIF2Bα, β and δ sequences from mammals and yeast, along with the sequence of the putative eIF2Bα subunit from *Caenorhabditis elegans* and eIF2Bδ from *Schizosaccharomyces pombe* shows that a large number of residues are identical or conserved between the C-terminal regions of all these sequences. This strong sequence conservation points to the likely functional importance of these residues. The implications of this are discussed in the light of results concerning the functions of the subunits of eIF2B in yeast and mammals. Our results also indicate that the large apparent differences in mobility on SDS/PAGE between eIF2Bβ and δ subunits from rat and rabbit are not due to differences in their lengths but reflect differences in amino acid composition. We have also examined the relative expression of mRNA species encoding the α, β, δ and ε subunits of eIF2B in a range of rat tissues by Northern blot analysis. As might be expected for mRNA species encoding subunits of a heterotrimeric protein, the ratios of expression levels of these subunits to one another did not vary between the different rat tissues examined (with the possible exception of liver). This represents the first analysis of the levels of expression of mRNA species encoding the different subunits of eIF2B.

INTRODUCTION

In eukaryotic protein synthesis the initiator Met-tRNA^{Met} is delivered to the 40 S ribosomal subunit as a ternary complex with the initiation factor eIF2 and GTP. Later in the initiation process the GTP is hydrolysed and eIF2 is released as the inactive binary complex [eIF2·GDP]. At physiological concentrations of Mg^{2+} a second factor, eIF2B (formerly called GEF, guanine nucleotide-exchange factor), is required to promote the exchange of GDP for GTP [1].

Mammalian eIF2B is a heteropentameric protein with apparent subunit masses for the rabbit reticulocyte factor of approx. 29 (α), 39 (β) 54 (γ), 66 (δ) and 84 kDa (ε) as judged by SDS/PAGE (see Table 1). Compared with other GEFs, which are generally monomeric, eIF2B is thus a relatively complex molecule. This added complexity might reflect its mechanism of action, multiple regulatory inputs, and/or other roles in the cell besides recycling eIF2. The only other known soluble GEF with more than one subunit is eEF1βγδ, the exchange factor for the eukaryotic elongation factor eEF1α. In this factor both the β and δ subunits are thought to possess GEF activity [2,3].

All other known GEFs seem to function via a substituted-enzyme mechanism {e.g. the bacterial protein synthesis elongation factor EFTs (the exchange factor for EFTu) [4] and CDC-25p (an exchange factor for one of the Ras family of proteins in yeast) [5]}. However, the details of the exchange reaction utilized by eIF2B are unclear (see [1]). Much evidence favours a ternary complex mechanism (the three components being eIF2B, [eIF2·GDP] and GTP). Certain biochemical data support the key requirement of this model, namely the presence of a GTP-binding site on eIF2B [6,7]. However, of the known eIF2B subunit sequences (all five from both yeast and mammals), none possesses consensus guanine nucleotide-binding domains.

Mammalian eIF2B is known to be subject to regulation by a variety of mechanisms on the basis of experiments *in vitro*, although its is not clear whether all of them are important for the control of eIF2B activity *in vivo*. The best-characterized regulatory mechanism involves the ability of the substrate [eIF2·GDP] to act as a competitive inhibitor of eIF2B when eIF2 is phosphorylated on its α subunit (reviewed in [1]). The residue in eIF2α involved here (Ser-51) is a target for highly specific protein kinases from mammalian cells (the haem-controlled and double-stranded RNA-activated kinases) and yeast (the protein kinase GCN2, believed to be activated under

Abbreviations used: eIF, eukaryotic initiation factor; GCD, general control derepressed; GCN, general control non-derepressed; GEF, guanine nucleotide-exchange factor; UTR, untranslated region.

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The nucleotide sequences reported will appear in EMBL and GenBank Nucleotide Sequence Databases under the accession numbers U31880 and Z48225.

Table 1 Properties of eIF2B subunits from rat and rabbit

The lengths cited include the initiator methionine. For rat and rabbit eIF2Bβ, the mature subunit is known to begin at Pro-2 in the sequence, i.e., the first methionine is removed (see Figure 1). All the other subunits of rat and rabbit eIF2B are N-terminally blocked. Predictions of molecular mass and pI are based on the full-length cDNA-derived protein sequence.

Subunit	Size on SDS/PAGE (Da)		Predicted molecular mass (Da)	Predicted length (amino acid residues)	pI		Sequence reference	Sequence accession number
	[11,21]	[22]			Predicted	Observed [11,22]		
Rat eIF2Bα	–	30900	33682	305	8.25	–	[23]	U05821
Rabbit eIF2Bα	30000	31000	–	–	–	–	–	–
Rat eIF2Bβ	42000	43200	38880	351	6.02	6.4	This study	U31880
Rabbit eIF2Bβ	39000	40000	39008	351	5.99	–	[24]	Z48222
Rat eIF2Bγ	–	55700	50440	452	6.8	6.9	[28]	U38253
Rabbit eIF2Bγ	54000	56000	–	–	–	–	–	–
Rat eIF2Bδ	61000	63100	57814	524	9.20	–	This study	Z48225
Rabbit eIF2Bδ	66000	66000	57126	523	9.31	Highly basic	[25]	X75451
Rat eIF2Bϵ	89000	89100	80230	716	4.82	$\sim$ 6.0	[34a]	U19511
Rabbit eIF2Bϵ	84000	83000	80175	721	5.10	–	[42]	U23037

conditions of amino acid deprivation [8,9]). The activity of mammalian eIF2B is subject to allosteric regulation by a variety of activators *in vitro* (e.g. polyamines, NADH, NADPH [10–12] or inhibitors {NAD^+, $NADP^+$ [10,11], ATP ([13]; B. L. Craddock and C. G. Proud, unpublished work) and heparin [12]}). Finally, eIF2B itself is subject to phosphorylation by a number of protein kinases *in vitro*, including kinases CK-1 and CK-2 and glycogen synthase kinase 3: phosphorylation by the first two is reported to activate eIF2B [14–16] whereas glycogen synthase kinase 3-mediated phosphorylation is inhibitory (G. I. Welsh, E. J. Foulstone, N. T. Price and C. G. Proud, unpublished work). All three phosphorylate the largest (ϵ) subunit of the factor and recent work has shown that this subunit is indeed phosphorylated *in vivo* ([17]; G. I. Welsh, E. J. Foulstone, N. T. Price and C. G. Proud, unpublished work).

To learn more about the structure, function and regulation of mammalian eIF2B, we have isolated cDNA species for its subunits. The present report focuses on the α, β and δ subunits. Studies in *Saccharomyces cerevisiae* have shown that these three subunits of yeast eIF2B possess considerable mutual amino acid sequence similarity [18–20], and suggest that they might have similar functional roles.

eIF2B subunits (in particular the β, δ and ϵ subunits) from rat and rabbit differ in size as judged by SDS/PAGE (Table 1) [21,22]. To address the basis of these differences in apparent size we have cloned cDNA species for these subunits from both species to allow comparisons of amino acid sequences. Here we report the sequences of eIF2Bβ and δ from rat.

We have isolated cDNA species for the α (rat [23]), β (rat, this paper; and rabbit [24]), δ (rat, this paper; and rabbit [25]) and ϵ [rat (K. M. Flowers, H. Mellor, R. Matts, S. R. Kimball and L. S. Jefferson, unpublished work)] subunits of mammalian eIF2B. The sequence for rabbit eIF2Bϵ ([19]; A. I. Asuru, H. Mellor, S. B. Thomas, Y. Lu, J. J. Chen, J. S. Crosby, S. D. Hartson, S. R. Kimball, L. S. Jefferson, and R. Matts, unpublished work), mouse eIF2Bδ [26] and *S. pombe* eIF2Bδ (nucleotide sequence databank accession number Q09924) are also available. In addition, as a result of the *Caenorhabditis elegans* genome sequencing project [27], a sequence for a putative eIF2Bα subunit is available. We have recently cloned and sequenced full-length cDNA species encoding mammalian eIF2Bγ and identified clones for *C. elegans* γ and ϵ. This work is described elsewhere [28].

MATERIALS AND METHODS

cDNA species for the rat eIF2Bβ and δ subunits were obtained from rat liver (λ ZAP II; catalogue number 936513) and rat brain (Uni-ZAP-XR vector; catalogue number 937502) cDNA libraries (Stratagene) respectively. Libraries were screened as described previously [23,25] by using the corresponding rabbit cDNA species as probes. cDNA species were sequenced on both strands by using a custom primer walking strategy, by a combination of automated (Du Pont Genesis 2000) or manual methods (Sequenase kit from U.S. Biochemical, Cleveland, OH, U.S.A.).

Transcription/translation *in vitro* was performed with the TnT coupled rabbit-reticulocyte lysate system (Promega) with [^{35}S]-methionine labelling. The templates were the cDNA species as excised from the libraries into pBluescript SK($-$) and were transcribed from the T3 or T7 promotors as appropriate. Poor expression of rabbit eIF2Bδ was initially observed. A shortened cDNA, containing only 15 nt from the 5′ untranslated region (UTR) was amplified by PCR and ligated into pCR II (TA cloning kit, Invitrogen). This allowed expression in the rabbit reticulocyte lysate system from the T7 promotor of pCRII. The rat eIF2Bδ cDNA insert possessed 8 nt of 5′ UTR (plus 13 nt of adapter). The rabbit eIF2β cDNA was expressed from pBluescript as described [24]. Translation products were separated by SDS/PAGE and analysed by autoradiography.

Preparation of poly(A)$^+$ RNA and Northern blot analysis were performed as described previously [29].

RESULTS AND DISCUSSION

Comparison of the β and δ subunits of eIF2B from rat and rabbit

Differences in subunit sizes for eIF2B from different species are observed in Western blotting of SDS/polyacrylamide gels with antibodies to the individual subunits [21], and on SDS/PAGE of the purified factors [11,22]. These differences are most pronounced for eIF2Bβ, δ and ϵ. The sizes of rabbit and rat eIF2B subunits on SDS/PAGE are reported in Table 1. In the present study we compare the cDNA-derived amino acid sequences of rabbit eIF2Bβ and δ with the newly reported sequences of the rat subunits.

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Rat     MPGAAAKGSELSERIESFVETLKRGGGRRTSEDMARETLGLLRRLITDHHWNNAGDLMDLIRREGRRMTAAHPPE 75
Rabbit  MPGATEKGSELSERIESFVEALKRGGGQRSSEDMARETLGLLRRIITDHRWSNAGELMELIRREGRRMMAAQPSE 75

Rat     TTVGNMVRRVLKIIREEYGRLHGRSDESDQQESLHKLLTSGGLSEDFSFHYAPLKSNIIEAINELLVELEGTTEN 150
Rabbit  TTVGNMVRRVLKIIREEYGRLHGRSDESDQQESLHKLLTSGGLSEDFSFHYAQLQSNIVEAINELLVELEGTTEN 150

Rat     IAAQALEHIHSNEVIMTIGFSRTVEAFLREAAQKRKFHVIAAECAPFCQGHEMAVNLSEAGIETTVMTDAAIFAV 225
Rabbit  IAAQALEHIHSNEVIMTIGLSRTVEAFLREAARKRKFHVIVAECAPFCQGHEMAVNLSKAGIETTVMTDAAIFAV 225

Rat     MSRVNKVIIGTKTILANGSLRAVAGTHTLALAAKHHSTPLIVCAPMFKLSPQFFSEEDSFHKFVAPEEVLPFTEG 300
Rabbit  MSRVNKVIIGTKTILANGALRAVTGTHTLALAAKHHSTPLIVCAPMFKLSPQFFNEEDSFHKFVAPEEVLPFTEG 300

Rat     DILEKVSVHCPVFDYVPPDLITLFISNIGGNAPSYVYRLMSELYHPDDHVL.                        352
Rabbit  DILDKVGCHCPVFDYVPPELITLFISNIGGNAPSYIYRLMSELYHPEDHVL.                        352
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Figure 1 Alignment of cDNA-derived amino acid sequences for eIF2Bβ from rat and rabbit

Non-identical positions in the sequences are highlighted. The sequences show 92% identity. The nucleotide sequence of rat eIF2Bβ is available in the GenBank and EMBL databases (see Table 1).

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Rat     MAAVAVAVREESRSEMKTELSPRPGAAGRELTQEEKLQLRKEKKQQKKKRKEEKGADQEIGSAVSAAQRQDPVRE 75
Rabbit  MAAVAVAVREDSGSGMKAELSARPGAGGKEMTQEEKLQLRKEKKQQKKKRKEEKGTDVDTASAVSAAQCPGPARE 75
Mouse   MAAVAVAVREESRSEMKTELSPRPGAAGRELTQEEKLQLRKEKKQQKKKRKEEKGADQEIGSAVSAAQRQDPIRE 75

Rat     LQGTGSQLGGTTGEKLPAGRSKAELRAERRAKQEAERALKQARKGEQGGPSPQACPSTAGEATSGVKRVPEHTQA 150
Rabbit  APGPGSQ-SSTPGEKVPAGRTKAELRAERRAKQEAERALKQARKGEQGGPPPQASPSTAGEAPAGGKRLTEHTQA 149
Mouse   LPGPGSQLGGTAGEKLPAGRSKAELRAERRAKQEAERALKQARKGEQGGVPPQACPSTAGETTSGVKRVPEHTPA 150

Rat     DDPTLLRRLLRKPDRQQVPTRKDYGSKVSLFSHLPQYSRQSSLTQYMSIPSSVIHPAMVRLGLQYSQGLVSGSNA 225
Rabbit  DDPTLLRRLVRKSERQQVPTRKDYGSKVSLFSHLPQYSRQNSLTQYMSIPSSVIHPAMVRLGLQYSQGLISGSNA 224
Mouse   DDPTLLRRLLRKPDRQQVPTRKDYGSKVSLFSHLPQYSRQSSLTQYMSIPSSVIHPAMVRLGLQYSQGLISGSNA 225

Rat     RCIALLHALQQVIQDYTTPPNEELSRDLVNKLKPYISFLTQCRPMSASMCNAIKFFNKEVTGMSSSKREEEAKSE 300
Rabbit  RCIALLRALQQVIQDYTTPPNEELSRDLVNKLKPYICFLTQCRPLSASMYNAIKFLNKEITGVSSTKREEEAKAE 299
Mouse   RCIALLHALQQVIQDYTTPPSEELSRDLVNKLKPYISFLTQCRPMSASMCNAIKFLTKEVTGMSSSKREEEAKSE 300

Rat     LKEAIDRYVQEKIVLASQAISRFASKKISDGDVILVYGCSSLVSRILQEAWVEGRRFRVVVVDSRPRLEGRHMLH 375
Rabbit  LQAAADRYVQEKIVLAAQAILRFASKKISNGDVILVYGCSSLVSRILQEAWSEGRKFRVVVVDSRPRLEGRHMLR 374
Mouse   LKEALDRYVQEKIVLAAQAISRFASTKISDGDVILVYGCSSLVSRILQEARVEGRRFRVVVVDSRPRLEGRHMLH 375

Rat     CLVRAGVPTSYLLIPAASYVLPEVSKVLLGAHALLANGSVMSRVGTAQLALVARAHNVPVLVCCETYKFCERVQT 450
Rabbit  FLVRAGVPASYLLIPAASYVLPEVSKVLLGAHALLANGSVMSRVGTAQLALVARAHNVPVLVCCETYKFCERVQT 449
Mouse   SLVRAGVPTSYLLIPAASYVLPEVSKVLLGAHALLANGSVMSRVGTAQLALVARAHNVPVLVCCETYKFCERVQT 450

Rat     DAFVSNELDDPDDLQCKRGDQVTLANWQNNSSLRLLNLVYDVTPPELVDLVITELGMIPCSSVPVVLRVKSSDQ. 525
Rabbit  DAFVSNELDDPDDLQCERGDHVALANWQSHPSLRLLNLVYDVTPPELVDLVITELGMIPCSSVPVVLRVKSSDQ. 524
Mouse   DAFVSNELDDPDDLQCKRGDQVALANWQSHPSLRLLNLVYDVTPPELVDLVITELGMIPCSSVPVVLRVKSSDQ. 525
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Figure 2 Alignment of cDNA-derived amino acid sequences for eIF2Bδ from rat, rabbit and mouse

Non-identical positions in the sequences are highlighted. The rat and rabbit sequences are 88% identical. The rat and mouse sequences show a higher level of identity (96%). The nucleotide sequence of rat eIF2Bδ is available in the GenBank and EMBL databases (see Table 1).

cDNA species for the β and δ subunits of rat eIF2B were obtained by screening rat cDNA libraries with the corresponding rabbit cDNA species [24,25]. Characteristics of the cDNA-derived amino acid sequences are also reported in Table 1. The derived amino acid sequences are shown in Figures 1 and 2. The nucleotide sequences (not shown) have been deposited in the Genbank and EMBL databanks (see Table 1).

Recently the sequence for murine eIF2Bδ cDNA has also been reported [26]. Surprisingly, cDNA species potentially encoding two isoforms were discovered. The significance of these two forms, which would differ only in their extreme N-terminal sequences, is as yet unknown. We have so far found no evidence for such heterogeneity in eIF2Bδ from rat or rabbit. Indeed, Henderson et al. [26] also isolated only the shorter form (which corresponds to our eIF2Bδ sequence) from rabbit reticulocyte and human spleen cDNA libraries.

The rat and rabbit eIF2Bβ cDNA-derived amino acid sequences (Figure 1) show no differences in length. N-terminal sequences obtained from the purified factor show that for both species, the mature eIF2Bβ sequence begins at residue 2 (proline), i.e. the first methionine is removed. The N-terminal sequence corresponds exactly to residues 2–29 for the rabbit β subunit [24], and to residues 2–24 for that from rat (results not shown). Thus differences in migration must be due to differences in either post-translational modification or, more probably, in amino acid composition.

For eIF2Bδ, the rat and rabbit cDNA-derived amino acid sequences differ in length by only one residue (Figure 2 and Table 1). The δ subunits of purified eIF2B from both species are N-terminally blocked, and thus the sequences of the N-termini of the mature proteins are unknown. However, the first in-frame AUG codon is in good start context for both rat and rabbit cDNA sequences. Both subunits migrate more slowly on SDS/PAGE than expected from their predicted molecular masses (Table 1). This is probably a consequence of their highly basic nature (the predicted pI values being 9.2 for rat and 9.3 for rabbit). In addition to this, the rabbit δ subunit migrates more slowly than that from rat.

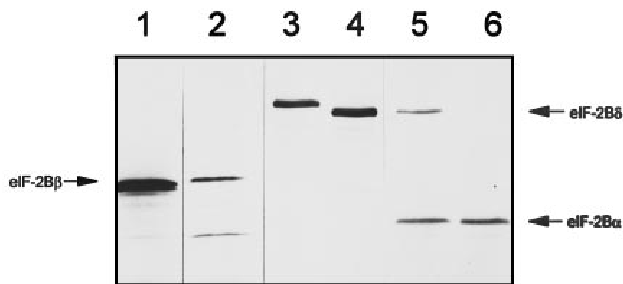

Figure 3 Transcription/translation *in vitro* for eIF2Bα, β and δ

cDNA species encoding the rat or rabbit α, β or δ subunits of eIF2B were transcribed and translated in the presence of [^{35}S]methionine *in vitro*. Lanes correspond to rabbit β (lane 1), rat β (lane 2), rabbit δ (lane 3), rat δ (lane 4), rabbit α and δ (lane 5) and rat α (lane 6). Arrows indicate the identities of the expressed polypeptides. The Figure shows an autoradiograph of the SDS/polyacrylamide gel. The rapidly migrating labelled band in lane 2 (also seen faintly in lane 1) represents an endogenous protein seen in nuclease-treated lysates not supplemented with exogenous mRNA.

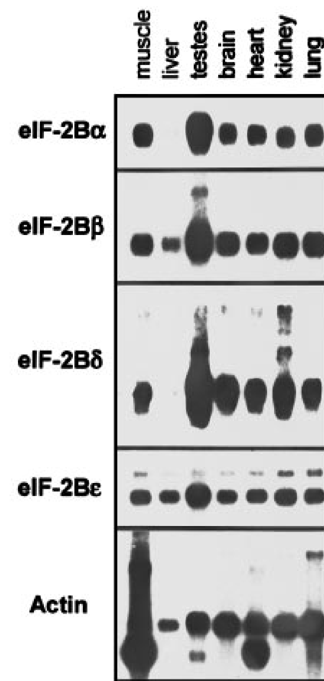

Figure 4 Northern blots for α, β and δ from rat tissues

Poly(A)$^{+}$ RNA samples were prepared from various rat tissues and subjected to Northern blotting. Blots were probed for β-actin and eIF2B subunits with ^{32}P-labelled probes. The Figure shows autoradiographs of the probed blots. The sizes of the major bands observed in each case were 1.6 kb (α), 1.5 kb (β), 1.8 kb (δ), and 2.7 and 3.5 kb (ε), as judged by the migration of RNA standards. Note that the loading of material in the lane containing the liver RNA was relatively low, as indicated by the signal obtained with the actin probe.

The eIF2Bε subunits also migrate less rapidly on SDS/PAGE than predicted from their predicted molecular masses (Table 1). In this case, the difference is likely to be due to the preponderance of acidic residues (predicted pI values 4.8 and 5.1 for rat and rabbit respectively). Here a difference in migration between the rat and rabbit proteins is also observed.

Several other translation initiation factor polypeptides also migrate anomalously on SDS/PAGE (e.g. eIF2β [30], eIF4G [31], eIF4B [32]), as seen for the δ and ε subunits of eIF2B (Table 1).

Expression of eIF2B subunits *in vitro*

The rat and rabbit eIF2Bβ and δ subunits were expressed in the rabbit reticulocyte lysate system by using coupled transcription/translation. Expression of the full rabbit eIF2Bδ cDNA (including 122 nt of 5′ UTR) was initially poor, with no strongly labelled band being observed on SDS/PAGE (results not shown). The 5′ UTR is GC-rich and has a high degree of predicted secondary structure, which probably interferes with translation [33]. Consistent with this, when a shortened version of this cDNA was constructed (with 15 nt of 5′ UTR remaining), good expression was achieved. The rat cDNA, which possessed only 8 nt of 5′ UTR and 13 nt of adapter, was well expressed.

For the eIF2Bβ subunit from rabbit, a closely migrating doublet was observed (Figure 3, lane 1). The upper band migrated the same distance on SDS/PAGE as the corresponding subunit in the purified factor, i.e. the rabbit β subunit expressed *in vitro* ran at an apparent molecular mass of 39 kDa (results not shown; see [21]). The relative intensities of the two components of the doublet did not vary with time, indicating that the differences are unlikely to be due to post-translational modification (results not shown). Immunoblotting of purified eIF2B from rabbit (or rat, guinea pig and human) with polyclonal or monoclonal antibodies [21] shows only a single band for eIF2Bβ, corresponding to the upper species resulting from translation *in vitro*. This suggests that the shorter polypeptide does not occur *in vivo* and is merely an artifact of this *in vitro* system. Rat eIF2Bβ gave a single band (Figure 3, lane 2) at 42 kDa as observed for this subunit of the purified factor from this species [21].

The rat polypeptide of the δ subunit ran at 61 kDa and the rabbit δ subunit gave a very closely migrating doublet at 66 kDa when expressed in reticulocyte lysates (Figure 3, lanes 4 and 3). Their apparent molecular masses correspond to those of the δ subunit of the purified factor from each species [21]. As with eIF2Bβ, only one major species of eIF2Bδ is observed on immunoblots from various mammalian species [21].

The product transcribed *in vitro* for rat eIF2Bα (Figure 3, lane 6) [23] migrates in the same position as the α subunit from the purified rat factor, with a molecular mass of 31 kDa. Rat eIF2Bα thus migrates more rapidly than predicted from its sequence (Table 1). The N-terminus of this subunit of the purified factor is blocked. However, antibodies raised to a peptide corresponding to the 17 residues of the predicted N-terminus recognize the α subunit of purified rat eIF2B, implying that the mature subunit is not subject to significant N-terminal shortening (results not shown).

Because the predicted molecular masses of eIF2Bβ and δ do not differ sufficiently between rat and rabbit to explain their differing migrations on SDS/PAGE, these differences seem likely to be due simply to their amino acid compositions. As these differences were seen when translated in the reticulocyte lysate system, and as the size of the expressed proteins did not vary with time, they are unlikely to reflect differences in post-translational modification.

Analysis of poly(A)$^{+}$ mRNA levels for eIF2B subunits in different tissues

One would expect that the expression of the mRNA species for the five subunits of eIF2B would be co-ordinated so as to allow efficient production and assembly of the eIF2B heteropentameric complex. Thus the messages would be expected to be expressed

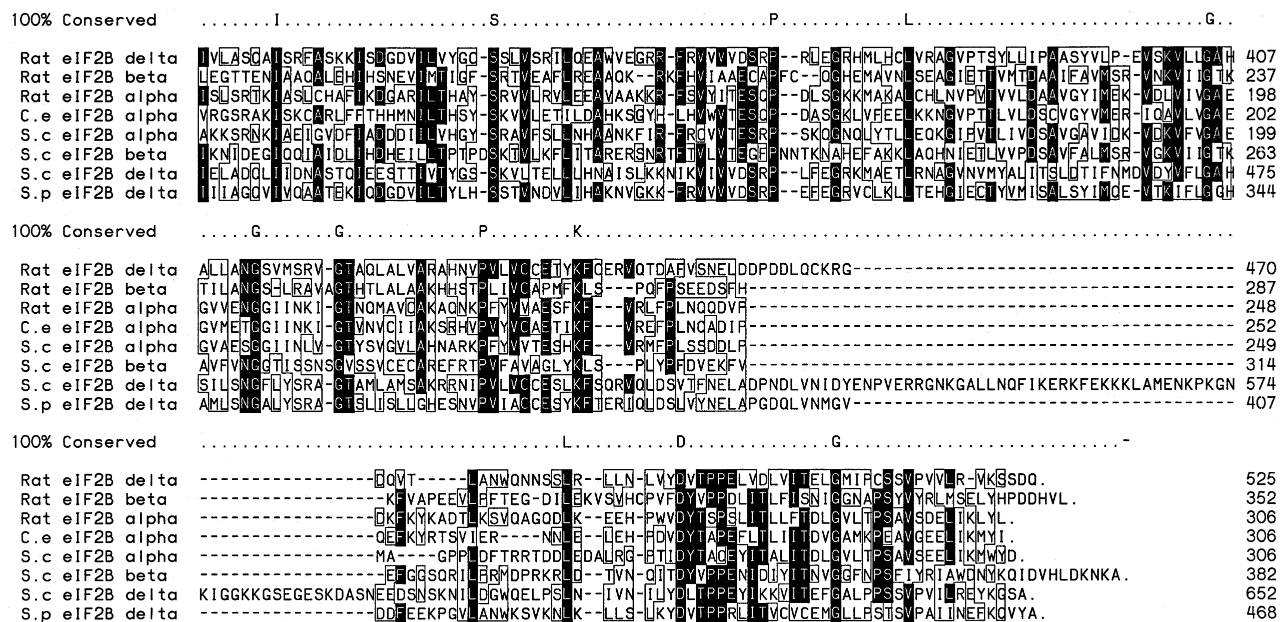

Figure 5 Alignment of eIF2Bα, β and δ sequences

In addition to the eIF2Bα, β and δ sequences from mammals (rat) and budding yeast, the putative *C. elegans* eIF2Bα (accession number Z22176 [27]) and the *S. pombe* eIF2Bδ (accession number Q09924) sequences have been included. Residues identical or conserved in four or more of the eight sequences are marked. Identical residues are shown in white text on black; conservative replacements are in boxes. Residues conserved in all eight sequences are shown above the alignment. The sequence similarity between all eight sequences does not extend further towards the N-terminus than shown. Sequences from other mammalian species are not included to avoid giving visual bias to the alignment. Sequences were aligned by the Clustal method with the Megalign package from DNAStar (Madison, WI, U.S.A.). Minor adjustments were made manually. Abbreviations: C.e., *C. elegans*; S.c., *S. cerevisiae*; S.p., *S. pombe*.

at the same levels relative to one another in different tissues, with differences in absolute mRNA levels reflecting their translational efficiencies or, perhaps, the rates of degradation of the corresponding polypeptides. These results represent the first study of the relative levels of expression of eIF2B subunit messages for any tissue. Sequences of the genes for rat eIF2Bα and ϵ have been determined and examined for elements that might be involved in regulating their transcription [34,34a].

Northern blot analyses were performed for each of the α, β, δ and ϵ subunits of eIF2B for a range of tissues from rat (Figure 4). For the δ subunit, only a single major hybridizing species was observed in all tissues tested. The existence of two alternative forms of the mRNA for this subunit in mouse has been reported [26]. The full-length probe used in our studies would be expected to detect both of these forms because they differed only at their 5′-termini. However, their relative sizes (difference only 36 nt) are such that they would not be distinguishable on these Northern blots. As previously reported, single major bands were also seen for the α [23] and β [24] subunits of eIF2B, whereas one major and one minor band were observed for the ϵ subunit [34a]. For each subunit the major bands were of the same apparent size in all tissues examined, consistent with the observation that these subunits are of the same apparent molecular mass in all tissues (from any one species) for which this analysis has been performed [21,22].

In rat, the relative levels of expression of the mRNA species encoding the individual eIF2B subunits were similar in any one tissue, as would be predicted for the individual subunits of a heteromeric protein. The only possible exception to this was liver, where the apparent levels of expression of the α and the δ mRNA species were rather low (even allowing for the lower signal for actin). The level of the eIF2Bγ mRNA was also found to be rather low in liver [28].

Particularly high levels of expression of the eIF2B mRNA (relative to β-actin) were observed in rat testis although this was less pronounced for the ϵ mRNA (Figure 4). The high levels of eIF2B mRNA species might indicate a generally high level of translation factors in testis, perhaps reflecting a high rate of protein synthesis in this tissue.

Sequence similarities between the α, β and δ subunits of mammalian eIF2B and the corresponding subunits of eIF2B in yeasts

The α–ϵ subunits of eIF2B in *S. cerevisiae* are encoded by the genes *GCN3*, *GCD7*, *GCD1*, *GCD2* and *GCD6* respectively [8]. Mutations in these genes were identified as phenotypes effecting the general control of amino acid biosynthesis and were shown to cause defects in protein synthesis initiation or in its regulation.

When the sequence of mammalian eIF2Bβ is aligned with that of GCD7, considerable similarity is apparent throughout the entire length of the sequences [24], with overall 36% identity between the two. This sequence similarity is also true of mammalian eIF2Bα and GCN3 [24]. In the eIF2Bδ/GCD2 pairing, the latter sequence has an insertion of 51 residues relative to mammalian eIF2Bδ (Figure 5) [25]. The *S. pombe* sequence does not contain this insert (Figure 5). Indeed, *S. pombe* eIF2Bδ is more similar (34.4%) to the rat subunit than is the sequence from budding yeast (31.4%). The two yeast sequences show 28.8% mutual identity.

Sequence similarities between the α, β and δ subunits of mammalian and *S. cerevisiae* eIF2B

Studies on yeast eIF2B have revealed considerable similarity between the eIF2Bα (GCN3), β (GCD7) and δ (GCD2) sequences. The greatest similarity is seen in the C-terminal regions

of these molecules, although alignments are published that extend for the full length of the sequences [18–20].

Thus we were interested in comparing the sequences of mammalian eIF2Bα, β and δ with one another and in comparing them with the sequences of the corresponding yeast subunits. As can be seen from Figure 5, there is considerable sequence similarity between the C-terminal regions of these mammalian subunits. Strikingly, the residues conserved between the mammalian sequences are also in many cases those conserved in their yeast counterparts, and are thus identical in all six sequences. Furthermore when the *C. elegans* eIF2Bα and fission yeast eIF2Bδ sequences are also included in the alignment, they can be seen to have a similar pattern of conserved residues. [The rabbit (eIF2Bβ and δ) and mouse (eIF2Bδ) sequences have been omitted from the alignment to avoid giving a visual bias towards the mammalian sequences: residues conserved between yeast and rat sequences are also conserved in the rabbit and mouse sequences (compare Figures 1, 2 and 5)]. The mutual identity between all eight of the eIF2B$\alpha/\beta/\delta$ sequences does not extend further towards the N-terminus than shown in Figure 5.

There are 12 positions where residues are identical in all eight sequences (11 including the rabbit and mouse sequences) (Figure 5). Moreover, there are many more positions where the majority of sequences are identical, and those that differ often involve only conservative replacements (e.g. Ile for Leu, Phe for Ile, Ile for Val, or Ser for Thr). Although many of the conserved regions show a marked periodicity, this does not seem to correlate with regions of predicted α-helix, which might have formed a basis for sequence repeats. Clearly, discussion based on this sequence alignment is highly speculative and awaits further structural studies. Our alignment, through the advantage of having the additional mammalian sequences available, should give a better indication of the conserved residues than those previously derived from only the yeast sequences. In most cases the present alignments agree with earlier ones for the yeast sequences, with many of the differences being ambiguous, depending where gaps are inserted, particularly in the regions flanking the insertion in GCD2.

The insertion in GCD2 relative to mammalian or fission yeast eIF2Bδ [25] is also an insertion relative to all the other sequences in the alignment (Figure 5). Mammalian and *S. pombe* eIF2Bδ have small (about ten residues) insertions in this region relative to the eIF2Bα/β sequences. The function of this region of GCD2 is unknown. It does not contain any known sequence motifs but is rich in charged residues.

Possible functions of the individual subunits of eIF2B

Very little is known about the functions of the α, β and δ subunits of the mammalian eIF2B. Examination of their sequences has not so far revealed any motifs that might suggest their roles although the γ and ϵ subunits do possess motifs indicating that they might be involved in nucleotide binding (see [28]). Whereas the genes encoding eIF2Bβ and eIF2Bδ are essential in *S. cerevisiae*, that encoding the α subunit (GCN3) is not [35,36], although this subunit does seem to be required for the nucleotide-exchange activity of the mammalian factor [37].

An interesting parallel with the similarity found between the C-terminal regions of eIF2Bα, β and δ can be drawn to eEF1$\beta\gamma\delta$, the GEF for protein-synthesis elongation factor eEF1α [the nomenclature for eEF1 is confusing: the substrate, the GTP-binding protein (eEF1α) and the exchange factor (eEF1$\beta\gamma\delta$) are viewed as being components of the same complex]. For eEF1, the C-terminal regions of the β and δ subunits show very strong amino acid sequence identity [38,39]. These regions have been shown to be responsible for the GTP/GDP exchange activity of the factor [2,3,39]. This, however, probably reflects the fact that two eEF1α polypeptides bind to the exchange complex, i.e. there is only one GEF polypeptide per guanine nucleotide-binding subunit [3] (at least in *Artemia*). This subunit stoichiometry might reflect the participation of two eEF1α molecules per ribosome in the elongation step of protein synthesis, which appears to be so for its prokaryotic counterpart, EFTu [40]. For eIF2B, however, only one eIF2B molecule is thought to bind to each eIF2 molecule (on the basis of the behaviour of the [eIF2·eIF2B] complex on gel filtration, and the relative intensity of Coomassie Blue staining of the bands on SDS/polyacrylamide gels [1]). It remains to be determined which subunit(s) of eIF2B possess exchange activity: it seems unlikely that the complex contains three such activities.

Delineation of the functional roles of individual subunits of eIF2B in its catalytic mechanism and its regulation will be aided by their individual expression and reconstitution into partial complexes. This work is in progress. Similarly, the roles of the conserved residues can be addressed by assessing the effects of their mutation, either in reconstituted complexes (for the mammalian and yeast factors) or in appropriate strains *in vivo*, for the yeast protein.

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