Eukaryotic initiation factor (eIF) 2B is a heteropentameric protein, which catalyzes a key step in translation initiation, i.e. the exchange of guanine nucleotides on initiation factor eIF2. eIF2 binds GTP and in this form can also interact with the initiator methionyl-tRNA (Met-tRNAi) to form the ternary complex eIF2-GTP-Met-tRNAi. Such complexes can then bind to the 40S ribosomal subunit to form pre-initiation complexes, bringing the Met-tRNAi into position to recognize the AUG start codon of the mRNA following ribosomal scanning. The eIF2-bound GTP molecule is then hydrolyzed to GDP and P_i in a process that requires a further factor, eIF5. The inactive eIF2-GDP complex is released from the ribosome. The rate of release of GDP from eIF2 is very slow under physiological conditions, and the exchange of bound GDP for GTP, to regenerate active eIF2-GTP, requires an additional factor, eIF2B. The activity of eIF2B plays a key role in regulating the initiation of translation and can be controlled in several ways. For example, phosphorylation of the α-subunit of eIF2 inhibits eIF2B and serves to inhibit translation under a range of stressful conditions (1). Conversely, eIF2B is activated by many stimuli, which stimulate protein synthesis, including insulin, and this appears to involve changes in the state of phosphorylation of the largest (e) subunit of eIF2B (3, 4). eIF2B has also been reported to be regulated, in vitro, by a number of allosteric modulators, including nicotinamide adenine nucleotides (5–9). Studies using photoactivatable nucleotide analogs suggested that eIF2B contains binding sites for GTP and ATP, although the functions of these sites are unclear.

eIF2B is an unusually complicated guanine nucleotide exchange factor (GEF), those acting on small G-proteins such as Ras, for example, being much smaller monomeric proteins. The reasons for this complexity and the roles of the five subunits are still not completely clear. A major step toward addressing these issues has been the cloning of cDNAs encoding subunits of eIF2B. This has now been achieved for several species, including yeast (see, e.g., Refs. 10 and 11), mammals (see, e.g., Refs. 12–16), and Drosophila (17). Studies for all three sources have shown that the e-subunit alone can catalyze nucleotide exchange on eIF2 (17–19), although its activity is enhanced by the others (18–20). eIF2Bα forms a catalytic subcomplex with eIF2βγ, with which it shares some sequence similarity. The other three subunits also display mutual sequence similarity and form a second subcomplex, which appears to have a regulatory function (19). There is some disagreement over the role of the α-subunit. This polypeptide is encoded by a non-essential gene in yeast (GCN3), although it is required for regulation of eIF2B by phosphorylation of eIF2α (21). Similarly, studies in which mammalian eIF2B polypeptides were expressed in insect cells concluded that eIF2Bα was dispensable for activity (22). However, others have found that preparations of eIF2B lacking the α-subunit showed reduced activity in nucleotide exchange (23), implying that this component was required for full activity.

There is also disagreement over the catalytic mechanism of eIF2B; early studies supported the so-called “ping-pong” or substituted enzyme mechanism (Mechanism A in Fig. 1; Ref. 24), which is the one utilized by another GEF involved in mRNA translation, the bacterial elongation factor EF-Ts (25) and
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Materials and Methods

Chemicals and Biochemicals—Chemicals and biochemicals were obtained from BDH-Merck (Poole, Dorset, United Kingdom (UK)) or Sigma–Aldrich (Gillingham, Dorset, UK), unless otherwise stated. [3H]GDP (10 Ci/mmol), [γ-32P]GTP (5000 Ci/mmol), and [γ-32P]ATP (30 Ci/mmol) were from Amersham International. X-ray film was from Konica. Restriction enzymes were from Promega.

Purification of eIF2B and eIF2B—These proteins were purified as described previously (8), but using HEK cell cytoplasm as the starting material (from Computer Cell Culture Center, Seneffe, Belgium). The cytoplasmic fraction was subjected to ammonium sulfate fractionation, and the material precipitating between 30% and 60% saturation was subjected to ion exchange chromatography successively on Q-Sepharose, S-Sepharose, MonoQ, and MonoS (all from Pharmacia Nycomed). The material (from Computer Cell Culture Center, Seneffe, Belgium) was devoid of eIF2, as assessed by Western blotting with a sensitive monoclonal antibody against eIF2α (Fig. 2B). Such preparations are highly active in catalyzing exchange of labeled GDP bound to eIF2 for unlabeled GTP (Fig. 2C).

We (8) and others (30, 31) have previously reported that eIF2B failed to catalyze the release of labeled GDP from eIF2-[3H]GDP complexes unless excess unlabeled GTP was present. In view of the aims of this study, it was important to show that this was also true for this preparation of eIF2B, which is indeed the case as shown in Fig. 2C; whereas rapid release of bound [3H]GDP was observed when unlabeled GTP was present, very little release was observed in the absence of added GTP. A small degree of release of [3H]GDP was seen when the higher concentration of eIF2B was used, and this effect is discussed in greater detail below. Two different models have been proposed to explain the catalytic mechanism of eIF2B. These are depicted in Fig. 1. Briefly, in the ping-pong or substituted enzyme mechanism, there is requirement for added guanine nucleotide in order to observe catalytic behavior (Fig. 2A). In this model, eIF2B is acting as a GDS. Complexes between eIF2, GDP, and eIF2B presumably do form, but only transiently, decaying as the GDP is released to yield eIF2-eIF2B binary complexes. The second model (sequential, Fig. 1B) involves the formation of a “ternary complex” containing eIF2-GDP, eIF2B, and GTP. The data showing a requirement for added guanine nucleotide in order to observe the release of bound GDP from eIF2B have been taken as support for the sequential mechanism (B in Fig. 1) (30, 31). However, consideration of the two mechanisms reveals that they are also entirely consistent with mechanism A. Indeed, such behavior is a prediction of this mechanism as displayed in Fig. 1A. eIF2B is included in the assays used by ourselves and others at catalytic rather than stoichiometric amounts; in the assays depicted in Fig. 2C, typically the ratio of eIF2:eIF2B is about 1000:1. Interpretation of the mechanism shown in Fig. 1A shows that for eIF2B to act catalytically, it must be released from the intermediate eIF2-eIF2B complex and that this requires free nucleotide. Free nucleotide would act to regenerate eIF2B, thus allowing it to function as a catalyst and thus to mediate further release of labeled GDP from the eIF2-[3H]GDP complexes, which are the substrate for this reaction.

Since eIF2 can bind either GDP or GTP, one would expect that, under this model, catalytic exchange would proceed with either free GTP or GDP present. This is indeed the case for

UV Cross-linking—UV cross-linking reactions were performed using a Stratalinker 1800 (Stratagene). Reactions were 10-fold and grown to an A492 of 0.6. Cultures were cooled on ice for 15 min, and exposure was induced with 1 mM isopropyl-β-D-galactoside for 2.5 h. Cells were harvested by centrifugation and lysed in 5% glycerol, 500 mM KCl, 20 mM Tris/HCl, pH 7.6, 3 mM MgCl2, 5 mM β-mercaptoethanol, 10 mM imidazole, 0.1% Triton X-100, 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μM protease inhibitors (leupeptin, pepstatin, aprotinin, and benzamidine) at ~80 °C. Following lysis cells were defrosted and sonicated to shear any DNA present. Purification of proteins was performed using nickel-nitrilotriacetic acid-agarose (Qiagen).

Results

Dependence of eIF2B on Free Nucleotide for Catalytic Guanine Nucleotide Exchange—eIF2B was purified from HEK cell extract using a slightly modified version of the method used earlier for its isolation from reticulocyte lysates (see “Materials and Methods”). This yielded protein displaying five polypeptides migrating on SDS-PAGE. These polypeptides displayed expected apparent molecular weights for subunits of mammalian eIF2B. This material was devoid of eIF2α, as assessed by staining with Coomassie Brilliant Blue (Fig. 2A) and by the much more stringent criterion of Western blotting with a sensitive monoclonal antibody against eIF2α (Fig. 2B). Such preparations are highly active in catalyzing exchange of labeled GDP bound to eIF2 for unlabeled GTP (Fig. 2C).

**Fig. 1. Proposed mechanisms for the eIF2B-mediated exchange of nucleotides on eIF2.** A. ping-pong or substituted enzyme mechanism; binding of eIF2B to eIF2-GDP brings about release of GDP. Subsequent binding of GTP to eIF2 leads to formation of active eIF2-GTP binary complexes and release of eIF2B, regenerating free eIF2B for further rounds of catalysis (dotted line). B, sequential mechanism; this involves formation of a ternary complex (eIF2-GDP-eIF2B-GTP) and subsequent release of GDP and active eIF2-GTP.

by factors that promote nucleotide exchange on other GTP-binding proteins (26–29). The methods used to identify these factors act to stimulate the release of bound guanine nucleotide and are thus termed “GDP dissociation stimulation proteins” (GDS proteins, Ref. 29). Other studies have suggested that eIF2B may operate via the alternative sequential mechanism (Mechanism B in Fig. 1; Refs. 30 and 31).

Here we demonstrate that the properties of eIF2B match the operation of the ping-pong mechanism and suggest that eIF2B acts as a GDS protein. We also show that eIF2β contains a binding site for purine nucleotides and that eIF2β is required for the full activity of mammalian eIF2B.
shown are the regions corresponding to eIF2 applied at differing dilutions to an SDS-polyacrylamide gel, which was used to resolve complexes of protein. Samples of purified eIF2 or eIF2B (as indicated) were applied to a Superose 6 column without GDP, and data not shown), as reported earlier for the yeast factor (11). Consistent with the earlier findings of Cigan et al. (11) for yeast eIF2B, ATP did not facilitate release of bound GDP from eIF2 (data not shown).

**Guanine Nucleotides Cause Dissociation of eIF2-eIF2B Complexes**—A prediction of mechanism A, the ping-pong mechanism, but not of mechanism B, is that incubation of eIF2-eIF2B complexes with guanine nucleotides should cause such complexes to dissociate. The eIF2-eIF2B complex was purified by ion-exchange chromatography on MonoQ (Fig. 3A) and then incubated with GDP, followed by addition of MgCl₂ and rechromatography on MonoQ. During this second run, eIF2 was resolved from eIF2B (which eluted later from the column; see also Ref. 8 and Fig. 3B and C)). Chromatography of complexes that had not been pre-treated with GDP did not result in separation of the two proteins, which instead again eluted as the eIF2-eIF2B complex (data not shown). To study this further, we performed similar experiments using gel permeation chromatography rather than ion exchange to resolve eIF2 or eIF2B from eIF2-eIF2B complexes. When eIF2-eIF2B complexes were applied to a Superose 6 column without GDP treatment, as expected, eIF2 and eIF2B eluted together (Fig. 3D). In contrast, when another sample of the eIF2-eIF2B complex was incubated with GDP and then applied to a Superose 6 column, eIF2 (seen in fractions 27–29) clearly eluted later than eIF2B (mainly in fractions 24–26), indicating that eIF2 and eIF2B had dissociated from one another (Fig. 3E). These data are again consistent with mechanism A, but not mechanism B, and support the contention that the ability of free GDP or GTP to facilitate the release of [³²P]GTP bound to eIF2 is due to the regeneration of free, active eIF2B. The data again support the idea that eIF2B acts as a GDS.

**Stoichiometric Amounts of eIF2B Can Mediate Nucleotide Release in the Absence of Free Nucleotide**—An additional prediction of mechanism A is that stoichiometric amounts of eIF2B should lead to extensive release of GDP even in the absence of free nucleotide. That this might be the case was suggested by the modest release of [³²P]GDP observed in Fig. 2C in the presence of the higher amount of eIF2B. To test this further, eIF2-[³²P]GDP complexes were formed and subjected to gel filtration to remove free nucleotide. Purified eIF2B was then added in increasing amounts similar to or greater than the amount of eIF2-[³²P]GDP complexes present. Under these conditions, release of labeled GDP was observed (Fig. 4A). Release was not complete, but in each case leveled out at a maximum extent (as the reaction attained equilibrium). As expected, the extent of release increased as larger amounts of eIF2B were used, since this will drive the equilibrium further toward completion. This is precisely the behavior predicted from the ping-pong mechanism, where eIF2B can bring about the release of amounts of GDP similar to the amount of eIF2B added, but cannot act catalytically as it becomes locked into the eIF2-eIF2B complexes. The release of eIF2B from these complexes requires free GDP or GTP, enabling the eIF2B now to act catalytically rather than stoichiometrically. Consistent with this, upon addition of free nucleotide, rapid complete loss of bound labeled GDP occurred, due to the catalytic action of the eIF2B (Fig. 4B).

**Cross-linking of Purine Nucleotides to eIF2Bβ**—Mechanism B requires that eIF2B contains a binding site for GTP and two eIF2B complexes. The release of eIF2B from these complexes requires free GDP or GTP, enabling the eIF2B to act catalytically rather than stoichiometrically. Consistent with this, upon addition of free nucleotide, rapid complete loss of bound labeled GDP occurred, due to the catalytic action of the eIF2B (Fig. 4B).
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**Fig. 3. Addition of GDP causes dissociation of eIF2-eIF2B complexes.** eIF2-eIF2B complexes were isolated as described under "Materials and Methods." A. Western blot of consecutive fractions from MonoS containing eIF2/eIF2B complexes as demonstrated using antisera for eIF2α and eIF2β (positions shown). B. A Coomassie-stained gel of the complex is shown (eIF2α/eIF2B). GDP (final concentration 200 μM) was added to the complex (in Buffer A: 20 mM HEPES-KOH, pH 7.4, 100 mM KCl, 5% (v/v) glycerol, 1 mM dithiothreitol), and this was incubated for 30 min at 30 °C. MgCl2 was then added (final concentration, 5 mM), and the material was applied to a MonoQ column attached to a fast protein liquid chromatography system equilibrated in the same buffer supplemented with GDP (1 μM) and MgCl₂ (5 mM). The column was developed with a salt gradient (KCl), and fractions were collected. Selected fractions were analyzed by SDS-PAGE followed by Coomassie staining or immunoblotting (shown in panel C). Fractions 20 and 29 correspond to KCl concentrations of 250 and 350 mM, respectively. In panel B, the migration positions of the subunits of eIF2 (α–γ) and eIF2B (α–ε) are shown. The single bands in lanes 23–26 are contaminating proteins resolved on the MonoQ column and are not subunits of eIF2 or eIF2B. Vertical lines indicate separate gels, which have been combined for presentation. It should be noted that eIF2αε stabilizes weakly with Coomassie Blue when compared with the other subunits of the complex. C. This panel shows immunoblots developed with antisera against eIF2βγ or eIF2α as indicated. Note that, on the immunoblot for eIF2βγ, the peak fraction (26) was loaded at one-tenth the amount of the others. The last lane in each case shows the signal from the initial eIF2-eIF2B complex run in parallel. eIF2 is eluted at 240–260 mM KCl, whereas eIF2B, free of eIF2, was eluted between 335 and 350 mM KCl. When a sample of eIF2-eIF2B was subjected to ion exchange on MonoQ without prior treatment with GDP, and without GDP in the buffer, as a control, it was eluted at ~280–315 mM KCl as the complex, and no separation of eIF2 from eIF2B was observed (data not shown). D. Purified eIF2-eIF2B complexes were applied to a Superose 6 column equilibrated in Buffer A containing 5 mM MgCl₂. Fractions were collected, and the figure shows a Coomassie Blue-stained gel of those containing eIF2 and eIF2B. E. A second sample of the same material was preincubated with GDP (1 μM) for 15 min on ice prior to addition of labeled by azido-GTP. However, in contrast to that study, labeling was reduced by the presence of cold ATP as well as by cold GTP (Fig. 5A). Similar labeling experiments using [γ-32P]ATP led to labeling of the same polypeptide, rather than eIF2βγ and -δ reported by Haley et al. (32). To rule out the possibility that labeling occurred by transfer of the γ-phosphate (e.g. by a contaminating kinase), reactions were also performed without UV irradiation. Under this condition, no labeling was indeed observed (Fig. 5B, lane 2). Labeling was decreased by addition of cold GTP or ATP (Fig. 5C), GTP being slightly more effective than ATP. This suggests that ATP and GTP bind to the same (or overlapping) sites in eIF2β.

To verify that GTP bound directly to eIF2β, we expressed this polypeptide in *E. coli* as a His-tagged protein, purified it on nickel-agarose, and tested whether [γ-32P]GTP became cross-linked to it. As shown in Fig. 5D (lane 2), UV irradiation did indeed result in cross-linking of labeled GTP to recombinant eIF2β. This confirms that GTP interacts directly with this subunit and thus that the cross-linking to this polypeptide observed for the complex is not a consequence of its binding to another subunit at a site adjacent to eIF2β in the holoprotein. As a negative control, reactions were again performed in the same way but without UV irradiation. In this case, no cross-linking was observed, once more ruling out the theoretical possibility that labeling by [γ-32P]GTP was due to phosphorylation of eIF2Bβ by a contaminating kinase (Fig. 5D, lanes 3 and 4).

eIF2B is also known to interact with NAD⁺ and related compounds (5, 8). To examine whether the binding site for these compounds was also located in eIF2β, we performed UV cross-linking experiments with radioactive NAD⁺. However, this failed to result in labeling of any of the polypeptides of eIF2, although lactate dehydrogenase, used as a positive control, was labeled (data not shown). In another approach, we examined whether NAD⁺ or NADPH competed with GTP or ATP in the UV cross-linking experiments (Fig. 5). It did not; in fact, a reproducible increase in cross-linking was observed. It therefore appears that the nucleotide-binding site in eIF2β is not the site responsible for binding NAD⁺, which presumably resides elsewhere in the protein. The ability of NAD⁺ to enhance labeling by [γ-32P]GTP or ATP may reflect allosteric interactions between the binding sites for these ligands.

**eIF2Bα Is Required for Full Activity of Mammalian eIF2B**—As reported previously (23), isolation of mammalian eIF2B from rabbit reticulocytes yields a proportion of the protein as a four-subunit complex lacking the α-subunit. The same is true of eIF2B prepared from HeLa cells (Fig. 6A). Chromatography on MonoS yielded two pools of eIF2B; the material eluting at 290–320 mM KCl showed only four subunits on SDS-PAGE (Fig. 6, A and B), whereas the material eluting at 330–370 mM showed five. The absence of eIF2Bα from the former material was confirmed using an antibody specific for the α-subunit of eIF2B (Fig. 6A). No signal for eIF2Bα was observed in these fractions, whereas a clear signal was seen for the factor displaying all five subunits on SDS-PAGE (Fig. 6B). When the activities of the four- and five-subunit preparations were compared, the former was found to display only about 20–25% of the activity of the five-subunit material in the standard nucleotide exchange assay (Fig. 6C). This is consistent with our earlier data for the four- and five-subunit prepa-
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**DISCUSSION**

The data presented here show that eIF2B can elicit release of \[^{3}H\]GDP from eIF2 even in the absence of GDP or GTP. This apparently contradicts earlier data from ourselves (8) and (1, 30) others; however, these earlier experiments were mostly performed using catalytic amounts of eIF2B (i.e. low amounts compared with the substrate eIF2[^1]H(GDP). We have reported previously that approximately stoichiometric amounts of eIF2B prepared from a different source did not elicit release of bound GDP from eIF2; in fact, the present data show that a roughly equal amount of eIF2B displaces approximately 50% of the nucleotide bound to eIF2. The extent release of bound GDP at any given ratio of eIF2B to eIF2 depends of course on the rate constants for the reaction eIF2-GDP + eIF2B ⇋ eIF2-eIF2B + GDP. The ping-pong mechanism does indeed predict that the catalytic function of eIF2B requires free nucleotide in order to regenerate eIF2B from the eIF2-eIF2B complexes, which are an intermediate in this mechanism (Fig. 1A). When added at stoichiometric amounts, as in this study, this mechanism predicts that GDP will be released from eIF2, as eIF2B displaces the nucleotide from eIF2. In complete accordance with this, we show that the addition of GDP to eIF2-eIF2B complexes causes them to dissociate, again as expected from the ping-pong mechanism. Neither of these effects is consistent with the other mechanism proposed for eIF2B (sequential mechanism, Fig. 1B). In our study we also failed to detect eIF2B bound to eIF2-GDP, an intermediate that may be formed in the substituted enzyme mechanism, but that cannot arise in the ping-pong mechanism. Thus, our data are in agreement with the ping-pong mechanism but do not, of course, prove that this is the mechanism by which eIF2B functions. Another GEF involved in translation, EF-Ts, also uses the substituted enzyme mechanism (25). RCC1 and Cdc25Mm, exchange factors for the small GTP-binding proteins Ran and Ras, respectively, have been shown to operate by an analogous mechanism (26–28), and recent data support such a mechanism for the guanine nucleotide dissociation factor that acts on Rho (29). It seems plausible that the substituted enzyme mechanism is a general one by which such proteins function, although the fine details of the mechanism at the structural level are likely to vary (37, 38). The data in Figs. 2 and 6 suggest that eIF2B acts as a GDS (by promoting the release of GDP from eIF2 rather than the exchange of GDP for GTP; GTP binds spontaneously to the unliganded eIF2).

Other studies have provided evidence that eIF2B can bind guanine nucleotides, which is a requirement of the substituted enzyme mechanism. Wahba and colleagues (30) reported GTP binding to mammalian eIF2B, although the stoichiometry was low. Similarly, recent data from Hannig’s group (31) for yeast eIF2B show only a stoichiometry of only 0.2 mol/mol, even at a nucleotide concentration almost 3 orders of magnitude above the apparent dissociation constant. In this work we were unable to detect significant binding of GTP to purified eIF2B in filter binding assays. In addition, eIF2B does not bind to GDP-agarose (data not shown), although this could reflect steric problems due either to the means of attachment of the GDP to the resin (via the ribose hydroxyls) or to the large size of the eIF2B complex. It is possible that either the off-rates for guanine nucleotides are very high, despite the low apparent \(K_d\) reported by others, so that little or no stable binding is obtained, or that the low stoichiometry of binding seen by others reflects the presence of a contaminating nucleotide-binding protein, although none was evident on the gels shown by earlier workers (1, 30). We do nonetheless observe cross-linking of GTP to eIF2B, specifically to eIF2Bβ. This suggests that the protein does indeed bind guanine nucleotides and that this subunit is involved in the interaction, as concluded by the earlier studies of Haley et al. (32), who used a photoaffinity-labeling reagent (8-azido-GTP) rather than normal GTP as used here. In our study, cross-linking of \(\gamma^{32P}\)GTP was competed by ATP, and, consistent with this, \(\gamma^{32P}\)ATP also became cross-linked to this subunit. These findings differ from the earlier results (32) where labeling by GTP was not affected by ATP, and where 8-azido-ATP became cross-linked to the \(\gamma\) and \(\delta\) subunits. It is not clear how this discrepancy is to be explained. Our data suggest that the binding site in eIF2Bβ...
interacts with both ATP and GTP. The observation that the isolated recombinant \( b \)-subunit is also labeled by \( g \)-32P\( \text{GTP} \) strongly supports the idea that this subunit directly binds guanine nucleotides, and that its labeling is not merely a consequence of the binding of GTP to an adjacent subunit in the complex. Since the sequence of eIF2B\( b \) contains no identifiable nucleotide-binding motifs, its interaction with GTP and ATP must involve a non-canonical binding site.

We found that addition of NAD\(^+\) did not reduce the labeling of eIF2B\( b \) by GTP or ATP, indicating that the binding site in eIF2B\( b \) is distinct from that through which nicotinamide adenine dinucleotides exert their allosteric effects upon eIF2B activity. We were unable to achieve labeling of eIF2B using radiolabeled NAD\(^+\) and were thus also unable to try to locate the binding site in eIF2B for this nucleotide.

As noted above, the substituted enzyme mechanism requires that eIF2B possesses a binding site for GTP. Although our data suggest that there may be such a site, it is important to note that this resides in eIF2B\( b \), and not in the catalytic e-subunit. This is a key point, since if eIF2B utilized the substituted enzyme mechanism, this site would necessarily be present in eIF2B\( e \), which has been shown to catalyze nucleotide exchange on its own, based on data from three different groups for the protein from yeast, insects, and mammals (17–19). These findings thus also argue against the substituted enzyme mechanism. Taken together, the present data suggest that eIF2B mediates release of GDP from eIF2, allowing the subsequent binding of GTP (the more abundant of these two nucleotides in the cell). If this is the case, then eIF2B acts as a GDS protein rather than a nucleotide exchange factor (a name which could be taken to imply catalysis of the GDP/GTP exchange step itself). The physiological role of eIF2B is of course to promote this exchange process, but our data suggest that this is a
consequence of its ability to bring about the release of GDP from eIF2, with binding of GTP occurring to the resulting nucleotide-free eIF2.

It has previously been suggested that purine nucleotides might act as allosteric modulators of eIF2B (39). Indeed, Kimball and Jefferson (9) have shown that ATP inhibits the activity of eIF2B. This effect is surprising in physiological terms, as one might expect enhanced cellular energy status, if anything, to activate eIF2B rather than inhibit it. Their data suggested that the effect might be mediated by antagonizing the activating effect of NADPH (9), which, based on our findings, is presumably mediated by a different binding site in eIF2B (since NAD+ did not reduce the labeling of eIF2Bβ by GTP). The function of nucleotide binding to eIF2Bβ therefore remains unclear.

A third area of conflicting data relating to eIF2B is the role of its smallest 32 (α) subunit. Earlier studies agree that eIF2α was not required for activity in vivo or in vitro, respectively. In contrast, our data for preparations of eIF2B that lack this subunit indicated it was necessary for full activity (23). The findings reported here confirm this for eIF2B isolated from a different source (HeLa cells versus reticulocyte lysate) and, most importantly, demonstrate that addition of recombinant eIF2Bα almost completely restores eIF2B activity. This eliminates the possibility that the low activity of four-subunit eIF2B is due to other defects in the protein and demonstrates that, under in vitro assay conditions, the mammalian factor does require eIF2Bα for full activity. Possible explanations for the differences between our data and those of earlier studies include (i) for the yeast studies, complementation of GCN3 by the product of another gene, or differences between the studies of eIF2B in vitro and in vivo and (ii) for the studies in Spodoptera cells, the presence of endogenous insect eIF2Bα in the complexes containing the four subunits of eIF2B from mammals, although no band is apparent at this position in the authors’ SDS-polyacrylamide gels.

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