The Crystal Structure of the Glutathione S-Transferase-like Domain of Elongation Factor 1Bγ from *Saccharomyces cerevisiae* 

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The crystal structure of the N-terminal 219 residues (domain 1) of the conserved eukaryotic translation elongation factor 1Bγ (eEF1Bγ), encoded by the *TEF3* gene in *Saccharomyces cerevisiae*, has been determined at 3.0 Å resolution by the single wavelength anomalous dispersion technique. The structure is overall very similar to the glutathione S-transferase proteins and contains a pocket with architecture highly homologous to what is observed in glutathione S-transferase enzymes. The *TEF3*-encoded form of eEF1Bγ has no obvious catalytic residue. However, the second form of eEF1Bγ encoded by the *TEF4* gene contains serine 11, which may act catalytically. Based on the x-ray structure and gel filtration studies, we suggest that the yeast eEF1 complex is organized as an [eEF1A:eEF1Bα:eEF1Bγ]2 complex. A 23-residue sequence in the middle of eEF1Bγ is essential for the stable dimerization of eEF1Bγ and the quaternary structure of the eEF1 complex.

The protein biosynthesis process is divided into initiation, elongation, and termination. During initiation Met-tRNA^Met is bound to the ribosomal P-site and base-paired with an initiator AUG codon on the mRNA. In the elongation cycle, the aminoacylated tRNA is brought to the ribosomal A-site by eukaryotic elongation factor 1 (eEF1) in the presence of a correct codon-anticodon match between the aminoacylated tRNA and the A-site codon of mRNA. eEF1A hydrolyzes its bound GTP, and eEF1A-GDP leaves the ribosome (reviewed in Ref. 1). The yeast elongation factor 1 complex (eEF1) consists of eEF1A and elongation factor 1B (eEF1B), which is the guanine nucleotide exchange factor for eEF1A. In all eukaryotes eEF1B contains at least two subunits, α and γ. In metazoans a third subunit, eEF1Bβ, which shares high sequence similarity to eEF1Bα, is present (2, 3). eEF1Bα and eEF1Bγ are catalytic subunits of the exchange factor, and eEF1Bα is essential for viability in *Saccharomyces cerevisiae* (4). The interaction between eEF1Bγ and eEF1α involves the N-terminal portion of both proteins (5). The eEF1Bγ subunit in *Artemia salina* enhances the activity of the eEF1α subunit by 100% when added in a 1:1 molar ratio in vitro (6).

There are unique aspects of eEF1Bγ function other than association with eEF1αα. In *A. salina* 5% of the eEF1Bα:eEF1Bγ complex in the cell is associated with membranes, and eEF1Bγ can associate with tubulin (6). Studies in human fibroblasts indicated that the eEF1 complex is predominantly associated with the endoplasmic reticulum, possibly anchored via eEF1Bγ (7). Association between eEF1Bαγ and mRNA has also been reported (8). eEF1Bγ in *S. cerevisiae* was identified through a screen for calcium-dependent membrane-binding proteins (9). From the protein sequence a gene was identified named *CAM1* (calcium and membrane binding), and its disruption does not affect the viability of the cell. The same gene was later identified as acting as a dosage extragenic suppressor of a cold-sensitive mutant, and named *drs2* (deficient for ribosomal subunits), which is deficient in the assembly of 40 S ribosomal subunits (10). The gene identified was named *TEF3* and the protein it encodes Tef3p. A second isoform of eEF1Bγ encoded by the *TEF4* gene in *S. cerevisiae* has also been identified (11). The sequence identity between Tef3p and Tef4p is only 64.5%, with the highest conservation in the C terminus. It is further believed that protein synthesis is under the control of the cell cycle during meiosis and mitosis. eEF1Bγ from *Xenopus laevis* and *Carassius auratus* was found to be a substrate both in vivo and in vitro for the cell division control M-phase promoting factor (12, 13). Taken together these results may indicate additional functions for this protein. Very recently Tef3p has been identified in a complex binding to the *msrA* promoter suggesting a function in regulation of expression of methionine sulfone-reductase (14).

The N-terminal domain of eEF1Bγ has sequence motifs characteristic of the Theta class glutathione transferases (GST) and, in accordance with this, was suggested to form homodimers and be enzymatically active (15). Recently, GST activity was observed toward the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) with the recombinant eEF1Bγ subunit from *Oryza sativa* expressed in *Escherichia coli* and for the full native eEF1B complex (16). Likewise, the silk worm (*Bombyx mori*) eEF1Bγ has been observed to bind to GSH-Sepharose (17).

Soluble GSTs (EC 2.5.1.18) are proteins involved in the cellular three-phase metabolism of exogenous and endogenous...
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Expression and Purification of SeMet-substituted Protein—Plasmin was transferred into electrocompetent BL21(DE3)/B834 E. coli cells. The SeMet medium contained 1 mg/liter vitamers (riboflavin, niacinamide, pyridoxine monohydrochloride, and thiamine), 40 mg/liter of all amino acids except methionine, 40 mg/liter of seleno-t-methionine, 25 mg/liter FeSO4, 4 g/liter glucose, 2 mM MgSO4, 2 g/liter NH4Cl, 6 g/liter KH2PO4, 25.6 g/liter Na2HPO4, 7.0 g/liter THO, and 100 μg/ml ampicillin. All amino acids, vitamins, and glucose were filtered through a 0.45-μm filter (Whatman). The rest of the ingredients were autoclaved prior to mixing (24). An overnight bacterial culture was used to inoculate SeMet media, and after 24 h of growth at 37 °C, an Ao of ~1.0 was achieved. Protein expression was induced with isopropyl-β-D-thiogalactopyranoside—final concentration of 0.5 mM for 6 h, and cells were harvested by centrifugation. All purification steps were performed at 0–6 °C. The cells were resuspended in lysis buffer (250 mM KCl, 50 mM Tris–HCl, pH 7.6, 1.0 mM DTT, and 0.1 mM PMSF). Samples with 200–300 μg of protein were centrifuged before loading on the column. The flow rate was 0.5 ml/min, and 0.5-ml fractions were collected.

Gel Filtration Assay—The proteins to be used in the assay were dialyzed overnight against PBS buffer (140 mM NaCl, 2.7 KCl, 10 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.3). The concentration of each protein was determined by the absorbance at 280 nm (ε = 8000). A 30-cm Superdex 75 HR column (Amersham Biosciences) was equilibrated in gel filtration buffer (250 mM KCl, 50 mM Tris–HCl, pH 7.6, 1.0 mM DTT, and 0.1 mM PMSF). The eluted protein was concentrated in a centrifugal filter. As a reference, the absorbance at 340 nm was measured using a bovine serum albumin standard (26). Crystals appeared in the flow-through.

RESULTS

Expression of eEF1β Fragments—In order to study the organization of the eEF1 complex in yeast, we initially expressed full-length TEF3-encoded eEF1β in E. coli. However, this protein showed undesirable properties for structural studies.
such as a strong tendency to aggregate at physiological salt conditions (results not shown). Based on limited trypsin digestion and sequence alignment, we constructed two deletion mutants. The smallest consisted of residues 1–219, domain 1, which has clear sequence homology to GST proteins (15). Based on sequence alignment with the human GST T2-2 sequence, we also expressed residues 1–242, domain 1. Both recombinant fragments of eEF1Bγ were highly soluble (data not shown).

In gel filtration assays domain 1 eluted at a position similar to carbonic anhydrase (29 kDa) indicating a monomer, whereas domain 1’ eluted at a position similar to bovine serum albumin (66 kDa) indicating a dimer (Fig. 1). These results suggest that residues 220–242 are required for the stable dimerization of Tef3p in S. cerevisiae. Proteolytic studies of the full-length S. cerevisiae Tef3p showed that the protein could be cleaved into two stable fragments of 26 and 21 kDa, respectively, as assessed by SDS-PAGE. The C-terminal domain 2, residues 237–415, elutes as a monomer from a gel filtration column (Fig. 1). Domain 2 does not interact with eEF1Bγ and can be separated from the N-terminal fragment by ion exchange (data not shown). Both domain 1 and 1’ were able to form stable complexes with eEF1Bα (data not shown). Although the amino acid sequence suggests homology to the GSH-binding motif of GST proteins, no such enzymatic activity was observed with domain 1 or 1’ toward the GST marker substrate CDNB, whereas activity was demonstrated for a recombinant GST (data not shown).

The Structure of Domain 1—Although large single crystals of domain 1’ were obtained, they diffracted only weakly to ~4.5 Å. Crystals of selenomethionated domain 1 diffracted better, and a three wavelength data set was collected at 3 Å resolution. The density map obtained from MAD phases was superior to that obtained from MAD phases, most likely due to radiation damage during data collection at the remote and inflection wavelengths. Initially the crystals were believed to be tetragonal, and 100 degrees of data were collected for each wavelength. Radiation damage was observed already within the peak wavelength collected first, and only the first 50 degrees of data were used, but due to the high symmetry this was sufficient to obtain a redundancy of 10.7 (Table I). The asymmetric unit contains one molecule of domain 1 and a sulfate ion and has a solvent content of 63%. The experimental map obtained after density modification was easily interpretable over almost the full length of the protein (Fig. 2). The mean residual real space correlation calculated with O between the refined model and the experimental map is 0.83. For comparison the correlation is 0.91 between the model and the final 2Fo − Fc map, but this map inevitably suffers to some degree from model bias, so the correlation to this map is likely to be artificially high. Visually, there is rather little difference between the two maps indicating a very high quality of the input MAD phases. These phases were used as MLHL restraints in all cycles of refinement. Although the same test sets were used in all refinement steps, there is an unusual small difference between R and Rfree (Table I). However, it has been demonstrated that there is a strong correlation between Rfree and the phase error, and with good experimental phases the MLHL target is superior for refinement (35). Our structure fully confirms the power of using good experimental phases as restraints with respect to obtaining low values of Rfree.

The refined crystal structure of S. cerevisiae eEF1Bγ domain 1 contains all 219 amino acids and a sulfate ion (Fig. 3A).
structure shows striking similarity to GST proteins, and one of the crystallographic dimers is organized as a known GST dimer (Fig. 3). Like GST enzymes the monomer consists of two subdomains. Subdomain 1N, residues 1-74, has a central four-stranded β-sheet flanked on one side by the parallel helices α1 and α3 and on the other solvent-exposed side by the α2 helix. A linker of 15 residues (75-89), of which residues 77-83 forms a helix, connects subdomain 1N to subdomain 1C containing residues 90-219. As in GST structures we named the helix in the linker α3B. This helix formation is not seen in the human GST T2-2 structure, the *Arabidopsis thaliana* GST Theta class structure, nor the Australian sheep blowfly *Lucilia cuprina* GST structure (20, 36, 37). However, a short 310 helix is found in the linker connecting subdomains 1N and 1C in the structures of maize GST 1-3 and GST 1-4 from the mosquito *Anopheles dirus* (38, 39).

Subdomain 1C contains five helices: α4, α5, α6, α6B, and α7 (Figs. 3 and 4). As in the human GST T2-2 structure, helix α4 is irregular and is more accurately described as three helices α4A, α4B, and α4C. The three helices of eEF1Bγ, however, are almost coaxial. Helix α5 runs antiparallel with almost the full length of α4 and is slightly bent. Helix α6 contains at its start the N-capping box (S/T/XXD) and a hydrophobic staple motif, both of which are highly conserved among GSTs and appear to greatly stabilize their fold (40). This helix runs parallel to helix α4 and bends away from the subdomain 1N of the protein at its C-terminal end. Compared with the human GST T2-2 structure, there is an additional 310 helix between helices α6 and α7, termed α6B. A similar helix is seen in the *A. thaliana* GST Theta class structure (37). Subdomain 1C has a hydrophobic core created by an intricate ring stacking of the side chains of residues Trp-99(α4A), Phe-143(α5), Phe-169(α6), Phe-173(α6), Trp-181(α6B), Trp-191(α7), and Phe-192(α7).

Although present in the crystallization solution and in the cryobuffer, no electron density for GSH was observed in the electron density maps. A large spherical piece of electron density surrounded by Arg-11, Arg-13, and Arg-171 was observed at the N terminus of helix α1, which cannot be attributed to protein. Given the fact that the protein was crystallized in ammonium sulfate, the basic environment, and that the human GST T2-2 and the maleylacetoacetate isomerase/glutathione transferase ζ, MAAI/GST Z1-1 structures have a sulfate ion in a similar position (36, 41), this density was modeled as a sulfate ion (Fig. 5).
The eEF1Bγ Dimer—Although in solution domain 1 does not dimerize, the crystallization conditions induce dimer formation around a crystallographic 2-fold axis (Fig. 3B). The A and B monomers interact primarily by contacts between β4 and α3 from the subdomain 1N of one subunit and α4 from the subdomain 1C of the adjacent subunit. This interaction decreases the solvent-accessible surface by 1237 Å² per monomer. The interactions between the monomers in the dimer are both hydrophobic and polar. The side chains of Leu-60 (α4), Ala-65 (α3), Tyr-68 (α3), and Tyr-69 (α3) form the hydrophobic core of the dimerization interactions with Leu-90, Gln-93, Ala-94, and Ile-97, all from α4A. The methylene groups of Glu-62 (α4), Met-64 (α3), Lys-72A (α3), and Arg-98 (α4A) all contribute to the dimerization through their side chain methylene groups at the periphery of this hydrophobic core. The polar and charged residues Tyr-68, Lys-72, Asp-87, and Gln-93 and their symmetrical equivalents from the other monomer all point toward the 2-fold symmetry axis of the dimer, creating a polar/charged pocket in the dimer interface. At the 3 Å resolution of the structure, water-mediated contacts cannot be observed. The hydrophobic “lock-and-key” motif described for mammalian GST structures was not present in eEF1Bγ Tef3p (42). This involves a hydrophobic key, usually Phe or Tyr, from the loop preceding β3 and a lock composed of residues from α4 and α5 of the other monomer. The hydrophobic key in this structure is represented by Leu-46, but the side chain of this residue is too short and is not near a putative lock motif. The bacterial (Proteus mirabilis) Beta and squid Sigma class GSTs both have a polar rather than a hydrophobic dimeric interface and also lack the lock and key motif (43, 44). In contrast to the classical V-shaped dimer interface observed in other GST structures, as well as the two Theta-like structures, a more close-packed

![Crystal Structure of the N-terminal Domain of Yeast eEF1Bγ](http://www.jbc.org/Downloaded from)

**Fig. 4.** A, amino acid sequence alignment between Tef3p eEF1Bγ domain 1 and hGST T2-2. The sequences have been aligned based on their structure. The secondary structure elements of the two proteins are indicated above and below the sequence, respectively. The eEF1Bγ sequence has been colored based on an alignment between 20 eEF1Bγ sequences made with ClustalW. Similar residues have a light gray background; residues identical in 50% or more of the sequences have a dark gray background; and strictly conserved residues have a black background. The active residue in hGST T2-2 has a black background, and three other residues important for the binding of GSH are boxed. The similarity between the two sequences is indicated below by : (similar) and * (identical). B, partial sequences of eEF1Bγ from different species with the putative eEF1Bγ recognition loop. Same coloring as in A.
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Figure 5. The putative active site of eEF1Bγ Teβ3p. The side chains of key residues in and around the active site of GST proteins are shown in ball and stick. See text for further discussion.

interface is observed in eEF1Bγ. Finally, the stacking of symmetry related arginine guanidinium groups in the dimer interface of Alpha, Mu, Pi, and Sigma GST classes (42, 44–46) is not observed in the structure presented here. This is consistent with the observations in the L. cuprina GST structure (20). The only Arg residue near the dimeric interface, Arg-98 from helix α4A, points away from the 2-fold axis of the dimer and toward the solvent. With respect to the interface and the lack of stacked residues, eEF1Bγ is similar to the bacterial GST Beta class protein (43).

Domain 1′ of eEF1Bγ has a lower level of identity (27.9%) compared with domain 2 (57.9%) when comparing the two S. cerevisiae forms with those from human, X. laevis, and A. salina (11). An alignment of the GST homology regions from 20 eEF1Bγ sequences resulted in the identification of 12 conserved residues (Fig. 4A). Two of the conserved residues, cis-Pro-50 and Glu-62 from S. cerevisiae eEF1Bγ, are conserved in the GST proteins and are involved in the positioning and binding of glutathione in the active site (47). Three other identical residues, Thr-151, Arg-190, and Thr-194 from subdomain 1C, form an accessible surface patch opposite to the dimer interface (Fig. 6). The largest region of conserved residues between the aligned eEF1Bγ sequences are found around the hydrophobic staple motif located between α5 and the start of α6 and are involved in the proper folding of this motif (40).

A consensus pattern derived for the Theta class GSTs showed that the residues unique to this class mainly cluster on the hydrophilic surface and flanking loops of helix α2 (47). This region contains the largest difference between GST classes, so it possibly plays a class-specific role in the reaction mechanism. The largest discrepancies between the eEF1Bγ sequences are seen in the region around the C terminus of helices α3 and α3B in the linker region between subdomains 1N and 1C. A short sequence between these two domains is common in mammalian GSTs. Another notable fact is the lack of sequence conservation at helix α4B, which in our structure forms a 310 helix. Surprisingly, there are no strictly conserved residues at the eEF1Bγ dimer interface. This feature has also been suggested to be a Theta class GST characteristic (48). The dimerization region contains only partly conserved residues, such as Ala-94 from α4A and Ala-65 from α3.

The Putative Active Site—The backbone density for the region homologous to the active site of GSTs is continuous in the initial SAD electron density map, but some of the side chain densities are weak. The side chain of a potential catalytic residue, Tyr-7, is oriented away from the active site, and no rotamer could bring it near the putative active site. Residues Arg-11 and Arg-13 have weak side chain density, which suggest high mobility. The side chain of Arg-13 is within hydrogen bonding distance of the backbone carboxyl group between Val-49 and cis-Pro-50 (Fig. 5). This carboxyl group is coordinating the amine group of the sulfhydryl moiety of active site-bound GSH in GSTs. The backbone density for Val-49 and Pro-50 is good, and only a cis-Pro could be modeled at this position in agreement with a cis-Pro in all other GST structures. The side chain of Lys-48, conserved as Lys-53 in the hGST T2-2 structure, has weak side chain density, indicating high mobility. In the presence of a ligand molecule such as GSH, it might recognize the side chain carbonyl group of the glutamyl moiety, as in the hGST T2-2 structure, or the glycine-carboxylate group of GSH. The density for Glu-62 was also of good quality and could easily be modeled. Residue Glu-62 lies in a generously allowed region of the Ramachandran plot. This residue is involved in the binding of GSH in GST proteins and lies in a similar position in the Ramachandran plots of all GSTs. Asp-106 from monomer B appears to be able to coordinate GSH in the active site of monomer A. This is as far as we know in accordance with all other GST structures except human Omega class, squid Sigma class, and the two Theta-like GSTs from A. thaliana and L. cuprina (20, 37, 44, 49).

In the hGST T2-2 structure a sulfate ion is bound in a tetrahedral fashion to Glu-12, Arg-107, Trp-115, and Arg-239 and a water molecule. In our structure it is coordinated to the backbone amide of the Ile-12 peptide bond and the guanidinium group of Arg-171 (Fig. 5). The positive dipole moment of helix α1 also contributes to the affinity for the ion at this position. Due to the low resolution of the structure, no water molecules could be modeled, but water molecules are likely to be involved in the coordination of this ion.

The hydrophobic binding pocket, or H-site, is the binding site for the secondary substrate in GSTs, i.e., the cellular toxic compound. Due to the coaxial nature of helices α4A, α4B, and α4C in our structure, the residues corresponding to helix α4C in the hGST T2-2 structure that are responsible for forming part of the H-site are not near a putative H-site in the isolated domain 1, and they are not conserved. The loop between β2 and α2 responsible for forming the other part of the H-site in hGST T2-2 is too short and positioned too far from a possible H-site in eEF1Bγ domain 1. Crystal contacts around the loop region preceding helix α2 may be responsible for dislocating this region.

DISCUSSION

The Quaternary Structure of eEF1—The characterization of an eEF1 complex in S. cerevisiae was first performed by Saha and Chakraburtty (2). They determined the ratio of eEF1A-eEF1Bγ-eEF1Bα subunits to be 2:1:1, and estimated the molecular mass of the complex to be ~200 kDa. Based on the crystal structure and the gel filtration studies presented here together with the structure of the eEF1α-eEF1Bβ complex (23), a model for eEF1 in yeast can be proposed (Fig. 7). This results in a 2:2 stoichiometric composition of the three yeast components eEF1A, eEF1Bα, and eEF1Bβ for the eEF1 complex, yielding a theoretical molecular mass of 240 kDa in agreement with the organization of the eEF1β-deficient eEF1 complex II from Artemia (50). In the hGST T2-2 dimer, the two C-terminal
Fig. 6. Surface representation of conservation as described in Fig. 4 mapped on the eEF1B \( \gamma \) domain 1 monomer. Residues less than 50% identical are colored red, and blue indicates 100% identity. Residues with between 50 and 100% identity are colored gray. A, eEF1B \( \gamma \) viewed from the dimer interface in the same orientation as Fig. 3A, B, view from the solvent-exposed face. The three 100% conserved residues Thr-151, Arg-190, and Thr-194 form a patch on the solvent-exposed side of the N terminus of eEF1B \( \gamma \).

Fig. 7. Hypothetical model of yeast eEF1 based on structural studies and gel filtration experiments. The three subunits eEF1A, eEF1B \( \alpha \), and eEF1B \( \gamma \) are proposed to form a [eEF1A\(\beta\)eEF1B\(\gamma\)] \( \gamma \) complex. The crystal structure of eEF1A in complex with the C-terminal (C-term) catalytic fragment of eEF1B \( \alpha \) from S. cerevisiae (23) is shown at the bottom left and right. The nucleotide-binding domain I (purple) of eEF1A and domain II (blue) interacts with eEF1B \( \gamma \) (green). Domain III of eEF1A is colored red. The GST-like domain of eEF1B \( \gamma \) is hypothesized to mediate the dimerization of the complex. The C-terminal of eEF1B \( \gamma \) and the N-terminal (N-term) of eEF1A are indicated by the dashed spheres. The suggested interaction site for eEF1B \( \gamma \) on eEF1B \( \gamma \), based on the high sequence identity in this region, is marked by *.

The theoretical molecular mass of the complex is 240 kDa.

helices are oriented in an anti-parallel fashion resulting in the two C-terminal residues from each monomer being almost in hydrogen bonding distance. If eEF1B \( \gamma \) in its full-length form adopts a similar secondary structure in this region, the stabilization of an eEF1B \( \gamma \) dimer could be due to a coiled-coil structure in this region possibly formed by one of the residues 220–242. The size of the N-terminal fragment originally isolated after limited trypsinolysis, and which formed a dimer in a gel filtration assay, seem to have an intermediate size between recombinant domain 1 and domain 1’ when analyzed by SDS-PAGE. It is therefore likely that not all of residues 220–242 are required for the stable dimerization.

Several other models for eEF1 have been proposed (50–54). The one that is most consistent with the model presented here is the A. salina model in which [eEF1A\(\alpha\)\(\beta\)eEF1B\(\gamma\)] \( \gamma \), with a molecular mass of 408 kDa, constitutes the eEF1 complex (50). To elaborate on this model, we can say that the GST-like domain 1’ of eEF1B \( \gamma \) mediates the dimerization of the complex and that the C-terminal part of eEF1B \( \alpha \) interacts with the N terminus of eEF1A (23). eEF1B \( \gamma \) has been suggested to mediate the dimerization in two mammalian models (51, 54). The only other model in which eEF1B \( \gamma \) is explicitly suggested to dimerize is in the rabbit eEF1 model (53), in which eEF1B \( \alpha \) also is suggested to dimerize. The Xenopus model, in which eEF1B \( \gamma \) is suggested to form a trimer that dimerizes (52), seems less likely based on the structure presented here.

The conserved patch on the solvent-exposed side of eEF1B \( \gamma \) may be part of an interaction area with eEF1B \( \alpha \) (Figs. 6 and 7). An interaction site for eEF1B \( \alpha \) close to the putative active site of eEF1B \( \gamma \) (see below) may help facilitate communication between the exchange activity and the putative GST-like activity of eEF1B \( \gamma \). However, until the proper substrate for the latter has been identified, in vitro experiments demonstrating such a linkage are not feasible. Based on sequence alignment, it appears that eEF1B \( \gamma \) from many species have a longer loop region between \( \beta \)2 and \( \alpha \)2 (Fig. 4B), and this appears to correlate with the presence of eEF1B \( \gamma \). Hence, this loop could be important for the interaction between eEF1B \( \gamma \) and eEF1B \( \beta \) in metazoans. This loop is well separated from the conserved surface patch, which might be involved in interaction with eEF1B \( \alpha \). Alternatively, it could form part of the supposedly active site in eEF1B \( \gamma \), possibly adding flexibility to the region and allowing induced fit upon substrate binding and catalysis. An induced fit mechanism has been suggested for the maize GST I enzyme (38), which was suggested to belong to the Theta class (55). This solvent-exposed loop region is involved in the formation of the H-site in GST proteins, so this region may also define specificity for the putative substrate. The consensus sequence derived from sequences containing the long loop is \(^{36}\text{FXXGX(T/S)}\text{N/KR}^{45}\) (Fig. 4B). The boldface letters indicate conserved residues; \( X \) indicates any residue, and the numbers correspond to the human sequence. The animal sequences seem to be slightly longer in this region as compared with plant sequences, but these seem to have slightly longer sequence around \( \alpha \)3B. One exception is eEF1B \( \gamma \) from the protozoans Trypanosoma cruzi and Leishmania infantum, which have a Cys residue instead of the conserved Phe residue at position 38.

Is eEF1B \( \gamma \) Catalytically Active?—The overall structure of the Tef3p dimer and especially the conserved \( \text{cis-Pro-50} \) and Glu-62 all suggest that eEF1B \( \gamma \) in yeast is catalytically active as a GST protein as demonstrated previously for recombinant rice eEF1B \( \gamma \) (16), despite our own failure to demonstrate this with the fragments of yeast eEF1B \( \gamma \). The evolutionary maintenance of such a specific structure for no reason seems very unlikely. Because the active site of eEF1B \( \gamma \) Tef3p has a high degree of
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homology to other GST active sites, and although no catalytic residue could be identified, a novel GST activity mechanism with a very specific secondary substrate should not be ruled out. Arginine is a conserved active site residue in class Alpha GSTs except in a chicken liver GST. The structural equivalent in eEF1β Tef3p is Arg-13, which is also conserved in the Tef4p form of yeast eEF1β. The conformation of Arg-13 in eEF1β Tef3p could explain why neither of the two Tef3p constructs were able to bind to a GSH affinity matrix because it overlaps with the putative GSH-binding site. Mutational studies of the equivalent Arg-15 from the human Alpha class GST, hGST A1-1, showed that alteration of this amino acid reduced the catalytic activity of the enzyme (56). A conformation of eEF1β Arg-13 similar to the one in hGST A1-1 could not only facilitate the binding of GSH in the active site but could also at the same time bring the charged δ-guanido group of Arg-13 closer to the sulfate ion, thereby stabilizing it further. The involvement of an Arg residue in the active site no longer seems to be α-class-specific. The squid Sigma class GST also has an Arg residue in the active site which may catalyze the binding of GSH in the active site (57). Their function seems far from similar, however. Whereas the squid GST model substrate CDNB, does not seem to possess a potential catalytic residue near the putative active site (16).

The GST-like domain of eEF1β GSTs except in a chicken liver GST. The structural equivalent Arg-15 from the human Alpha class GST, hGST A1-1, showed that alteration of this amino acid reduced the catalytic activity of the eEF1B complex. Thus, this domain may play a novel role in regard to the activity of the eEF1B complex and the response to oxidative stress.

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