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Clues to the mechanism of action of eIF2B, the guanine-nucleotide-exchange factor for translation initiation

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Abstract

A variety of cellular processes rely on G-proteins, which cycle through active GTP-bound and inactive GDP-bound forms. The switch between these states is commonly regulated by GEFs (guanine-nucleotide-exchange factors) and GAPs (GTPase-activating proteins). Although G-proteins have structural similarity, GEFs are very diverse proteins. A complex example of this system is seen in eukaryotic translation initiation between eIF (eukaryotic initiation factor) 2, a G-protein, its five-subunit GEF, eIF2B, and its GAP, eIF5. eIF2 delivers Met-tRNA$_i$ (initiator methionyl-tRNA) to the 40S ribosomal subunit before mRNA binding. Upon AUG recognition, eIF2 hydrolyses GTP, aided by eIF5. eIF2B then re-activates eIF2 by removing GDP, thereby promoting association of GTP. In the present article, we review data from studies of representative G-protein–GEF pairs and compare these with observations from our research on eIF2 and eIF2B to propose a model for how interactions between eIF2B and eIF2 promote guanine nucleotide exchange.

eIF2 and eIF2B in translation initiation

Protein synthesis initiation is the multistep process necessary to form a complex between Met-tRNA$_i$ (initiator methionyl-tRNA) and the correct AUG start codon of a selected mRNA within the P-site (peptidyl site) of an 80S ribosome. This is facilitated and regulated by a series of eIFs (eukaryotic translation initiation factors) [1]. One of the key protein factors is eIF2, which recruits Met-tRNA$_i$ to the 40S ribosomal subunit and plays a role in start site selection. eIF2 displays several features characteristic of a classic G-protein: it is active in its GTP-bound form and inactive when bound to GDP. Similarly to other G-proteins, the switch between these nucleotide-bound states is regulated by other factors. GTP hydrolysis by the GAP (GTPase-activating protein) eIF5 occurs only once the eIF2-bound Met-tRNA$_i$ forms a codon–anticodon pair with the AUG start codon. eIF2 · GDP and eIF5 are then released from the ribosome-bound Met-tRNA$_i$.

For released eIF2 to participate in further rounds of translation initiation, the GEF (guanine-nucleotide-exchange factor) eIF2B regenerates active eIF2 · GTP. This step is tightly controlled and is a major point for regulation of protein synthesis in all eukaryotic systems studied. eIF2B can be controlled directly through changes in its phosphorylation status, or indirectly via phosphorylation of eIF2. Both of these regulatory mechanisms have been reviewed extensively elsewhere [2,3], so are not discussed here. It is clear that, under a variety of physiological conditions and cell stresses, changes in eIF2 phosphorylation occur that can modulate the rates of protein synthesis. In mammals, this can affect responses to viral infection, diet and memory formation, among others [4]. One important finding is that, although most mRNA translation is down-regulated by these controls, specific mRNAs are known to escape repression and can become actively translated. Two well-studied mRNAs encode transcription factors: yeast *GCN4* and mammalian ATF4 (activating transcription factor 4) both contain regulatory upstream open reading frames that enable their own translational control [5–7].

Interest in the GEF eIF2B has also increased following the discovery that inherited mutations in eIF2B genes cause a fatal brain disorder. This disease has various forms and multiple names including: CACH (childhood ataxia with central nervous system hypomyelination), VWM (leukoencephalopathy with vanishing white matter), or eRDs (eIF2B-related disorders) [8–10]. As the disorder names suggest, the principally affected cells are white-matter oligodendrocytes and astrocytes within the brain, which disappear or vanish over time to be replaced with cerebrospinal fluid. These cells normally insulate axons with myelin sheaths, and their damage and loss prevents neuronal signals reaching their destinations; this causes ataxia. In addition, affected individuals also suffer seizures. Onset and progression is very varied, ranging from severe rapidly progressing infant forms to slower progressing adult-onset variants [10].

Key words: childhood ataxia with central nervous system hypomyelination (CACH), eukaryotic initiation factor 2B (eIF2B), G-protein, guanine-nucleotide-exchange factor (GEF), leukoencephalopathy with vanishing white matter (VWM), translation initiation.

Abbreviations used: AA-box, motif of largely acidic and aromatic residues; aIF2, archaeal initiation factor 2; ARF, ADP-ribosylation factor; ARNO, ARF nucleotide-binding-site opener; CACH, childhood ataxia with central nervous system hypomyelination, CTD, C-terminal domain; EF, translation elongation factor; eIF, eukaryotic initiation factor; eRD, eIF2B-related disorder; GAP, GTPase-activating protein; GEF, guanine-nucleotide-exchange factor; Met-tRNA$_i$, initiator methionyl-tRNA; P-loop, phosphate-binding loop; Sw, switch; VWM, leukoencephalopathy with vanishing white matter.

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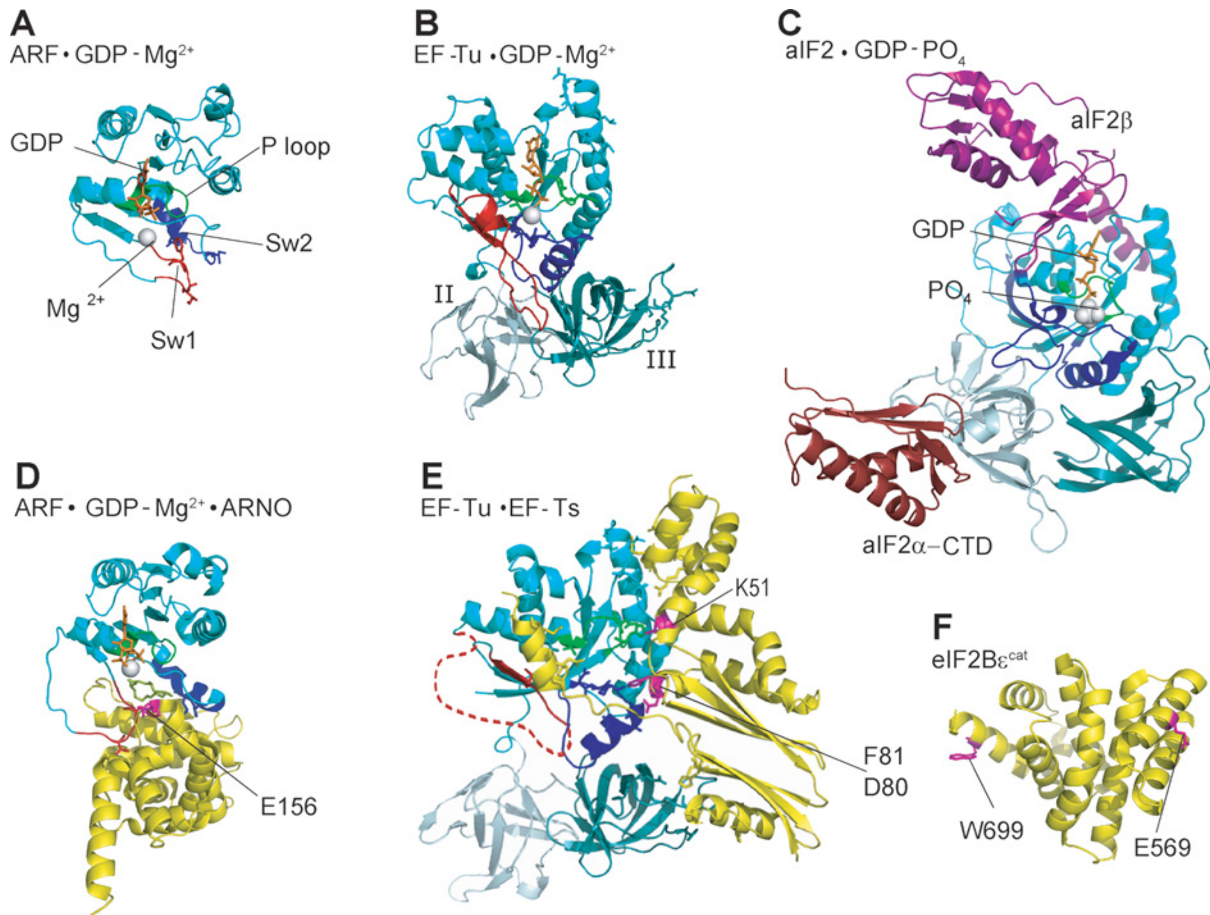
Figure 1 | Structural features of G-proteins and GEFs

Ribbon cartoons of selected G-proteins (**A**–**C**), G–GEF co-structures (**D** and **E**) and eIF2Bε^{cat} (**F**). Uniform labelling colours are used throughout: G-domain (cyan) with P-loop (green), Sw1 (red), Sw2 (blue), GDP (orange) and Mg^{2+} ion (white sphere). In (**B**) and (**C**), additional G-protein domains are in pale cyan (II) and deep teal (III). Extra aIF2 subunits are coloured pink and rust and are labelled in (**C**). In this structure PO_4 replaces Mg^{2+}. GEFs are shown in (**D**–**F**) (yellow), with residues important for nucleotide exchange indicated (magenta). In (**D**), GDP–Mg^{2+} intermediate is stabilized by brefeldin A (lime). The Figure was drawn with PyMOL software (http://pymol.sourceforge.net) using PDB codes 1U81, 1R8Q, 1TUI, 1EFU, 2QMU and 1PAQ.



It is not clear how mutations in eIF2B, a ubiquitous protein, can cause such a cell-type-specific defect. Where measured, eIF2B activity is reduced in patient lymphocytes and some cellular models of disease. It is also unclear how the cell specificity observed is caused. Two alternative ideas are that mutations alter translation of a glial-cell-specific eIF2B-regulated gene. Alternatively eIF2B protein levels or activity may be intrinsically low in these cells normally, so that they have heightened sensitivity to cell stress. In the latter case, it is possible that the mutations lower eIF2B activity below a threshold critical for cell survival, leading to the disease observed [11–15].

Because eIF2 and eIF2B are essential proteins within all eukaryotic cells, one strand of research in our laboratory has been to probe the mechanism of eIF2B-catalysed guanine nucleotide exchange. In the following sections, we review our progress and that of other researchers and compare these studies with other related G-proteins and their GEFs.

Similarities between eIF2 and other G-proteins

In common with all members of the large family of G-proteins, eIF2 activity is regulated by alteration of bound guanine nucleotide. All are active in a GTP-bound state and inactive when GTP is hydrolysed to GDP. Although their functions are diverse, G-proteins share common structural features in their nucleotide-binding G-domain (containing elements G1–G5). A small representative sample of determined G-protein structures is shown in Figures 1(A)–1(C), where the small G-protein ARF (ADP-ribosylation factor) [16] is shown alongside EF (translation elongation factor)-Tu [17] and the archaeal homologue of eIF2 [aIF2

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(archaeal initiation factor 2)] [18]. In Figure 1, the G-domains are coloured cyan. Additional protein domains required for the function of specific G-proteins are often located within other domains or separate proteins of multisubunit G-protein complexes. For the prokaryotic EF-Tu and aIF2/eIF2, these are shown in different colours in Figure 1.

Common motifs in all G-proteins include the nucleotide-base-binding G4 motif (N/TKXD), and the P-loop (phosphate-binding loop) sequence (GXXXXGKS/T; coloured green in Figure 1), which interacts with the α- and β-phosphates to stabilize nucleotide binding. Sw (switch) regions 1 and 2 (red and blue in Figure 1) are critical to G-protein function and alter (or switch) conformation depending on whether GTP or GDP is bound. Sw2 interacts with the γ-phosphate of GTP. Finally, critical for stabilizing nucleotide binding to G-proteins is an Mg^{2+} ion (white spheres in Figure 1). Magnesium is variously co-ordinated in different G-proteins to residues in Sw1 and/or Sw2 and to the nucleotide itself.

eIF2 binds multiple ligands during translation initiation

The main difference between translation factor G-proteins and small G-proteins is that they contain multiple additional domains, and, in the case of eIF2, extra subunits, that are essential to their functions. For example, EF-Tu shown in Figure 1(B) has two additional domains: II and III. These make contacts with aminoacylated tRNA [19], its GEF (EF-Ts; Figure 1E) [20] and the ribosome [21].

eIF2 has three subunits (α–γ). Its nucleotide-binding subunit eIF2γ is a close sequence and structural homologue of EF-Tu and its eukaryotic counterpart, eEF1A [22]. In addition to nucleotides, EF-Tu · GTP and its eukaryotic equivalent eEF1A · GTP bind all aminoacylated elongator tRNAs. By analogy and from analysis of mutations, eIF2γ · GTP is thought to make the major contribution to Met-tRNA$_i$-binding eIF2 [23,24]. Macromolecular modelling suggests that a pocket in eIF2γ formed by the position of Sw1 in the GTP form provides a site for the aminoacylated 3′-end of Met-tRNA$_i$. Loss of eIF2α has been shown to influence Met-tRNA$_i$ affinity [25], and the eIF2α CTD (C-terminal domain) may contact Met-tRNA$_i$ directly [23,24]. The eIF2 · GTP · Met-tRNA$_i$ ternary complex interacts with initiation factors eIF5 (the GAP), eIF3 and eIF1 and also with the 40S ribosome [26]. Direct contact between eIF2β and eIF1A [27], eIF5 [28] and eIF3 [29] has been demonstrated and mutations in eIF2β destabilize these interactions. A separate interaction between eIF2γ and eIF5 is proposed to mediate the GAP activity upon AUG codon recognition [30].

In its GDP-bound form, eIF2 interacts with the GEF eIF2B, which binds all three eIF2 subunits. eIF2α is a target for the kinase-regulated inhibition of eIF2B described above [31,32]. However, both eIF2β and eIF2γ interactions are implicated in nucleotide exchange (as described below). Recently, a complex of eIF2 with eIF5 has been described that lacks Met-tRNA$_i$ [33,34]. It is proposed that this represents an eIF2 · GDP/eIF5 complex released from the ribosome after GTP hydrolysis, and genetic evidence suggests that this complex can antagonize the function of eIF2B [33,34].

GEFs: mechanistic information from structural and biochemical studies

All GEFs function to promote GDP and Mg^{2+} release from their cognate G-protein to allow formation of active GTP-bound forms. Despite the common structural elements found within all G-domains, each GEF studied varies dramatically in structure and interaction with its cognate G-protein. There are some excellent reviews of the literature describing both similarities and differences between mechanisms [35–37]. In general, GEF interactions cause structural rearrangements of Sw1 and Sw2 domains, disrupt Mg^{2+} co-ordination and destabilize the nucleotide-binding motifs. In the present article, we focus on a few key examples where both structural and biochemical information is available. We use these to compare directly with studies of the more complex eIF2 and eIF2B.

ARF · ARNO (ARF nucleotide-binding-site opener)

GEFs specific for ARF1, including the yeast Gea1p and the human ARNO, each possess a Sec7 domain that carries out the exchange. The ARNO Sec7 domain has an α-helical structure, and mutagenesis of residues at the ARF1-binding site have shown that a glutamic acid residue, $ARNO^{E156}$, is critical for GDP-release. As shown in Figure 1(D), ARNO interacts with and displaces both Sw1 and Sw2 [38]. Both are extensively remodelled, reducing the affinity of the bound Mg^{2+} and GDP. (Note that in the structure shown, Mg^{2+} and GDP are stabilized by the binding of the small inhibitor molecule brefeldin A.) GEF assays with $ARNO^{E156A}$ mutant showed that Glu^{156} is critical for ARNO function.

EF-Tu · EF-Ts

Unlike the ARF · ARNO example, EF-Ts makes more extensive contacts with EF-Tu, including G-domain and domain III contacts (Figure 1E). An interaction between $EF\text{-}Ts^{F81}$ and Sw2 causes displacement of Sw2 $EF\text{-}Tu^{D80-C81-P82}$, reducing affinity for Mg^{2+} and GDP [20].

Mutational analysis revealed that combining both $EF\text{-}Ts^{D80}$ and $EF\text{-}Ts^{F81}$ mutations reduced activity 10-fold [39]. This observation, and kinetic data on the relatively low dissociation rate of GDP from EF-Tu in the absence of Mg^{2+}, both support the idea that EF-Ts must use a mechanism in addition to Mg^{2+} displacement for nucleotide exchange. Nucleotide base attack is proposed [40]. Another key observation from the kinetic analysis was that, although GTP/GDP binding to EF-Tu alone is slow, the binding of nucleotides to an EF-Tu · EF-Ts complex increases 10-fold, therefore binding of EF-Ts opens up the nucleotide-binding pocket, allowing rapid association/dissociation of nucleotides with EF-Tu [40].

eIF2B

eIF2B is a heteropentamer. Subunits α, β and δ form a regulatory subcomplex that binds to and recognizes phosphorylated eIF2α and down-regulates the activity of the eIF2B catalytic

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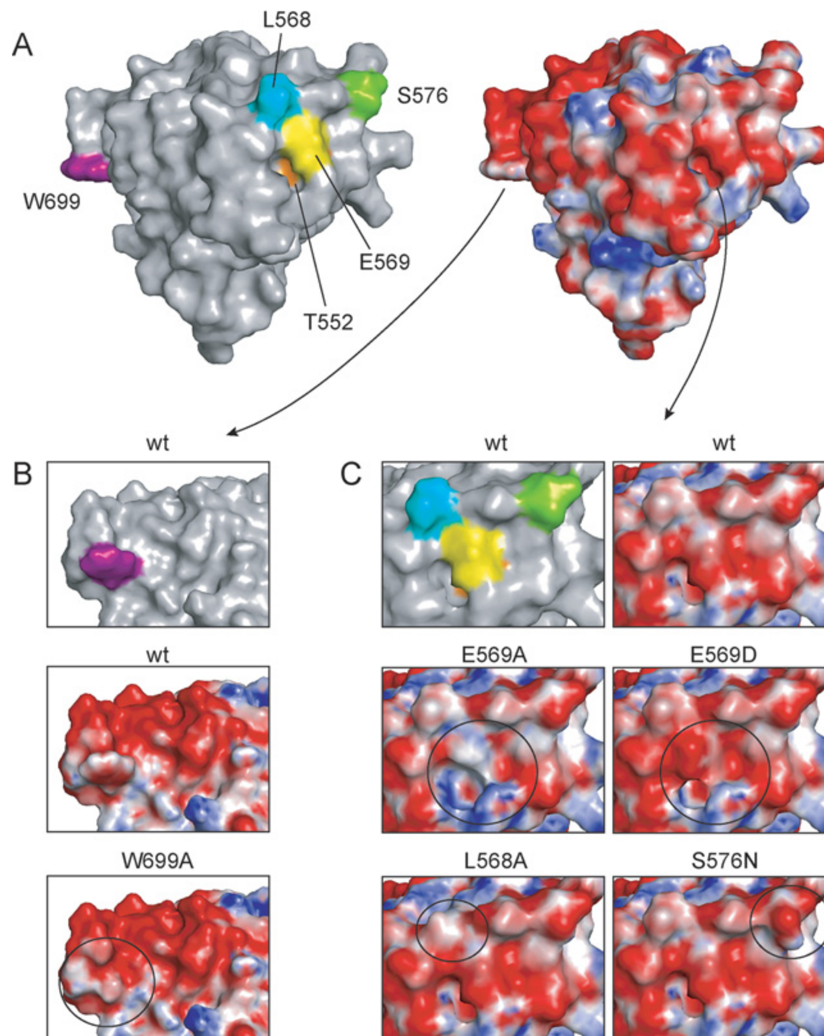
Figure 2 | Effect of eIF2Bε^{cat} mutations on surface electrostatic potential

(**A**) Left: surface representation of wild-type eIF2Bε CTD with key residues coloured. Right: electrostatic potential mapped on the surface of wild-type eIF2Bε CTD with negative potential in red, positive potential in blue and neutral potential in white. (**B** and **C**) Close-ups of either wild-type (wt) or mutant eIF2Bε CTD, with key surface changes circled. The Figure was generated using PyMOL with APBS (adaptive Poisson–Boltzmann solver) (http://apbs.sourceforge.net).



subcomplex (subunits γ and ε) [2]. eIF2Bε is the subunit that carries out nucleotide exchange [31]. The N-terminus of eIF2Bε mediates interactions between eIF2B subunits [13,41,42], while both nucleotide exchange and substrate binding have been located to the CTD (residues 518–712; numbering relates to the *Saccharomyces cerevisiae* sequence) hereafter denoted ε^{cat} [43].

ε^{cat} structure

The crystal structure of the isolated ε^{cat} domain revealed that it is an all-helical domain (Figure 1F) [44], later shown to be highly similar to the eIF5 CTD [45,46]. This suggested that eIF5 and ε^{cat} interact with eIF2 in similar ways and may compete for binding. Within this domain, two conserved motifs of largely acidic and aromatic residues (AA-boxes) at the extreme C-terminus have been identified. In both proteins, AA-box 2 mutations disrupt eIF2β binding [28]. In addition, ε^{cat} can also bind to eIF2γ [30,47]. A second region of conserved residues specific to ε^{cat} is found at its N-terminus. It has been termed the 'catalytic centre', as it is essential for nucleotide exchange [43].

Residues critical for eIF2B GEF function

Trp^{699}

Observations of the crystal-stacking interactions between eIF2B$\varepsilon^{544-702}$ proteins drew attention to the conserved tryptophan residue at position 699 [44]. Trp^{699} lies in one AA-box region and protrudes from the surface (Figures 1F and 2A). We demonstrated the necessity of this residue for eIF2 binding in yeast, as the lethal mutation W699A

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weakens binding to both eIF2β and eIF2γ and consequently prevents nucleotide exchange [47] without destabilizing the helical structure, as determined by CD experiments. Macromolecular modelling of the W699A mutant structure agrees with this result (Figure 2B). Together, these results show that this surface of the protein is pivotal for maintaining substrate interaction.

Catalytic centre residues

The opposite face on the structure contains the catalytic centre. Our research has also highlighted the importance of four residues in the catalytic centre: Thr^{552}, Leu^{568}, Glu^{569} and Ser^{576}. The last three of these cluster on the surface of the protein, whereas Thr^{552} is buried just beneath the critical Glu^{569} residue (Figure 2A). Mutation of Glu^{569} was considerably detrimental to eIF2B function; changes to alanine, glutamine, lysine and even the conservative acidic aspartic acid all conferred lethality on yeast cells. In one sensitive *in vivo* assay for activity, eIF2Bε^{E569D} demonstrated some residual activity, therefore it appears that retaining a negative charge at this site is important for GEF function. Interestingly, protein interaction studies between eIF2 subunits and ε^{cat} confirmed that the latter interacts independently with both eIF2β and eIF2γ. All Glu^{569} mutants, except E569A, reduced eIF2γ binding, showing that this surface is important for the $\varepsilon^{cat}\cdot$eIF2γ interface [47].

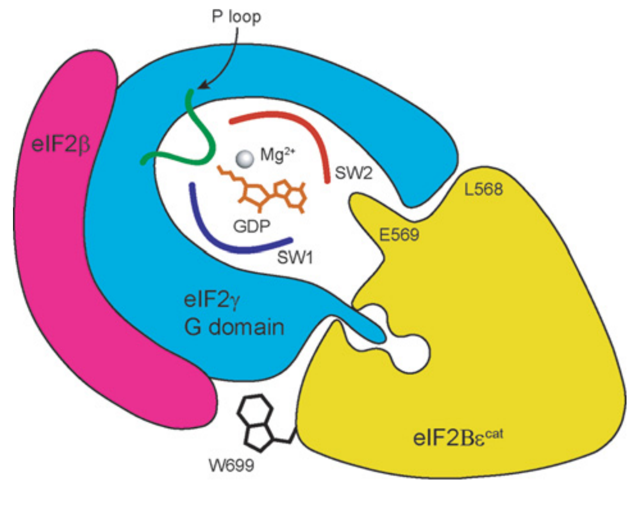
Residues surrounding Glu^{569} also make important contributions for eIF2B function. Non-lethal mutants eIF2Bε^{T552I} and eIF2Bε^{S576N} are slow-growing in yeast and, together with a third mutant, L568A, all exhibit cold sensitivity, which is an indicator of protein interaction defects [41,47]. *In vitro* studies found that these mutant proteins have reduced GEF activity [41] and can weaken eIF2–eIF2B interactions [47]. Because of these results and the fact that the residues form part of an acidic patch on the ε^{cat} surface (Figure 2A), we propose that they form an interacting surface for eIF2γ and GEF activity.

Macromolecular modelling of the effects of catalytic centre mutations on the structure and surface charge (Figure 2C) show that each mutation has a small effect on the overall structure. Only local changes are predicted, consistent with our CD studies [47]. Leu^{568} protrudes from the surface, whereas mutation to alanine (a smaller residue) eliminates this, without affecting the overall charge. Ser^{576} is conserved as a small alanine residue in other species. Its mutation to asparagine introduces a much larger side chain. It is likely that these changes destabilize close contact between eIF2γ and the eIF2Bε catalytic centre.

Glu^{569} is the residue that appears to be most critical for nucleotide exchange. E569D and E569A mutations are both lethal, but only E569D reduces binding to eIF2 [47]. The predicted structural change caused by aspartic acid suggests that a surface pore just under residue 569 becomes concealed by the aspartic acid side chain. In contrast, the alanine substitution increases the size of this surface pore, and reduces the overall acidic charge (red colour in Figure 2A) of this region.

Figure 3 | Model for the interaction between eIF2 and eIF2Bε^{cat}

A schematic diagram based on the experimental data described in the text. Protein domains are coloured as in Figure 1(C) and 1(F). The GDP, Mg^{2+}, Sw regions and the P-loop are labelled, as are residues in eIF2Bε^{cat} that are critical for guanine nucleotide exchange.



Model for eIF2–eIF2B interaction and exchange

By comparing structural and mutagenic data with studies of how different GEFs affect their G-proteins, we can speculate how eIF2Bε^{cat} interacts with eIF2 and achieves nucleotide exchange. Our proposed model accommodates the following observations.

(i) That the W699A mutant in this region has reduced binding to both eIF2β and eIF2γ. This suggests that Trp^{699} binds somewhere along the interface between these two subunits.

(ii) Residues within the catalytic centre (Leu^{568}, Glu^{569} and Ser^{576}) are critical for interaction with eIF2γ. We therefore predict that these residues either function directly to remove the nucleotide from eIF2γ or help to stabilize a reorganized folding of the G-domain.

(iii) E569D mutation reduces eIF2γ affinity, whereas E569A does not.

(iv) Modelling of the effects of catalytic centre mutations on the surface of ε^{cat}.

(v) The GEFs discussed above all appear to insert residues directly into the G-domain and cause rearrangement of the switch regions and/or P-loop.

Our model (Figure 3) proposes that the surface patch containing Leu^{568}, Glu^{569} and Ser^{576} contacts residues directly on the G-domain of eIF2, and the acidic residue Glu^{569} inserts further and is required to disrupt either the switch regions or the P-loop, or both, to destabilize the bound nucleotide and Mg^{2+} ion.

Future directions

Our model on eIF2Bε GEF activity is largely based on the mutagenic and structural data of the catalytic domain.

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In order to confirm our theories, we need to extend our analyses to the G-domain of eIF2γ. Currently, we are limited to using structural data from the archaeal eIF2 homologue, aIF2, to base our model of eIF2B nucleotide exchange on. Ideally, a three-dimensional structure of eIF2 in complex with eIF2B would put studies into this G-protein–GEF relationship at the level of the other examples described here. This would allow more precise models for eIF2B-catalysed exchange. In addition, it would allow modelling of the effects of mutations causing CACH/VWM/eRD that may help to better understand these diseases. This would, however, mean obtaining a detailed structure for an eight-protein complex. A structure of the eIF2γ G-domain interacting with eIF2Bε^{cat} may be a more realistic goal.

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