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ADVANCED REVIEW

# Regulation of translation initiation factor eIF2B at the hub of the integrated stress response

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Phosphorylation of the translation initiation factor eIF2 is one of the most widely used and well-studied mechanisms cells use to respond to diverse cellular stresses. Known as the integrated stress response (ISR), the control pathway uses modulation of protein synthesis to reprogram gene expression and restore homeostasis. Here the current knowledge of the molecular mechanisms of eIF2 activation and its control by phosphorylation at a single-conserved phosphorylation site, serine 51 are discussed with a major focus on the regulatory roles of eIF2B and eIF5 where a current molecular view of ISR control of eIF2B activity is presented. How genetic disorders affect eIF2 or eIF2B is discussed, as are syndromes where excess signaling through the ISR is a component. Finally, studies into the action of recently identified compounds that modulate the ISR in experimental systems are discussed; these suggest that eIF2B is a potential therapeutic target for a wide range of conditions.

This article is categorized under:
Translation > Translation Regulation

**KEYWORDS**
eIF2 phosphorylation, integrated stress response, ISRIB, translational control, vanishing white matter disease

## 1 | INTRODUCTION

The integrated stress response (ISR) is the name attributed to a series of related stress sensing pathways that regulate gene expression and converge to regulate protein synthesis via a common mechanism (Figure 1; Harding et al., 2003). The signaling mechanisms allow a wide range of diverse signals to activate a common pathway that involves a widespread reprogramming of protein synthesis including both a global repression of mRNA translation as well as the activation of translation on specific mRNAs. Activated mRNAs encode proteins with specific roles in overcoming cell stress to restore homeostasis. They typically contain one or more upstream open reading frame (uORF) that normally function in unstressed cells to limit the flow of ribosomes to the main coding ORF. However, following stress signaling, translation of the main ORF is upregulated facilitating cellular stress responses (Hinnebusch, Ivanov, & Sonenberg, 2016; Young & Wek, 2016).

## 2 | OVERVIEW OF THE ISR

The signaling mechanism known as the ISR was first described for the activation of the yeast transcription factor Gcn4 following amino acid starvation in a series of elegant papers from the Hinnebusch laboratory, that established that translational control by phosphorylation of eIF2α could confer a rapid cellular response to stress by reprogramming gene expression, a

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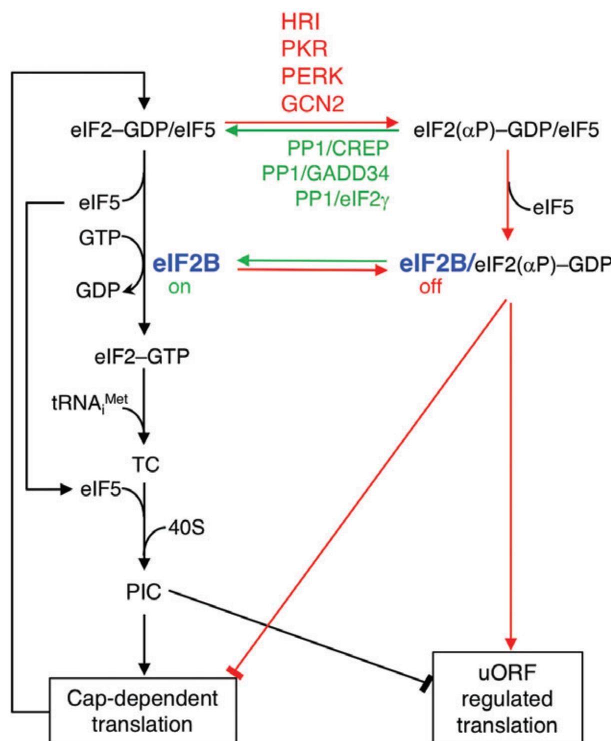


**FIGURE 1** Overview of translational control of eIF2 during the ISR. Left, main steps in activation of eIF2 for translation that typically repress uORF mediated translation. Right, ISR by reversible phosphorylation of eIF2α (red arrows) mediated by specific kinases (red text and arrows) to promote uORF translation and that is reversed by the action of PP1 phosphatase complexes (green text and arrows)

response that is known as general amino acid control (Dever et al., 1992; Hinnebusch, 2005). It was subsequently shown that similar response pathways act in mammalian cells, where multiple upstream activators were found to converge to downregulate global protein synthesis and at the same time upregulate expression of the transcriptional activator ATF4 via a similar mechanism involving altered translation of upstream ORFs (uORFs), leading the idea of an integrated common response to stress or ISR (Harding et al., 2003; Lu, Harding, & Ron, 2004; Vattem & Wek, 2004). We now know that ATF4 is one of several mammalian mRNAs activated by this stress response mechanism (see below) (Hinnebusch et al., 2016; Young & Wek, 2016).

Here an overview of the ISR and its importance in both mammals and yeast is described. As there are other excellent recent reviews that focus on the upstream signaling and downstream outputs (Baird & Wek, 2012; Donnelly, Gorman, Gupta, & Samali, 2013; Pakos-Zebrucka et al., 2016), instead here a greater emphasis is placed on describing the mechanism of control of the activity of the translation initiation factor eIF2B, which acts as a central point, or hub, of the regulatory mechanism because phosphorylation of its substrate eIF2 blocks eIF2B activity causing changes in the initiation of protein synthesis. When unbalanced, inappropriate activation or a failure to activate the ISR response can contribute to a wide range of conditions.

## 2.1 | Kinases respond to different stresses by phosphorylating eIF2α

The ISR relies on a signaling relay, such that once stimulated, a protein kinase is activated that attenuates global protein synthesis, but enables gene-specific translational activation. These ISR protein kinases each phosphorylate the same single serine residue (known as $ser^{51}$) on the alpha subunit of the global eukaryotic translation initiation factor 2 (eIF2). This initiates the ISR.

In yeast *Saccharomyces cerevisiae*, a single kinase Gcn2 (general amino acid control nonderepressible 2) responds to a range of stresses including amino acid starvation, purine starvation, oxidative stress and salt stress to phosphorylate eIF2α. Phosphorylated eIF2 [eIF2(αP)] attenuates eIF2B and causes both the reduction in translation initiation globally, but activation of *GCN4* mRNA translation. Gcn4 stimulates a diverse transcription program that includes activation of genes that will enable yeast cells to tackle the cellular stress imposed and resume normal growth (Hinnebusch, 2005). Once the stress is resolved, $ser^{51}$ is dephosphorylated by the action of protein phosphatase 1 (PP1), Glc7 (Dever, Kinzy, & Pavitt, 2016; Hinnebusch, 2005).

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In metazoa, the ISR is expanded to include four protein kinases with diverse regulatory domains, but with related catalytic domains (Donnelly et al., 2013; Pakos-Zebrucka et al., 2016; Taniuchi, Miyake, Tsugawa, Oyadomari, & Oyadomari, 2016). In humans, the genes have systematic names *EIF2AK1–4*, but the proteins retain additional more common use names. GCN2 (EIF2AK4) is named as its yeast counterpart and senses the level of tRNA amino-acylation. PKR (double-stranded [ds] RNA-activated protein kinase or EIF2AK2) responds to dsRNA produced during viral infections. Heme-Regulated Inhibitor (HRI or EIF2AK1) responds to heme deficiency and PKR-like endoplasmic reticulum (ER) kinase (PERK or EIF2AK3) is part of the widely studied unfolded protein response that senses unfolded proteins in the ER (Baird & Wek, 2012; Walter & Ron, 2011). The kinases are expressed across different tissues in latent forms where altered interactions with ligands contribute to kinase activation. For example, the binding of deacylated tRNA activates GCN2 (Dong, Qiu, Garcia-Barrio, Anderson, & Hinnebusch, 2000; Qiu, Dong, Hu, Francklyn, & Hinnebusch, 2001), while viral dsRNA binding activates PKR (Dar, Dever, & Sicheri, 2005; Dey, Cao, et al., 2005). In contrast ligand binding inhibits HRI. HRI is expressed in erythroid cells where it binds a regulatory heme that inhibits its kinase activation; hence heme limitation stress releases heme from the kinase to activate HRI (Chen, 2007). Finally, PERK resides across the ER membrane and is thought to bind either to ER-resident chaperones that repress activation or conversely to unfolded proteins that stabilize active PERK multimers (Walter & Ron, 2011). Each kinase is active as a homodimer and trans-autophosphorylation promotes their activation prior to eIF2α phosphorylation (Dey, Cao, et al., 2005; Dey, Cao, Sicheri, & Dever, 2007). The eIF2 kinases recognize sequences around $ser^{51}$ as well as a separate conserved motif 20 Å away on the surface of eIF2α to facilitate $ser^{51}$ phosphorylation (Dey, Trieselmann, et al., 2005). As phosphorylation of eIF2 can lead to a global shut-down of cytoplasmic protein synthesis, the ISR is under tight control.

Phosphorylation of eIF2α at $ser^{51}$ has been described as applying a brake to protein synthesis (Sidrauski et al., 2013). It does this by reducing the levels of the active form of eIF2 (eIF2–GTP). Active eIF2 delivers initiator tRNA ($tRNA_i^{Met}$) to ribosomes as an eIF2–GTP–$tRNA_i^{Met}$ complex known as ternary complex (TC). In addition to ribosome binding, TC facilitates AUG start codon recognition. Hence eIF2 is critical for global protein synthesis and its structure and functions are described in more detail below. Following AUG recognition, eIF2 is released from the ribosome in an inactive GDP-bound form. eIF2 interaction with two regulatory initiation factors eIF2B and eIF5 control eIF2 activity. It is the ability of eIF2B to act as a guanine nucleotide exchange factor (GEF) which stimulates the release of GDP and reactivation of eIF2 that promotes protein synthesis (Figure 1). ISR signaling and $ser^{51}$ phosphorylation inhibit eIF2B GEF effectively because eIF2B levels are lower than eIF2 and thereby limits TC levels. These events are discussed in more detail in separate sections below, where a model for the combined actions of eIF5 and eIF2B in eIF2 activation and the ISR are described.

## 2.2 | uORF-regulated mRNAs in the ISR

### 2.2.1 | Global attenuation of protein synthesis

eIF2(αP) accumulation and eIF2B inhibition are critical for two opposing translational control elements of the ISR. First, the global reduction in protein synthesis and, second, gene-specific activation of translation. Global inhibition of translation initiation can be observed by fractionating extracts of growing cells on sucrose gradients-termed polysome profiles. Actively growing cells maintain a large population of mRNAs engaged with multiple ribosomes called polysomes. Inhibition of translation initiation causes fewer ribosomes to engage productively with mRNAs and consequently as elongating ribosomes reach stop codons and disengage the total population of actively translating ribosomes falls causing a reduction in polysome abundance and instead a peak corresponding to inactive 80S monosomes is typically enhanced (Figure 2a). This indicates that globally across a cell population translation activity of most mRNAs is reduced (Figure 2b). Increased eIF2(αP) is one prominent cause of global changes in translation initiation in response to stress (Simpson & Ashe, 2012; Spriggs, Bushell, & Willis, 2010). Codon resolution measures of ribosome engagement with individual mRNAs ± stress can be measured by ribosome footprinting (Ingolia, Ghaemmaghami, Newman, & Weissman, 2009; Sidrauski, McGeachy, Ingolia, & Walter, 2015). During periods of acute stress translationally stalled mRNAs can accumulate into stress granules within the cytoplasm that also include a range of RNA-binding proteins and some translation factors (Buchan, Yoon, & Parker, 2011; Kedersha, Ivanov, & Anderson, 2013). These are thought to be sites in which mRNAs are stored awaiting signals for resumed growth or possibly for engagement with RNA-decay factors. Prolonged co-occurrence of stress granules and eIF2(αP) have been associated with neurodegeneration (Kim et al., 2014).

### 2.2.2 | ISR-activated mRNAs

In addition to the widespread translational repression, some mRNAs are translationally maintained or induced. Some can be translated in an eIF2-independent manner, when eIF2 is inactivated. Translation mechanisms include Internal Ribosome Entry Sites (IRESes), sequences within mRNA 5′ leaders that facilitate a cap-independent mode of translation initiation (Kim, Park, Park, Keum, & Jang, 2011; Spriggs et al., 2010; Thakor & Holcik, 2012). Non-AUG codon initiation has also been observed

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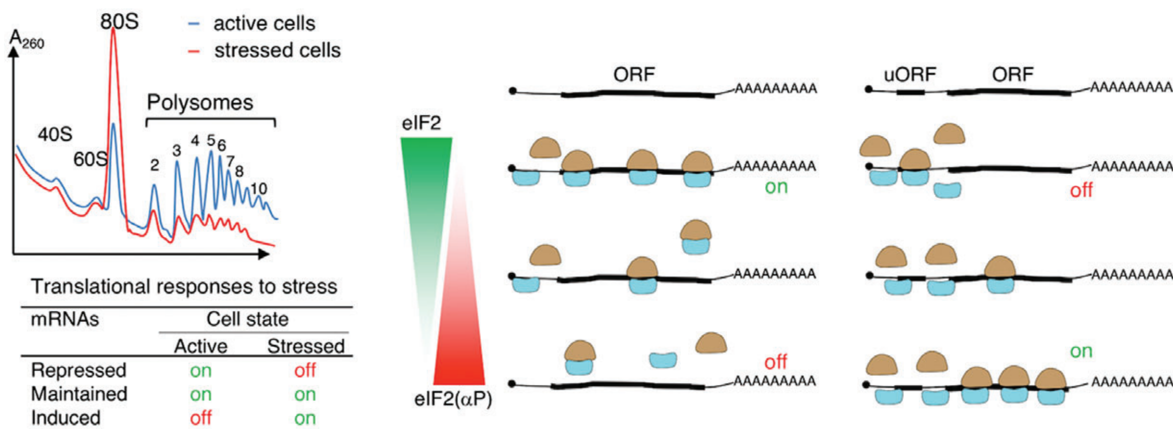


**FIGURE 2** Effects of ISR on mRNA-ribosome interactions. (a) Typical polysome profiles ($A_{260}$ profile) from cell extracts sedimented on 15–50% sucrose gradients from active and stressed cells. Cartoon of ribosome engagement with single ORF mRNA (b) or uORF bearing mRNA (c) under variable levels of eIF2 phosphorylation during the ISR. Table summarizes translation state for different mRNA classes

to increase following stress (Ingolia et al., 2009). For some mRNAs, this may be via an alternative initiation factor, eIF2A, which was found necessary for hepatitis C virus translation in infected cells (Kim et al., 2011) as well as some cellular transcripts (Starck et al., 2012, 2016). However, one major class of ISR-activated mRNAs require eIF2. These contain one or more uORFs, that is coding regions within the 5′ leader of an mRNA, upstream and often out of frame with the main coding ORF (Figure 2c). It is now recognized that as many as 50% of mouse and human genes have uORF coding potential (Calvo, Pagliarini, & Mootha, 2009) uORFs lower expression of the main ORF because the normal scanning mechanism of translation initiation favors initiation at the 5′ AUG codon (Dever et al., 2016; Hinnebusch, 2017). Ribosome footprinting confirmed that many uORFs are occupied by ribosomes and revealed that non-AUG codons can also be used to initiate uORF translation (Ingolia et al., 2009; Lee et al., 2012; Starck et al., 2016).

### 2.2.3 | Ribosome reinitiation control

*GCN4* in yeast was the first mRNA shown to possess uORFs that regulate its translation in response to ISR signaling (Dever et al., 2016; Hinnebusch, 2005). *GCN4* mRNA contains four short uORFs with different functions in control. Analyses show that *GCN4* has both reinitiation promoting uORFs (1 and 2) that retain 40S ribosomes following stop codon recognition and inhibitory reinitiation-preventing uORFs (3 and 4) that terminate translation and release ribosomes. Reinitiation promoting uORFs enable 40S ribosomes to resume scanning downstream to facilitate further initiation events on the same mRNA. In contrast reinitiation-preventing uORFs terminate translation and release ribosomes in the normal way. These elements combine to repress *GCN4* translation during conditions of low eIF2 phosphorylation and high TC availability (by translating uORF1 (or 2) and uORF3 (or 4). Following stress and reduction of TC levels a higher proportion of reinitiating ribosomes (after translating uORFs 1 or 2) lack TC and bypass the inhibitory uORFs 3 and 4, but if they acquire TC before reaching the *GCN4* AUG they can initiate there and increase dramatically the expression of Gcn4 to reprogram transcription (Dever et al., 2016; Hinnebusch, 2005).

Minimally one reinitiation promoting followed by one reinitiation preventing uORF are required for the regulated reinitiation mechanism to operate. Regulated reinitiation mechanisms that are operationally equivalent to *GCN4* have been demonstrated for uORF-containing transcripts in mammalian cells (Hinnebusch et al., 2016; Young & Wek, 2016) including *ATF4* (Vattem & Wek, 2004), the α isoform of *ATF5* (Zhou et al., 2008), that each contain two uORFs one of each type in the same order as found in *GCN4*. *ATF4* is the most intensely studied mammalian ISR gene, as recently reviewed (Pakos-Zebrucka et al., 2016).

### 2.2.4 | Regulated ribosome stalling

Different mechanisms by which uORFs can effect ISR activation have also been characterized. C/EBP-homologous protein (CHOP, also called GADD153) mRNA possesses a single inhibitory uORF, that contains peptide sequences that stall elongating ribosomes to prevent reinitiation at the *CHOP* ORF. The uORF is bypassed during the ISR to promote CHOP expression (Palam, Baird, & Wek, 2011; Young, Palam, Wu, Sachs, & Wek, 2016). *GADD34* mRNA contains uORFs and is also translated via regulated ribosome bypass (Young, Willy, Wu, Sachs, & Wek, 2015). A weak initiation codon context was proposed to contribute to *CHOP* uORF bypass and this may contribute to other uORF control.

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It should be emphasized that while uORFs are generally inhibitory to translation of the main ORF, not all uORFs enable ISR-regulated translational control. *CReP* encodes the constitutive PP1 regulatory subunit (also called PPP1R15B) and its mRNA has two uORFs that do not impart ISR control, instead both impair CReP expression under both stressed and unstressed conditions (Young et al., 2015).

#### 2.2.5 | Other elements contribute to uORF control

As the presence of uORFs alone is not predictive of control, then other 5′ leader elements must contribute. For *GCN4*, the best studied system, reinitiation-promoting sequence motifs have been identified upstream and downstream of uORFs 1 and 2 that help retain 40S ribosomes and eIF3 to facilitate control (Gunisova & Valasek, 2014; Mohammad, Munzarova Pondelickova, Zeman, Gunisova, & Valasek, 2017; Munzarova et al., 2011; Szamecz et al., 2008). *ATF4* uORF1 also retains eIF3 and ribosomes to promote reinitiation (Hronova et al., 2017). Hence eIF3 retention signals are likely to be generally important for controlled reinitiation events following the translation of a short uORF. In addition, it was recently suggested that variable mRNA 5′ leader methylation, specifically $N^6$-methyladenosine (m6A), contributes to *ATF4*-regulated reinitiation. m6A at a site in uORF2 enhances ribosome stalling and uORF2 recognition in unstressed cells and this modification is diminished in amino acid-starved cells to facilitate ribosome skipping of uORF2 and enhanced *ATF4* translation (Zhou et al., 2018). Hence there remain questions concerning the precise mechanisms of uORF control by either ribosome bypass or regulated reinitiation and new examples of uORF-regulated genes are being characterized, some of which likely respond in tissue-specific ways (Onofre, Tome, Barbosa, Silva, & Romao, 2015).

#### 2.2.6 | uORF mutations and polymorphisms

It should be noted that an increasing number of uORF mutations have been associated with disorders and with human polymorphisms (Calvo et al., 2009). In general, adding an extra uORF reduces translation of the main ORF, while loss of a uORF may enhance translation. Both loss and gain of uORFs have been associated with altered main ORF translation rates that contribute to human genetic conditions as recently reviewed (Barbosa, Peixeiro, & Romao, 2013). In addition to rare mutations, common polymorphisms also create or remove uORFs. In one recent example, a uORF-creating polymorphism widespread in world populations was shown to confer ISR sensitivity to ERCC5 expression levels. Because chemotherapy treatment induced the ISR, and as ERCC5 promotes DNA–repair pathways, the polymorphism variant with two uORFs was associated with a poorer cancer chemotherapy treatment survival rate in ependymoma, a form of glioma (Somers et al., 2015).

#### 2.2.7 | ISR-activated RNAs restore homeostasis

mRNAs that are preferentially translated by the ISR typically act to facilitate cellular recovery to restore homeostasis. In the yeast ISR, Gcn4 activates transcription of over 500 genes in response to amino acid limitation, including biosynthetic enzymes for 19/20 common amino acids (Natarajan et al., 2001). Similarly in metazoa ATF4 and ATF5 activate transcriptional programs that differ by cell type (Baird & Wek, 2012; Fusakio et al., 2016; Harding et al., 2003). Several transcriptionally induced mRNAs bear uORFs facilitating temporal activation of different mRNAs in the ISR. For example, one mRNA activated transcriptionally by ATF4 is *GADD34* that is then ISR activated translationally by its 5′ leader (Young et al., 2015). GADD34 (also called PPP1R15A) encodes a PP1 eIF2α phosphatase regulatory subunit that acts to reset ISR control as stress is overcome. However, if stress cannot be managed and normal homeostasis is not restored, cells may enter a chronic stress state (Guan et al., 2017) or induce cell death pathways including apoptosis, as recently reviewed (Pakos-Zebrucka et al., 2016). A failure to induce the ISR or a failure to restore homeostasis and downregulate the ISR can cause serious consequences as described in a separate section below.

### 2.3 | eIF2 phosphoprotein phosphatases reset the ISR

Once a stress has been resolved eIF2 must be dephosphorylated so that normal protein synthesis and cellular activity can resume. Protein phosphatases are typically composed of generic catalytic subunits and specific regulatory subunits that provide substrate specificity to the dephosphorylation reaction. In the mammalian ISR, a PP1 catalytic subunit interacts with two separate regulatory subunits GADD34 (ISR-induced) and CReP (constitutively expressed) to downregulate eIF2(αP) (Connor, Weiser, Li, Hallenbeck, & Shenolikar, 2001; Jousse et al., 2003; Novoa, Zeng, Harding, & Ron, 2001) (Figure 1). The critical role of these proteins in controlling eIF2α phosphorylation and mammalian development was elegantly demonstrated by mouse knockout studies. Cells deleted for CReP increased both basal and ISR activated eIF2(αP) levels higher than controls. The mice had severe growth retardation and impaired erythropoiesis (Harding et al., 2009). GADD34 knockout cells only affect ISR activated eIF2(αP) levels (Brush, Weiser, & Shenolikar, 2003; Kojima et al., 2003) and knockout mice have mildly impaired erythropoiesis among other defects. Combining both GADD34/CReP knock outs conferred early embryonic lethality (Harding et al., 2009). Hence regulated phosphorylation of eIF2α is a major function of CReP and GADD34, and both

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hyperphosphorylation of eIF2α or its hypophosphorylation are lethal, as eIF2α S51A homozygous mice die from hypoglycaemia soon after birth (Scheuner et al., 2001). As indicated above, GADD34 is both transcriptionally and translationally regulated by the ISR, such that its expression timing coincides with the cellular requirement to downregulate the ISR.

In the yeast ISR, the PP1 catalytic subunit homolog Glc7 has been implicated as a eIF2α phosphatase for a long time (Wek, Cannon, Dever, & Hinnebusch, 1992), but it was only recently that its eIF2-targeting mechanism was uncovered. Yeast cells lack clear homologs of CReP and GADD34 and instead Glc7 binds to PP1 via a unique amino terminal domain (NTD) extension found in the yeast eIF2 gamma subunit that shares motifs with other PP1 regulatory subunit interaction motifs (Rojas, Gingras, & Dever, 2014). Thus, the yeast targeting/regulatory protein is actually a domain of the PP1 substrate eIF2. The eIF2γ domain was proposed to function as a constitutive targeting domain analogous to CReP function in mammalian cells rather than the stress inducible GADD34 (Rojas et al., 2014).

In summary, the ISR provides a regulatory loop to modulate translation and transcription events in response to diverse cell stresses to control cellular activity and restore homeostasis. When stress is prolonged, or cannot be resolved it results in a range of different diseases. At the hub of this regulatory mechanism is the phosphorylation of eIF2 that controls translation initiation, reducing the rate of global 5′ cap-dependent events and activating translation of ISR-responsive genes (Figure 1). This regulation requires a series of interactions between eIF2, eIF5, and eIF2B that are modulated by the change in phosphorylation status of eIF2. In the next sections of this review, the current understanding of the roles and interactions of these factors is described along with a speculative step-by-step model for eIF2 activation and its control in the ISR.

## 3 | THE ROLE OF eIF2 IN THE INITIATION OF PROTEIN SYNTHESIS

Accurate initiation of protein synthesis is critical for cells to express the correct proteins. eIF2 is central to this process as it binds to initiator tRNA ($tRNA_i^{Met}$) in the cytoplasm of all eukaryotic cells and delivers it to ribosomes, where it helps locate the AUG codon on a bound mRNA. Cells specify separate initiator and elongator methionyl tRNAs that can base-pair with AUG codons. eIF2 binds specifically to $tRNA_i^{Met}$. This tRNA specificity is determined by a combination of differences between the elongator and initiator tRNA sequences and RNA base modifications that ensure eIF2 binds to $tRNA_i^{Met}$ and elongation factor eEF1A does not (Kolitz & Lorsch, 2010). eIF2 is a guanine-nucleotide binding protein and its GDP/GTP-bound status dramatically alters its affinity for $tRNA_i^{Met}$, such that the GTP-bound form has high affinity for $tRNA_i^{Met}$ (1 nM), while the eIF2–GDP complex has 50- to 100-fold lower affinity (Algire, Maag, & Lorsch, 2005; Jennings, Kershaw, Adomavicius, & Pavitt, 2017). The eIF2–GTP–$tRNA_i^{Met}$ complex, typically called the TC, is a key intermediate in the translation initiation pathway (Figures 1 and 3). eIF2 itself is a heterotrimer of α, β, and γ subunits. eIF2γ is the core subunit that binds GDP/GTP and $tRNA_i^{Met}$, while eIF2α assists with $tRNA_i^{Met}$-binding and AUG codon recognition. eIF2β makes multiple interactions with eIF2 ligands as well as other translation factors important for eIF2 function in translation and its control (Figure 3; Dever et al., 2016; Hinnebusch, 2017; Hussain et al., 2014; Llacer et al., 2015).

In the cap-dependent initiation pathway, eIF2 binds to the small ribosomal subunit (40S) to form a preinitiation complex (PIC) with eIF1, eIF1A, eIF3, and eIF5. This PIC binds an eIF4F-bound mRNA near to the 5′ methylguanosine cap structure.

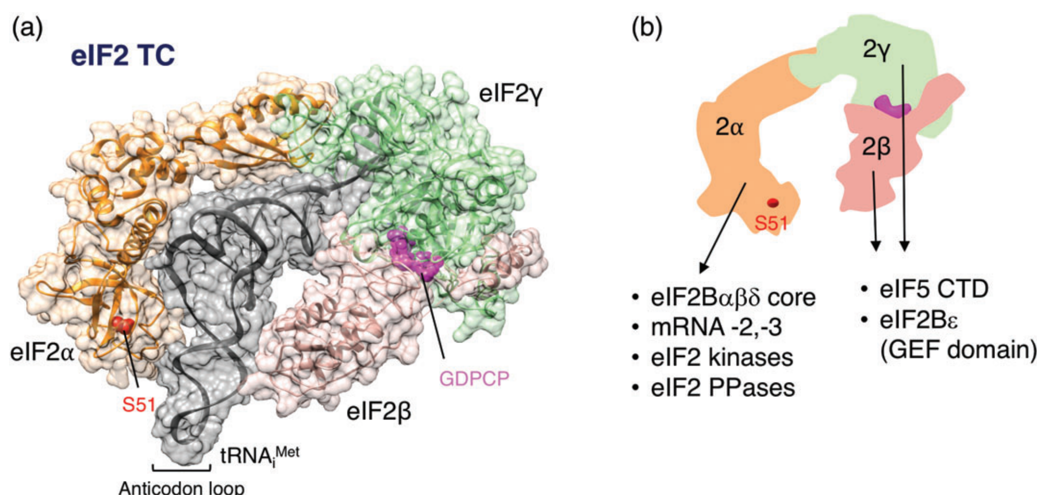


**FIGURE 3** The structure of eIF2 TC. (a) eIF2 bound to $tRNA_i^{Met}$ and the GTP analog GDPCP, drawn with Chimera software using the PDB file 3JAP. A transparent surface and secondary structure elements (SSE) are shown. $Ser^{51}$ is highlighted in space-fill (red). eIF2 shown is taken from a larger partial yeast PIC structure (PDB file 3JAP) (Llacer et al., 2015). (b) Cartoon image indicating major protein interaction partners important in the ISR

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eIFs 4A and 4B help eliminate mRNA secondary structure to promote PIC–mRNA interactions (Hinnebusch, 2014; Merrick & Pavitt, 2018). Recent evidence from in vitro reactions suggests that the PIC is threaded on to the mRNA so that $tRNA_i^{Met}$ can recognize AUG codons close to the 5′ cap (Kumar, Hellen, & Pestova, 2016). From here, eIFs 1, 1A, 3, 4A, and 4B promote "scanning" of the PIC along the mRNA 5′ leader seeking an AUG codon in an appropriate context for base-pairing and initiation. Typically the first encountered AUG in a good context is used to initiate protein synthesis. Optimal AUG codons include a purine nucleotide at the −3 position. eIF2α makes contacts here that help in AUG recognition (Hussain et al., 2014; Llacer et al., 2015; Pisarev et al., 2006). eIFs 1, 2, 3, and 5 as well as the 40S ribosome all contribute to accurate AUG recognition. This has been demonstrated in yeast by the isolation of mutations in each of these factors (termed $Sui^-$ mutations) that enable enhanced initiation at UUG and reduced AUG recognition (Dever et al., 2016). Importantly, structural analyses have demonstrated the residues mutated in each factor typically mediate important interprotein or protein–RNA contacts critical for accurate recognition of AUG codons, highlighting the critical contribution of each factor to this process (Hinnebusch, 2017).

On start codon recognition there is a major rearrangement of factors, 40S and $tRNA_i^{Met}$ that accompany relocation and subsequent release of eIF1 (Cheung et al., 2007). These distinct conformations are called "open" (scanning) and "closed" (AUG recognition) states. Recent structural analysis of partial yeast initiation complexes has revealed many of the conformational changes in molecular detail (Hussain et al., 2014; Llacer et al., 2015). In either state, the GTP bound to eIF2 is hydrolysed to GDP + phosphate (Pi) and upon AUG recognition the complex reorganization likely acts as a signal to permit Pi release. The resulting eIF2–GDP has significantly reduced affinity for $tRNA_i^{Met}$, prompting eIF2–GDP release from $tRNA_i^{Met}$ and the PIC. These events likely commit the ribosome to initiating at the bound AUG by prompting 60S joining so that the translation elongation phase can proceed (Hinnebusch, 2014; Hinnebusch, 2017; Merrick & Pavitt, 2018). Released factors participate in new rounds of initiation on the same or other mRNAs.

## 4 | TRANSLATIONAL CONTROL BY THE COMBINED ACTIONS OF EIF2B AND EIF5

Key to both continued translation initiation and its regulation by the ISR is the regulation of the activity of eIF2. eIF2 is a member of the conserved G protein superfamily and its nucleotide status is modulated by the translation factors eIF5 and eIF2B that function analogously to regulators of other G proteins.

### 4.1 | eIF5

eIF5 acts as a GTPase-activating protein (GAP) to promote hydrolysis of eIF2–GTP to eIF2–GDP. eIF5 GAP only acts within the context of a 40S-bound TC during scanning or at AUG codons. It does not hydrolyse GTP within free TC/eIF5 complexes (Algire et al., 2005; Majumdar & Maitra, 2005; Paulin, Campbell, O'Brien, Loughlin, & Proud, 2001), suggesting that other factors and/or the 40S ribosome contribute to GAP action. GAP activity requires the NTD of eIF5 ($eIF5^{NTD}$) (Algire et al., 2005; Majumdar & Maitra, 2005; Paulin et al., 2001). The position of eIF5 within the 48S PIC was not well resolved in available structures (Hussain et al., 2014; Llacer et al., 2015; Simonetti et al., 2016). Indeed eIF5 may change position within the complex between scanning and AUG recognition steps prior to its release from initiating ribosomes with eIF2–GDP (Obayashi et al., 2017).

eIF5 binds to both eIF2β and γ subunits (Alone & Dever, 2006; Asano, Krishnamoorthy, Phan, Pavitt, & Hinnebusch, 1999; Das, Maiti, Das, & Maitra, 1997; Jennings & Pavitt, 2010a) and with equal affinity for both eIF2–GDP and TC forms (Algire et al., 2005) (Figure 3b). Hence eIF2–GDP is released from the PIC in complex with eIF5 (Singh et al., 2006) Although eIF2 binds GDP more tightly than GTP (Erickson & Hannig, 1996; Jennings et al., 2016), eIF5 further enhances the affinity of yeast eIF2 for GDP and therefore prevents premature eIF2B-independent release of GDP from eIF2γ (Jennings et al., 2016; Jennings & Pavitt, 2010b). This GDP dissociation inhibitor (GDI) activity requires both the eIF5 carboxy-terminal HEAT domain (CTD) and conserved residues within the linker region (termed the DWEAR motif) that joins the independently folded $eIF5^{NTD}$ and $eIF5^{CTD}$ (Jennings & Pavitt, 2010b). eIF2β also contributes to the GDI activity, as a missense mutation in eIF2 beta mimics GDI mutations that inactivate eIF5 GDI (Jennings et al., 2016). In yeast, GDI mutants bypass the normally tight control of translation initiation and impair the activation of *GCN4* translation during stress. Thus, the GDI function is important for tight phosphoregulation of eIF2 during the ISR because it likely limits unregulated eIF2B-independent release of GDP from eIF2. It is not yet clear if eIF2–GDP/eIF5 interactions are important in the mammalian ISR. GDP is more tightly bound to human eIF2 than the yeast complex and was not further stabilized by eIF5 in vitro (Sokabe, Fraser, & Hershey, 2012), but the DWEAR motif is conserved (Jennings & Pavitt, 2010b), and overexpression of eIF5 in human cells activates ATF4 reporter plasmids, in a manner similar to prior findings overexpressing eIF5 in yeast cells (Asano et al., 1999; Kozel et al., 2016; Singh et al., 2006). These data and recent modeling approaches suggest that eIF2–GDP/eIF5

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interactions are critical for the yeast ISR and may be relevant to mammalian translation and the ISR (Bogorad, Lin, & Marintchev, 2018; Khan, Spurgeon, & von der Haar, 2018).

### 4.2 | eIF2B

To activate eIF2 and promote translation, eIF2–GDP/eIF5 complexes are actively dissociated by free eIF2B, enabling nucleotide exchange to form eIF2–GTP (Jennings, Zhou, Mohammad-Qureshi, Bennett, & Pavitt, 2013). Thus, eIF2B is both a GDI-displacement factor (or GDF) and a guanine-nucleotide exchange factor (GEF) for eIF2 (Jennings & Pavitt, 2014). In yeast, both activities are necessary to activate eIF2 to promote TC formation for each round of protein synthesis initiation as mutations that primarily affect GDF (Jennings et al., 2013) or GEF (Gomez & Pavitt, 2000; Mohammad-Qureshi, Haddad, Hemingway, Richardson, & Pavitt, 2007) activities are known. Only the GEF function has been studied for human eIF2B, where mutations reducing eIF2B GEF cause the fatal leukoencephalopathy childhood ataxia with central nervous system hypomyelination/ vanishing white matter disease (CACH/VWM; OMIM 603896) (Fogli & Boespflug-Tanguy, 2006; Li, Wang, Van Der Knaap, & Proud, 2004; Scheper, Proud, & van der Knaap, 2006), although not all CACH/VWM mutations appear to impair GEF activity in vitro (Liu et al., 2011). CACH/VWM is discussed further in a separate section.

eIF2B is a large protein complex with subunits designated α-ε encoded by five genes (*EIF2B1–5* in humans) that was recently shown to be a decamer complex (a dimer of pentamers) approaching 600 KDa (Gordiyenko et al., 2014; Kashiwagi et al., 2016; Wortham, Martinez, Gordiyenko, Robinson, & Proud, 2014). The crystal structure of *Schizosaccharomyces pombe* eIF2B shows that it has a "regulatory" hexameric core in which an eIF2B βδ tetramer is bound to an α dimer that sits on the "back", forming two symmetrical heterotrimers, each capable of binding eIF2α (Kashiwagi et al., 2016; Figure 4a and b), in agreement with prior structural studies of the eIF2Bβδ interactions (Bogorad et al., 2014; Kuhle, Eulig, & Ficner, 2015). A wealth of genetic and biochemical studies implicate the eIF2Bαβδ core subunits in direct binding of eIF2α (Figure 4c; Dev et al., 2010; Dey, Trieselmann, et al., 2005; Kashiwagi et al., 2016; Kimball, Fabian, Pavitt, Hinnebusch, & Jefferson, 1998; Krishnamoorthy, Pavitt, Zhang, Dever, & Hinnebusch, 2001; Pavitt, Ramaiah, Kimball, & Hinnebusch, 1998; Pavitt, Yang, & Hinnebusch, 1997; Vazquez de Aldana, Dever, & Hinnebusch, 1993). Binding to eIF2α is enhanced by $ser^{51}$ phosphorylation (Kashiwagi et al., 2016; Krishnamoorthy et al., 2001; Pavitt et al., 1998) and this is likely critical for the ISR, as eIF2Bαβδ mutants weakening binding of eIF2 impair the ISR in yeast and mammalian models (Elsby et al., 2011; Kimball et al., 1998; Krishnamoorthy et al., 2001).

Linked to the regulatory hexameric core of eIF2B are two "arms" each composed of single ε and γ subunits (Figure 4a,c). It is these "arms" that are responsible for eIF2B GEF and GDF activities via interactions with eIF2βγ (Alone & Dever, 2006; Jennings et al., 2013). For both yeast and mammalian eIF2B, it is the largest subunit, eIF2Bε, that is both necessary and sufficient for GEF action (Fabian, Kimball, & Jefferson, 1998; Pavitt et al., 1998). The eIF2Bε CTD forms HEAT repeats (Boesen et al., 2004) and is minimally sufficient to promote GDP release from eIF2 (de Almeida et al., 2013; Gomez, Mohammad, & Pavitt, 2002), so is here called the GEF domain ($2B\varepsilon^{GEF}$). GEF activity is stimulated by interaction of eIF2Bε with the other eIF2B subunits (Fabian et al., 1998; Pavitt et al., 1998). Remarkably, the decameric structure shows that each eIF2Bε subunits makes extensive direct contacts with one of each of the two eIF2B β, γ, and δ subunits within the decamer (Figure 4c, top). Additionally, there is contact between each 2Bε subunit and both 2Bα subunits via the 2Bε beta-helical domain. Hence the extensive network of protein–protein interactions that link the eIF2B complex together (Figure 4c) are likely important for promotion and inhibition of eIF2 recycling in the ISR.

Minimally, the yeast eIF2Bγε sub-complex that forms a single "catalytic arm" of the structure is fully active in both GEF and GDF assays (Jennings et al., 2013; Pavitt et al., 1998) as is a larger eIF2Bβγδε complex. This latter complex lacks the alpha subunit and is defective for ISR control of eIF2B, but is a stable octamer (dimer of tetramers) with full GEF and GDF activities (Gordiyenko et al., 2014; Jennings et al., 2013). In contrast data for mammalian eIF2B subunit requirements for GEF action is more varied. Although rat eIF2Bβγδε complexes (lacking 2Bα) purified from insect cells behaved similarly to the yeast protein (Fabian et al., 1998), human eIF2Bβγδε isolated from HeLa cells was found to have lower activity than the full eIF2B complex (Williams, Price, Loughlin, & Proud, 2001). Human eIF2Bβγδε can dimerise, like the yeast complex, but its octamer form appears less stable during purification and the tetramer complex binds less well to eIF2 than the full decamer, which likely contributes to its lower GEF activity (Wortham et al., 2014). The compound ISRIB acts to promote eIF2B activity in part through stabilizing the decamer form (Sidrauski, Tsai, et al., 2015; see next section).

Evolutionarily conserved eIF2Bε residues critical for GEF activity include a glutamate in $2B\varepsilon^{GEF}$ (yeast E569 or human E577) (Boesen et al., 2004; de Almeida et al., 2013; Wang & Proud, 2008). The $2B\varepsilon^{GEF}$ domain was not resolved in the decamer crystal structure and so how it interacts with the decamer and eIF2 complexes remain unclear. One clue comes from an "NFD" motif of adjacent conserved residues in the eIF2Bε NTD. Although GEF activity is normally boosted by eIF2B complex formation, mutations at the NFD motif impair or prevent enhanced activity (Gomez & Pavitt, 2000). The *S. pombe* crystal structure (Figure 4a) shows that the NFD residues are solvent exposed on the arms. It is possible that they either contact the

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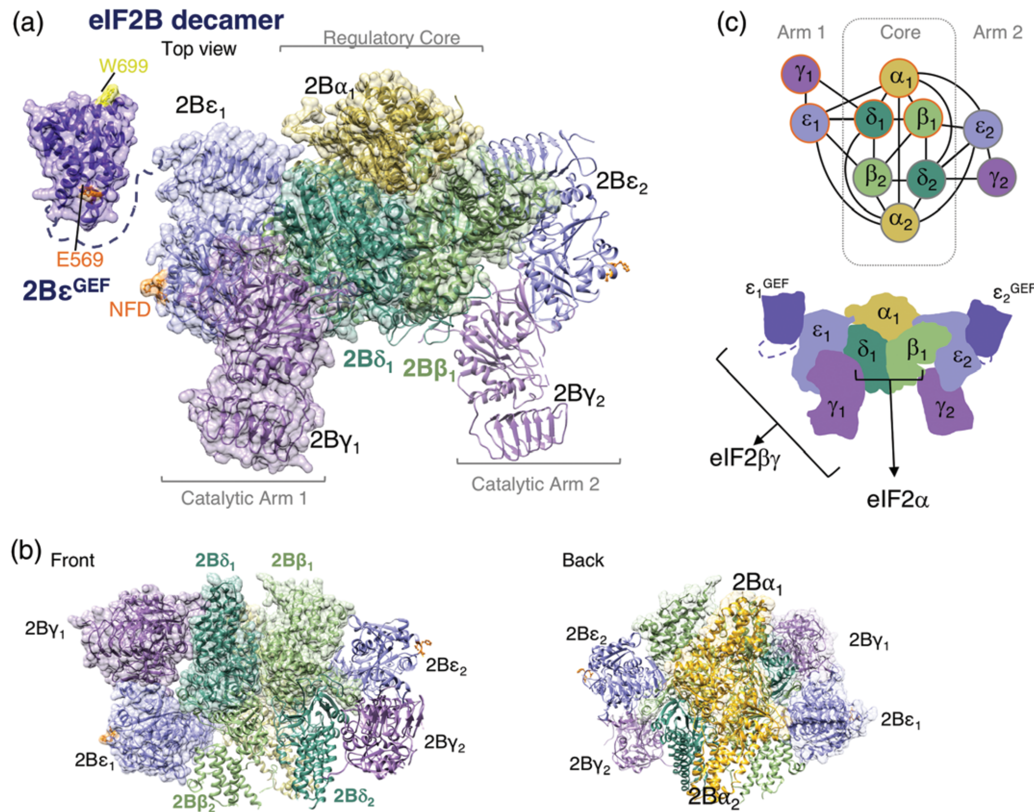


**FIGURE 4** The structure of eIF2B. (a) "Top view" of the eIF2B decamer from *Schizosaccharomyces pombe* (PDB file 5B04) (Kashiwagi et al., 2016) showing one half of the regulatory core and catalytic arm 1 as both transparent surface and SSE and catalytic arm 2 as SSE only. One copy of the independent $2B\varepsilon^{GEF}$ domain from *Saccharomyces cerevisiae* (PDB file 1PAQ) (Boesen, Mohammad, Pavitt, & Andersen, 2004) is also shown with SSE and transparent surface. Residues discussed in the text are highlighted. (b) Rotated views of eIF2B decamer as in panel (a), with one half in SSE only view. "Front" view (left) showing the $(2B\beta\delta)_2$ core and "back" view (right) showing the 2Bα dimer. (c) Cartoons showing intersubunit interactions as lines connecting circles representing each subunit within the decamer (top) and eIF2 interaction regions (bottom)

GEF domain or eIF2 to enhance GEF activity of the complex. Together these observations indicate that eIF2B is a large decamer complex with a central eIF2α-binding core and two lateral arms that enable eIF2 activation for protein synthesis initiation (Figure 4).

## 5 | A MODEL FOR ISR CONTROL OF TC FORMATION

It remains unclear precisely how eIF2B stimulates TC formation or how eIF2α $ser^{51}$ phosphorylation prevents this. In the current scheme (Figures 1 and 5), eIF2–GDP/eIF5 complexes bind eIF2B ejecting eIF5. GDP release from eIF2γ and its replacement by GTP is the major regulated reaction, enabling eIF2–GTP to bind $tRNA_i^{Met}$ to form TC. TC is stabilized by binding to eIF5 in a step that prevents eIF2B reversing these reactions (Jennings et al., 2017). The precise order and molecular events are beginning to be resolved. A model to explain these events is presented here.

### 5.1 | Initial binding of eIF2

Key to the inhibition of eIF2B by eIF2(αP) is how eIF2B interprets the phosphorylation status of eIF2. Multiple studies show that the eIF2B core regulatory subunits bind to both phosphorylated and unphosphorylated eIF2, hence an early phosphosensing 2α/2Bαβδ interaction should occur (Krishnamoorthy et al., 2001; Pavitt et al., 1997, 1998). Recent cross-linking experiments also indicate that the 2α/2Bαβδ interactions are not dependent on $ser^{51}$ phosphorylation (Kashiwagi et al., 2016). Precisely how eIF2α binds eIF2B is not yet resolved, although various possibilities are suggested based upon the structural and cross-linking data available (Bogorad, Lin, & Marintchev, 2017; Kashiwagi et al., 2016; Kuhle et al., 2015). In addition recent NMR analyses show that phosphorylation stabilizes an extended open conformation of eIF2α that could contribute to control (Bogorad et al., 2017). Importantly the affinity of eIF2B for eIF2(αP) is enhanced over its affinity for unphosphorylated eIF2 by approximately 10- to 100-fold depending on assay conditions (Jennings et al., 2017; Rowlands, Panniers, & Henshaw, 1988). eIF2(αP) stabilizes eIF2α binding to the 2Bαβδ regulatory complex (Krishnamoorthy et al., 2001; Pavitt

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et al., 1998) and is a competitive inhibitor of nucleotide exchange (Rowlands et al., 1988). Hence phosphorylation both prevents the bound eIF2(αP) engaging productively with the GEF domain in eIF2Bε as well as interferes with exchange of non-phosphorylated eIF2–GDP.

There may be little difference in the initial binding of both forms of eIF2 to eIF2B. If eIF2(αP) has a slower off-rate than eIF2 from eIF2B, this could contribute to control. It is not clear if eIF2α also directly contacts the eIF2Bγε catalytic arm, or if eIF2 binding to the regulatory core causes a conformational change in either eIF2 or eIF2B that is important for GEF function or ISR control, mediated in part perhaps via altered contacts between eIF2Bε and the other eIF2B subunits. Finally, as there are two regulatory binding sites for eIF2α in the eIF2B decamer it is likely that two molecules of eIF2 bind simultaneously. In an attempt to depict one possible scheme for eIF2 activation and its control by the ISR a sequence of cartoons is shown in Figure 5 outlining steps in the interaction between one eIF2 and one eIF2B decamer. Once freed from the constraints of $tRNA_i^{Met}$ interactions within the TC (Figure 3), eIF2 may enjoy conformational flexibility enabling an opening out of eIF2 to facilitate its interaction with the regulatory core of eIF2B (Figure 5; steps 1 and 7).

## 5.2 | eIF5 release and guanine nucleotide exchange

Active release of eIF5 from a transient eIF2–GDP/eIF5/eIF2B complex (GDF action) requires eIF23Bγε and occurs both for phosphorylated and unphosphorylated eIF2 (Jennings et al., 2013). This could occur before, concurrently with eIF2α binding to eIF2B, or immediately following it (Figure 5, steps 2 and 8). As $2Bε^{GEF}$ and $eIF5^{CTD}$ share similar structures (Bieniossek et al., 2006; Boesen et al., 2004; Wei, Xue, Xu, & Gong, 2006) and eIF2βγ binding sites (Alone & Dever, 2006; Asano et al., 1999; Das et al., 1997; Jennings & Pavitt, 2010a), hence eIF5 likely occupies a position on eIF2 that would prevent productive $2Bε^{GEF}$ interaction. While eIF5-binding to eIF2 can stabilize GDP binding (GDI activity), $2Bε^{GEF}$ has the opposite impact and facilitates rapid GDP release (Jennings et al., 2013) (step 3). In simpler GTP-binding proteins, diverse GEFs bind directly to their cognate G-protein enabling deformation of the conserved “switch I” and “switch II” regions that contribute to both nucleotide and magnesium ion binding. This promotes GDP release and enables GTP binding (Bos, Rehmann, & Wittinghofer, 2007; Sprang & Coleman, 1998; Thomas, Fricke, Scrima, Berken, & Wittinghofer, 2007). However, as both eIF2 and eIF2B have additional subunits, normal rules may not fully apply. In the partial 48S TC structure eIF2β abuts the eIF2γ bound

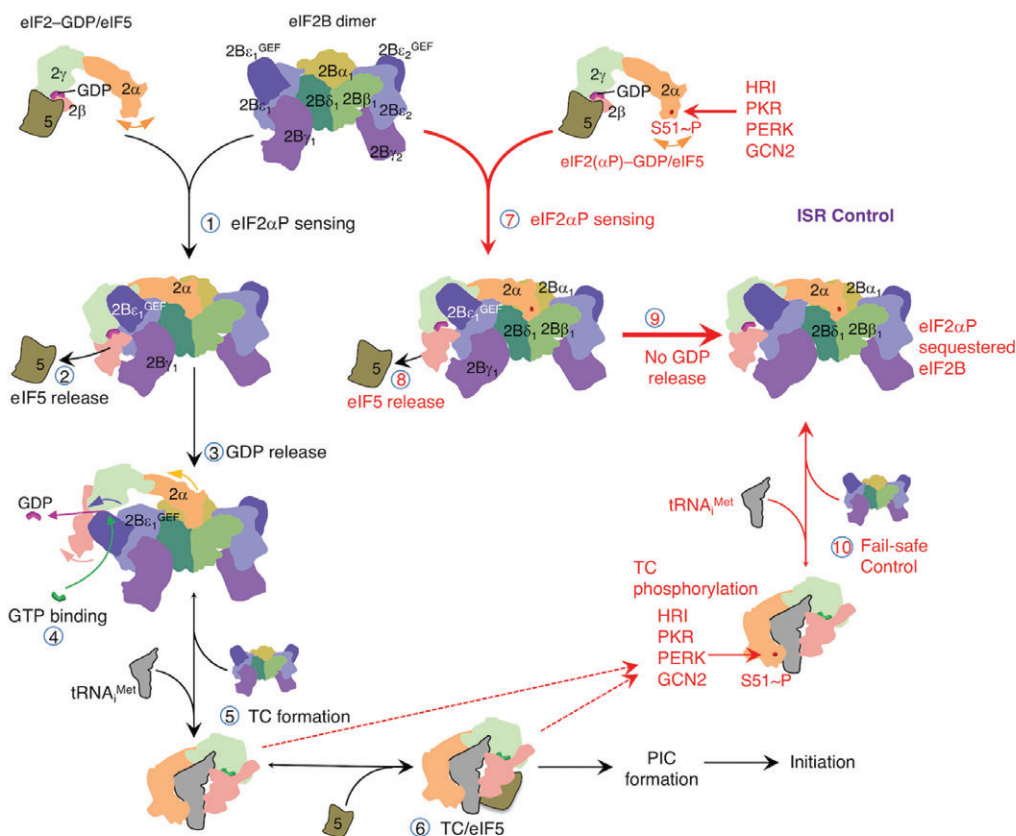


**FIGURE 5** Model for eIF2/eIF2B interactions during eIF2 activation and in the ISR. Cartoon model of eIF2 activation (black arrows, steps 1–6) and the ISR (red arrows, steps 7–10) based on the structures shown in Figures 3 and 4 and the interactions described in detail in the text. Subunit-colored curved arrows are used to indicate movement or conformational flexibility

GTP analog (Figure 3), but adopts different positions in the "open" (scanning) and "closed" (AUG recognition) conformations (Llacer et al., 2015). In addition, a mutation in a yeast eIF2β residue (S264Y) adjacent to the bound nucleotide, promotes enhanced eIF5-independent GTP hydrolysis (Huang, Yoon, Hannig, & Donahue, 1997), indicating that eIF2β–2γ interactions modulate nucleotide affinity. Other observations show that eIF2β is critical for both eIF5 and eIF2B functions (Asano et al., 1999). Together various findings suggest that altering eIF2β–2γ interactions contributes to eIF2B GEF action. This is shown in Figure 5 (steps 3 and 4) as $2B\varepsilon^{GEF}$ promoted movement of eIF2β away from eIF2γ to expose the bound GDP and promote its release.

### 5.3 | How does phosphorylation inhibit eIF2B GEF?

We know that it is the guanine nucleotide exchange that is inhibited by the ISR, just not precisely how. Phosphorylation enhances eIF2 binding to eIF2B and missense mutations that weaken this interaction, either within the eIF2B regulatory core or within eIF2α itself can impair ISR regulation without significantly reducing the rate of GDP release (Dey, Trieselmann, et al., 2005; Kimball et al., 1998; Krishnamoorthy et al., 2001). Hence tight binding to phosphorylated eIF2α is a major factor preventing GEF action with eIF2(αP). As even a conservative E569D mutation in yeast eIF2Bε eliminates GEF activity with eIF2 (Mohammad-Qureshi et al., 2007), this suggests that only a small conformational change between the binding of eIF2 and eIF2B induced by phosphorylation may be sufficient to prevent GEF activity. This may involve one of two options. Phosphorylation may cause eIF2 to adopt a bound conformation where eIF2γ is held away from $2B\varepsilon^{GEF}$, or perhaps the opposite is true and eIF2(αP) is held too close to the main eIF2B decamer structure such that there is not enough space for $2B\varepsilon^{GEF}$ to engage productively with eIF2βγ. This latter idea is shown in Figure 5, step 9.

### 5.4 | TC/eIF5 formation, the final steps in nucleotide exchange?

Following nucleotide exchange, eIF2–GTP has a high affinity for $tRNA_i^{Met}$ to form TC (Algire et al., 2005). However, eIF2B can also remain bound to eIF2–GTP and eIF2B can compete with $tRNA_i^{Met}$ for binding to eIF2, thereby effectively reducing the rate of TC formation (Jennings et al., 2017). Hence eIF2B can undo the eIF2 activation step it promotes! This could potentially act as a regulatory point in the ISR, particularly if there are low levels of $tRNA_i^{Met}$ or other initiation factors that engage with TC available locally. Earlier studies suggested that efficient GTP binding to eIF2/eIF2B complexes required $tRNA_i^{Met}$, implying that $tRNA_i^{Met}$ binding may precede eIF2B release (Gross, Rubino, & Hessefort, 1991). However, recent experiments suggest that nucleotides have only minor impact on overall eIF2/eIF2B affinity (Jennings et al., 2017). Hence the precise order of $tRNA_i^{Met}$ binding and eIF2B release to form TC requires further investigation (Figure 5, step 5). The binding of eIF5 to TC to form TC/eIF5 complexes occurs with high affinity and effectively prevented eIF2B from disrupting TC (Jennings et al., 2017). Together these results suggest that TC/eIF5 complexes likely represent the true final product of eIF2B promoted nucleotide exchange and eIF2 activation for protein synthesis (Figure 5, step 6). There is therefore a distinction between eIF2–GDP/eIF5 complexes and TC/eIF5 complexes, as the former can be rapidly dissociated by eIF2B and the latter are resistant to eIF2B (Jennings et al., 2013, 2017). As eIF2 and eIF5 are typically equimolar in cells, with eIF2B present in lower amounts, the data suggest that there is little if any free eIF2 or free TC and that it is always bound to either eIF5 or eIF2B.

### 5.5 | Fail-safe control of the ISR

In the ISR inhibition of eIF2B GEF action is the major regulatory event. However, observations that eIF2B can bind to both eIF2–GTP and TC, prompted an investigation of its potential to regulate additional complexes as part of the ISR. It was found that eIF2B could disrupt both TC and TC/eIF5 complexes when eIF2 was prephosphorylated at ser[51]. The observations suggest that any free eIF2B not fully bound by eIF2(αP)–GDP is able to disrupt TC and TC/eIF5 complexes containing phosphorylated eIF2. This was termed "fail-safe control" and represents a route for eIF2B to capture additional forms of eIF2. For example, any eIF2 that is phosphorylated while already part of TC or TC-containing complexes may be a substrate for fail-safe control. This adds a new layer to the ISR (Figure 5, step 10).

As this model shows, considerable progress has been made in uncovering the molecular basis of ISR control of eIF2B in recent years, but that important questions remain to be resolved. An understanding of precisely how eIF2 and eIF2B interact at different steps would help place the existing genetics and biochemistry in a better context.

## 6 | DISORDERS WITH AN ELEVATED ISR

Mutations in both eIF2 and eIF2B have been found to cause distinct inherited human disorders that affect brain functions eIF2γ mutations cause MEHMO syndrome (OMIM 300148) after its broad range of symptoms: mental retardation, epileptic

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seizures, hypogonadism and hypogenitalism, microcephaly, and obesity. In contrast, mutations in any eIF2B subunit cause CACH/VWM (Leegwater et al., 2001; van der Knaap et al., 2002). Both of these disorders affect the brain, but primarily different cell types. Each disorder has the potential to cause a chronic or heightened ISR response. In contrast, mutations in the Perk kinase gene *EIF2AK3* cause Wolcott–Rallison syndrome (WRS; OMIM 226980), a severe form of insulin-dependent diabetes (Julier & Nicolino, 2010). Mutations in the Gcn2 gene *EIF2AK4* cause pulmonary venoocclusive disease-2 (PVOD2; OMIM 234810), a form of pulmonary hypertension (Best et al., 2014; Eyries et al., 2014). The eIF2 kinase mutations reduce or eliminate eIF2 kinase activity and so limit the ability of affected cells to respond to specific ISR stresses and hence cause a chronic reduction in the ISR in response to specific signals. These genetic disorders highlight that distinct pathologies result when the ISR is not appropriately controlled and that ISR defects can affect a wide range of tissues. Here MEHMO, CACH/VWM and conditions where elevated eIF2(αP) and ISR activity contribute to pathology are discussed.

## 6.1 | MEHMO

Mutations in eIF2γ gene (*EIF2S3*) cause MEHMO syndrome. It was also termed severe X-linked intellectual disability syndrome (Borck et al., 2012; Moortgat et al., 2016; Skopkova et al., 2017). MEHMO is very rare and only described in males who have inherited a defective allele from their symptomless mothers. The symptoms are consistent with neurons being the primary affected cells. The mutations have been shown to impair eIF2 complex formation and interactions with eIF2B, that lead to inappropriate activation of the ISR in a yeast cell model, and in patient fibroblasts (Borck et al., 2012; Skopkova et al., 2017). Hence the cell phenotypes and biochemistry are consistent with the idea that MEHMO mutations cause constitutively reduced eIF2 activity, albeit to various degrees, which cause an elevated basal level of the ISR and may also cause other defects such as reducing the fidelity of AUG start codon recognition, as found in yeast. As eIF2γ alleles are only recently described, there are limited studies of this human disorder to date.

## 6.2 | CACH/VWM

Mutations in any of the five eIF2B genes (*eIF2B1–5*) cause CACH/VWM (Leegwater et al., 2001; van der Knaap et al., 2002). MRI imaging of CACH/VWM patients typically reveals widespread loss of white matter, a likely cause of ataxia (Leegwater et al., 2001). White matter glial cells include oligodendrocytes, which form myelin sheaths that wrap axons of neurons and astrocytes. Both these glial cell types are affected. In some patients CACH/VWM is linked to premature ovarian failure (Fogli et al., 2003), while multiorgan defects have been observed in severe cases that have antenatal- or early-infantile onset (van der Knaap et al., 2003). CACH/VWM has autosomal recessive inheritance and appears more common than MEHMO, with approaching 200 different mutations described in the Human Gene Mutation Database (www.hgmd.cf.ac.uk). Biochemical analyses have revealed that mutations can affect eIF2B complex integrity, eIF2 interaction, and/or GEF activity (de Almeida et al., 2013; Fogli & Boespflug-Tanguy, 2006; Li et al., 2004; Richardson, Mohammad, & Pavitt, 2004; Scheper et al., 2006; Wortham & Proud, 2015). However, some mutations cause severe disease without apparently affecting these roles, as assayed in vitro (Liu et al., 2011; Wortham & Proud, 2015). It remains possible that eIF2B activity is compromised in vivo in ways not accounted for in assays performed with purified factors. For example, whether CACH/VWM mutations that do not affect GEF activity have defects in the displacement of eIF5 from eIF2–GDP/eIF5 complexes, as shown for some yeast eIF2Bγ mutants (Jennings et al., 2013), has not yet been tested.

In affected patient glial cells, both astrocytes and oligodendrocytes, there are elevated ISR and UPR responses that likely cause aberrant cell morphology and apoptosis (van der Voorn et al., 2005; van Kollenburg et al., 2006). Other primary cells from patients are also particularly sensitive to stress and elicit a heightened ISR response (Kantor et al., 2005). Intriguingly, recent studies suggest that each cell type is critical for CACH/VWM. Mutant myelin sheaths wrapping axons are thin (hypomyelination) and the cells have abnormal appearance. An excess of abnormal oligodendrocytes with foamy cytoplasm was observed in patient cerebral tissue (Wong et al., 2000). Similar observations were made when eIF2B activity was reduced in an experimental mouse model where PERK expression was specifically and highly induced only in oligodendrocytes. The brains of these animals reproduced features of CACH/VWM including hypomyelination, foamy cytoplasm, and myelin loss. The disease-like effects were only observed when PERK expression was induced in young mice. In adult animals impaired eIF2B activity had minimal effects on glial cell morphology or function (Lin et al., 2014). These results point to CACH/VWM being a developmental disorder. Presumably higher eIF2B activity is required in oligodendrocytes within the developing brain when both neurons and glial cells are growing and forming important connections, than in later life when these networks have been established. Hence hyperactivation of the ISR caused by excess PERK, or by eIF2B mutations leads to similar oligodendrocyte pathology.

In tandem with the effects on oligodendrocytes, astrocytes are also affected and important in CACH/VWM (Rodriguez, 2013). Where analyzed, preserved white matter has a paucity of astrocytes. Few primary astrocytes could be cultured from

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CACH/VWM patient-derived neural progenitor cultures, and those cells obtained had abnormal morphology, and reducing eIF2Bε expression by RNAi of *EIF2B5* in glial progenitor cells similarly affected astrocyte development (Dietrich et al., 2005). In mouse models of CACH/VWM where severe human mutations were mimicked in eIF2Bδ and eIF2Bε, mice developed symptoms similar to human CACH/VWM (Dooves et al., 2016). Here white matter astrocytes were abnormal and appeared immature. It was shown that CACH/VWM astrocytes could inhibit the maturation of oligodendrocyte progenitor cells in culture. Hyaluronan, one of many factors secreted by astrocytes inhibits oligodendrocyte maturation. The main hyaluronan synthesizing enzyme, HAS2, levels were elevated in CACH/VWM mutant astrocytes. This study therefore suggests that CACH/VWM may be initiated by defective astrocyte gene expression leading to a secondary oligodendrocyte maturation defect (Dooves et al., 2016).

### 6.3 | Why do eIF2 and eIF2B mutations cause distinct pathologies?

A recent study examining primary fibroblasts and astrocytes from a milder eIF2Bε mutant mouse (R132H) found that eIF2B mutations can cause defects in mitochondrial function and abundance altering cell respiration (Raini et al., 2017). As eIF2B and cytoplasmic translation is required to synthesize mitochondrial-localized proteins, it was proposed that impaired mitochondrial function caused by reduced eIF2B activity contributes to CACH/VWM pathology (Raini et al., 2017). This study points to the broad role of eIF2B (and eIF2) across all cytoplasmic translation, not just ISR targets and shows that a wide range of mRNAs and have differential sensitivity to reduced eIF2B activity.

Because both eIF2 and eIF2B function together in the same pathway and both MEHMO and CACH/VWM likely cause a constitutive reduction in active eIF2 levels, it is surprising that these diseases primarily impact different cell types and have distinct pathologies. However, they do share some overlapping symptoms, as reported in at least some patients (Skopkova et al., 2017). There are some parallels between the tissue-specific effects of both MEHMO and CACH/VWM and the ribosomeopathies, diseases such as Diamond–Blackfan anemia (DBA), where nine different ribosomal proteins are mutated (Boria et al., 2010). Explanations proposed to account for tissue-specific DBA defects include the idea that individual mRNAs are differentially dependent on ribosome numbers for their appropriate expression in different tissues (Mills & Green, 2017). Similar ideas may explain all these conditions. It seems plausible that different cell transcriptomes have variable sensitivity to defects in each component of the core translation machinery. For example, in one study eIF2B GEF activity was shown to be variably, but significantly reduced in immortalized lymphocytic cells from CACH/VWM patients, but the same cells had normal ISR/UPR responses, indicating these cells were relatively resistant to the lower eIF2B GEF activity (Horzinski et al., 2010). The data suggest that in these immortalized cells eIF2B expression levels were not limiting, hence although eIF2B GEF activity was reduced the ISR was not aberrantly activated. Such observations likely underlie the tissue-specific defects observed in these disorders.

### 6.4 | Pathologies associated with elevated eIF2 phosphorylation

Altered eIF2 phosphorylation and ISR activation contribute to a wider range of pathological conditions than those described above. These include a range of disorders where memory is altered or are neurodegenerative, such as schizophrenia (Trinh et al., 2012), Alzheimer's (Ma et al., 2013), prion disease (Moreno et al., 2012), amyotrophic lateral sclerosis (Kim et al., 2014), and head or other nerve trauma (Chou et al., 2017; Larhammar et al., 2017). Typically defects in the normal regulation of eIF2 phosphorylation or the expression of ISR-responsive mRNAs such as *ATF4* have been described. Many long-term memory defects have also been studied in mouse models of different diseases exhibiting ISR defects including Alzheimer's and prion diseases (Moreno et al., 2012). These mouse models further demonstrate the importance of a balanced ISR for memory in the brain (Batista, Johnson, Dominguez, Costa-Mattioli, & Pena, 2016; Buffington, Huang, & Costa-Mattioli, 2014; Costa-Mattioli et al., 2007; Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009) and have been useful to assess the therapeutic potential of ISR modifying compounds in whole animal models, as discussed below.

The ISR also plays roles in other diseases such as forms of cancer and during infections. Some findings appear contradictory, for example reduced levels of eIF2(αP) were found in osteosarcoma tumors, which was attributed to lower interferon signaling (Wimbauer et al., 2012). In contrast, increased Perk and eIF2(αP) levels were associated with acute myeloid leukemia (Kusio-Kobialka et al., 2012). Wide variations such as highlighted by these examples likely result from the distinct patterns of gene expression required in each tissue and tumor type.

In many studies, it is not clear whether the global lowering of protein synthesis brought about by eIF2B inhibition or the associated activation of ISR activated mRNAs is the more critical response. In the case of RNA virus infection, many viruses have evolved diverse mechanisms to interfere with PKR activation or its ability to phosphorylate eIF2. Multiple studies point to the requirement of viruses to utilize the host translation machinery to produce new viral particles and the cells response to limit this via inhibiting translation in infected cells (Walsh, Mathews, & Mohr, 2013). However, for enveloped viruses, Perk

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activation and the UPR are also important for cells to cope with high viral protein folding load. Similarly, any virus that can use an eIF2-independent mode of translation will also benefit from high levels of eIF2(αP) which will downregulate host mRNA translation (Walsh et al., 2013).

In a recent study, the issue of the relative contribution of global translational repression versus ISR mRNA activation was addressed experimentally. In malignant melanoma glutamine limitation was investigated as a nutrient that becomes limiting in solid tumors. Glutamine limitation promoted tumor invasion and activated the ISR and ATF4 expression. Following a wide range of experiments, it was found that when ATF4 induction was uncoupled from eIF2B inhibition it was not sufficient to drive tumor invasion, and that translational repression was also needed to promote cancer invasion (Falletta et al., 2017). These studies show that translational repression can be as important to the cellular stress response as activation of ATF4 and other downstream mRNA targets.

## 7 | COMPOUNDS THAT MODIFY THE ISR

Compounds have been identified that can modulate the activity of elements of the ISR. These include eIF2 kinase and phosphatase inhibitors as well as recently identified activators of eIF2B.

### 7.1 | eIF2 kinase inhibitors

Inhibitors of specific ISR kinases impair the ISR activation in response to specific stress signals. Among the compounds developed are Inhibitors of PERK and PKR activity. GSK2606414 (Axten et al., 2012) and GSK2656157 (Atkins et al., 2013) are inhibitors of PERK kinase activity. They are ATP site inhibitors that prevent activation the PERK arm of the ISR in response to UPR stress. These kinase inhibitors have been widely used in cell and disease model studies and have prevented disease symptoms developing in experimental models including in prion and Parkinson's disease models (Mercado et al., 2018; Moreno et al., 2013). However, they can cause pancreatic toxicity as seen when Perk is inactivated in WRS. It was recently shown that both compounds independently target and inhibit another kinase, RIPK1, blocking cell death and proinflammatory responses (Rojas-Rivera et al., 2017). These recent findings complicate conclusions drawn from the use of the molecules. A PKR inhibitor (C16) is also available. Similar to the PERK inhibitors it is an ATP-binding site directed inhibitor that has been used to attenuate PKR function in rodents (Xiao, Tan, Li, & Luo, 2016; Zhu et al., 2011).

### 7.2 | eIF2 phosphatase inhibitors

In contrast to kinase inhibitors, compounds that target eIF2 or eIF2B should moderate ISR activation in response to multiple signals. Salubrinal (Boyce et al., 2005), Guanabenz, and Sephin1 (Das et al., 2015; Tsaytler, Harding, Ron, & Bertolotti, 2011) are three PP1 inhibitors. As indicated previously, the CReP/PP1 complex acts as a constitutive phosphatase, while GADD34/PP1 is ISR induced. Salubrinal treatment elevates eIF2(αP) levels in both stressed and unstressed cells (Boyce et al., 2005) and has been widely used in experimental studies, although it also targets the antiapoptotic protein Bcl-2 (Kessel, 2006). In contrast, Guanabenz and Sephin1 are related compounds that were found to inhibit the stress-induced eIF2α PP1 by targeting its regulatory subunit GADD34 (Das et al., 2015; Tsaytler et al., 2011). By targeting only the ISR-induced PP1 subunit, these inhibitors prolong the time that eIF2(αP) is elevated, without affecting unstressed cells. As they do not target the activity of the constitutive CReP/PP1 complex, they can be used in vivo. ISR gene expression is altered by either PP1 inhibitor treatment. For example, early ISR responsive genes such as ATF4 are maintained, but later activated proteins including BiP and GADD34 itself were repressed by the reduced levels of protein synthesis (Das et al., 2015). Sephin1 was found to suppress neurodegeneration in a mouse model of disease, while guanabenz has been used to treat hypertension (Holmes, Brogden, Heel, Speight, & Avery, 1983). However, recent results examining PP1 activities in vitro question whether either of these two compounds directly inhibit GADD34/PP1 complexes (Crespillo-Casado, Chambers, Fischer, Marciniak, & Ron, 2017) and leave open the question of how these compounds act in vivo.

### 7.3 | ISRIB, an eIF2B activator

In contrast to the PP1 inhibitors, the ISR inhibitor ISRIB was isolated in a screen for compounds that reduced rather than activated the ISR (Sidrauski et al., 2013). In mice, ISRIB was shown to improve spatial and fear-associated learning (Sidrauski et al., 2013). In addition, ISRIB administration to mice can protect against neurodegeneration caused by prion disease (Halliday et al., 2015), or perhaps most surprisingly, cognitive impairment due to traumatic brain injury, even when a single dose was administered after injury (Chou et al., 2017). It was initially proposed that ISRIB may lower the

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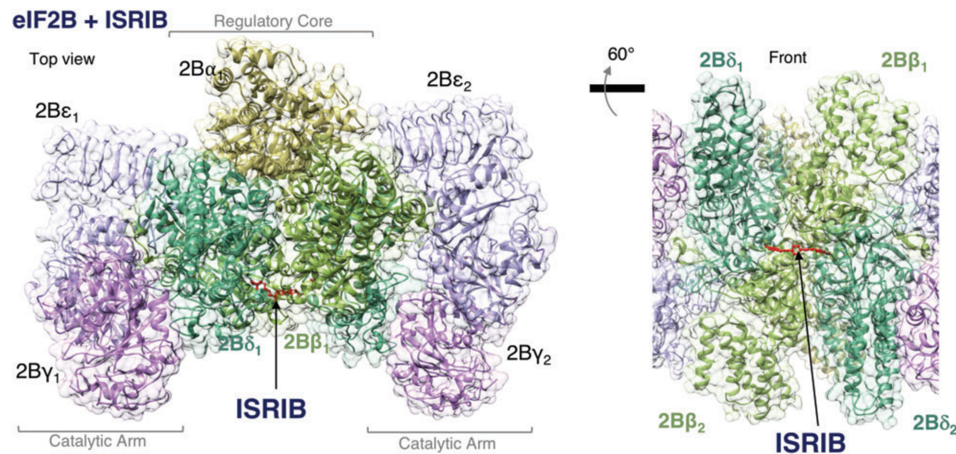


**FIGURE 6** ISRIB binds at the eIF2Bβδ dimer interface. Top view (a) and front zoom view (b) of ISRIB (red) binding to human eIF2B decamer, from cryo-EM analyses (PDB file 6CAJ) (Tsai et al., 2018)

sensitivity of eIF2B to eIF2(αP), rather than alter activity with nonphosphorylated eIF2 (Sidrauski et al., 2013), but more recent studies indicate ISRIB can boost eIF2B activity with nonphosphorylated eIF2 (Sekine et al., 2015; Sidrauski, Tsai, et al., 2015; Tsai et al., 2018). ISRIB-resistant mutants in eIF2Bδ were isolated (Sekine et al., 2015) and recent cryo-electron microscopy shows that ISRIB binds into a pocket on the surface of eIF2B at the interface between the eIF2Bβδ subunits that form a tetramer at the regulatory eIF2B core (Figure 6; Tsai et al., 2018; Zyryanova et al., 2018). The binding site is both distinct and distant from the proposed binding interfaces for eIF2α and $2B\varepsilon^{GEF}$ (Figures 4 and 5), indicating that ISRIB is an allosteric activator. ISRIB appears to act, at least in part, by promoting or stabilizing eIF2B decamer formation (Sidrauski, Tsai, et al., 2015; Tsai et al., 2018). Human eIF2B may exist in cells in different complexes with the full decamer complex being the most active and pentamer complexes or complexes lacking eIF2Bα having reduced activity (Williams et al., 2001; Wortham et al., 2014). ISRIB stabilizes the most active form and also enhanced GEF activity of a form lacking eIF2Bα (Tsai et al., 2018). ISRIB can also rescue defects in eIF2B activity caused by a range of CACH/VWM mutations, where it also blocks an aberrant ISR response (Wong et al., 2018). How these effects of ISRIB combine to alter the sensitivity of eIF2B to enhanced eIF2(αP) in vivo during the ISR is not yet fully clear. In cells with modest levels of eIF2(αP), ISRIB may simply boost the activity of noninhibited eIF2B and thereby reduce the ISR; however, more complicated explanations are possible.

### 7.4 | Trazodone and dibenzoylmethane

Two further compounds have been identified that may also activate eIF2B. Trazodone and dibenzoylmethane (DBM) were both identified in a cellular UPR reporter screen similar to that which isolated ISRIB (Halliday et al., 2017). These compounds also, like ISRIB, cross the blood–brain barrier and were shown to reverse or prevent the onset of symptoms of neurodegeneration in mouse models of Alzheimer's and prion diseases (Halliday et al., 2017). In these disease models, persistent elevated eIF2(αP) is observed and this is not altered by compound treatment; however, protein synthesis levels rise. These point to a mechanism of action downstream of eIF2(αP) and hence modulation of eIF2B activity. Both trazodone and DBM are licensed for human use, with trazodone being an antidepressant (Halliday et al., 2017). It is not clear if or how either compound activates eIF2B, but they are likely via a different mode to stabilizing an eIF2B decamer (Halliday et al., 2017). Trazodone inhibits the kinase GSK3β and this may be relevant to its mode of action here. It was shown that GSK3β phosphorylates human eIF2Bε at $ser^{540}$, adjacent to the $2B\varepsilon^{GEF}$ domain, to reduce eIF2B activity (Welsh, Miller, Loughlin, Price, & Proud, 1998). Hence trazadone may lead to lower eIF2Bε $ser^{540}$ phosphorylation and indirectly boost eIF2B activity. This idea should be tested experimentally. DBM also impacts kinase signaling and may act via glucose uptake, and AMP-activated protein kinase (Kim et al., 2015). If or how this links to eIF2B activity independently of the ISR is not clear.

In summary, recent studies have cast doubt on whether GSK2606414 and GSK2656157 are selective inhibitors of PERK kinase activity (Rojas-Rivera et al., 2017), or whether guanabenz and sephin1 directly inhibit GADD34/PP1 complexes (Crespillo-Casado et al., 2017). In addition, guanabenz can independently target other pathways, again questioning its selectivity for GADD34/PP1 (Perego et al., 2018). In contrast, new compounds that directly or indirectly target eIF2B activity have shown activity in a range of animal models of disease, suggesting eIF2B is a good target for intervening to ameliorate an aberrantly active ISR. Given that eIF2B is active throughout the body, translating compounds into the clinic may present the usual

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challenges of "side effects." However, as trazadone and DBM are licensed for human use, it will be interesting to assess them for conditions where aberrant ISR activity contributes to disease.

## 8 | CONCLUSION

Great progress has been made in recent years in our molecular understanding of the roles of eIF2 and eIF2B in translation and its control in the ISR. Yet as the speculative nature of the model outlined in Figure 5 and accompanying text indicates, there are many steps that require deeper understanding. These include how the eIF2B decamer and its linked 2BεGEF domains are arranged and communicate the fact that eIF2(αP) has bound rather than eIF2. Perhaps structural biology will provide future insights in to this important control mechanism.

The studies of the yeast ISR imply that eIF5 plays important roles (Asano et al., 1999; Jennings & Pavitt, 2010a; Singh et al., 2006) which should be further explored in mammalian ISR contexts. Human eIF5 levels are autoregulated via uORFs with poor AUG codon contexts such that the uORFs act to autoregulate eIF5 synthesis (Loughran, Sachs, Atkins, & Ivanov, 2012). Mammalian cells also express homologs of eIF5 termed eIF5-mimic proteins (5MP1 and 5MP2, also known as BZW2 and BZW1 respectively) that can compete with eIF5 for interaction with eIF2, but because they lack an eIF5 GAP domain, they downregulate the standard translation initiation pathway (Singh et al., 2011), as well as enhancing ATF4 expression (Kozel et al., 2016). Hence the precise role variations in eIF5 levels and the 5MP1 and 5MP2 proteins play in the ISR in different cells is not yet clear.

More broadly, an increase in our understanding of the wide range of conditions where an elevated ISR is found shows that this control mechanism has been widely adopted. It remains important to assess the relative importance of global translation repression versus the activation of ISR mRNA translation for each situation. The characterization of chemical modifiers of eIF2B is an important development as they offer new prospects for identifying those conditions where ISR modulation may be therapeutic. These or as yet undiscovered compounds may prove to be useful in treating the rare genetic conditions directly affecting eIF2 and eIF2B, as a recent study suggests (Wong et al., 2018), as well as some more widespread conditions where there is an elevated eIF2(αP) and ISR response. Hopefully, the next few years will continue to bring insight into this central and fascinating pathway.

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### CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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### REFERENCES

Algire, M. A., Maag, D., & Lorsch, J. R. (2005). Pi release from eIF2, not GTP hydrolysis, is the step controlled by start-site selection during eukaryotic translation initiation. *Molecular Cell*, *20*(2), 251–262.

Alone, P. V., & Dever, T. E. (2006). Direct binding of translation initiation factor eIF2gamma-G domain to its GTPase-activating and GDP-GTP exchange factors eIF5 and eIF2B epsilon. *The Journal of Biological Chemistry*, *281*(18), 12636–12644. https://doi.org/10.1074/jbc.M511700200

Asano, K., Krishnamoorthy, T., Phan, L., Pavitt, G. D., & Hinnebusch, A. G. (1999). Conserved bipartite motifs in yeast eIF5 and eIF2Bepsilon, GTPase-activating and GDP-GTP exchange factors in translation initiation, mediate binding to their common substrate eIF2. *The EMBO Journal*, *18*(6), 1673–1688. https://doi.org/10.1093/emboj/18.6.1673

Atkins, C., Liu, Q., Minthorn, E., Zhang, S. Y., Figueroa, D. J., Moss, K., … Kumar, R. (2013). Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Research*, *73*(6), 1993–2002. https://doi.org/10.1158/0008-5472.CAN-12-3109

Axten, J. M., Medina, J. R., Feng, Y., Shu, A., Romeril, S. P., Grant, S. W., … Gampe, R. T. (2012). Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *Journal of Medicinal Chemistry*, *55*(16), 7193–7207. https://doi.org/10.1021/jm300713s

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Baird, T. D., & Wek, R. C. (2012). Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. *Advances in Nutrition*, *3*(3), 307–321. https://doi.org/10.3945/an.112.002113

Barbosa, C., Peixeiro, I., & Romao, L. (2013). Gene expression regulation by upstream open reading frames and human disease. *PLoS Genetics*, *9*(8), e1003529. https://doi.org/10.1371/journal.pgen.1003529

Batista, G., Johnson, J. L., Dominguez, E., Costa-Mattioli, M., & Pena, J. L. (2016). Translational control of auditory imprinting and structural plasticity by eIF2alpha. *eLife*, *5*, e17197. https://doi.org/10.7554/eLife.17197

Best, D. H., Sumner, K. L., Austin, E. D., Chung, W. K., Brown, L. M., Borczuk, A. C., … Elliott, C. G. (2014). EIF2AK4 mutations in pulmonary capillary hemangiomatosis. *Chest*, *145*(2), 231–236. https://doi.org/10.1378/chest.13-2366

Bieniossek, C., Schutz, P., Bumann, M., Limacher, A., Uson, I., & Baumann, U. (2006). The crystal structure of the carboxy-terminal domain of human translation initiation factor eIF5. *Journal of Molecular Biology*, *360*(2), 457–465. https://doi.org/10.1016/j.jmb.2006.05.021

Boesen, T., Mohammad, S. S., Pavitt, G. D., & Andersen, G. R. (2004). Structure of the catalytic fragment of translation initiation factor 2B and identification of a critically important catalytic residue. *The Journal of Biological Chemistry*, *279*(11), 10584–10592. https://doi.org/10.1074/jbc.M311055200

Bogorad, A. M., Lin, K. Y., & Marintchev, A. (2017). Novel mechanisms of eIF2B action and regulation by eIF2alpha phosphorylation. *Nucleic Acids Research*, *45*(20), 11962–11979. https://doi.org/10.1093/nar/gkx845

Bogorad, A. M., Lin, K. Y., & Marintchev, A. (2018). eIF2B mechanisms of action and regulation: A thermodynamic view. *Biochemistry*, *57*, 1426–1435. https://doi.org/10.1021/acs.biochem.7b00957

Bogorad, A. M., Xia, B., Sandor, D. G., Mamonov, A. B., Cafarella, T. R., Jehle, S., … Marintchev, A. (2014). Insights into the architecture of the eIF2Balpha/beta/-delta regulatory subcomplex. *Biochemistry*, *53*(21), 3432–3445. https://doi.org/10.1021/bi500346u

Borck, G., Shin, B. S., Stiller, B., Mimouni-Bloch, A., Thiele, H., Kim, J. R., … Basel-Vanagaite, L. (2012). eIF2gamma mutation that disrupts eIF2 complex integrity links intellectual disability to impaired translation initiation. *Molecular Cell*, *48*(4), 641–646. https://doi.org/10.1016/j.molcel.2012.09.005

Boria, I., Garelli, E., Gazda, H. T., Aspesi, A., Quarello, P., Pavesi, E., … Dianzani, I. (2010). The ribosomal basis of diamond-Blackfan anemia: Mutation and database update. *Human Mutation*, *31*(12), 1269–1279. https://doi.org/10.1002/humu.21383

Bos, J. L., Rehmann, H., & Wittinghofer, A. (2007). GEFs and GAPs: Critical elements in the control of small G proteins. *Cell*, *129*(5), 865–877. https://doi.org/10.1016/j.cell.2007.05.018

Boyce, M., Bryant, K. F., Jousse, C., Long, K., Harding, H. P., Scheuner, D., … Yuan, J. (2005). A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. *Science*, *307*(5711), 935–939. https://doi.org/10.1126/science.1101902

Brush, M. H., Weiser, D. C., & Shenolikar, S. (2003). Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. *Molecular and Cellular Biology*, *23*(4), 1292–1303.

Buchan, J. R., Yoon, J. H., & Parker, R. (2011). Stress-specific composition, assembly and kinetics of stress granules in *Saccharomyces cerevisiae*. *Journal of Cell Science*, *124*(Pt 2), 228–239. https://doi.org/10.1242/jcs.078444

Buffington, S. A., Huang, W., & Costa-Mattioli, M. (2014). Translational control in synaptic plasticity and cognitive dysfunction. *Annual Review of Neuroscience*, *37*, 17–38. https://doi.org/10.1146/annurev-neuro-071013-014100

Calvo, S. E., Pagliarini, D. J., & Mootha, V. K. (2009). Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(18), 7507–7512. https://doi.org/10.1073/pnas.0810916106

Chen, J. J. (2007). Regulation of protein synthesis by the heme-regulated eIF2alpha kinase: Relevance to anemias. *Blood*, *109*(7), 2693–2699. https://doi.org/10.1182/blood-2006-08-041830

Cheung, Y. N., Maag, D., Mitchell, S. F., Fekete, C. A., Algire, M. A., Takacs, J. E., … Hinnebusch, A. G. (2007). Dissociation of eIF1 from the 40S ribosomal subunit is a key step in start codon selection *in vivo*. *Genes & Development*, *21*(10), 1217–1230. https://doi.org/10.1101/gad.1528307

Chou, A., Krukowski, K., Jopson, T., Zhu, P. J., Costa-Mattioli, M., Walter, P., & Rosi, S. (2017). Inhibition of the integrated stress response reverses cognitive deficits after traumatic brain injury. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(31), E6420–E6426. https://doi.org/10.1073/pnas.1707661114

Connor, J. H., Weiser, D. C., Li, S., Hallenbeck, J. M., & Shenolikar, S. (2001). Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1. *Molecular and Cellular Biology*, *21*(20), 6841–6850. https://doi.org/10.1128/MCB.21.20.6841-6850.2001

Costa-Mattioli, M., Gobert, D., Stern, E., Gamache, K., Colina, R., Cuello, C., … Sonenberg, N. (2007). eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. *Cell*, *129*(1), 195–206. https://doi.org/10.1016/j.cell.2007.01.050

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron*, *61*(1), 10–26. https://doi.org/10.1016/j.neuron.2008.10.055

Crespillo-Casado, A., Chambers, J. E., Fischer, P. M., Marciniak, S. J., & Ron, D. (2017). PPP1R15A-mediated dephosphorylation of eIF2alpha is unaffected by Sephin1 or Guanabenz. *eLife*, *6*, e26109. https://doi.org/10.7554/eLife.26109

Dar, A. C., Dever, T. E., & Sicheri, F. (2005). Higher-order substrate recognition of eIF2alpha by the RNA-dependent protein kinase PKR. *Cell*, *122*(6), 887–900. https://doi.org/10.1016/j.cell.2005.06.044

Das, I., Krzyzosiak, A., Schneider, K., Wrabetz, L., D'Antonio, M., Barry, N., … Bertolotti, A. (2015). Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science*, *348*(6231), 239–242. https://doi.org/10.1126/science.aaa4484

Das, S., Maiti, T., Das, K., & Maitra, U. (1997). Specific interaction of eukaryotic translation initiation factor 5 (eIF5) with the beta-subunit of eIF2. *The Journal of Biological Chemistry*, *272*(50), 31712–31718.

de Almeida, R. A., Fogli, A., Gaillard, M., Scheper, G. C., Boesflug-Tanguy, O., & Pavitt, G. D. (2013). A yeast purification system for human translation initiation factors eIF2 and eIF2Bepsilon and their use in the diagnosis of CACH/VWM disease. *PLoS One*, *8*(1), e53958. https://doi.org/10.1371/journal.pone.0053958

Dev, K., Qiu, H., Dong, J., Zhang, F., Barthlme, D., & Hinnebusch, A. G. (2010). The beta/Gcd7 subunit of eukaryotic translation initiation factor 2B (eIF2B), a guanine nucleotide exchange factor, is crucial for binding eIF2 in vivo. *Molecular and Cellular Biology*, *30*(21), 5218–5233. https://doi.org/10.1128/MCB.00265-10

Dever, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F., & Hinnebusch, A. G. (1992). Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. *Cell*, *68*(3), 585–596.

Dever, T. E., Kinzy, T. G., & Pavitt, G. D. (2016). Mechanism and regulation of protein synthesis in *Saccharomyces cerevisiae*. *Genetics*, *203*(1), 65–107. https://doi.org/10.1534/genetics.115.186221

Dey, M., Cao, C., Dar, A. C., Tamura, T., Ozato, K., Sicheri, F., & Dever, T. E. (2005). Mechanistic link between PKR dimerization, autophosphorylation, and eIF2alpha substrate recognition. *Cell*, *122*(6), 901–913. https://doi.org/10.1016/j.cell.2005.06.041

Dey, M., Cao, C., Sicheri, F., & Dever, T. E. (2007). Conserved intermolecular salt bridge required for activation of protein kinases PKR, GCN2, and PERK. *The Journal of Biological Chemistry*, *282*(9), 6653–6660. https://doi.org/10.1074/jbc.M607897200

Dey, M., Trieselmann, B., Locke, E. G., Lu, J., Cao, C., Dar, A. C., … Dever, T. E. (2005). PKR and GCN2 kinases and guanine nucleotide exchange factor eukaryotic translation initiation factor 2B (eIF2B) recognize overlapping surfaces on eIF2alpha. *Molecular and Cellular Biology*, *25*(8), 3063–3075.

Dietrich, J., Lacagnina, M., Gass, D., Richfield, E., Mayer-Proschel, M., Noble, M., … Proschel, C. (2005). EIF2B5 mutations compromise GFAP+ astrocyte generation in vanishing white matter leukodystrophy. *Nature Medicine*, *11*(3), 277–283. https://doi.org/10.1038/nm1195

17577012, 2018, 6, Downloaded from https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1491 by The University Of Manchester, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Dong, J., Qiu, H., Garcia-Barrio, M., Anderson, J., & Hinnebusch, A. G. (2000). Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. *Molecular Cell*, *6*(2), 269–279.

Donnelly, N., Gorman, A. M., Gupta, S., & Samali, A. (2013). The eIF2alpha kinases: Their structures and functions. *Cellular and Molecular Life Sciences*, *70*(19), 3493–3511. https://doi.org/10.1007/s00018-012-1252-6

Dooves, S., Bugiani, M., Postma, N. L., Polder, E., Land, N., Horan, S. T., … van der Knaap, M. S. (2016). Astrocytes are central in the pathomechanisms of vanishing white matter. *The Journal of Clinical Investigation*, *126*(4), 1512–1524. https://doi.org/10.1172/JCI83908

Elsby, R., Heiber, J. F., Reid, P., Kimball, S. R., Pavitt, G. D., & Barber, G. N. (2011). The alpha subunit of eukaryotic initiation factor 2B (eIF2B) is required for eIF2-mediated translational suppression of vesicular stomatitis virus. *Journal of Virology*, *85*(19), 9716–9725. https://doi.org/10.1128/JVI.05146-11

Erickson, F. L., & Hannig, E. M. (1996). Ligand interactions with eukaryotic translation initiation factor 2: Role of the gamma-subunit. *The EMBO Journal*, *15*(22), 6311–6320.

Eyries, M., Montani, D., Girerd, B., Perret, C., Leroy, A., Lonjou, C., … Soubrier, F. (2014). EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. *Nature Genetics*, *46*(1), 65–69. https://doi.org/10.1038/ng.2844

Fabian, J. R., Kimball, S. R., & Jefferson, L. S. (1998). Reconstitution and purification of eukaryotic initiation factor 2B (eIF2B) expressed in Sf21 insect cells. *Protein Expression and Purification*, *13*(1), 16–22. https://doi.org/10.1006/prep.1998.0860

Falletta, P., Sanchez-Del-Campo, L., Chauhan, J., Effern, M., Kenyon, A., Kershaw, C. J., … Goding, C. R. (2017). Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes & Development*, *31*(1), 18–33. https://doi.org/10.1101/gad.290940.116

Fogli, A., & Boespflug-Tanguy, O. (2006). The large spectrum of eIF2B-related diseases. *Biochemical Society Transactions*, *34*(Pt 1), 22–29. https://doi.org/10.1042/BST20060022

Fogli, A., Rodriguez, D., Eymard-Pierre, E., Bouhour, F., Labauge, P., Meaney, B. F., … Boespflug-Tanguy, O. (2003). Ovarian failure related to eukaryotic initiation factor 2B mutations. *American Journal of Human Genetics*, *72*(6), 1544–1550. https://doi.org/10.1086/375404

Fusakio, M. E., Willy, J. A., Wang, Y., Mirek, E. T., Al Baghdadi, R. J., Adams, C. M., … Wek, R. C. (2016). Transcription factor ATF4 directs basal and stress-induced gene expression in the unfolded protein response and cholesterol metabolism in the liver. *Molecular Biology of the Cell*, *27*(9), 1536–1551. https://doi.org/10.1091/mbc.E16-01-0039

Gomez, E., Mohammad, S. S., & Pavitt, G. D. (2002). Characterization of the minimal catalytic domain within eIF2B: The guanine-nucleotide exchange factor for translation initiation. *The EMBO Journal*, *21*(19), 5292–5301.

Gomez, E., & Pavitt, G. D. (2000). Identification of domains and residues within the epsilon subunit of eukaryotic translation initiation factor 2B (eIF2Bepsilon) required for guanine nucleotide exchange reveals a novel activation function promoted by eIF2B complex formation. *Molecular and Cellular Biology*, *20*(11), 3965–3976.

Gordiyenko, Y., Schmidt, C., Jennings, M. D., Matak-Vinkovic, D., Pavitt, G. D., & Robinson, C. V. (2014). eIF2B is a decameric guanine nucleotide exchange factor with a gamma2epsilon2 tetrameric core. *Nature Communications*, *5*, 3902. https://doi.org/10.1038/ncomms4902

Gross, M., Rubino, M. S., & Hessefort, S. M. (1991). The conversion of eIF-2.GDP to eIF-2.GTP by eIF-2B requires Met-tRNA(fMet). *Biochemical and Biophysical Research Communications*, *181*(3), 1500–1507.

Guan, B. J., van Hoef, V., Jobava, R., Elroy-Stein, O., Valasek, L. S., Cargnello, M., … Hatzoglou, M. (2017). A unique ISR program determines cellular responses to chronic stress. *Molecular Cell*, *68*(5), 885–900. https://doi.org/10.1016/j.molcel.2017.11.007

Gunisova, S., & Valasek, L. S. (2014). Fail-safe mechanism of GCN4 translational control--uORF2 promotes reinitiation by analogous mechanism to uORF1 and thus secures its key role in GCN4 expression. *Nucleic Acids Research*, *42*(9), 5880–5893. https://doi.org/10.1093/nar/gku204

Halliday, M., Radford, H., Sekine, Y., Moreno, J., Verity, N., le Quesne, J., … Mallucci, G. R. (2015). Partial restoration of protein synthesis rates by the small molecule ISRIB prevents neurodegeneration without pancreatic toxicity. *Cell Death & Disease*, *6*, e1672. https://doi.org/10.1038/cddis.2015.49

Halliday, M., Radford, H., Zents, K. A. M., Molloy, C., Moreno, J. A., Verity, N. C., … Mallucci, G. R. (2017). Repurposed drugs targeting eIF2aP-mediated translational repression prevent neurodegeneration in mice. *Brain*, *140*(6), 1768–1783. https://doi.org/10.1093/brain/awx074

Harding, H. P., Zhang, Y., Scheuner, D., Chen, J. J., Kaufman, R. J., & Ron, D. (2009). Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2alpha) dephosphorylation in mammalian development. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(6), 1832–1837. https://doi.org/10.1073/pnas.0809632106

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., … Ron, D. (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Molecular Cell*, *11*(3), 619–633.

Hinnebusch, A. G. (2005). Translational regulation of GCN4 and the general amino acid control of yeast. *Annual Review of Microbiology*, *59*, 407–450. https://doi.org/10.1146/annurev.micro.59.031805.133833

Hinnebusch, A. G. (2014). The scanning mechanism of eukaryotic translation initiation. *Annual Review of Biochemistry*, *83*, 779–812. https://doi.org/10.1146/annurev-biochem-060713-035802

Hinnebusch, A. G. (2017). Structural insights into the mechanism of scanning and start codon recognition in eukaryotic translation initiation. *Trends in Biochemical Sciences*, *42*, 589–611. https://doi.org/10.1016/j.tibs.2017.03.004

Hinnebusch, A. G., Ivanov, I. P., & Sonenberg, N. (2016). Translational control by 5′-untranslated regions of eukaryotic mRNAs. *Science*, *352*(6292), 1413–1416.

Holmes, B., Brogden, R. N., Heel, R. C., Speight, T. M., & Avery, G. S. (1983). Guanabenz. A review of its pharmacodynamic properties and therapeutic efficacy in hypertension. *Drugs*, *26*(3), 212–229.

Horzinski, L., Kantor, L., Huyghe, A., Schiffmann, R., Elroy-Stein, O., Boespflug-Tanguy, O., & Fogli, A. (2010). Evaluation of the endoplasmic reticulum-stress response in eIF2B-mutated lymphocytes and lymphoblasts from CACH/VWM patients. *BMC Neurology*, *10*, 94. https://doi.org/10.1186/1471-2377-10-94

Hronova, V., Mohammad, M. P., Wagner, S., Panek, J., Gunisova, S., Zeman, J., … Valasek, L. S. (2017). Does eIF3 promote reinitiation after translation of short upstream ORFs also in mammalian cells? *RNA Biology*, *14*, 1–8. https://doi.org/10.1080/15476286.2017.1353863

Huang, H. K., Yoon, H., Hannig, E. M., & Donahue, T. F. (1997). GTP hydrolysis controls stringent selection of the AUG start codon during translation initiation in Saccharomyces cerevisiae. *Genes & Development*, *11*(18), 2396–2413.

Hussain, T., Llacer, J. L., Fernandez, I. S., Munoz, A., Martin-Marcos, P., Savva, C. G., … Ramakrishnan, V. (2014). Structural changes enable start codon recognition by the eukaryotic translation initiation complex. *Cell*, *159*(3), 597–607. https://doi.org/10.1016/j.cell.2014.10.001

Ingolia, N. T., Ghaemmaghami, S., Newman, J. R., & Weissman, J. S. (2009). Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science*, *324*(5924), 218–223. https://doi.org/10.1126/science.1168978

Jennings, M. D., Kershaw, C. J., Adomavicius, T., & Pavitt, G. D. (2017). Fail-safe control of translation initiation by dissociation of eIF2alpha phosphorylated ternary complexes. *eLife*, *6*, e24542. https://doi.org/10.7554/eLife.24542

Jennings, M. D., Kershaw, C. J., White, C., Hoyle, D., Richardson, J. P., Costello, J. L., … Pavitt, G. D. (2016). eIF2beta is critical for eIF5-mediated GDP-dissociation inhibitor activity and translational control. *Nucleic Acids Research*, *44*(20), 9698–9709. https://doi.org/10.1093/nar/gkw657

Jennings, M. D., & Pavitt, G. D. (2010a). eIF5 is a dual function GAP and GDI for eukaryotic translational control. *Small GTPases*, *1*(2), 118–123. https://doi.org/10.4161/sgtp.1.2.13783

Jennings, M. D., & Pavitt, G. D. (2010b). eIF5 has GDI activity necessary for translational control by eIF2 phosphorylation. *Nature*, *465*(7296), 378–381. https://doi.org/10.1038/nature09003

17577012, 2018, 6, Downloaded from https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1491 by The University Of Manchester, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Jennings, M. D., & Pavitt, G. D. (2014). A new function and complexity for protein translation initiation factor eIF2B. *Cell Cycle*, *13*(17), 2660–2665. https://doi.org/10.4161/15384101.2014.948797

Jennings, M. D., Zhou, Y., Mohammad-Qureshi, S. S., Bennett, D., & Pavitt, G. D. (2013). eIF2B promotes eIF5 dissociation from eIF2*GDP to facilitate guanine nucleotide exchange for translation initiation. *Genes & Development*, *27*(24), 2696–2707. https://doi.org/10.1101/gad.231514.113

Jousse, C., Oyadomari, S., Novoa, I., Lu, P., Zhang, Y., Harding, H. P., & Ron, D. (2003). Inhibition of a constitutive translation initiation factor 2alpha phosphatase, CReP, promotes survival of stressed cells. *The Journal of Cell Biology*, *163*(4), 767–775. https://doi.org/10.1083/jcb.200308075

Julier, C., & Nicolino, M. (2010). Wolcott-Rallison syndrome. *Orphanet Journal of Rare Diseases*, *5*, 29. https://doi.org/10.1186/1750-1172-5-29

Kantor, L., Harding, H. P., Ron, D., Schiffmann, R., Kaneski, C. R., Kimball, S. R., & Elroy-Stein, O. (2005). Heightened stress response in primary fibroblasts expressing mutant eIF2B genes from CACH/VWM leukodystrophy patients. *Human Genetics*, *118*(1), 99–106. https://doi.org/10.1007/s00439-005-0024-x

Kashiwagi, K., Takahashi, M., Nishimoto, M., Hiyama, T. B., Higo, T., Umehara, T., … Yokoyama, S. (2016). Crystal structure of eukaryotic translation initiation factor 2B. *Nature*, *531*(7592), 122–125. https://doi.org/10.1038/nature16991

Kedersha, N., Ivanov, P., & Anderson, P. (2013). Stress granules and cell signaling: More than just a passing phase? *Trends in Biochemical Sciences*, *38*(10), 494–506. https://doi.org/10.1016/j.tibs.2013.07.004

Kessel, D. (2006). Protection of Bcl-2 by salubrinal. *Biochemical and Biophysical Research Communications*, *346*(4), 1320–1323. https://doi.org/10.1016/j.bbrc.2006.06.056

Khan, M. F., Spurgeon, S., & von der Haar, T. (2018). Origins of robustness in translational control via eukaryotic translation initiation factor (eIF) 2. *Journal of Theoretical Biology*, *445*, 92–102. https://doi.org/10.1016/j.jtbi.2018.02.020

Kim, H. J., Raphael, A. R., LaDow, E. S., McGurk, L., Weber, R. A., Trojanowski, J. Q., … Bonini, N. M. (2014). Therapeutic modulation of eIF2alpha phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nature Genetics*, *46*(2), 152–160. https://doi.org/10.1038/ng.2853

Kim, J. H., Park, S. M., Park, J. H., Keum, S. J., & Jang, S. K. (2011). eIF2A mediates translation of hepatitis C viral mRNA under stress conditions. *The EMBO Journal*, *30*(12), 2454–2464. https://doi.org/10.1038/emboj.2011.146

Kim, N., Kim, H. M., Lee, E. S., Lee, J. O., Lee, H. J., Lee, S. K., … Kim, H. S. (2015). Dibenzoylmethane exerts metabolic activity through regulation of AMP-activated protein kinase (AMPK)-mediated glucose uptake and adipogenesis pathways. *PLoS One*, *10*(3), e0120104. https://doi.org/10.1371/journal.pone.0120104

Kimball, S. R., Fabian, J. R., Pavitt, G. D., Hinnebusch, A. G., & Jefferson, L. S. (1998). Regulation of guanine nucleotide exchange through phosphorylation of eukaryotic initiation factor eIF2alpha. Role of the alpha- and delta-subunits of eiF2b. *The Journal of Biological Chemistry*, *273*(21), 12841–12845.

Kojima, E., Takeuchi, A., Haneda, M., Yagi, A., Hasegawa, T., Yamaki, K., … Isobe, K. (2003). The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: Elucidation by GADD34-deficient mice. *The FASEB Journal*, *17*(11), 1573–1575. https://doi.org/10.1096/fj.02-1184fje

Kolitz, S. E., & Lorsch, J. R. (2010). Eukaryotic initiator tRNA: Finely tuned and ready for action. *FEBS Letters*, *584*(2), 396–404. https://doi.org/10.1016/j.febslet.2009.11.047

Kozel, C., Thompson, B., Hustak, S., Moore, C., Nakashima, A., Singh, C. R., … Asano, K. (2016). Overexpression of eIF5 or its protein mimic 5MP perturbs eIF2 function and induces ATF4 translation through delayed re-initiation. *Nucleic Acids Research*, *44*(18), 8704–8713. https://doi.org/10.1093/nar/gkw559

Krishnamoorthy, T., Pavitt, G. D., Zhang, F., Dever, T. E., & Hinnebusch, A. G. (2001). Tight binding of the phosphorylated alpha subunit of initiation factor 2 (eIF2alpha) to the regulatory subunits of guanine nucleotide exchange factor eIF2B is required for inhibition of translation initiation. *Molecular and Cellular Biology*, *21*(15), 5018–5030. https://doi.org/10.1128/MCB.21.15.5018-5030.2001

Kuhle, B., Eulig, N. K., & Ficner, R. (2015). Architecture of the eIF2B regulatory subcomplex and its implications for the regulation of guanine nucleotide exchange on eIF2. *Nucleic Acids Research*, *43*(20), 9994–10014. https://doi.org/10.1093/nar/gkv930

Kumar, P., Hellen, C. U., & Pestova, T. V. (2016). Toward the mechanism of eIF4F-mediated ribosomal attachment to mammalian capped mRNAs. *Genes & Development*, *30*(13), 1573–1588. https://doi.org/10.1101/gad.282418.116

Kusio-Kobialka, M., Podszywalow-Bartnicka, P., Peidis, P., Glodkowska-Mrowka, E., Wolanin, K., Leszak, G., … Piwocka, K. (2012). The PERK-eIF2α phosphorylation arm is a pro-survival pathway of BCR-ABL signaling and confers resistance to imatinib treatment in chronic myeloid leukemia cells. *Cell Cycle*, *11*(21), 4069–4078. https://doi.org/10.4161/cc.22387

Larhammar, M., Huntwork-Rodriguez, S., Jiang, Z., Solanoy, H., Sengupta Ghosh, A., Wang, B., … Watkins, T. A. (2017). Dual leucine zipper kinase-dependent PERK activation contributes to neuronal degeneration following insult. *eLife*, *6*, e20725. https://doi.org/10.7554/eLife.20725

Lee, S., Liu, B., Lee, S., Huang, S. X., Shen, B., & Qian, S. B. (2012). Global mapping of translation initiation sites in mammalian cells at single-nucleotide resolution. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(37), E2424–E2432. https://doi.org/10.1073/pnas.1207846109

Leegwater, P. A., Vermeulen, G., Konst, A. A., Naidu, S., Mulders, J., Visser, A., … van der Knaap, M. S. (2001). Subunits of the translation initiation factor eIF2B are mutant in leukoencephalopathy with vanishing white matter. *Nature Genetics*, *29*(4), 383–388. https://doi.org/10.1038/ng764

Li, W., Wang, X., Van Der Knaap, M. S., & Proud, C. G. (2004). Mutations linked to leukoencephalopathy with vanishing white matter impair the function of the eukaryotic initiation factor 2B complex in diverse ways. *Molecular and Cellular Biology*, *24*(8), 3295–3306.

Lin, Y., Pang, X., Huang, G., Jamison, S., Fang, J., Harding, H. P., … Lin, W. (2014). Impaired eukaryotic translation initiation factor 2B activity specifically in oligodendrocytes reproduces the pathology of vanishing white matter disease in mice. *The Journal of Neuroscience*, *34*(36), 12182–12191. https://doi.org/10.1523/JNEUROSCI.1373-14.2014

Liu, R., van der Lei, H. D., Wang, X., Wortham, N. C., Tang, H., van Berkel, C. G., … Proud, C. G. (2011). Severity of vanishing white matter disease does not correlate with deficits in eIF2B activity or the integrity of eIF2B complexes. *Human Mutation*, *32*(9), 1036–1045. https://doi.org/10.1002/humu.21535

Llacer, J. L., Hussain, T., Marler, L., Aitken, C. E., Thakur, A., Lorsch, J. R., … Ramakrishnan, V. (2015). Conformational differences between open and closed states of the eukaryotic translation initiation complex. *Molecular Cell*, *59*(3), 399–412. https://doi.org/10.1016/j.molcel.2015.06.033

Loughran, G., Sachs, M. S., Atkins, J. F., & Ivanov, I. P. (2012). Stringency of start codon selection modulates autoregulation of translation initiation factor eIF5. *Nucleic Acids Research*, *40*(7), 2898–2906. https://doi.org/10.1093/nar/gkr1192

Lu, P. D., Harding, H. P., & Ron, D. (2004). Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *The Journal of Cell Biology*, *167*(1), 27–33. https://doi.org/10.1083/jcb.200408003

Ma, T., Trinh, M. A., Wexler, A. J., Bourbon, C., Gatti, E., Pierre, P., … Klann, E. (2013). Suppression of eIF2alpha kinases alleviates Alzheimer's disease-related plasticity and memory deficits. *Nature Neuroscience*, *16*(9), 1299–1305. https://doi.org/10.1038/nn.3486

Majumdar, R., & Maitra, U. (2005). Regulation of GTP hydrolysis prior to ribosomal AUG selection during eukaryotic translation initiation. *The EMBO Journal*, *24*(21), 3737–3746. https://doi.org/10.1038/sj.emboj.7600844

Mercado, G., Castillo, V., Soto, P., Lopez, N., Axten, J. M., Sardi, S. P., … Hetz, C. (2018). Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. *Neurobiology of Disease*, *112*, 136–148. https://doi.org/10.1016/j.nbd.2018.01.004

Merrick, W. C., & Pavitt, G. D. (2018). Protein synthesis initiation in eukaryotic cells. *Cold Spring Harbor Perspectives in Biology*, a033092. https://doi: 10.1101/cshperspect.a033092

Merrick, W. C., Weis, F., Faille, A., Alard, A. A., Crespillo-Casado, A., Sekine, Y., … Ron, D. (2018). Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. *Science*, *359*(6383), 1533–1536. https://doi.org/10.1126/science.aar5129

Mills, E. W., & Green, R. (2017). Ribosomopathies: There's strength in numbers. *Science*, *358*(6363), eaan2755. https://doi.org/10.1126/science.aan2755

17577012, 2018, 6, Downloaded from https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1491 by The University Of Manchester, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mohammad, M. P., Munzarova Pondelickova, V., Zeman, J., Gunisova, S., & Valasek, L. S. (2017). *In vivo* evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation. *Nucleic Acids Research*, *45*, 2658–2674. https://doi.org/10.1093/nar/gkx049

Mohammad-Qureshi, S. S., Haddad, R., Hemingway, E. J., Richardson, J. P., & Pavitt, G. D. (2007). Critical contacts between the eukaryotic initiation factor 2B (eIF2B) catalytic domain and both eIF2beta and -2gamma mediate guanine nucleotide exchange. *Molecular and Cellular Biology*, *27*(14), 5225–5234. https://doi.org/10.1128/MCB.00495-07

Moortgat, S., Desir, J., Benoit, V., Boulanger, S., Pendeville, H., Nassogne, M. C., … Maystadt, I. (2016). Two novel EIF2S3 mutations associated with syndromic intellectual disability with severe microcephaly, growth retardation, and epilepsy. *American Journal of Medical Genetics. Part A*, *170*(11), 2927–2933. https://doi.org/10.1002/ajmg.a.37792

Moreno, J. A., Halliday, M., Molloy, C., Radford, H., Verity, N., Axten, J. M., … Mallucci, G. R. (2013). Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Science Translational Medicine*, *5*(206), 206ra138. https://doi.org/10.1126/scitranslmed.3006767

Moreno, J. A., Radford, H., Peretti, D., Steinert, J. R., Verity, N., Martin, M. G., … Mallucci, G. R. (2012). Sustained translational repression by eIF2alpha-P mediates prion neurodegeneration. *Nature*, *485*(7399), 507–511. https://doi.org/10.1038/nature11058

Munzarova, V., Panek, J., Gunisova, S., Danyi, I., Szamecz, B., & Valasek, L. S. (2011). Translation reinitiation relies on the interaction between eIF3a/TIF32 and progressively folded cis-acting mRNA elements preceding short uORFs. *PLoS Genetics*, *7*(7), e1002137. https://doi.org/10.1371/journal.pgen.1002137

Natarajan, K., Meyer, M. R., Jackson, B. M., Slade, D., Roberts, C., Hinnebusch, A. G., & Marton, M. J. (2001). Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Molecular and Cellular Biology*, *21*(13), 4347–4368. https://doi.org/10.1128/MCB.21.13.4347-4368.2001

Novoa, I., Zeng, H., Harding, H. P., & Ron, D. (2001). Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *The Journal of Cell Biology*, *153*(5), 1011–1022.

Obayashi, E., Luna, R. E., Nagata, T., Martin-Marcos, P., Hiraishi, H., Singh, C. R., … Asano, K. (2017). Molecular landscape of the ribosome pre-initiation complex during mRNA scanning: Structural role for eIF3c and its control by eIF5. *Cell Reports*, *18*(11), 2651–2663. https://doi.org/10.1016/j.celrep.2017.02.052

Onofre, C., Tome, F., Barbosa, C., Silva, A. L., & Romao, L. (2015). Expression of human Hemojuvelin (HJV) is tightly regulated by two upstream open reading frames in HJV mRNA that respond to iron overload in hepatic cells. *Molecular and Cellular Biology*, *35*(8), 1376–1389. https://doi.org/10.1128/MCB.01462-14

Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., & Gorman, A. M. (2016). The integrated stress response. *EMBO Reports*, *17*(10), 1374–1395. https://doi.org/10.15252/embr.201642195

Palam, L. R., Baird, T. D., & Wek, R. C. (2011). Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. *The Journal of Biological Chemistry*, *286*(13), 10939–10949.

Paulin, F. E., Campbell, L. E., O'Brien, K., Loughlin, J., & Proud, C. G. (2001). Eukaryotic translation initiation factor 5 (eIF5) acts as a classical GTPase-activator protein. *Current Biology*, *11*(1), 55–59.

Pavitt, G. D., Ramaiah, K. V., Kimball, S. R., & Hinnebusch, A. G. (1998). eIF2 independently binds two distinct eIF2B subcomplexes that catalyze and regulate guanine-nucleotide exchange. *Genes & Development*, *12*(4), 514–526.

Pavitt, G. D., Yang, W., & Hinnebusch, A. G. (1997). Homologous segments in three subunits of the guanine nucleotide exchange factor eIF2B mediate translational regulation by phosphorylation of eIF2. *Molecular and Cellular Biology*, *17*(3), 1298–1313.

Perego, J., Mendes, A., Bourbon, C., Camosseto, V., Combes, A., Liu, H., … Pierre, P. (2018). Guanabenz inhibits TLR9 signaling through a pathway that is independent of eIF2alpha dephosphorylation by the GADD34/PP1c complex. *Science Signaling*, *11*(514), eaam8104. https://doi.org/10.1126/scisignal.aam8104

Pisarev, A. V., Kolupaeva, V. G., Pisareva, V. P., Merrick, W. C., Hellen, C. U., & Pestova, T. V. (2006). Specific functional interactions of nucleotides at key −3 and +4 positions flanking the initiation codon with components of the mammalian 48S translation initiation complex. *Genes & Development*, *20*(5), 624–636. https://doi.org/10.1101/gad.1397906

Qiu, H., Dong, J., Hu, C., Francklyn, C. S., & Hinnebusch, A. G. (2001). The tRNA-binding moiety in GCN2 contains a dimerization domain that interacts with the kinase domain and is required for tRNA binding and kinase activation. *The EMBO Journal*, *20*(6), 1425–1438. https://doi.org/10.1093/emboj/20.6.1425

Raini, G., Sharet, R., Herrero, M., Atzmon, A., Shenoy, A., Geiger, T., & Elroy-Stein, O. (2017). Mutant eIF2B leads to impaired mitochondrial oxidative phosphorylation in vanishing white matter disease. *Journal of Neurochemistry*, *141*(5), 694–707. https://doi.org/10.1111/jnc.14024

Richardson, J. P., Mohammad, S. S., & Pavitt, G. D. (2004). Mutations causing childhood ataxia with central nervous system hypomyelination reduce eukaryotic initiation factor 2B complex formation and activity. *Molecular and Cellular Biology*, *24*(6), 2352–2363.

Rodriguez, D. (2013). Leukodystrophies with astrocytic dysfunction. *Handbook of Clinical Neurology*, *113*, 1619–1628. https://doi.org/10.1016/B978-0-444-59565-2.00030-7

Rojas, M., Gingras, A. C., & Dever, T. E. (2014). Protein phosphatase PP1/GLC7 interaction domain in yeast eIF2gamma bypasses targeting subunit requirement for eIF2alpha dephosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), E1344–E1353. https://doi.org/10.1073/pnas.1400129111

Rojas-Rivera, D., Delvaeye, T., Roelandt, R., Nerinckx, W., Augustyns, K., Vandenabeele, P., & Bertrand, M. J. M. (2017). When PERK inhibitors turn out to be new potent RIPK1 inhibitors: Critical issues on the specificity and use of GSK2606414 and GSK2656157. *Cell Death and Differentiation*, *24*(6), 1100–1110. https://doi.org/10.1038/cdd.2017.58

Rowlands, A. G., Panniers, R., & Henshaw, E. C. (1988). The catalytic mechanism of guanine nucleotide exchange factor action and competitive inhibition by phosphorylated eukaryotic initiation factor 2. *The Journal of Biological Chemistry*, *263*(12), 5526–5533.

Scheper, G. C., Proud, C. G., & van der Knaap, M. S. (2006). Defective translation initiation causes vanishing of cerebral white matter. *Trends in Molecular Medicine*, *12*(4), 159–166. https://doi.org/10.1016/j.molmed.2006.02.006

Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., Gillespie, P., … Kaufman, R. J. (2001). Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Molecular Cell*, *7*(6), 1165–1176.

Sekine, Y., Zyryanova, A., Crespillo-Casado, A., Fischer, P. M., Harding, H. P., & Ron, D. (2015). Stress responses. Mutations in a translation initiation factor identify the target of a memory-enhancing compound. *Science*, *348*(6238), 1027–1030. https://doi.org/10.1126/science.aaa6986

Sidrauski, C., Acosta-Alvear, D., Khoutorsky, A., Vedantham, P., Hearn, B. R., Li, H., … Walter, P. (2013). Pharmacological brake-release of mRNA translation enhances cognitive memory. *eLife*, *2*, e00498. https://doi.org/10.7554/eLife.00498

Sidrauski, C., McGeachy, A. M., Ingolia, N. T., & Walter, P. (2015). The small molecule ISRIB reverses the effects of eIF2alpha phosphorylation on translation and stress granule assembly. *eLife*, *4*, e05033. https://doi.org/10.7554/eLife.05033

Sidrauski, C., Tsai, J. C., Kampmann, M., Hearn, B. R., Vedantham, P., Jaishankar, P., … Walter, P. (2015). Pharmacological dimerization and activation of the exchange factor eIF2B antagonizes the integrated stress response. *eLife*, *4*, e07314. https://doi.org/10.7554/eLife.07314

Simonetti, A., Brito Querido, J., Myasnikov, A. G., Mancera-Martinez, E., Renaud, A., Kuhn, L., & Hashem, Y. (2016). eIF3 peripheral subunits rearrangement after mRNA binding and start-codon recognition. *Molecular Cell*, *63*(2), 206–217. https://doi.org/10.1016/j.molcel.2016.05.033

Simpson, C. E., & Ashe, M. P. (2012). Adaptation to stress in yeast: To translate or not? *Biochemical Society Transactions*, *40*(4), 794–799. https://doi.org/10.1042/BST20120078

17577012, 2018, 6, Downloaded from https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1491 by The University Of Manchester, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Singh, C. R., Lee, B., Udagawa, T., Mohammad-Qureshi, S. S., Yamamoto, Y., Pavitt, G. D., & Asano, K. (2006). An eIF5/eIF2 complex antagonizes guanine nucleotide exchange by eIF2B during translation initiation. *The EMBO Journal*, *25*(19), 4537–4546. https://doi.org/10.1038/sj.emboj.7601339

Singh, C. R., Watanabe, R., Zhou, D., Jennings, M. D., Fukao, A., Lee, B., … Asano, K. (2011). Mechanisms of translational regulation by a human eIF5-mimic protein. *Nucleic Acids Research*, *39*(19), 8314–8328. https://doi.org/10.1093/nar/gkr339

Skopkova, M., Hennig, F., Shin, B. S., Turner, C. E., Stanikova, D., Brennerova, K., … Kalscheuer, V. M. (2017). EIF2S3 mutations associated with severe X-linked intellectual disability syndrome MEHMO. *Human Mutation*, *38*(4), 409–425. https://doi.org/10.1002/humu.23170

Sokabe, M., Fraser, C. S., & Hershey, J. W. (2012). The human translation initiation multi-factor complex promotes methionyl-tRNAi binding to the 40S ribosomal subunit. *Nucleic Acids Research*, *40*(2), 905–913. https://doi.org/10.1093/nar/gkr772

Somers, J., Wilson, L. A., Kilday, J. P., Horvilleur, E., Cannell, I. G., Poyry, T. A., … Willis, A. E. (2015). A common polymorphism in the 5' UTR of ERCC5 creates an upstream ORF that confers resistance to platinum-based chemotherapy. *Genes & Development*, *29*(18), 1891–1896. https://doi.org/10.1101/gad.261867.115

Sprang, S. R., & Coleman, D. E. (1998). Invasion of the nucleotide snatchers: Structural insights into the mechanism of G protein GEFs. *Cell*, *95*(2), 155–158.

Spriggs, K. A., Bushell, M., & Willis, A. E. (2010). Translational regulation of gene expression during conditions of cell stress. *Molecular Cell*, *40*(2), 228–237. https://doi.org/10.1016/j.molcel.2010.09.028

Starck, S. R., Jiang, V., Pavon-Eternod, M., Prasad, S., McCarthy, B., Pan, T., & Shastri, N. (2012). Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. *Science*, *336*(6089), 1719–1723. https://doi.org/10.1126/science.1220270

Starck, S. R., Tsai, J. C., Chen, K., Shodiya, M., Wang, L., Yahiro, K., … Walter, P. (2016). Translation from the 5′ untranslated region shapes the integrated stress response. *Science*, *351*(6272), aad3867. https://doi.org/10.1126/science.aad3867

Szamecz, B., Rutkai, E., Cuchalova, L., Munzarova, V., Herrmannova, A., Nielsen, K. H., … Valasek, L. (2008). eIF3a cooperates with sequences 5′ of uORF1 to promote resumption of scanning by post-termination ribosomes for reinitiation on GCN4 mRNA. *Genes & Development*, *22*(17), 2414–2425. https://doi.org/10.1101/gad.480508

Taniuchi, S., Miyake, M., Tsugawa, K., Oyadomari, M., & Oyadomari, S. (2016). Integrated stress response of vertebrates is regulated by four eIF2alpha kinases. *Scientific Reports*, *6*, 32886. https://doi.org/10.1038/srep32886

Thakor, N., & Holcik, M. (2012). IRES-mediated translation of cellular messenger RNA operates in eIF2alpha-independent manner during stress. *Nucleic Acids Research*, *40*(2), 541–552. https://doi.org/10.1093/nar/gkr701

Thomas, C., Fricke, I., Scrima, A., Berken, A., & Wittinghofer, A. (2007). Structural evidence for a common intermediate in small G protein-GEF reactions. *Molecular Cell*, *25*(1), 141–149. https://doi.org/10.1016/j.molcel.2006.11.023

Trinh, M. A., Kaphzan, H., Wek, R. C., Pierre, P., Cavener, D. R., & Klann, E. (2012). Brain-specific disruption of the eIF2alpha kinase PERK decreases ATF4 expression and impairs behavioral flexibility. *Cell Reports*, *1*(6), 676–688. https://doi.org/10.1016/j.celrep.2012.04.010

Tsai, J. C., Miller-Vedam, L. E., Anand, A. A., Jaishankar, P., Nguyen, H. C., Renslo, A. R., … Walter, P. (2018). Structure of the nucleotide exchange factor eIF2B reveals mechanism of memory-enhancing molecule. *Science*, *359*(6383), eaaq0939. https://doi.org/10.1126/science.aaq0939

Tsaytler, P., Harding, H. P., Ron, D., & Bertolotti, A. (2011). Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science*, *332*(6025), 91–94. https://doi.org/10.1126/science.1201396

van der Knaap, M. S., Leegwater, P. A., Konst, A. A., Visser, A., Naidu, S., Oudejans, C. B., … Pronk, J. C. (2002). Mutations in each of the five subunits of translation initiation factor eIF2B can cause leukoencephalopathy with vanishing white matter. *Annals of Neurology*, *51*(2), 264–270.

van der Knaap, M. S., van Berkel, C. G., Herms, J., van Coster, R., Baethmann, M., Naidu, S., … Pronk, J. C. (2003). eIF2B-related disorders: Antenatal onset and involvement of multiple organs. *American Journal of Human Genetics*, *73*(5), 1199–1207. https://doi.org/10.1086/379524

van der Voorn, J. P., van Kollenburg, B., Bertrand, G., Van Haren, K., Scheper, G. C., Powers, J. M., & van der Knaap, M. S. (2005). The unfolded protein response in vanishing white matter disease. *Journal of Neuropathology and Experimental Neurology*, *64*(9), 770–775.

van Kollenburg, B., van Dijk, J., Garbern, J., Thomas, A. A., Scheper, G. C., Powers, J. M., & van der Knaap, M. S. (2006). Glia-specific activation of all pathways of the unfolded protein response in vanishing white matter disease. *Journal of Neuropathology and Experimental Neurology*, *65*(7), 707–715. https://doi.org/10.1097/01.jnen.0000228201.27539.50

Vattem, K. M., & Wek, R. C. (2004). Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(31), 11269–11274. https://doi.org/10.1073/pnas.0400541101

Vazquez de Aldana, C. R., Dever, T. E., & Hinnebusch, A. G. (1993). Mutations in the alpha subunit of eukaryotic translation initiation factor 2 (eIF-2 alpha) that overcome the inhibitory effect of eIF-2 alpha phosphorylation on translation initiation. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(15), 7215–7219.

Walsh, D., Mathews, M. B., & Mohr, I. (2013). Tinkering with translation: Protein synthesis in virus-infected cells. *Cold Spring Harbor Perspectives in Biology*, *5*(1), a012351. https://doi.org/10.1101/cshperspect.a012351

Walter, P., & Ron, D. (2011). The unfolded protein response: From stress pathway to homeostatic regulation. *Science*, *334*(6059), 1081–1086. https://doi.org/10.1126/science.1209038

Wang, X., & Proud, C. G. (2008). A novel mechanism for the control of translation initiation by amino acids, mediated by phosphorylation of eukaryotic initiation factor 2B. *Molecular and Cellular Biology*, *28*(5), 1429–1442. https://doi.org/10.1128/MCB.01512-07

Wei, Z., Xue, Y., Xu, H., & Gong, W. (2006). Crystal structure of the C-terminal domain of S.Cerevisiae eIF5. *Journal of Molecular Biology*, *359*(1), 1–9. https://doi.org/10.1016/j.jmb.2006.03.037

Wek, R. C., Cannon, J. F., Dever, T. E., & Hinnebusch, A. G. (1992). Truncated protein phosphatase GLC7 restores translational activation of GCN4 expression in yeast mutants defective for the eIF-2 alpha kinase GCN2. *Molecular and Cellular Biology*, *12*(12), 5700–5710.

Welsh, G. I., Miller, C. M., Loughlin, A. J., Price, N. T., & Proud, C. G. (1998). Regulation of eukaryotic initiation factor eIF2B: Glycogen synthase kinase-3 phosphorylates a conserved serine which undergoes dephosphorylation in response to insulin. *FEBS Letters*, *421*(2), 125–130.

Williams, D. D., Price, N. T., Loughlin, A. J., & Proud, C. G. (2001). Characterization of the mammalian initiation factor eIF2B complex as a GDP dissociation stimulator protein. *The Journal of Biological Chemistry*, *276*(27), 24697–24703. https://doi.org/10.1074/jbc.M011788200

Wimbauer, F., Yang, C., Shogren, K. L., Zhang, M., Goyal, R., Riester, S. M., … Maran, A. (2012). Regulation of interferon pathway in 2-methoxyestradiol-treated osteosarcoma cells. *BMC Cancer*, *12*(1), 93. https://doi.org/10.1186/1471-2407-12-93

Wong, K., Armstrong, R. C., Gyure, K. A., Morrison, A. L., Rodriguez, D., Matalon, R., … Schiffmann, R. (2000). Foamy cells with oligodendroglial phenotype in childhood ataxia with diffuse central nervous system hypomyelination syndrome. *Acta Neuropathologica*, *100*(6), 635–646.

Wong, Y. L., LeBon, L., Edalji, R., Lim, H. B., Sun, C., & Sidrauski, C. (2018). The small molecule ISRIB rescues the stability and activity of vanishing white matter disease eIF2B mutant complexes. *eLife*, *7*, e32733. https://doi.org/10.7554/eLife.32733

Wortham, N. C., Martinez, M., Gordiyenko, Y., Robinson, C. V., & Proud, C. G. (2014). Analysis of the subunit organization of the eIF2B complex reveals new insights into its structure and regulation. *The FASEB Journal*, *28*(5), 2225–2237. https://doi.org/10.1096/fj.13-243329

Wortham, N. C., & Proud, C. G. (2015). Biochemical effects of mutations in the gene encoding the alpha subunit of eukaryotic initiation factor (eIF) 2B associated with vanishing white matter disease. *BMC Medical Genetics*, *16*, 64. https://doi.org/10.1186/s12881-015-0204-z

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Xiao, J., Tan, Y., Li, Y., & Luo, Y. (2016). The specific protein kinase R (PKR) inhibitor C16 protects neonatal hypoxia-ischemia brain damages by inhibiting Neuroinflammation in a neonatal rat model. *Medical Science Monitor*, *22*, 5074–5081.

Young, S. K., Palam, L. R., Wu, C., Sachs, M. S., & Wek, R. C. (2016). Ribosome elongation stall directs gene-specific translation in the integrated stress response. *The Journal of Biological Chemistry*, *291*(12), 6546–6558. https://doi.org/10.1074/jbc.M115.705640

Young, S. K., & Wek, R. C. (2016). Upstream open reading frames differentially regulate gene-specific translation in the integrated stress response. *The Journal of Biological Chemistry*, *291*(33), 16927–16935. https://doi.org/10.1074/jbc.R116.733899

Young, S. K., Willy, J. A., Wu, C., Sachs, M. S., & Wek, R. C. (2015). Ribosome Reinitiation directs gene-specific translation and regulates the integrated stress response. *The Journal of Biological Chemistry*, *290*(47), 28257–28271. https://doi.org/10.1074/jbc.M115.693184

Zhou, D., Palam, L. R., Jiang, L., Narasimhan, J., Staschke, K. A., & Wek, R. C. (2008). Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. *The Journal of Biological Chemistry*, *283*(11), 7064–7073. https://doi.org/10.1074/jbc.M708530200

Zhou, J., Wan, J., Shu, X. E., Mao, Y., Liu, X. M., Yuan, X., … Qian, S. B. (2018). *N*(6)-Methyladenosine guides mRNA alternative translation during integrated stress response. *Molecular Cell*, *69*(4), 636–647 e637. https://doi.org/10.1016/j.molcel.2018.01.019

Zhu, P. J., Huang, W., Kalikulov, D., Yoo, J. W., Placzek, A. N., Stoica, L., … Costa-Mattioli, M. (2011). Suppression of PKR promotes network excitability and enhanced cognition by interferon-gamma-mediated disinhibition. *Cell*, *147*(6), 1384–1396. https://doi.org/10.1016/j.cell.2011.11.029

Zyryanova, A. F., Weis, F., Faille, A., Alard, A. A., Crespillo-Casado, A., Sekine, Y., … Ron, D. (2018). Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. *Science*, *359*(6383), 1533–1536. https://doi.org/10.1126/science.aar5129

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