Eukaryotic translation initiation factor 2B (eIF2B), a heterodecameric complex of two sets of the α, β, γ, δ, and ε subunits, is the guanine nucleotide exchange factor (GEF) specific for eIF2, a heterotrimeric G protein consisting of the α, β, and γ subunits. The eIF2 protein binds GTP on the γ subunits and delivers an initiator methionyl-tRNA (Met-tRNA\textsubscript{Met}) to the ribosome. The GEF activity of eIF2B is inhibited by stress-induced phosphorylation of Ser51 in the α subunit of eIF2, which leads to lower amounts of active eIF2 and a limited quantity of Met-tRNA\textsubscript{Met} for the ribosome, resulting in global repression of translation. However, the structural mechanism of the GEF activity inhibition remained enigmatic, and therefore the three-dimensional structure of the entire eIF2B molecule had been awaited. Recently, we determined the crystal structure of Schizosaccharomyces pombe eIF2B. In this Structural Snapshot, we present the structural features of eIF2B and the mechanism underlying the GEF activity inhibition by the phosphorylation of eIF2α, elucidated from structure-based \textit{in vitro} analyses.

**Introduction**

Eukaryotic translation initiation factor 2 (eIF2), a heterotrimeric G protein consisting of the α, β, and γ subunits, primarily functions to deliver Met-tRNA\textsubscript{Met} to the small ribosomal subunit in a GTP-dependent manner. During the translation initiation process, the ribosome releases eIF2 in an inactive GDP-bound form. For the next round of translation initiation, the GDP on eIF2γ must be replaced by GTP with the help of the eIF2-specific guanine nucleotide exchange factor, eIF2B, a heterodecameric complex consisting of two copies each of the α, β, γ, δ, and ε subunits [1,2]. The nucleotide exchange activity of eIF2B requires the formation of the catalytic γε subcomplex [3]. Especially, the consecutive Asn-Phe residues called the NF motif and the HEAT domain, which are both in the ε subunit, are critical for the activity. Although the HEAT

**Abbreviations**

AGP, ADP-glucose pyrophosphorylase; CACH, childhood ataxia with central nervous system hypomyelination; eIF, eukaryotic translation initiation factor; Gcn\textsubscript{-}, general control nondepressible; GEF, guanine nucleotide exchange factor; ISRIB, integrated stress response inhibitor; Met-tRNA\textsubscript{Met}, initiator methionyl-tRNA; P-eIF2α, eIF2α phosphorylated at Ser51; pBpa, p-benzoyl-L-phenylalanine; R15P, ribose-1,5-bisphosphate isomerase; R15P, ribose-1,5-bisphosphate; VWM, leukoencephalopathy with vanishing white matter.
domain alone possesses weak nucleotide exchange activity, it is greatly accelerated by the NF motif in the catalytic subcomplex [4,5]. The other three subunits of eIF2B form the regulatory αβδ subcomplex, which binds tightly to eIF2α phosphorylated at Ser51 (P-eIF2α) [3,6]. In mammals, four eIF2α kinases responding to various stress stimuli have been identified: mGCN2, PKR, PERK, and HRI [7]. The strong interaction between eIF2B and phosphorylated eIF2 inhibits the nucleotide exchange on eIF2γ [6,8], and thus limits the amount of Met-tRNA<sup>Met</sup> available to the ribosome, which consequently represses translation globally. Importantly, many mutations in the human eIF2B subunits have been identified as causing the neurodegenerative disease called leukoencephalopathy with vanishing white matter (VWM) or childhood ataxia with central nervous system hypomyelination (CACH) [9,10]. In VWM/CACH patients, white matter lesions severely deteriorate and neurological disorders are exacerbated after stresses, and their eIF2B guanine nucleotide exchange activities are generally lower than normal [11], even though the severity of the disease does not correlate with the deficits in the activity [11,12]. To date, some partial structures of eIF2B have been solved: the HEAT domain [13,14], the α<sub>3</sub> homodimer [15], and the (βδ)<sub>2</sub> heterotetramer [16]. However, the overall structure of eIF2B had remained unknown, and the structural bases of the eIF2B-mediated translational control and the pathogenesis of VWM/CACH disease are poorly understood.

**The overall structure of Schizosaccharomyces pombe eIF2B**

We successfully established a system to produce a large amount of *S. pombe* eIF2B by coexpressing all subunits in *Escherichia coli*, and the purified eIF2B displayed the characteristic biochemical properties of natural eIF2B [17,18]. Using this recombinant protein, we produced high quality crystals of eIF2B, and determined its three-dimensional structure at 3.0-Å resolution. We assigned almost all regions except the HEAT domain of the ε subunit, and revealed the arrangement of the eIF2B subunits: the α<sub>3</sub>β<sub>2</sub>δ<sub>3</sub> hexameric regulatory subcomplex is sandwiched between the two γε heterodimeric catalytic subcomplexes (Fig. 1A). Mapping of the residues corresponding to the missense mutations causing VWM disease [9] revealed that many mutations are located within or around the subunit interface [18], indicating that the correct assembly into the decameric structure of eIF2B is important for its activity. The arrangement of the regulatory subunits is similar to that in the homohexameric structure of ribose-1,5-bisphosphate isomerase (R15Pi) [19], which shares sequence homology with the eIF2B regulatory subunits (Fig. 1B). The catalytic γ and ε subunits also share structural homology, and their structures resemble the subunit structure of the homotetrameric ADP-glucose pyrophosphorylase (AGP) [20] (Fig. 1C). The HEAT domain at the very C terminus of the ε subunit was disordered in the crystal, probably because of its high mobility. The visible C terminus of the ε subunit extends out from the main body of eIF2B, and therefore the subsequent HEAT domain can move around the 'distal' region of eIF2B (Fig. 1A,C). The NF motif, the other motif important for nucleotide exchange, also resides on this distal face of eIF2B (Fig. 1C), suggesting that nucleotide exchange on eIF2γ occurs on the distal face of eIF2B.

**Identification of interfaces for eIF2 by surface-scanning photo-cross-linking**

In order to examine the interaction between eIF2B and eIF2γ on the distal face experimentally, we performed surface-scanning photo-cross-linking experiments. The photo-reactive unnatural amino acid, p-benzoyl-L-phenylalanine (pBpa), was incorporated at various desired positions in eIF2B, using the *E. coli* RFzero strain [21,22]. When the incorporated pBpas in eIF2B and eIF2γ are located in close proximity, the ultraviolet irradiation produces the cross-linked molecule, which is detected by western blotting. The eIF2γ cross-linked positions detected in eIF2B were distributed over a large area on the distal face, confirming that eIF2γ binds on this face (Fig. 2A). In addition, several VWM mutations are mapped around these cross-linked residues, supporting the idea that nucleotide exchange is performed on the distal face (Fig. 2A). We revealed that the phosphorylation of eIF2 retarded the cross-linking to the pBpa sites near the NF motif [18], indicating that the phosphorylation restricts the access of eIF2γ around the NF motif. We also showed that the retardation of cross-linking was not observed at the sites distant from the NF-motif [18]. These phosphorylation-insensitive cross-links presumably reflect the recently reported eIF2B activity to displace eIF5 from the eIF2–eIF5 complex, in which eIF5 inhibits GDP dissociation from eIF2 [23,24].

In the same manner, we next searched for the P-eIF2α-binding site of the eIF2B regulatory subcomplex. The experiments revealed that the 'central cavity', formed around the center of one set of the three regulatory subunits, is the P-eIF2α-binding site (Fig. 2A). This central cavity includes some residues with mutations that have been isolated as Gcn<sup>−</sup> (general control
(25,26), which prevent cells from inducing translational control upon eIF2 phosphorylation. We also found that the central cavity binds to unphosphorylated eIF2α in a similar manner to P-eIF2α, but with a lower affinity [18]. Since fewer missense VWM mutations were identified in the central cavity [18], the VWM disease does not seem to correlate strongly with the recognition of the phosphorylation of eIF2.

In order to determine how eIF2α fits within the central cavity, we introduced ρBpa in the N-terminal domain (NTD) of eIF2α, and detected the photo-cross-links with the regulatory subunits of eIF2B [18]. The results provided information about the orientation of eIF2α in the central cavity, and we successfully constructed the docking model of eIF2 and eIF2B, using the crystal structure of aIF2, the archaeal homolog of eIF2 [27] (Fig. 2B). This docking model indicated that it is difficult for the eIF2 to be captured by the central cavity and to interact with the NF motif simultaneously. Therefore, the eIF2-eIF2B complex state in which the eIF2α is captured in the central cavity is the ‘nonproductive’ state, which is distinct from the ‘productive’ state for efficient nucleotide exchange mediated by the NF motif (Fig. 2C) [3,28]. When eIF2α is not phosphorylated, the nonproductive state is not stable, because the eIF2α is captured only weakly by the central cavity. Consequently, the interaction mode between eIF2 and eIF2B easily moves to the productive state (Fig. 2C). However, once eIF2α is phosphorylated, the eIF2-eIF2B complex can hardly escape from the nonproductive state, as it is stabilized by the strong binding between the eIF2B central cavity and the P-eIF2α, which results in the robust inhibition of the nucleotide exchange and the global translation repression (Fig. 2C).

**Possible regulation of eIF2B by small molecule ligands**

The GTP binding to eIF2B [29] has attracted keen interest, because its binding site may provide clues to a
mechanism of nucleotide exchange. So far, two competing hypotheses have been proposed: a familiar substitution mechanism and a sequential mechanism involving the eIF2-GDP-eIF2B-GTP quaternary complex [30]. A recent mass spectrometric analysis [31] showed that GTP specifically binds to eIF2B, favoring the latter mechanism. The structural conservation between AGP [20] and the catalytic subunits prompted us to examine whether GTP binds to a corresponding site in eIF2B. However, our structure revealed that the pocket structure is not conserved between eIF2B and AGP, and it does not seem to have nucleotide-binding capacity (Fig. 3A). Consistent with a previous mutational analysis [32], the corresponding region in eIF2Be is also distorted and seemingly devoid of this capacity (Fig. 3A). Therefore, the GTP-binding site on eIF2B and the mechanism of nucleotide exchange remain elusive.

Other small molecules also reportedly bind to eIF2B. eIF2Be was shown to bind GMP or AMP within its interdomain pocket [16], and this event was speculated to regulate the activity of eIF2B independently of eIF2 phosphorylation by inducing the movement of the NTDs of the regulatory subunits, as observed in R15Pi [19]. Our structure revealed that this movement is applicable only to eIF2Be, because the association with the catalytic subcomplex restricts the movements of eIF2Bβ and eIF2Bδ (Fig. 1A). In our structure, the NTD of eIF2Be is in an intermediate position between the apo and ligand-bound conformations of R15Pi (Fig. 3B). The domain movement by GMP/AMP binding opens the central cavity more widely and may affect the recognition of (P-)eIF2α.

Recently, a small molecule called ISRIB (integrated stress response inhibitor) was found to render cells insensitive to the effects of eIF2 phosphorylation, by increasing the nucleotide exchange activity of eIF2B [33–35]. This molecule was shown to prevent neurodegeneration in prion disease [36] and to mitigate the chemoresistance of pancreatic ductal adenocarcinoma [37]. Our structure revealed that the residues whose corresponding mutations in human eIF2B interfere with the response to ISRIB [34] are located near the pseudo twofold rotational axis of eIF2B (Fig. 3C). Therefore, the symmetric ISRIB molecule probably bridges between the two δ subunits and prevents the eIF2B decamer from splitting into βγδε tetramers; this role has also been proposed for mammalian eIF2Be [38].
Concluding remarks

The crystallographic and biochemical studies of eIF2B provided not only the structural framework for the stress-induced eIF2B-mediated translational control but also the probable mechanism of the VWM disease. In order to elucidate the molecular mechanism of eIF2B completely, atomic-level structural studies of the eIF2–eIF2B complex in the productive and non-productive states will be required. Especially, the manner in which eIF2B recognizes the phosphorylation of eIF2 is of great interest, since the identified eIF2B central cavity is highly negatively charged, and thus may not bind tightly to the phosphate group. In addition, the molecular mechanism by which eIF2B displaces eIF5 from eIF2 is still poorly understood. We earnestly hope that this study provides some clues to unveil these mechanisms.

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Author contributions

All authors wrote the manuscript and KK prepared the figures.
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