Crystal structure of eukaryotic translation initiation factor 2B

The eukaryotic translation initiation factor 2B (eIF2B) is the guanine nucleotide exchange factor for eIF2. eIF2 carries the methionylated initiator tRNA (Met-tRNA$_i$) to the ribosome in a GTP-dependent manner and is released from the ribosome as the GDP-bound form after the recognition of an initiation codon. To participate in the next round of translation initiation events, eIF2 must be converted into the GTP-bound form by eIF2B (Fig. 1) [1]. However, this nucleotide exchange activity of eIF2B is inhibited by the phosphorylation of eIF2. The phosphorylation of eIF2 is known to be rapidly induced by eIF2 kinases under various stress conditions such as viral infection and amino acid deprivation, and the phosphorylated eIF2 forms a tight inactive complex with eIF2B. As a result of this inhibition, the cellular level of the active GTP-bound eIF2 is lowered and the supply of Met-tRNA$_i$ to the ribosomes is limited. Thus, protein synthesis under stress is globally restricted and stress response genes that contain some special elements on their mRNAs are selectively translated.

eIF2B is a heterodecameric protein composed of two sets of five non-identical subunits (α-ε), while eIF2 is a heterotrimer composed of three subunits (α-γ). Yeast eIF2B is known to be divided into two types of subcomplexes [2]. The α, β and δ subunits of eIF2B form a regulatory subcomplex that is responsible for the recognition of stress-induced phosphorylation on the α subunit of eIF2 (eIF2α) and a formation of the tight inactive eIF2-eIF2B complex. On the other hand, the γ and ε subunits of eIF2B form the catalytic subcomplex that is responsible for the exchange of GDP on the γ subunit of eIF2 (eIF2γ). In the last few decades, genetic and biochemical studies have identified residues in the

eIF2B subunits that are essential for these regulatory and catalytic interactions with eIF2. However, the molecular mechanisms of these interactions have hardly been described since the tertiary structure of the eIF2B decamer was unknown.

Toward structural analysis, our group established a bacterial expression system for the large-scale production of *Schizosaccharomyces pombe* eIF2B, which then was successfully crystallized [3]. X-ray diffraction data of the eIF2B crystals were collected at SPring-8 **BL32XU** and **BL41XU** and at Photon Factory beamlines. The initial phases were determined from the selenomethionine-derivative data set by the single-wavelength anomalous dispersion method [4]. The crystal structure of eIF2B was finally refined to a resolution of 3.0 Å, revealing its decameric architecture. A hexameric regulatory subcomplex composed of the α-α homodimer and two β-δ heterodimers occupies the central part of the structure and two catalytic subcomplexes composed of the γ-ε heterodimer are attached to the regulatory subcomplex on its β-δ heterodimer faces (Fig. 2). Both the HEAT domain and the NF motif in the ε subunit, the key elements required for an efficient nucleotide exchange reaction, reside in the "distal" side of the catalytic subcomplex.

Furthermore, we identified the interfaces for eIF2α and eIF2γ on the molecular surface of eIF2B by the site-directed incorporation of *p*-benzoyl-L-phenylalanine (*p*Bpa), a photoreactive synthetic amino acid, and photo-cross-linking with eIF2. Photo-cross-links with eIF2α were detected when *p*Bpa was incorporated in the cavity region at the center of the regulatory subcomplex, while those with eIF2γ were detected on the distal face of the catalytic subcomplex, adjacent to the NF motif (Fig. 3(a)). These results show that the phosphorylation status of eIF2α is recognized at the central cavity, while the catalysis of nucleotide exchange on eIF2γ is performed on the distal face. Intriguingly, our docking model of eIF2 and eIF2B revealed that it is difficult for a single molecule of eIF2 to interact simultaneously with these two interfaces. Therefore, we proposed that these two types of eIF2-eIF2B interactions are mutually exclusive and that two or more distinct states are present in the eIF2-eIF2B complex. When eIF2γ binds on the distal face of the catalytic subcomplex, the complex is in the "productive" state, where nucleotide exchange is catalyzed by the HEAT domain and the NF motif. On the other hand, when eIF2α is captured at the central cavity in the regulatory subcomplex, the
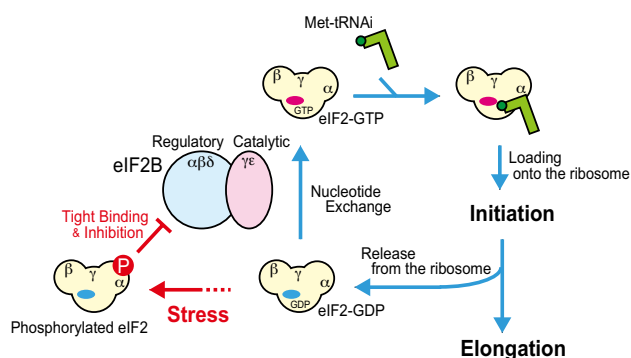

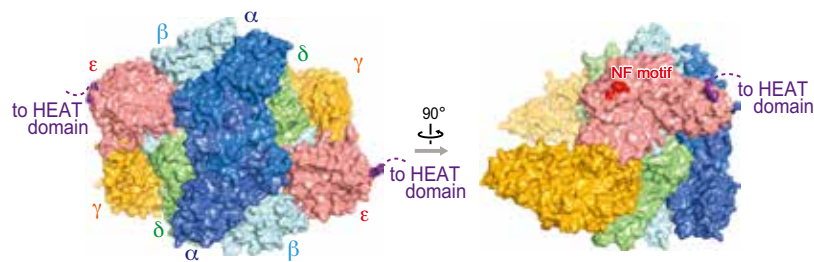Fig. 1. Schematic representation of eIF2B-mediated translational control.

Fig. 2. Overall structure of *S. pombe* eIF2B. The α, β, γ, δ and ε subunits are colored blue, cyan, orange, green and salmon pink, respectively. The NF motifs in the ε subunits are shown in red. The HEAT domains at the C-terminus of the ε subunits were not observed in our structure and the observed C-terminal residues of the ε subunits are colored purple.

complex is in the "non-productive" state, where nucleotide exchange is not performed. Since the stress-induced phosphorylation of eIF2α tightens the interaction with the regulatory subunits of eIF2B and stabilizes the non-productive state of the eIF2-eIF2B complex, nucleotide exchange reactions are restricted (Fig. 3(b)).

Our study first revealed the decameric architecture of eIF2B and paves the way for the mechanistic understanding of stress-induced, eIF2B-mediated translational control. Future structural studies are expected to unveil the molecular mechanisms of nucleotide exchange and the recognition of eIF2α phosphorylation.

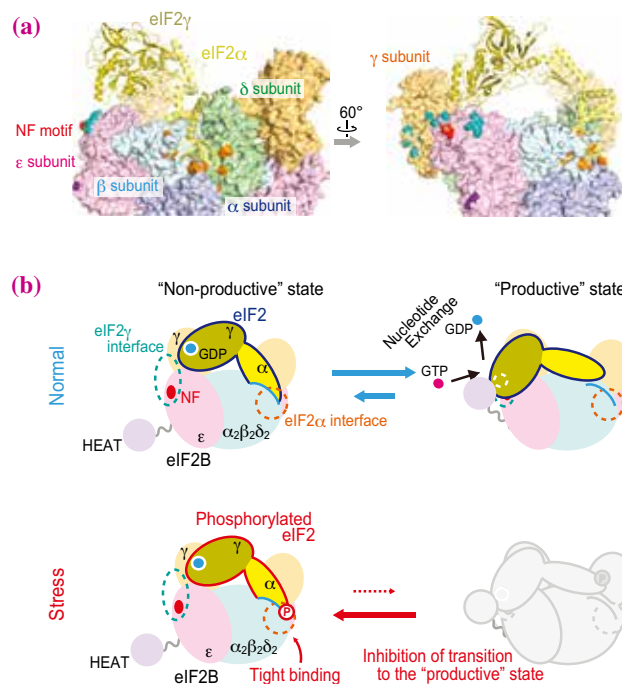Fig. 3. (a) Mapping of the photo-cross-linked eIF2B sites and the docking of eIF2 and eIF2B. The sites that cross-linked with eIF2α and eIF2γ are colored in orange and teal, respectively. In the docking, the structures of aIF2α and γ from PDB ID: 2QMU [5] were used instead of eIF2, and the N-terminal domain of a/eIF2α was positioned in the central cavity of eIF2B. (b) Schematic representations of the proposed mechanism of eIF2B inhibition by phosphorylated eIF2.

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