Mechanism of stress-induced inhibition of eukaryotic translation initiation factor 2B

The regulation of the eukaryotic initiation factor 2B (eIF2B) is central to the stress-induced control of protein synthesis. eIF2B is the guanine nucleotide exchange factor specific for another initiation factor eIF2, which delivers the methionylated initiator tRNA to the ribosomes in a GTP-dependent manner. eIF2B catalyzes the conversion of inactive GDP-bound eIF2 to active GTP-bound eIF2 [1]. When eukaryotic cells detect stress, eIF2 is rapidly phosphorylated by eIF2 kinases, and this phosphorylation inhibits the activity of eIF2B. As a result, the decreased cellular level of the GTP-bound eIF2 limits the supply of initiator tRNA to the ribosomes and globally attenuates translation. Various types of stress signals, such as nutrient starvation or viral infection, induce this response, and therefore it is called the "integrated" stress response (ISR) [2]. Although the factors involved in ISR are well characterized, the structural basis of this process is largely unknown. In particular, how eIF2B catalyzes the exchange reaction on eIF2 and how this activity is inhibited by the phosphorylation of eIF2 have remained undescribed.

eIF2 and eIF2B, the key factors of ISR, are both multimeric proteins. eIF2 is a heterotrimeric protein (α to γ); the α subunit is phosphorylated under stress, and the γ subunit binds GTP or GDP. eIF2B is a heterodecameric protein composed of two copies each of five different subunits (α to ε). The minimal region required for the nucleotide exchange is the HEAT domain, which resides at the C-terminus of the ε subunit. We prepared the various complexes of eIF2 and eIF2B, and analyzed them by cryo-electron microscopy (cryo-EM) and X-ray crystallography. X-ray diffraction data were collected at SPring-8 **BL41XU** [3].

We determined the cryo-EM structures of eIF2B complexed with phosphorylated or unphosphorylated eIF2. They revealed that the binding mode of eIF2 is completely different depending on its phosphorylation status (Fig. 1), and the catalytic HEAT domain of eIF2B is observed only in the unphosphorylated eIF2•eIF2B complex. Since the HEAT domain is bound to eIF2γ, the nucleotide-binding subunit, this complex seems to represent the "productive" state in which the nucleotide is being exchanged by eIF2B. On the other hand, in the phosphorylated eIF2•eIF2B complex, eIF2γ is located apart from eIF2Bε, representing the "nonproductive" state in which no nucleotide exchange reaction occurs.

The details of the recognition of eIF2 phosphorylation were obtained from the crystal structures of eIF2B complexed with the phosphorylated or unphosphorylated eIF2α subunit. Because of the absence of the interaction mediated by eIF2γ, the binding modes of eIF2α resemble the nonproductive mode regardless of their phosphorylation status (Fig. 2(a)). The difference between these structures is limited to the neighborhood of the phosphorylation residue Ser51. In the complex with the phosphorylated eIF2α, the phosphorylated Ser51 residue itself is not recognized directly by eIF2B, but instead, the loop region adjacent to this residue is inserted toward the eIF2B subunits (Fig. 2(b)). Compared with the unphosphorylated structure, this loop is inserted more deeply and undergoes more interaction with eIF2B subunits. Therefore, this change makes the "nonproductive" binding mode more stable than the productive mode when eIF2 is phosphorylated and prevents the nucleotide exchange from the phosphorylated eIF2.

In addition to the above prevention, the binding of phosphorylated eIF2 appears to have an additional role in the inhibition of eIF2B. Our cryo-EM structures

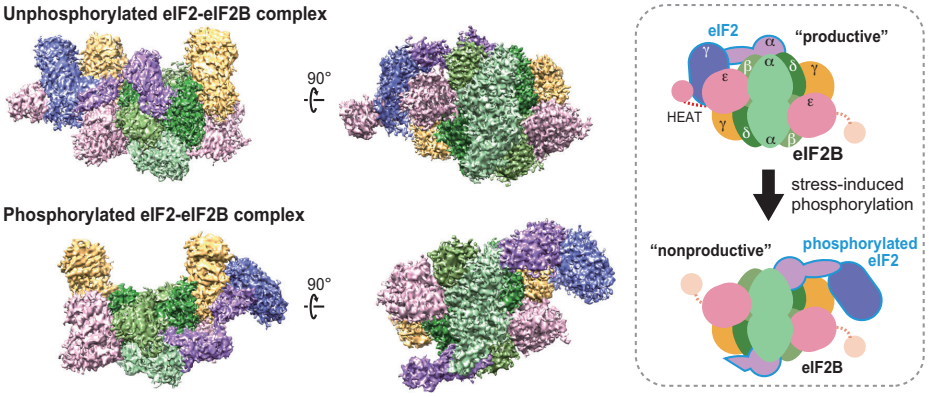

Fig. 1. Cryo-EM structures of eIF2B complexed with unphosphorylated eIF2 (upper) and phosphorylated eIF2 (below), and their schematic representations.

indicate that the simultaneous binding of one phosphorylated eIF2 and one unphosphorylated eIF2 to the opposite sides of eIF2B is impossible owing to the steric clash between the HEAT domain of eIF2Bε and the γ subunit of the phosphorylated eIF2 (Fig. 3(a)). Therefore, the binding of the phosphorylated eIF2 also blocks the nucleotide exchange reaction at the opposite side of eIF2B. On the basis of these findings, we propose the following mechanism of eIF2B inhibition under stress. Under the normal condition, eIF2B forms the productive complex with the unphosphorylated eIF2 and performs nucleotide exchange. Upon stress, the phosphorylated eIF2 appears and binds to eIF2B in the nonproductive manner. Not only does the phosphorylated eIF2 prevent nucleotide exchange by keeping itself away from the catalytic elements of eIF2B, it also hinders the catalysis at the opposite side. Eventually, the phosphorylated eIF2 occupies both sides of eIF2B and completely stops the exchange activity of eIF2B (Fig. 3(b)).

Our structures provide the structural basis for the stress-induced translational control mediated by eIF2B. eIF2B is now attracting growing interest as a potential drug target for traumatic brain injury and neurodegeneration [4,5]. The small molecule called ISRIB, which boosts the catalytic activity of eIF2B and reverses ISR, has shown promising effects in mouse models. Our structures may also provide valuable information for understanding the mechanistic action of ISRIB and the development of a novel treatment for traumatic brain injury and neurodegeneration.

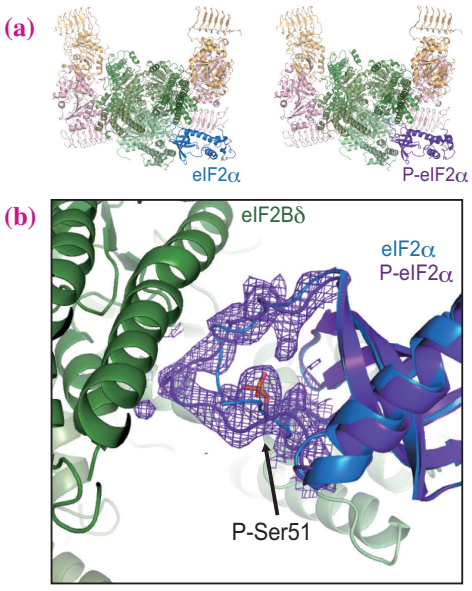

Fig. 2. **(a)** Crystal structures of eIF2B complexed with unphosphorylated eIF2α (left) and phosphorylated eIF2α (P-eIF2α, right). **(b)** Loop regions adjacent to the phosphorylation residue Ser51 (P-Ser51). The electron density in the loop region of phosphorylated eIF2α is also shown.

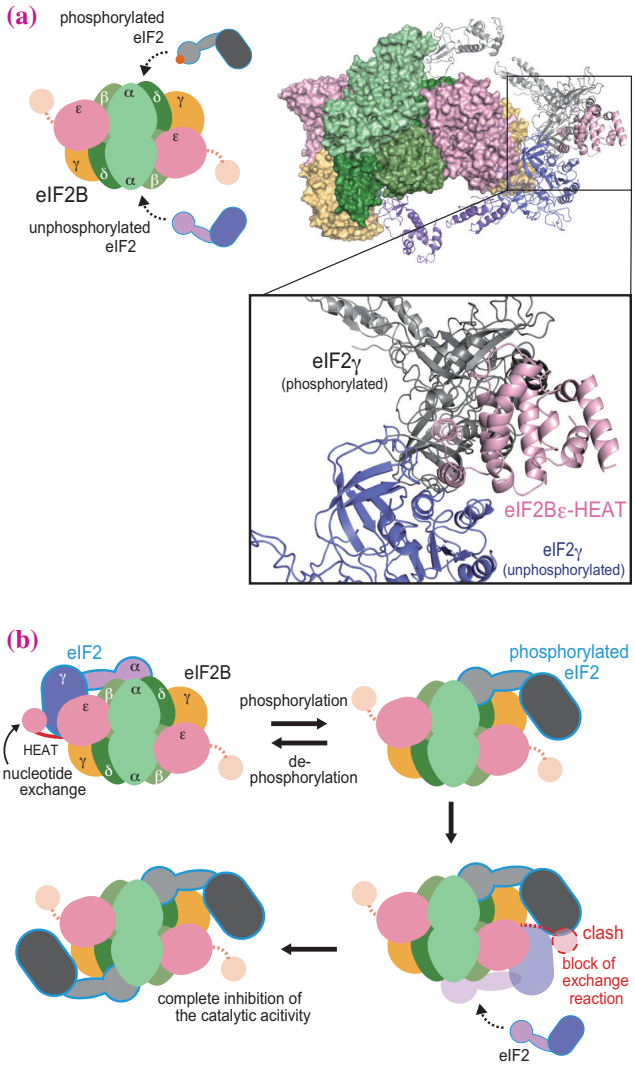

Fig. 3. **(a)** Model in which phosphorylated eIF2 (gray) and unphosphorylated eIF2 (violet) are bound to either side of eIF2B. The position of the eIF2Bε HEAT domain (pink) overlaps with that of the γ subunit of phosphorylated eIF2. **(b)** Proposed model of inhibition of eIF2B by phosphorylated eIF2.

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