How does ribosomal P stalk recognize and deliver elongation factor EF-2?

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The ribosome is molecular machinery that converts a sequence of nucleic acids to that of the corresponding protein(translation) in all organisms. In this translation on the ribosome, a large amount of energy is necessary for synthesizing proteins, which is provided by the guanosine-tri-phosphate (GTP) bound to the translational GTPases (trGTPases). As an essential ribosomal functional center, a GTPase-associated center of ribosome located at the sarcin/ricin loop (SRL) of 23S rRNA of the large subunit, recruits trGTPases for promoting the GTP hydrolysis. This center is also known to help new elongator aminoacylated-tRNAs to recruit precisely to the A site of the ribosome. For supplying the energy from the GTP hydrolysis continuously, the lateral protuberant protein complex, termed ribosomal stalk exists at the GTPase-associated center. This stalk functionally conserved in all ribosomes in all domains of life. It is composed of a scaffold protein (P0 in eucaryote and archaea, and L10 in bacteria) and a couple of sets of dimerized proteins (two sets of P1/P2 heterodimer in eukaryote, three sets of P1 homodimer in archaea, and two-four sets of L12 homodimer in bacteria).

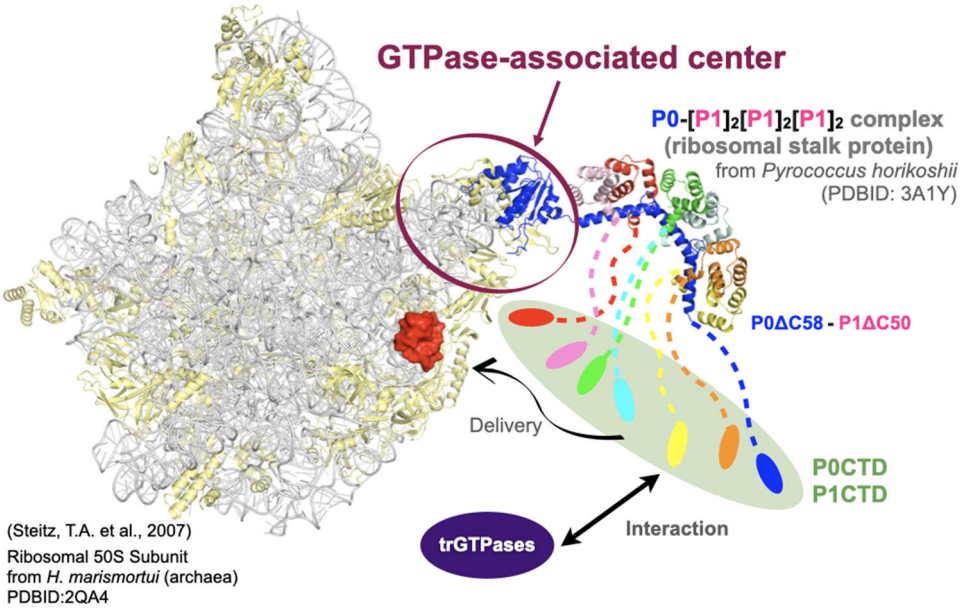

Figure 1. Model of archaeal GTPase-associated center. Archaeal ribosomal P stalk (PDB ID: 3A1Y) was fitted to the 50S ribosomal large subunit (PDB ID: 2QA4) of archaea. The C-terminal regions of the P stalk were drawn by broken lines.

The C-terminal regions are highly flexible and could not visualize by X-ray crystallography and cryogenic electron microscopic methods. Therefore, it is mysterious how the stalk recognizes and delivers trGTPases to the GTPase-associated center. In the previous study, our group determined the crystal structure of archaeal P stalk complex P0-$[P1]_2[P1]_2[P1]_2$ where we truncated the flexible C-terminal 58 residues of P0 and 50 residues of P1 for crystallization (Figure 1) [1]. Furthermore, based on the results of the binding assay, we know that the three residues, L103, L106, and F107 of the C-terminal domain of P1 (P1CTD) are key to interact with trGTPases [2]. On the other hand, it is characteristic and intriguing that amino acid sequences of the individual binding regions of trGTPases such as an initiation factor IF5B, elongation factors EF-1α and EF-2, and so on, are not conserved at all.

In this study, we determined a series of crystal structures based on archaeal elongation factor 2 from *Pyrococcus horikoshii* (*Pho*EF-2): Apo form (*Pho*EF-2-Apo), GMPPCP form (*Pho*EF-2-GMPPCP), GDP form (*Pho*EF-2-D2-GDP), and *Pho*EF-2-GMPPCP bound with the C-terminal 11 residues of P1 (*Pho*EF-2-GMPPCP-P1C11) at resolutions of 2.3, 2.3, 1.6, and 3.1 Å, respectively (Figure 2a). Additionally, we built the docking model of *Pho*EF-2-GDP with P1C11 (*Pho*EF-2-GDP-P1C11) using molecular dynamics simulation based on their crystal structures.

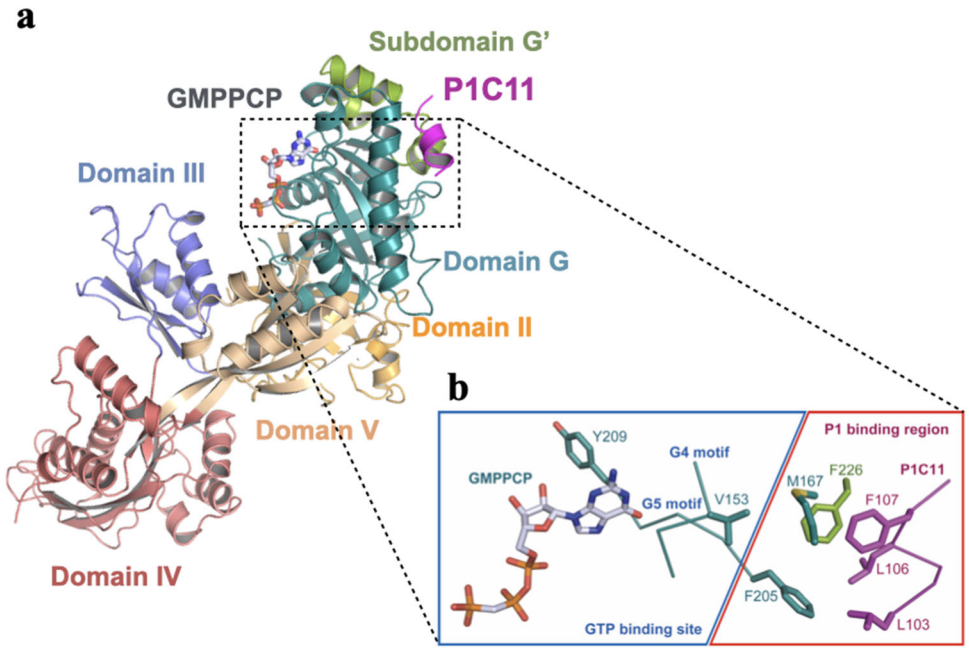

Figure 2. Crystal structure of *Pho*EF-2-GMPPCP-P1C11. (**a**) overall structure. (**b**) The structural relationships between GTP binding site and P1-binding region.

The P1C11 peptide formed an α-helical structure and bound to the hydrophobic groove between domain G and subdomain G' of *Pho*EF-2 where is in the opposite side of the GTP binding site (Figure 2a). This binding region was different from that of *Pho*EF-1α in terms of domain, position, and sequence, implying that such binding characteristic may be requested how trGTPase behave on the ribosome and P1CTD possesses a partner-function-dependent recognition manner. The results of the P1-binding assays of *Pho*EF-2 mutants showed that three residues (M167 and F205 of the domain G, and F226 of the subdomain G') played a role in the interaction with the P1CTD. The P1-binding position exists in close by the GTP binding site rather than when we look over the entire structure of the *Pho*EF-2-GMPPCP-P1C11 (Figure 2). Furthermore, V153 of the G4-motif (N151-D154) in the GTP-binding site forms a hydrophobic core with M167, F205 and F226, and F205 exists in the G5-motif (S207-Y209) (Figure 2b). These structural relationships between GTP- and P1-binding site indicate that the P1-binding may be affected allosterically by the GTP/GDP-binding, supported by a quantitative P1-binding experiment by surface plasmon resonance (SPR). The P1-binding affinity of GMPPCP and GDP was almost equal, while that of Apo form slightly decreased.

Moreover, we performed the molecular dynamics simulation of *Pho*EF-2-GDP-P1C11 for discussing the detailed interaction between *Pho*EF-2-GDP and P1C11. The docked model implies that *Pho*EF-2 was probably dissociated from the ribosome after the GTP hydrolysis. By structural comparison among five types of *Pho*EF-2, we found that their P1-binding regions formed different conformations. Taken all together, we propose that the *Pho*EF-2 responses to the processes of recruiting and releasing trGTPases through three states: 'closed', 'open', and 'release' of the P1-binding groove (Figure 3). The P1-binding region of trGTPases are not conserved among several trGTPases that P1CTD recognizes and delivers to the GTPase-associated center, suggesting that the P1 interacts with a favourable position of each trGTPase may be requested by how trGTPases perform their functions in individual positions and orientations on the ribosome.

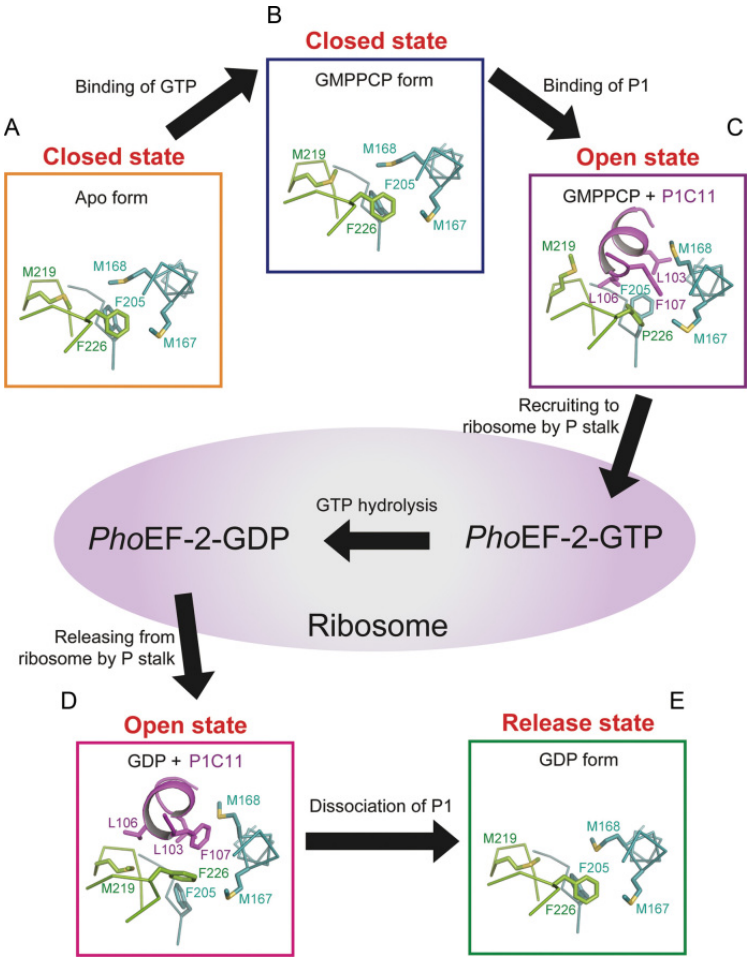

Figure 3. The response of the P1-binding groove of *Pho*EF-2 during recruitment process.

References

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