Structure and function of a protein mimicking transfer RNA revealed from the structure of translation elongation factor P complexed with an aminoacyl–tRNA synthetase paralog, GenX

For accurate protein synthesis, the 20 canonical amino acids must be joined properly according to the genetic code. Aminoacyl–tRNA synthetases (aaRSs), enzymes that ligate a specific amino acid to its cognate tRNA, are essential components of protein synthesis. Besides the classical aaRS proteins, aaRS-related proteins lacking tRNA aminoacylation activity exist in various species, and several of these aaRS paralogs participate in amino acid biosynthesis, cofactor biosynthesis, and tRNA modification. Among the aaRS paralogs, GenX (PoxA, YjeA) is homologous to the C-terminal catalytic domain of lysyl–tRNA synthetase (LysRS). The absence of the tRNA anticodon-binding domain suggests that GenX by itself does not act as a classical aaRS. To obtain clues about the function of GenX, we determined the crystal structure of *E. coli* GenX in complex with a LysRS inhibitor [1]. A superposition of the GenX structure on that of LysRS revealed that the active site residues are highly conserved with those of LysRS (Fig. 1). In GenX, the active site pocket is slightly wider than that in LysRS, and it has sufficient space to accommodate tRNA. However, GenX did not aminoacylate *E. coli* tRNAs with lysine [1].

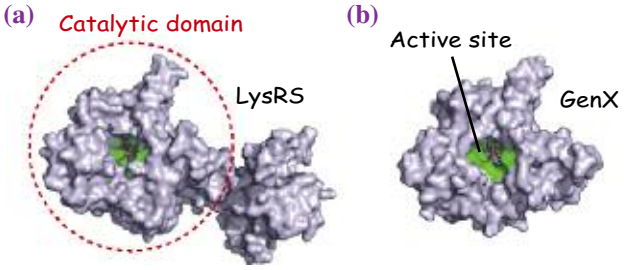

Fig. 1. Comparison of the structures of LysRS and GenX. LysRS **(a)** and GenX **(b)** are represented as surface models. The GenX residues that are conserved with those of LysRS are colored green. The structure of the catalytic domain of LysRS is similar to that of GenX.

We searched for a "tRNA-like" molecule in *Escherichia coli*, and noticed that translation elongation factor P (EF-P) assumes an L-shaped structure, which mimics that of tRNA [2]. EF-P reportedly binds to the ribosome, and stimulates the ribosomal peptidyl transferase activity [3]. A comparative genomic analysis predicted that GenX is functionally relevant to EF-P and the YjeK protein, encoded next to EF-P in the *E. coli* genome. Thus, we investigated whether EF-P is the GenX substrate, and whether EF-P is a binding partner of GenX. We successfully crystallized GenX complexed with EF-P, and determined the crystal structure of the GenX•EF-P complex at 2.5 Å resolution [1], using diffraction data collected at beamline **BL41XU**. The GenX•EF-P complex forms a $GenX_2$•$EF\text{-}P_2$ heterotetramer (Fig. 2(a)). The GenX•EF-P interactions are mediated mainly by the active site loops wrapping around the EF-P domain 1. The structure resembles that of the aspartyl–tRNA synthetase (AspRS)•$tRNA^{Asp}$ complex, because a conserved lysine residue (Lys34) in the exposed loop of EF-P appears to be located at the 3′-adenosine of the tRNA. The GenX•EF-P complex is an aaRS•tRNA mimic, and Lys34 appears to react with the lysine moiety. We examined whether GenX ligates lysine to EF-P, and found that GenX ligates Lys34 of EF-P with lysine [1]. This is a novel example of a post-translational protein modification, and GenX is the first aaRS paralog that has been shown to modify a protein side chain with an amino acid. Our crystallographic and biochemical analyses suggest that the lysyl modification of EF-P by GenX mimics the aminoacylation of tRNA by an aaRS ("aaRS•tRNA mimicry") (Fig. 2(b)).

In bacteria, EF-P is post-translationally modified with lysine. In eukaryotes, a conserved lysine residue (Lys50) of translation initiation factor 5A (eIF5A), a distant ortholog of EF-P, undergoes a unique post-translational hypusine modification (Fig. 3) [4]. Hypusine is introduced in a two-step reaction catalyzed by deoxyhypusine synthase (DHS) and deoxyhypusine

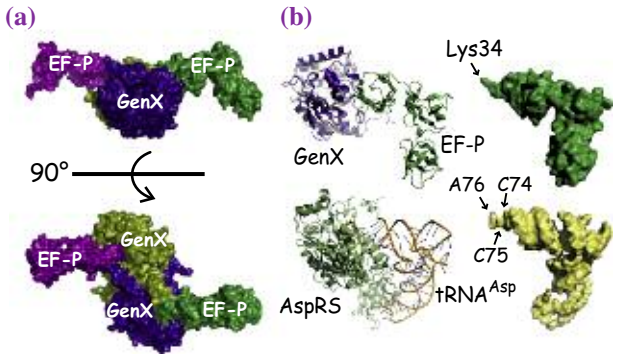

Fig. 2. **(a)** Structure of the GenX•EF-P complex. The GenX dimer (gold and blue) complexed with EF-Ps (violet and green) is represented as a surface model. The lower panel represents a view after a 90° rotation about the horizontal axis from the upper panel. **(b)** Structural comparison between the EF-P•GenX complex and the tRNA•aaRS complex. The complex of aspartic acid tRNA and aspartyl–tRNA synthetase ($tRNA^{Asp}$•AspRS) is shown, as an example of a tRNA•aaRS complex. The structures of the EF-P•GenX complex (upper left) and the tRNA•aaRS complex (lower left) are very similar. The Lys34 residue of EF-P corresponds to the CCA terminus (A76) where an amino acid binds to tRNA.

hydroxylase (DOHH). The conserved lysine residues at the tip of eIF5A and EF-P are both modified, and the modified residues (hypusine in eIF5A and lysyl–lysine in EF-P) are structurally similar. However, the modification enzymes (DHS and DOHH in eIF5A and GenX in EF-P) are phylogenetically unrelated to each other.

During the last decade, tRNA-protein mimicry has been proposed for several translation factors possessing structural features resembling those of tRNA. A recent crystallographic analysis revealed that EF-P binds to a new functional site between the peptidyl (P)- and exit (E)-sites of the ribosome. EF-P stabilizes the P-site-binding of fMet–tRNA^{fMet}, and thereby promotes the entry of the aminoacyl-tRNA into its binding site (A-site) [5]. Actually, the shape of the putative aminoacyl–EF-P resembles that of an aminoacyl–tRNA. The *Thermus thermophilus* EF-P Arg32 residue (corresponding to the modified Lys34 in *E. coli* EF-P), at the tip of the ribosome-bound EF-P, points toward the P site (50S ribosome). In the hypothetical structure of lysyl–Lys34, obtained by substitution of the Arg residue, the lysyl–lysine side chain might be able to reach the peptidyl transferase center (Fig. 4). Therefore, it would be interesting to determine how the putative aminoacyl–EF-P functions in the peptidyl transferase active site.

Our *in vivo* analyses revealed that YjeK (lysine 2,3-aminomutase paralog) enhanced the EF-P lysylation by GenX [1], and might convert (S)-α-lysyl–EF-P to (R)-β-lysyl–EF-P (Fig. 3(b)). Furthermore, *in vivo* analyses revealed that the EF-P modification by GenX and YjeK is essential for cell survival [1]. These results indicated that lysyl–EF-P is a functional form *in vivo*, and the lysyl modification at Lys34 in EF-P is important *in vivo*. Further details about the post-translational EF-P modification still remain unclear (e.g., what is the target gene of lysyl–EF-P?; what is the physiological role of the lysyl–EF-P *in vivo*?). Next, we will attempt to elucidate the answers to these questions.

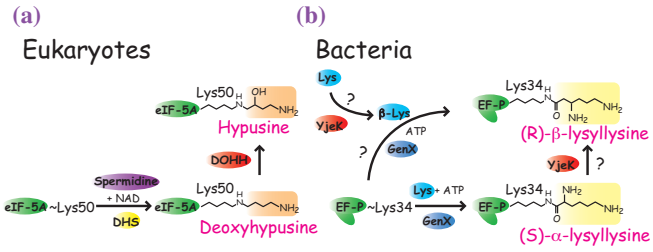

Fig. 3. Post-translational modifications of eIF5A and EF-P. **(a)** Deoxyhypusine modification of eukaryotic eIF5A, and the following hydroxylation of deoxyhypusine-modified eIF5A. **(b)** Lysyl modification of bacterial EF-P. GenX produces (S)-α-lysyl–EF-P from (S)-α-lysine (Lys), ATP, and EF-P, and then YjeK may catalyze the isomerization of (S)-α-lysyl–EF-P to (R)-β-lysyl–EF-P *in vivo*. β-Lys represents (R)-β-lysine.

In the present study, we crystallized the complex of EF-P and GenX and determined its structure, which is very similar to that of the tRNA-aaRS complex. Based on this finding, we elucidated that EF-P accepts the amino acid lysine from GenX, in a reaction similar to that of a tRNA. This is the first discovery of the striking similarities in both the structure and reaction between a nucleic acid and a protein, although they are completely different molecules. This phenomenon seems to be analogous to convergent evolution, in which different living organisms acquire similar shapes and living behaviors through evolution. GenX exists only in bacteria, such as *E. coli* and *Salmonella*, and not in eukaryotic organisms, such as humans. Therefore, GenX is a promising target for developing new antimicrobial agents for pathogenic bacteria and antimicrobial-agent-resistant bacteria, without adverse side effects.

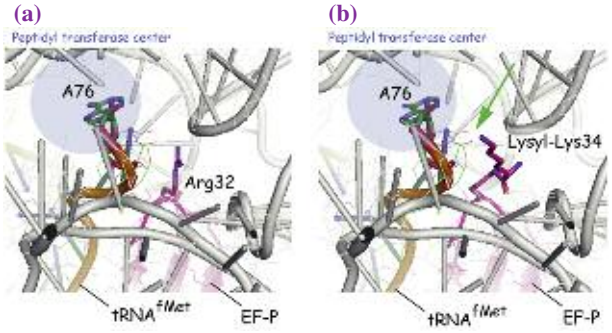

Fig. 4. Lysyl–Lys34 of EF-P docked with the EF-P–bound ribosome. **(a)** The Arg32 residue (corresponding to the modified Lys34 in *E. coli* EF-P), at the tip of the ribosome-bound *T. thermophilus* EF-P, points toward the P-site of the 50S ribosome. **(b)** In the hypothetical structure, obtained by substituting the Arg residue with lysyl–Lys34, the lysyl–lysine side chain could potentially reach the peptidyl transferase center (a green arrow).

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