Crystal structure of glutamine transamidosome reveals how two enzymes bound to one tRNA assume alternative conformations for consecutive reactions

Transfer RNA (tRNA) is the adaptor molecule between a codon in messenger RNA and the corresponding amino acid used in protein synthesis. Generally, each amino acid is attached to its cognate tRNA by its specific aminoacyl-tRNA synthetase, and the aminoacyl-tRNAs thus produced are transferred to the protein production machine, the ribosome. Therefore, faithful aminoacyl-tRNA formation is the prerequisite for accurate translation. However, most bacteria and all archaea lack glutaminyl-tRNA synthetase, the aminoacyl-tRNA synthetase specific to glutamine. Instead, such organisms use a two-step pathway to produce Gln-tRNA^{Gln}, as follows. The first step is the glutamylation of tRNA^{Gln} by a non-discriminating GluRS, which is also responsible for the formation of Glu-tRNA^{Glu}. The second step is the amidation of Glu-tRNA^{Gln} by a Glu-tRNA^{Gln} amidotransferase. The bacterial Glu-tRNA^{Gln} amidotransferase is the heterotrimeric GatCAB, consisting of GatA, GatB and GatC. However, the mechanism by which GluRS and GatCAB cooperate with each other in Gln-tRNA^{Gln} synthesis has not been elucidated.

To clarify the cooperation mechanism of GluRS and GatCAB, we first used a gel mobility shift assay to investigate whether bacterial GluRS, tRNA$^{Gln}_{CUG}$ and GatCAB form the ternary complex. We successfully identified the formation of the GluRS–tRNA^{Gln}–GatCAB ternary complex, also known as the bacterial 'glutamine transamidosome'. Next, to determine the mechanism at an atomic level, we solved the crystal structures of the GluRS–tRNA$^{Gln}_{CUG}$ binary complex and the glutamine transamidosome from *T. maritima* at 2.9-Å and 3.35-Å resolutions, respectively (Figs. 1(a) and 1(b)) [1]. The X-ray diffraction data were recorded at beamline **BL41XU**. The most notable characteristics of the elucidated structures are present in the glutamine transamidosome. tRNA$^{Gln}_{CUG}$ is recognized by both GluRS and GatCAB simultaneously. The coordinates of GluRS and tRNA$^{Gln}_{CUG}$ in the glutamine transamidosome are almost the same as those in the GluRS–tRNA$^{Gln}_{CUG}$ binary complex, except for the GatCAB-interacting region in tRNA$^{Gln}_{CUG}$, as detailed below.

GatCAB uses the tail body, which consists of the helical and tail domains located at the carboxy (C)-terminal region of the subunit GatB, to recognize the outer corner of tRNA$^{Gln}_{CUG}$. The GatCAB catalytic body, which consists of GatA, GatC and the amino (N)-terminal cradle domain of GatB, acts as one globule, and is located close to, but not in contact with, the acceptor arm of tRNA$^{Gln}_{CUG}$. In the present structure, GatCAB does not recognize the U1–A72 pair in the acceptor arm, although the recognition is indispensable for the amidation by GatCAB. Instead, the acceptor arm is in the catalytic site in GluRS, and the minor groove side of the acceptor stem is tightly recognized by the Rossmann-fold domain of GluRS. Furthermore, the 2′-hydroxyl group of A76 at the CCA end is located next to the co-crystallized L-glutamyl-sulfamoyl adenosine (Glu-SA), a non-hydrolyzable analogue of glutamyl-AMP. Thus, the glutamine transamidosome in the crystal represents the glutamylation state of tRNA^{Gln}. The GluRS molecule is in the productive form, whereas the GatCAB molecule is in the non-productive form.

The structures of C16 and U20 in the D-loop of tRNA$^{Gln}_{CUG}$ change upon the interaction with the tail body of GatCAB, as revealed by the superposition

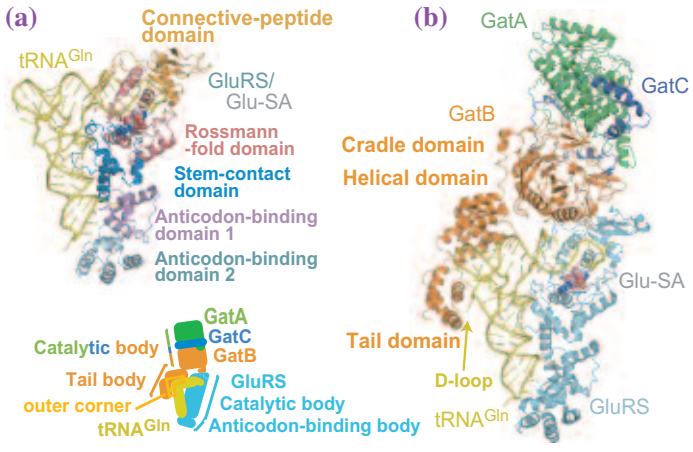

Fig. 1. Crystal structures of the GluRS–tRNA^{Gln} binary complex and the glutamine transamidosome. **(a)** Structure of the GluRS–tRNA^{Gln} binary complex from *T. maritima*. The overall structure is represented by a ribbon model, and the GluRS-bound Glu-SA is shown as a CPK model. The domain structure of GluRS is also indicated. **(b)** Structure of the glutamine transamidosome from *T. maritima*. The overall structure is represented by a ribbon model, and the GluRS-bound Glu-SA and the GatB-bound zinc ion are shown by CPK models. The names of the elements of GluRS and GatCAB are indicated on the left side.

of the structures of $tRNA^{Gln}_{CUG}$ in the glutamine transamidosome and the GluRS–$tRNA^{Gln}$ binary complex. Since the structural features of C16 and U20 in the D loop are specific to $tRNA^{Gln}$, the tail domain of GatCAB interacts mainly with the $tRNA^{Gln}$-specific structure. To validate this $tRNA^{Gln}$-selection mechanism by GatCAB, we constructed $tRNA^{Glu}_{CUC}$ variants that resemble $tRNA^{Gln}$, and tested their ability to form Gln-$tRNA^{Glu}$ with GluRS and GatCAB. Interestingly, the variant with both the U1–A72 pair substitution and the C20a deletion was able to form Gln-$tRNA^{Glu}$ to some extent, and the addition of the C20U substitution augmented the ability. These results clearly showed that the tertiary structure of the D loop and the first U1–A72 pair in $tRNA^{Gln}$ are the two major identity elements for the amidation by GatCAB.

The superposition of several GatCAB structures revealed that GatB possesses two flexible hinges at the domain boundaries. One is between the cradle and helical domains of GatB, or between the catalytic and tail bodies of GatCAB. The other is between the GatB helical and tail domains, within the tail body of GatCAB. As for GluRS, one hinge was identified between the anticodon-binding domains 1 and 2, when the structures in the transamidosome and the tRNA-free form were superimposed according to the C-terminal anticodon-binding domain 2. Consequently, GluRS can be divided into two bodies: the N-terminal catalytic body and the C-terminal anticodon-binding body [2].

These hinges in both GluRS and GatCAB may provide the flexibility required for the cooperative movements of the catalytic bodies in the functional glutamine transamidosome, as follows. First, when $tRNA^{Gln}$, GluRS and GatCAB cooperatively form the transamidosome, the anticodon-binding body of GluRS binds to the $tRNA^{Gln}$ anticodon, whereas the tail body of GatCAB binds to the outer corner of $tRNA^{Gln}$. When the GluRS catalytic body assumes the productive form to interact with the acceptor arm of $tRNA^{Gln}$, the GatCAB catalytic body in the non-productive form is located near the catalytic body of GluRS. The present transamidosome structure represents this 'glutamylation state' (step Ia in Fig. 2).

After the synthesis of Glu-$tRNA^{Gln}$ (step Ib in Fig. 2), the GluRS catalytic body is likely to leave the acceptor arm immediately, by a pivoting movement using the hinge, while the anticodon-binding body remains bound to the anticodon. As a result, the folded-back 3'-end moiety of Glu-$tRNA^{Gln}$ can leave the catalytic site of GluRS. This state is characterized by GluRS and GatCAB in the non-productive states (step II in Fig. 2). In this state, the space between the two enzymes allows the single-stranded 3'-end moiety of Glu-$tRNA^{Gln}$ to assume the normal, extended conformation, and it is thereby redirected towards the catalytic site of GatB (step III in Fig. 2).

In the next step, the pivoting transition of GatCAB from the non-productive form to the productive form probably brings the catalytic body closer to the acceptor arm of Glu-$tRNA^{Gln}$. The base-paired stem slightly bends by induced fitting, for the recognition of the determinant base pair U1–A72 by GatB. Thus, the 3'-end moiety of Glu-$tRNA^{Gln}$ reaches the amidation catalytic site of GatCAB (step IVa in Fig. 2). In this 'amidation state', Glu-$tRNA^{Gln}$ is converted to Gln-$tRNA^{Gln}$ (step IVb in Fig. 2).

The 'alternative-conformation' mechanism of the glutamine transamidosome, shown in Fig. 2, clearly explains how GluRS and GatCAB are able to bind to the acceptor arm of $tRNA^{Gln}$ in turn without steric hindrance, and how the misacylated intermediate Glu-$tRNA^{Gln}$ can be immediately corrected to Gln-$tRNA^{Gln}$ by GatCAB, with a low risk of releasing the misacylated product. The novel mechanism revealed in this study may serve as the structural basis for future studies on the expansion of the genetic code, in which glutamine and asparagine were incorporated at a later stage in the evolution of life.

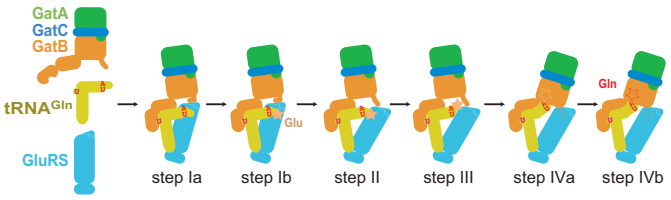

Fig. 2. Model for the pathway of Gln-$tRNA^{Gln}$ formation. Proposed model for the pathway of Gln-$tRNA^{Gln}$ formation by GluRS and GatCAB. The steps in the pathway are indicated under the schematic models.

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