Crystal Structure of Glutamine Transamidosome: Two Enzymes Bound to One Transfer RNA Function Cooperatively for Consecutive Reactions

In most bacteria and all archaea, Gln-tRNA^{Gln} is produced by two reactions: Glu-tRNA^{Gln} synthesis by glutamyl-tRNA synthetase (GluRS), and subsequent conversion to Gln-tRNA^{Gln} by a tRNA-dependent amidotransferase. We determined the crystal structure of the 'glutamine transamidosome', consisting of tRNA^{Gln}, GluRS and the heterotrimeric amidotransferase GatCAB (Glu-tRNA^{Gln} amidotransferase subunits C, A and B), from the bacterium *Thermotoga maritima* at 3.35-Å resolution. In the elucidated structure, GluRS and GatCAB interact with tRNA^{Gln} simultaneously. Together with further analysis, we proposed a mechanism that explains how the transamidosome efficiently performs the two consecutive steps of Gln-tRNA^{Gln} formation.

Most bacteria lack glutaminyl-tRNA synthetase, and instead use a two-step pathway to produce Gln-tRNA^{Gln}. 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. In bacteria, the heterotrimeric GatCAB, consisting of GatA, GatB and GatC, functions as the Glu-tRNA^{Gln} amidotransferase. GatB produces Gln-tRNA^{Gln} from Glu-tRNA^{Gln}, ATP and the ammonia generated by GatA.

In the study in ref. 1, we first used gel mobility shift assays to investigate whether the complex for the Gln-tRNA^{Gln} production is formed by bacterial GluRS, tRNA$^{Gln}_{CUG}$ and GatCAB from *T. maritima*. The experiments confirmed the formation of the GluRS–tRNA^{Gln}–GatCAB ternary complex, namely the bacterial glutamine transamidosome. Next, we successfully solved the crystal structures of the GluRS–tRNA$^{Gln}_{CUG}$ binary complex and the glutamine transamidosome at 2.9-Å and 3.35-Å resolutions, respectively (Fig. 1). In order to determine the structures, the beamline AR-NE3A was used. In the elucidated structure of 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$^{Gln}_{CUG}$.

GatCAB uses the tail body to recognize the outer side of tRNA$^{Gln}_{CUG}$. The GatCAB catalytic body acts as one globule, and is located close to, but not in contact with, the acceptor arm of tRNA$^{Gln}_{CUG}$. Therefore, GatCAB does not recognize the U1–A72 pair in the acceptor arm, one of the elements determining the amidation by GatCAB. Instead, the acceptor arm points towards the catalytic site in GluRS. Thus, the glutamine transamidosome in the crystal represents the glutamylation state of tRNA^{Gln}. Therefore, the GluRS molecule is in the productive form, whereas the GatCAB molecule is in the non-productive form.

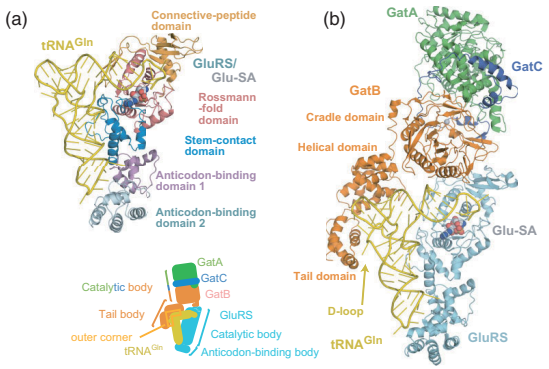

Figure 1
Crystal structures of the GluRS–tRNA^{Gln} binary complex and the glutamine transamidosome. (a), Structure of the GluRS–tRNA^{Gln} binary complex from *T. maritima*. The overall structure is represented by a ribbon model, and the GluRS-bound Glu-SA is shown as a CPK model. The domain structure 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.

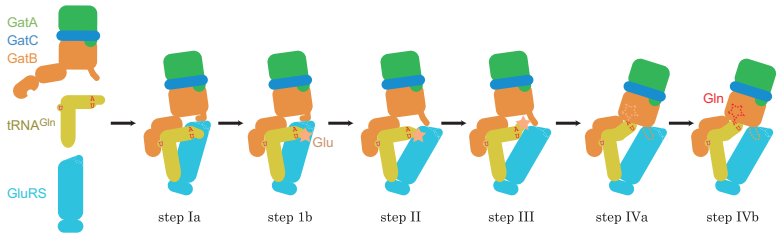

Figure 2
Model for the pathway of Gln-tRNA^{Gln} formation. Proposed model for the pathway of Gln-tRNA^{Gln} formation by GluRS and GatCAB. The steps in the pathway are indicated under the schematic models.

A comparison of the structure of tRNA$^{Glu}_{CUC}$ with that of tRNA$^{Gln}_{CUG}$ revealed the characteristic structures of each tRNA in the D loop. Furthermore, superposition of the structures of tRNA$^{Gln}_{CUG}$ in the glutamine transamidosome and the GluRS–tRNA^{Gln} binary complex from *T. maritima* clearly revealed the structural changes around C16 and U20 in the D loop of tRNA$^{Gln}_{CUG}$ upon interaction with the tail body of GatCAB. Because 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. In addition, the biochemical experiments 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 both ends of the helical domain. As already mentioned above, GatCAB is divided into two bodies: the catalytic and tail bodies, as shown in Fig. 1. As for GluRS, one hinge was identified between the anticodon-binding domains 1 and 2, and GluRS is also divided into two bodies: the catalytic and anticodon-binding bodies, as shown in Fig. 1. 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 tRNA^{Gln} outer corner. 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 GluRS catalytic body. 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). 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 most likely 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 thus 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.

REFERENCE

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BEAMLINE

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