Mechanism of Template-Independent RNA Polymerization by tRNA Nucleotidyltransferase

CCA addition onto the 3'-termini of tRNAs, by CCA-adding enzyme, is required for protein synthesis. CCA-adding enzyme adds the CCA without a nucleic acid template, using CTP and ATP as substrates in a single active pocket. However, the mechanism of CCA addition by eubacterial/eukaryotic enzymes has remained elusive. This report presents several crystal structures of *Aquilex aeolicus* CC-adding enzyme complexed with various tRNA primers. The sequence structures, representing the CC addition by the CC-adding enzyme, have clarified the molecular basis for CC addition onto tRNA using a single active pocket, as well as the mechanism for termination of nucleotide addition.

Every tRNA has the CCA sequence at its 3'-terminus (CCA-3' at positions 74 – 76). The invariant CCA-3' is required for amino acid attachment onto the 3'-end of the tRNA by aminoacyl-tRNA synthases, and for peptide-bond formation on ribosome. The invariant 3'-CCA of tRNA is synthesized and repaired by CCA-adding enzymes, using CTP and ATP as substrates, but without a nucleic acid template [1]. The CCA-adding enzymes are classified into two classes: class-I (archaeal CCA-adding enzymes) and class-II (eubacterial/eukaryotic CCA-adding enzymes). There are no significant amino acid similarities between the two classes.

Previous biochemical studies suggested that, during CCA synthesis, the tRNA neither translocates nor rotates relative to the enzyme, but the CCA addition proceeds in a single active pocket [2-4]. Results obtained by detailed crystallographic analyses of the CCA addition by the class-I archaeal CCA-adding enzyme explained the previous biochemical results [5-7]. On the other hand, since no complex structure of a class-II eubacterial/eukaryotic CCA-adding enzyme with tRNA has been available, the detailed mechanisms of CCA synthesis by eubacterial/eukaryotic enzymes have remained unclear [8-10]. In most organisms, 3'-CCA is synthesized by a single enzyme that adds CCA at one time. However, in some eubacteria, such as *Aquifex aeolicus*, the 3'-CCA is synthesized by two distinct, but closely related, class-II CC-adding and A-adding enzymes in a collaborative manner [11]. The mechanism by which the CC-adding enzyme synthesizes only the C74C75 sequence, without using a nucleic acid template, and the means by which the enzyme terminates RNA polymerization without adding a 3'-terminal A76, were obscure. Moreover, the molecular basis of the different specificities of nucleotides and tRNAs between the A-adding and CC-adding enzymes was unknown.

We determined the crystal structures of *A. aeolicus* CC-adding enzyme (AaS) and its complexes with tRNA primers, representing snapshots of each step of RNA polymerization [12]. Diffraction data were collected at beamlines BL-17A and BL-1A.

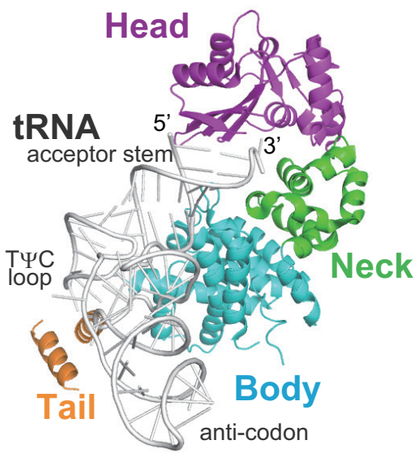

Figure 1: Complex structure of *A. aeolicus* CC-adding enzyme and tRNA lacking CCA.

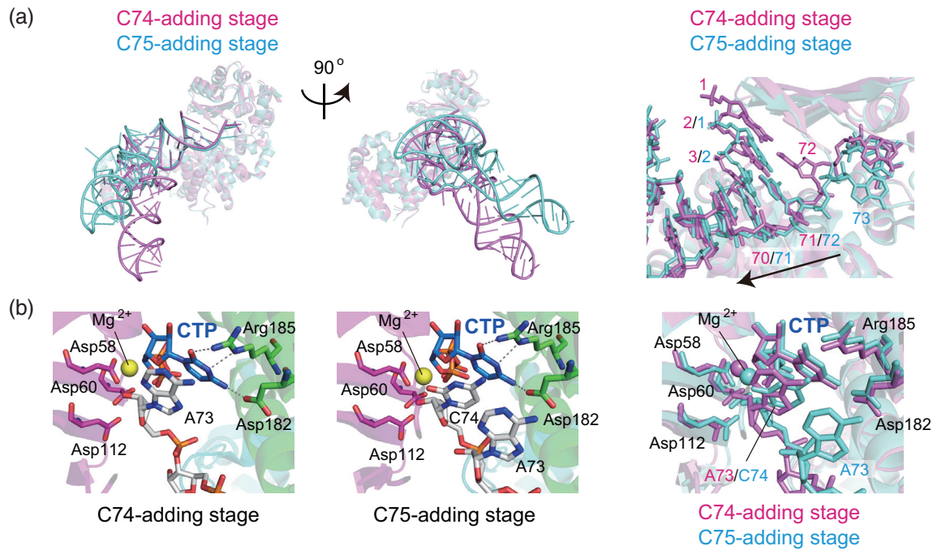

Figure2: Translocation and rotation of tRNA during C74C75-adding reaction. (a) The superimposition of the two reaction stage structures, the C74-adding stage (magenta) and the C75-adding stage (cyan). (b) The structure of the catalytic pocket at the C74-adding stage (left) and C75-adding stage (middle). The superimposition of the two structures (right).

AaS adopts a seahorse-shaped structure, and consists of four domains: the head, neck, body and tail domains. It recognizes the top-half region of the tRNA, and does not interact with the anticodon region. The TΨC loop and D-loop of the tRNA interact with the tail domain of AaS (Fig. 1). We determined structures of AaS representing C74-adding and C75-adding reactions. The comparison of structures representing C74-adding and C75-adding reactions revealed the translocation and rotation of the tRNA, relative to the enzyme, during the CC addition. After C74 addition and pyrophosphate release, the tRNA translocates backward by one nucleoside and rotates relative to the enzyme [Fig. 2(a)]. The C75 addition occurs using the same active pocket as for C74 addition. At both the C74-adding and C75-adding stages, CTP is selected by Watson-Crick-like hydrogen bonds between the cytosine of CTP and the conserved amino acid residues Asp and Arg in the active pocket, in the same manner [Fig. 2(b)]. These results also suggested that after C74C75 addition and pyrophosphate release, the tRNA translocates backward further and drops off the enzyme, and thus the CC-adding enzyme terminates RNA polymerization.

The present study has clarified the molecular mechanism of CC addition by tRNA nucleotidyltransferase, and has solved the long-standing classical problems of eubacterial/eukaryotic CCA-adding enzymes [13]. In addition, the present study is the first to visualize the translocation and rotation of the primer tRNA during the template-independent CC addition, as a movie. Previous biochemical studies using class-II CCA-adding enzymes showed that C74 addition, like C75 and A76 addition, does not involve tRNA translocation and rotation. The mechanisms of CC addition by the CC-adding and CCA-adding enzymes might be different. Further analyses of the complex structure of the class-II eubacterial/eukaryotic CCA-adding enzyme, representing CCA addition, would solve this issue.

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BEAMLINES

BL-17A and BL-1A

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