

## Structures of a Class I CCA-Adding Enzyme and Its Nucleotide Complexes

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**Introduction:** Mature tRNA molecules contain a universally conserved 3'-terminal CCA sequence. Although this sequence plays a crucial role in protein biosynthesis, it is not encoded in many eubacterial and archaeal tRNA genes and nearly all eukaryotic tRNA genes. The maturation of tRNA therefore requires an essential polymerase, the CCA-adding enzyme (tRNA nucleotidyltransferase) that catalyzes the post-transcriptional addition of the CCA sequence using CTP and ATP as substrates.

In general, polynucleotide polymerases require a template strand that specifies the sequence of nucleotides to be incorporated, with the most notable exception of the CCA-adding enzyme because it functions without a nucleotide template. Also remarkable is the occurrence of two classes of CCA-adding enzymes that do not show homologous regions outside of the catalytic domain [1], suggesting that this enzyme activity might have arisen twice in evolution.

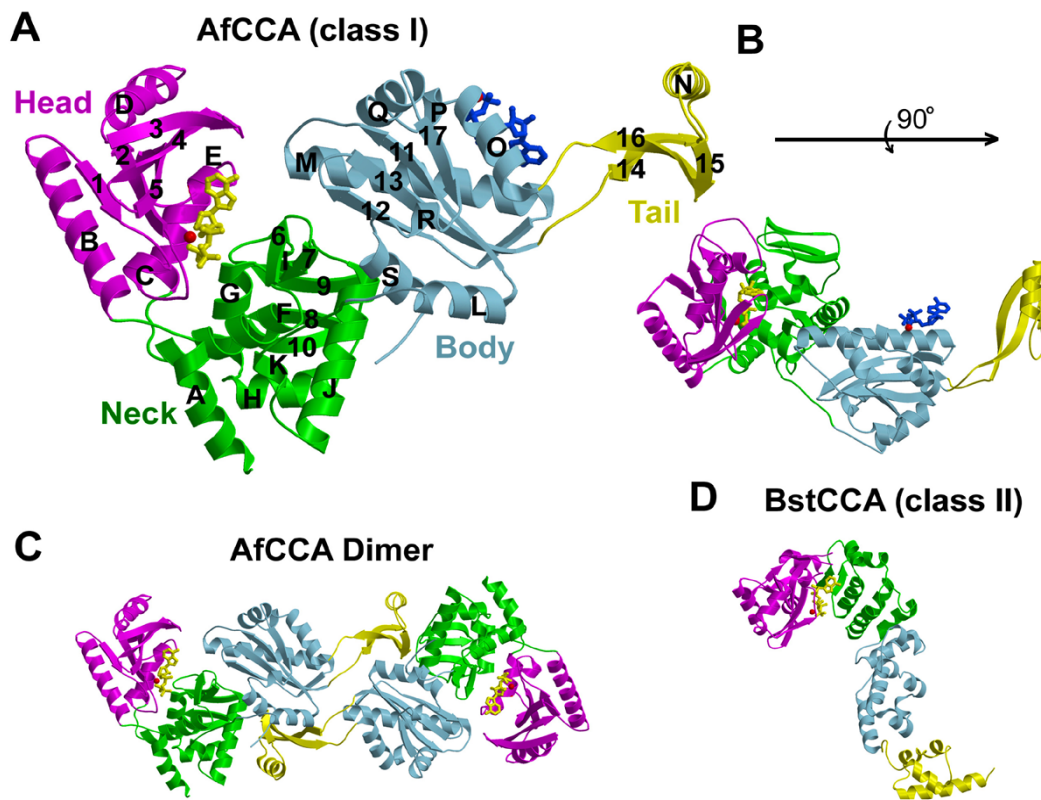
Following on our structural elucidation of a class II CCA-adding enzyme from the eubacterium *Bacillus stearothermophilus* (BstCCA) [1], we now have determined the crystal structures of a class I CCA-adding enzyme from *Archeoglobus fulgidus* (AfCCA), and its nucleotide complexes with ATP, CTP or UTP.

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### Overall structure:

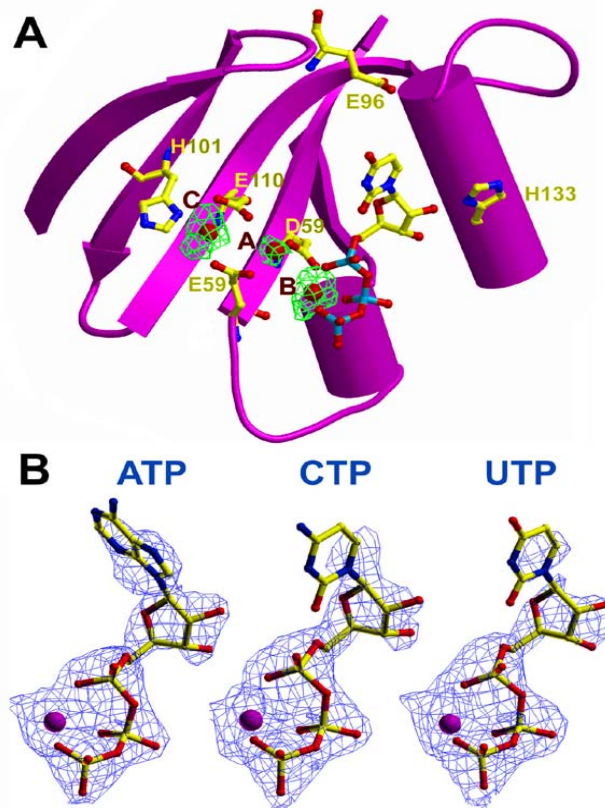
The AfCCA structure consists of four domains named the head, neck, body and tail (Figure 1), analogous to those observed in the class II BstCCA [1]. The domain architecture of AfCCA exhibits an elongated cleft, with the head and tail domains forming the end walls and the body domain lining the bottom (Figure 1B). The tail domain is stabilized by dimer interactions with a symmetry-related molecule in the unit cell (Figure 1C).

Although both the AfCCA and BstCCA enzymes have similar dimensions and numbers of domains, their structural homology is limited to the head domains. The neck, body and tail domains of AfCCA contain numerous  $\beta$ -strands, in contrast to the exclusively  $\alpha$ -helical domains in the class II BstCCA. In addition, the orientation of the long axis of the elongated body and tail domains with respect to the head domain differs by nearly 90° between the two classes of CCA-adding enzymes (Figures 1B and 1D).



**Fig 1:** The structure of the AfCCA enzyme.  
 (A) A ribbon representation of the monomer complexed with ATP.  
 (B) A view that is rotated by 90° about a horizontal axis compared to (A).  
 (C) The AfCCA dimer with its 2-fold axis in the center and oriented perpendicular to the plane.  
 (D) A ribbon representation of the Class II BstCCA enzyme monomer. [3]

**Fig 2:** Nucleotide binding to AfCCA. (A) A ribbon representation of selected protein side chains with UTP positioned as derived from the map in B. The anomalous difference electron densities ( $3\sigma$ ) arising from the  $Mn^{2+}$  ions (brown) are shown in green. (B) The final  $2Fo-Fc$  electron densities ( $1\sigma$ ) for ATP, CTP and UTP are shown superimposed on the nucleotide structures. [3]



**Apo-AfCCA Recognizes the Triphosphate and Sugar Moieties of NTP:**

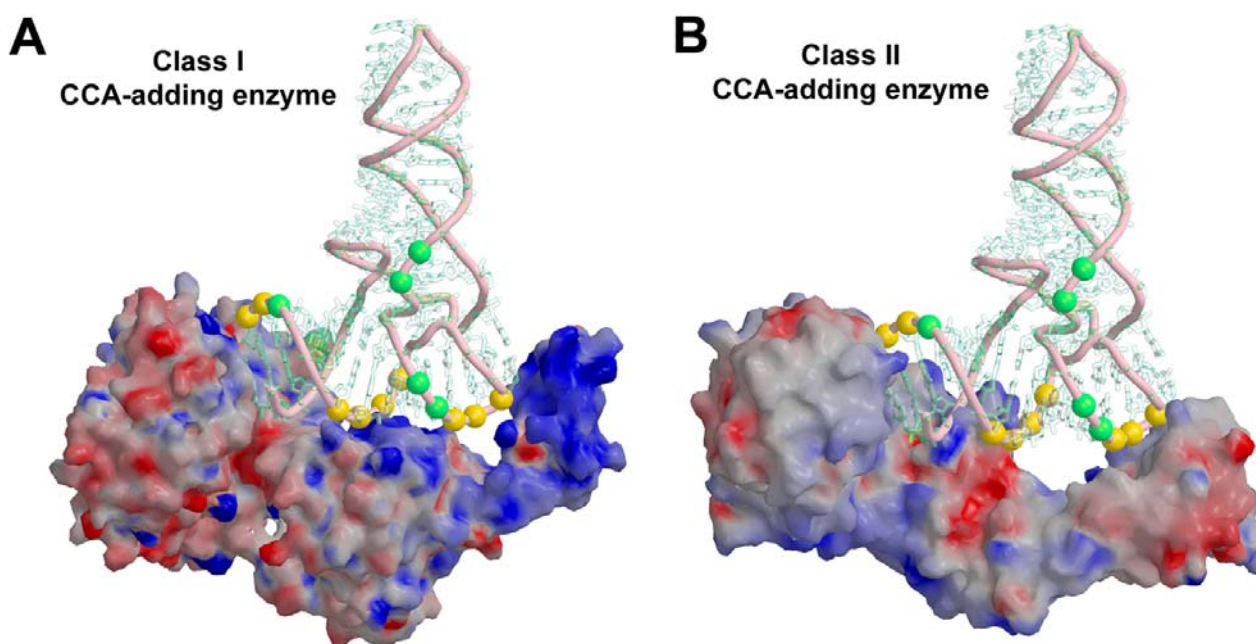
The apo-AfCCA does not recognize the base of an incoming NTP and consequently is unable to discriminate between correct and incorrect NTP's. We have determined the structures of complexes between AfCCA and ATP, CTP or UTP. The first two NTPs are substrates for the enzyme, but UTP is not. For all three nucleotides, the electron density is well defined for the triphosphate and the sugar moieties, while the base is not well ordered (Figure 2), presumably as a consequence of the lack of base specific interactions. In contrast, the class II BstCCA apo-enzyme is capable of discriminating against UTP or GTP [1].

### A Model of tRNA Bound to the Body and Tail Domains:

A model of tRNA bound to the AfCCA enzyme has been built that accounts for numerous biochemical and structural observations (Figure 3). A tRNA substrate was modeled onto the AfCCA enzyme to accommodate the charge and shape complementarities (Figure 3A). The acceptor and T-stems of tRNA were placed into the extended cleft of the enzyme, with the CCA terminus in the active site and the T-loop contacting the tail domain, and the anticodon stem and loop was pointed away from the enzyme.

This model is strongly supported by biochemical protection and interference experiments. Phosphates of the acceptor and T-stems of the tRNA are protected from alkylation by ethylnitrosourea when the tRNA is bound to the AfCCA enzyme [2]. These phosphates lie largely on one side of the tRNA helices and are mostly in contact with the enzyme in the modeled complex (Figure 3A). In spite of its different structure, a similar model for tRNA-binding can also be constructed for the class II enzymes in a manner that accommodates the shape and charge complementarity (Figure 3B).

The structures have been deposited in the Protein Data Bank with access codes of 1R8A, 1R8B, 1R89 and 1R8C for the apo enzyme, and ATP, CTP and UTP complexes, respectively.



**Fig 3:** Models of tRNA bound to monomers of the two classes of CCA-adding enzymes that account for tRNA protection experiments. (A) The AfCCA enzyme is shown in a surface representation and colored blue for negative electrostatic potential and red for positive electrostatic potential. The yellow spheres indicate those phosphates that are protected from alkylation by tRNA binding to the enzyme. The green balls indicate phosphates whose alkylation interferes with CCA-adding activity. (B) A model of the BstCCA complex with tRNA that has been color coded the same as in (A). [3]

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- [3] Xiong, Y., Li, F., Wang, J., Weiner A.M. and Steitz T.A. (2003). "Crystal Structures of an Archaeal Class I CCA-Adding Enzyme and Its Nucleotide Complexes". *Molecular Cell* **12**, 1165-1172.