

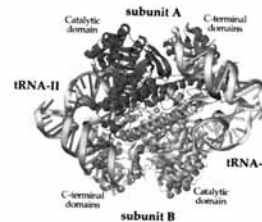
## X-ray crystallography of archaeal tRNA-guanine transglycosylase complexed with tRNA

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Archae bacteria in common have a characteristic post-transcriptionally-modified base, archaeosine, at the position of 15 in the D-loop of all the tRNAs. tRNA guanine transglycosylase (TGT) catalyzes the base exchange reaction between guanosine and preQ0, a preproduct of the archaeosine. TGT widely exists from prokaryote to eukaryote. Prokaryotic and eukaryotic TGTs post-transcriptionally modify the anticodon loop of specific tRNAs, while archaeal TGT modify the D-loop of all the tRNA. Archaeal enzyme has 300 a.a. extra domain as compared to prokaryotic and eukaryotic enzymes, which is suggested to function in the recognition of the tRNA L-shape.

To elucidate how archaeal TGT recognizes tRNA and how it catalyzes the base exchange reaction, we crystallized TGT from hyperthermophile, *Pyrococcus horikoshii* in a complex with tRNA. The crystals belong to a tetragonal space group *R*3 with unit-cell parameters  $a = b = c = 161 \text{ \AA}$ ,  $\alpha = \beta = \gamma = 89.2^\circ$ . The crystals diffract X-ray upto 3.3  $\text{\AA}$  resolution in the BL41XU. In this beamtime, we collected data upto 3.3  $\text{\AA}$  resolution, and we determined the crystal structure of the ArcTGT•tRNA complex, as shown in the right figure.



## Crystallographic analysis of alpha-synuclein

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$\alpha$ -Synuclein is a 140 amino acid protein. Although  $\alpha$ -synuclein is highly expressed in the brain, function of this protein is still unknown. It has been proposed that  $\alpha$ -synuclein is involved in synaptic transmission, based on the phenotype of the knockout mice and enrichment in presynaptic terminals. Induction of  $\alpha$ -synuclein expression in canary brains during the song-learning period suggests that it may function in neuronal plasticity.  $\alpha$ -Synuclein is a major component of the Parkinson's disease (PD), a movement disorder pathologically characterized by the loss of dopaminergic neurons. The link between  $\alpha$ -synuclein and PD was first suggested by the identification of autosomal dominant mutations in the  $\alpha$ -synuclein gene in early onset familial PD cases. Subsequently,  $\alpha$ -synuclein was identified in its fibrillar form as one of the major components of neuronal inclusions, named Lewy bodies (LBs), in PD brain. It suggests a possible etiological significance of the protein in these neurologic conditions. In neuronal cells, it is thought that  $\alpha$ -synuclein assumes a random coil formation. However, it has been observed that most of  $\alpha$ -synuclein accumulated in LBs forms  $\beta$ -sheet structure by X-ray fiber diffraction, electron diffraction and circular dichroism spectroscopy. To understand the mechanism of the accumulation of  $\alpha$ -synuclein, further structural analysis is expected.

$\alpha$ -Synuclein, which was overexpressed by *E.coli*, was crystallized by hanging drop vapor

diffusion method. The dimensions of the obtained crystals were approximately  $20 \times 20 \times 20 \mu\text{m}^3$ ; it was difficult to collect the diffraction data of the crystal with an in-house X-ray data collection system. The data collection of the  $\alpha$ -synuclein was carried out using a synchrotron radiation at BL41XU of SPring-8 under cryo-condition (100K). The crystal was mounted on a cryo-loop and flash-frozen in a cold  $\text{N}_2$  flow. The diffraction data were collected by oscillation method with  $1^\circ$  oscillation per frame. The exposure time was 15 - 20 seconds per frame. The collected diffraction data were processed using the program MOSFLM and scaled with the program SCALA in CCP4 program suite [1]. The analysis shows that the crystal of  $\alpha$ -synuclein belongs to a cubic space group of *F*432 with cell parameters  $a=b=c=180.7 \text{ \AA}$ . The best data was 100% completeness at 4.0  $\text{\AA}$  resolution with Rmerge of 19.8%.

### [References]

[1] CCP4 (1994). *Acta Cryst. D.*, **50**, 760-763.