X-ray crystallography of the complex of centromeric Abp1 protein and its target DNA

[1,2]Osamu Nureki (0003440), [1]Shuya Fukai (0003519), [1]Ryuichiro Ishitani (4145)

[1]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; [2]Cellular Signaling Lab., RIKEN

The centromere plays a major role in segregation of eukaryotic chromosomes in mitosis and meiosis, by serving as the site for kinetochore assembly and sister chromatid attachment. Moreover, the centromere is considered to regulate the cell cycle checkpoint for the metaphase-anaphase transition. The yeast *Saccharomyces cerevisiae* Abp1 protein has a central role in the chromosome segregation and has a sequence homology with human centromere binding protein B (CENP-B). In this study, we tried to collect the native data of Abp-1 protein•DNA complex. The crysrals belong to the space group P212121 with unit cell parameters a=117.2, b=180.3, c=110.2Å. However, the reproduction of the high-resolution crystals is quite difficult. In this beamtime, we collected 3.0Å resolution native data.

Crystallographic analysis of Archaeal-type class-I lysyl-tRNA synthetase

[1,2]Osamu Nureki (0003440), [2]Takaho Terada (0005670), [1]Ryu-ichiro Ishitani (0004145),

[1]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; [2]Cellular Signaling Lab., RIKEN

Aminoacyl-tRNA synthetases (aaRSs) specifically recognize their cognate amino acid and tRNAs to synthesize a correct aminoacyl-tRNA, which is a crucial step for the accurate protein biosynthesis. Most cells from prokaryote to eukaryote have 20 aaRSs corresponding to natural 20 amino acids. The 20 aaRSs are divided into two exclusive classes, classI I and class II, which might have evolved from distinct ancestors. However, recent genome analyses revealed that some archaea have less than 20 aaRSs. In prokaryotic and eukaryotic cells, lysyl-tRNA synthetase (LysRS) belongs to class I, whereas, in archaea, completely different proteins, belonging to class I, functions in the Lys-tRNA(Lys) synthesis. To elucidate the molecular evolution of aaRSs, we crystallized the class I LysRS from *Pyrococcus horikoshii*. The crystals belong to a monoclinic space group $P2_1$ with unit-cell parameters a = 59 Å, b = 75 Å, c = 157 Å, β=90.2°. In this beamtime, we collected MAD data of the SeMet derivative crystals. We picked up 15 out of 20 Se sites with the program SnB, and calculated the phase with SHARP. Density modification with the program RESOLVE and DM significantly improved the phase, and model building is now in progress.