Preliminary Results

Structural study for the complex between DNMT1, PCNA, PAF15Ub2 and DNA. DNA methylation is an important epigenetic mechanism that critically regulates chromatin structure and function. Dysregulation of DNA methylation has been associated with genomic instability and silencing of tumor suppressor genes, leading to various diseases, such as cancer and neurological disorders. In mammals, DNA methylation is maintained by DNA methyltransferase 1 (DNMT1) in a replication-dependent manner. Recent studies have indicated that DNMT1 is targeted to the replication foci through its direct contact with PCNA, as well as through the interaction between the RFTS domain of DNMT1 and PCNA-associated factor 15 (PAF15) ubiquitylated at lysine 15 and 24 (PAF15Ub2). This interaction between DNMT1 and the replication fork allows DNMT1 to methylate the nascent DNA strand immediately following DNA replication (Fig. 1A). However, the molecular basis of the interplay between DNMT1, PCNA and PAF15Ub2 remains unclear.

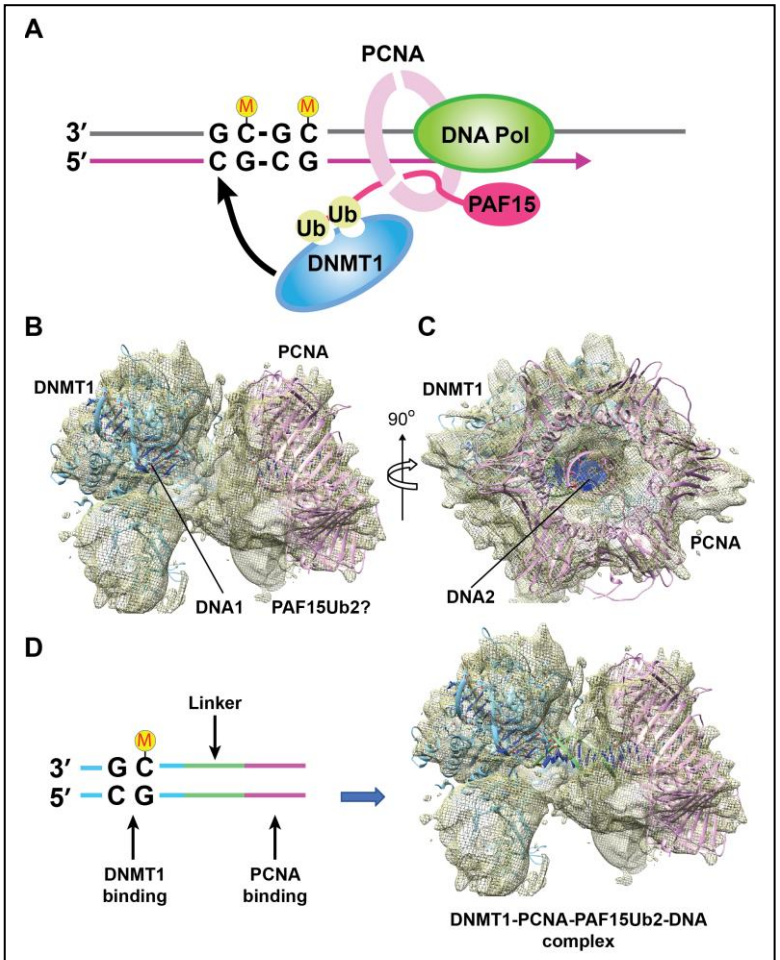

Figure 1. Preliminary study of the DNMT1-PCNA-PAF15Ub2-DNA complex. (**A**) Model for the replication-dependent DNA methylation by DNMT1. (B, C) Two orthogonal views of the density map for the DNMT1 in complex with DNA, PCNA and two-mono-ubiquitylated PAF15 (PAF15Ub2) at 5.5 Å resolution. The structures of PCNA-DNA and DNMT1-DNA were modeled using PDB entries 6EHT and 4DA4. In addition, one blob of density was putatively assigned to PAF15Ub2. (D) Work plan in determining high-resolution cryo-EM structure of the DNMT1-PCNA-PAF15Ub2-DNA complex. We will include a new DNA duplex for complex formation, which will bridge the DNMT1 and PCNA subcomplexes to reduce the flexibility of the entire complex. The linker DNA was modeled on the density map on the right panel.

In the previous cycle, we were awarded with one shift of data collection time for Titan Krios at NCCAT. The cryoEM sample was prepared by mixing the covalent DNMT1-DNA complex with PCNA-PAF15Ub2 complex, followed by size-exclusion chromatography and chemical crosslinking. After data collection, the images were processed using the Cryosparc program and the density map was analyzed using Chimera. Our preliminary 3D classification and refinement of the particles has successfully led to generation of a 5.5 Å-resolution density map corresponding to the DNMT1-PCNA complex (Fig. 1B, C). The relatively low resolution of the density map allows us to build a partial model of the DNMT1-PCNA-DNA complex, with the PAF15Ub2 left unmodeled. Nevertheless, it is apparent that DNMT1 is packed against the outer surface of the PCNA trimer, creating a continuous DNA-binding site. Further analysis of the density map also reveals short DNA fragments that bind to PCNA and/or DNMT1. On the other hand, our 2D and 3D classification analyses also suggest that the particles are associated with large conformational heterogeneity, which presumably limit the resolution of the current density map. To reduce the conformational flexibility of the DNMT1-PCNA-PAF15Ub2-DNA complex and to improve its structural resolution, we have a designed a new DNA duplex, which bridges the DNA molecule bound to DNMT1 (DNA1, Fig. 1B) with the DNA molecule bound to PCNA (DNA2, Fig. 1C), which is expected to stabilize the entire into a compact conformation (Fig. 1D).

In the coming cycle, we plan to collect the cryoEM data for the newly prepared DNMT1-PCNA-PAF15Ub2-DNA complex, which promises to reveal the structural basis for replication-dependent DNA methylation maintenance.