Preliminary Results

Structural study for the complex between DNMT1 and epigenetically modified nucleosome. 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, maintenance of DNA methylation is mainly mediated by DNA methyltransferase 1 (DNMT1), which contains a large regulatory region preceding the catalytic MTase domain (Fig. 1A). Our recent study has demonstrated that the DNMT1 RFTS domain preferentially binds to histone H3 ubiquitylated at lysine 18/23 and trimethylated at lysine 9 (H3Ub/H3K9me3), which allosterically stimulates the DNA methylation activity of DNMT1 (Fig. 1B, C) for efficient DNA methylation maintenance in cells. However, how these interactions interplay with other chromatin cues remains unclear.

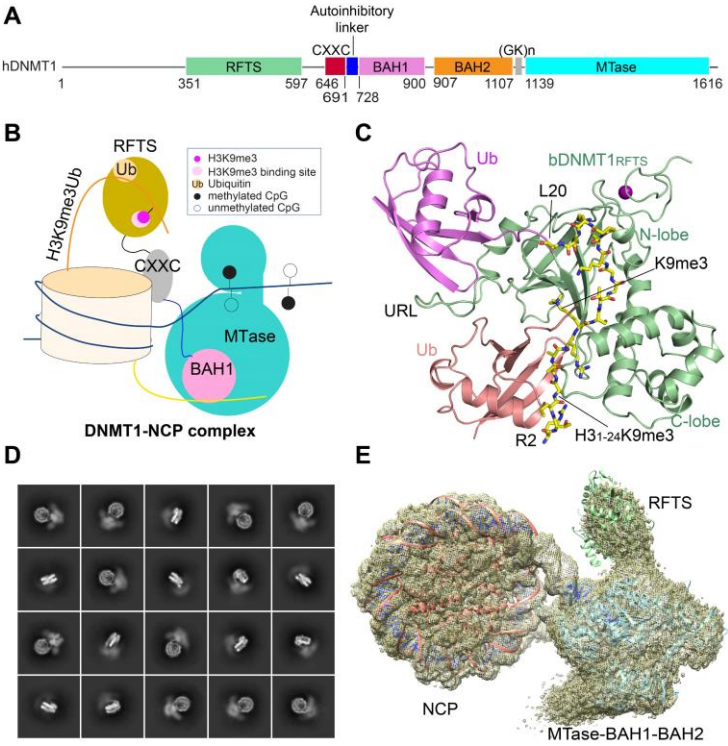

Figure 1. Structural study of the DNMT1-NCP complex. (**A**) Domain architecture of human DNMT1. (**B**) Model for the allosteric regulation of DNMT1 by epigenetically modified NCP. (**C**) Crystal structure of the DNMT1 RFTS domain (green) bound to histone H3K9me3 (yellow sticks) and ubiquitin (magenta and salmon) molecules. URL: ubiquitin recognition loop. (**D**) 2D classification of a DNMT1-NCP complex. (**E**) Preliminary density map (~3.5 Å) for a DNMT1-NCP complex, fit with DNMT1 (MTase, BAH1, BAH2 and RFTS domains) and NCP structures. No sharpening was applied to the map.

To understand the molecular basis underlying the functional crosstalk between DNMT1-mediated DNA methylation and histone modifications, we plan to solve the cryoEM structure of DNMT1 in complex with nucleosome core particle (NCP) labeled with histone modifications. The complex has a size of ~350 kDa and well suited for cryoEM structural study. In the previous cycle, we prepared the enzymatically crosslinked DNMT1-NCP complex and collected one data set at NCCAT. The images were processed using the Cryosparc and Relion programs. About 100,000 particles were picked for final refinement (Fig. 1D), which led to a ~3.5 Å-resolution composite map that is currently subject to further refinement and structural modeling (Fig. 1E). It is apparent that DNMT1 associates with the nucleosome via multiple protein-protein and protein-DNA interactions, invoking a large conformational change from the nucleosome-free state. Building on this progress, we plan to prepare additional DNMT1-NCP complexes with different length of the linker DNA, following the same protocol as that used for the previous complex. Structural study of the DNMT1-NCP complexes with various NCP linker sequences will permit us to capture the dynamic conformation and NCP contacts of DNMT1. These preliminary studies have placed us in an ideal position us to perform the proposed research.