Preliminary Results

Structural insight into UHRF1-mediated histone ubiquitylation. DNA methylation is one of the major epigenetic mechanisms that critically regulate gene expression and genome stability. Aberrant DNA methylation is associated with cancers and many other human diseases. Maintenance of DNA methylation in mammals is critically regulated by UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1) protein. UHRF1 is a multi-domain protein that contains an N-terminal ubiquitin-like UBL domain, a tandem Tudor domain (TTD), a plant homeodomain (PHD), a SET- and RING-associated (SRA) domain, and a C-terminal RING finger domain. It has been established that UHRF1 specifically recognizes hemimethylated CpG site and histone H3K9me3 modification during S phase, which allosterically stimulates UHRF1-medidated ububiquitylation of histone H3 lysine 18 and/or 23 (H3Ub). Subsequently, H3Ub serves as a platform to recruit DNA methyltransferase 1 (DNMT1) to the replication foci for maintenance of DNA methylation. The cell cycle-dependent activity of UHRF1 needs to be tightly reguatled. Overexpression of UHRF1 in cancers led to silencing of tumor suppressor genes, which makes UHRF1 an attractive target for cancer therapy. However, due to the lack of structural knowledge, the molecular basis for UHRF1-mediated histone ubiquitylation remains unclear.

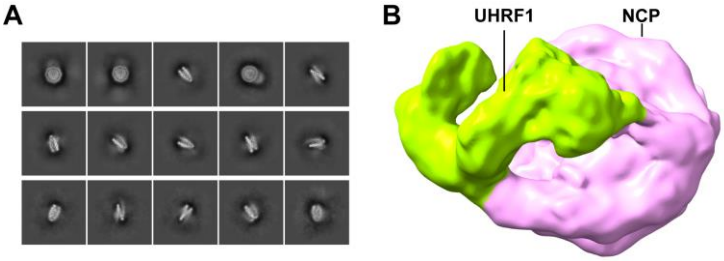

Figure 1. Structural study of UHRF1 in complex with nucleosome core particle (NCP). (**A**) 2D classification analysis of a preliminary data set. (**B**) Cryo-EM map of the UHRF1-NCP complex at 6.5 Å resolution, obtained from the preliminary data set. The map is unsharpened. The regions corresponding to the UHRF1 protein and NCP are labeled.

We propose to solve the structure of UHRF1 in complex with nucleosome core particle (NCP) bearing two distinct epigenetic marks: hemimethylated CpG and H3K9me3. Structural elucidation of the UHRF1-NCP complex will provide mechanistic insights into the UHRF1-mediated histone ubiquitylation, thereby paving the road for development of novel therapeutic target against cancer. Toward this, we have prepared the complex of full-length UHRF1 with epigenetically modified NCP. Electrophoretic mobility shift assay (EMSA) and negative staining analysis both confirmed the formation of the UHRF1-NCP complex. Furthermore, we submitted the complex sample to NCCAT during the previous cycle for cryo-EM data collection. In total 4178 movies were collected and subject to data processing using CryoSparc software. Our preliminary 2D classifications analysis of the particles revealed prominent features of the UHRF1-NCP complex (Figure 1A). Finally, 26,210 particles were selected for non-uniform refinement, which led to a density map of 6.5 A resolution (Figure 1B). The density map reveals that UHRF1 binds to the linker DNA with its SRA domain, while engaging close contacts with the histone octamer. However, due to limited resolution, we are currently unable to perform accurate modeling of the UHRF1-NCP complex. Guided by the preliminary data, we have now optimized the chemical crosslinking condition to stabilize the complex, as well as the sample loading condition. We expect that a 2-3 days of data collection of this optimized sample, if available, will lead to a great increase in particle number and map resolution.

Structure determination of the UHRF1-NCP complex by cryo-EM will provide important insights into the molecular basis of UHRF1-mediated histone ubiquitylation, which is of great interest to the broad field of DNA methylation, epigenetics and cancer biology.