Preliminary Results

Structural study for the complex between histone methyltransferase Clr4 and epigenetically modified nucleosome. Histone modifications represent one of the major epigenetic mechanisms that are essential for cell differentiation and development. Dysregulation of histone modifications has been linked to various human diseases, in particular cancer. This project focuses on molecular understanding of how histone H3K9 methyltransferase Clr4 is regulated by histone H3K14 ubiquitylation (H3K14Ub). To elucidate the mechanism by which H3K14Ub regulates Clr4, we have prepared the complex of Clr4 with recombinant nucleosome core particle (NCP) installed with the H3K14Ub mark.

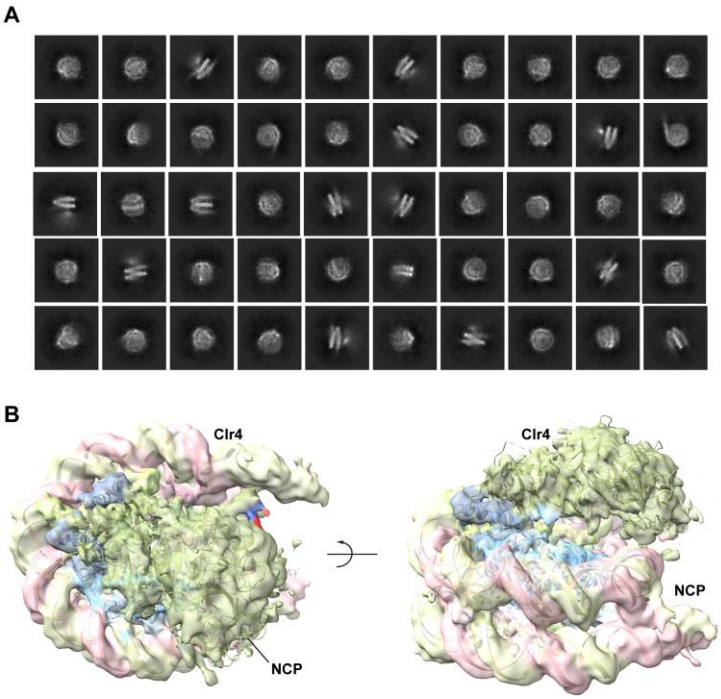

Figure 1. Structural study of the Clr4-NCP complex. (**A**) 2D classification of the DNMT1-NCP complex. (**B**) Preliminary density map (~4 Å) for the Clr4-NCP complex. No sharpening was applied to the map.

In preliminary studies, we have generated the H3K14Ub-modified NCP following a previously reported protocol. On this basis, we mixed the H4K14Ub-modified NCP with the recombinant Clr4 protein, followed by size-exclusion chromatography and controlled chemical crosslinking. Furthermore, we have collected one cryoEM dataset for the complex sample from the NCI cryoEM facility and performed data processing using the Cryosparc program. Our preliminary analysis of the 2D and 3D classifications reveals the formation of the Clr4-NCP complex (Fig. 1A). In comparison with the free NCP particles, the population of the Clr4-NCP complex is relatively low, presumably due to the dynamic dissociation of the complex under the cryogenic condition. Nevertheless, these preliminary data led us to obtain a medium-resolution density map (~6 angstrom; Fig. 1B), providing initial insights into the interaction between Clr4 and NCP. Remarkably, the Clr4 protein covers one face of the NCP, prompting partial unwrapping the nucleosome particle. To further improve the structural resolution of the Clr4-NCP complex, we have improved the sample preparation through optimizing the complex formation and crosslinking conditions. SDS-PAGE analysis of our new samples indicate a higher population of the Clr4-NCP complex. We expect that additional data collection of the Clr4-NCP sample at NCCAT will help us refine the structure to a near-atomic resolution. Based on our preliminary results, we expect that a 4-5 days of data collection at Titan Krios will be sufficient for the structural study.

Structure determination of the Clr4-NCP complex by cryo-EM will provide important insights into the functional regulation of Clr4, which is of great interest to the broad field of epigenetics and cancer biology.