Cryo-EM Reveals Structural Basis for Activation Mechanism of DNMT1

Mammalian cytosine methylation in CpG sequence is involved in diverse biological processes. A DNA methyltransferase DNMT1 plays a pivotal role in maintenance of DNA methylation in which hemimethylated DNA is recovered to full methylation state. Here, we report the Cryo-EM structure of DNMT1 in complex with ubiquitinated H3 and hemimethylated DNA, which reveals a novel activation mechanism of DNMT1. Our results not only clarify a fundamental activation mechanism of DNMT1 but also provide a basis for therapeutic drug for targeting DNMT1.

5th carbon atom of cytosine residue in CpG sequence of mammalian genome is frequently methylated by DNA methyltransferases. In differentiated cells, approximately 60~80% of CpG sequence are methylated to determine the cell-type specific gene expression pattern [1]. Thus, DNA methylation regulates the cell fate of each cell in tissues. CpG sequence is a palindrome, therefore cytosines in both DNA strands are symmetrically methylated, which is called as fully methylation. After each DNA replication, hemimethylated DNA, where only cytosine in a newly synthesized strand is methylated, is transiently produced. To upfold the cell fate of the differentiated cells, hemimethylated DNA must be converted to fully-methylated DNA. This process is known as maintenance of DNA methylation. A DNA methyltransferase DNMT1 is an essential for maintenance of DNA methylation. DNMT1 catalyzes the conversion of hemimethylated DNA to fully-methylated DNA. Knock-out mice of DNMT1 shows embryonic lethal at early stage of embryogenesis. Importantly, aberrant DNA methylation pattern caused by DNMT1 is relevant to carcinogenesis, thus DNMT1 is an attractive target for therapeutic drugs of several cancers.

DNMT1 consists of five functional domains, RFTS, CXXC, BAH1, BAH2 and catalytic domains (Fig. 1(a)). Excellent X-ray crystallography have revealed the structural-functional relationship of DNMT1 [2–5]. Structural study of apo-form DNMT1 shows auto-inhibitory state of DNMT1; N-terminal RFTS domain is inserted into the DNA-binding surface of the catalytic domain. Thus, when DNMT1 is activated, RFTS domain must be dissociated from the catalytic domain to bind to the hemimethylated DNA. Crystal structure of DNMT1 harboring BAH1-BAH2-catalytic domains, in which RFTS domain is removed, bound to hemimethylated DNA shows the activating structure, providing the substrate DNA recognition. However, the N-terminal auto-inhibitory domains of DNMT1 is deleted for crystallization, thus it is still unclear the structural rearrangement of the N-terminal domains during activation of DNMT1. In 2017, we have reported that ubiquitinated histone H3 (H3ub) is emerged as an activator of DNMT1; H3ub binds to the RFTS domain and subsequently it enhances the DNA methylation activity of DNMT1 [6]. However, molecular mechanism by which H3ub enhances the DNA methylation activity of DNMT1 is unknown.

To clarify the activation mechanism of DNMT1 upon binding of H3ub, we aimed to cryogenic electron microscopy (cryo-EM) analysis of the ternary complex, DNMT1:H3ub:hemimethylated DNA [7]. 2.5 Å resolution of the cryo-EM map of ternary complex clearly showed the activation mechanism of DNMT1: Cryo-EM map of RFTS domain bound to H3ub is not observed, which indicates that RFTS domain is thoroughly forced-out from the catalytic domain and highly dynamic state. Cryo-EM structure also unveiled a novel activation mechanism of DNMT1. The two key structures are important for activation of DNMT1: An Activating Helix between RFTS and CXXC domains and activation/inactivation regulatory pocket in catalytic domain (hereafter Toggle Pocket) (Fig. 1(a)). The Toggle Pocket consists of hydrophobic residues, Phe1229, Val1248, Phe1263, Leu1265, Phe1274, Val1279, and Leu1282. In the inactive state, the DNA Recognition Helix (residues 1236-1259) in the catalytic domain is kinked and Phe1235 of the catalytic loop and Phe1243 of the DNA Recognition Helix are invaded in the Toggle Pocket. Tyr1240 within DNA Recognition Helix forms hydrophobic interactions with Phe628, Phe631 and Phe632 from the Activating Helix (residues 620-635). In contrast to the inactive state, Phe631 and Phe632 in Activating Helix invade the Toggle Pocket in the activating state. The DNA Recognition Helix is freed from the Toggle Pocket and springs into a straight conformation, which allows access of Phe1235, Arg1238 and Tyr1240 to the DNA (Fig. 1(b)).

To examine the functional importance of the Phe631 and 632, we performed *in vitro* assay using the mutant DNMT1 including substitutions of the F631/F632 to alanine residues. *In vitro* DNA methylation measurement and DNA-binding assay demonstrated that the DNA-binding ability of the mutant DNMT1 was dramatically reduced, leading to the less DNA methylation activity in the presence of H3ub. The functional importance of the F631/F632 was also validated in mammalian cell; the cells expressing the DNMT1 mutant failed to maintain the DNA methylation level. Notably, F631/F632 in the Activating Helix are highly conserved among vertebrate DNMT1. Thus, the F631/F632 in the activating helix identified in this study are crucial role in activation of DNMT1 and the maintenance of DNA methylation in the mammalian cell.

DNA methylation pattern is rigorously inherited to the daughter cells after each DNA replication and cell division. Dysregulation of DNA methylation maintenance is caused to various diseases, especially cancers. Our structural study reveals that the activation of DNMT1 is regulated by complicated mechanism, which confer the robustness for DNA methylation maintenance in cells. We also succeeded in identification of the regulatory motif for DNMT1 activation. This will provide the information for the development of a novel drug targeting the regulatory motif of DNMT1.

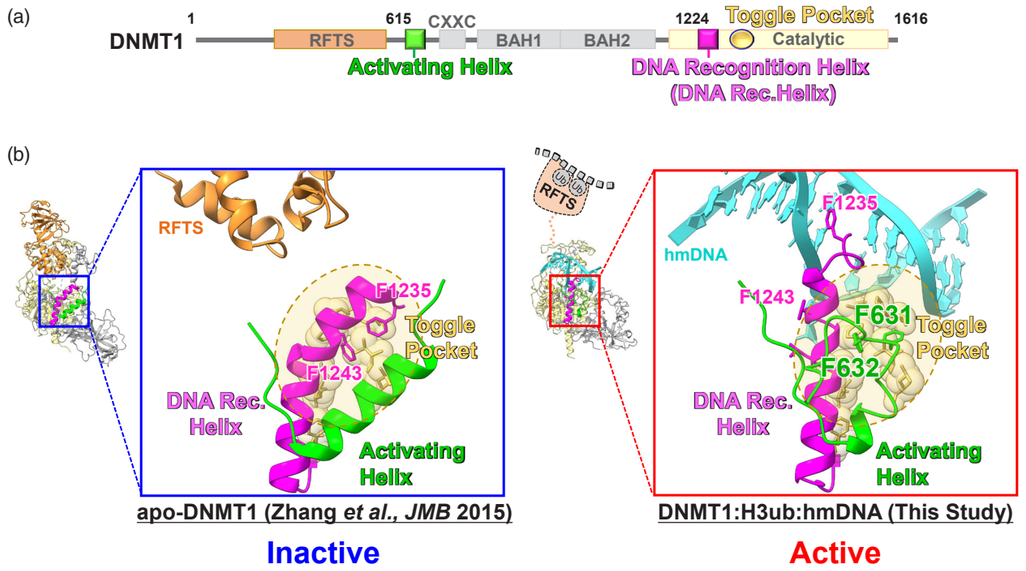

Figure 1: (a) Domain structure of human DNMT1. (b) Structure of apo-DNMT1 (left, PDB:4WXX) and the ternary complex (right). Insets in each figure show the schematic structure of Toggle Pocket.

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BEAMLINES

BL-10C and KEK Cryo-EM

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