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 full methylation. After each DNA replication, hemimethylated DNA, where only cytosine in a newly synthesized strand is methylated, is transiently produced. To uphold 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 domain (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 catalytic domain to bind to the hemimethylated DNA. apo-DNMT1 (Zhang et al., JMB 2016) (Fig. 1(b)). 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 loop and Phe1243 of the DNA Recognition 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.

References


A. Kikuchi and K. Arita (Yokohama City Univ.)