A Novel Mechanism for Inheritance of Cellular Memory: DNA Methylation

Recruitment of a DNA methyltransferase, DNMT1, to hemi-methylated DNA sites is crucial for DNA methylation maintenance. However, the molecular mechanism of the process remains unclear. Biochemical and structural biological processes have demonstrated that dual mono-ubiquitylation of histone H3 functions as a unique signal that recruits DNMT1 to the hemi-methylation sites. Further, the ubiquitylated histone H3 enhances the DNA methylation activity of DNMT1. The proper location and timing of DNMT1 activation are regulated by the ubiquitylated histone H3 to maintain DNA methylation fidelity.

An adult human body consists of about 60 trillion cells, all derived from one fertilized egg. One of the basic principles of biology is the inheritance of genomic sequences via semiconservative replication. The number of somatic cell types in our body are between 200–230, which essentially have the same set of genes in the genome. Whereas our somatic cells have same genomic information, the function and morphology are different depending on the cell types, and are maintained after several rounds of cell division. How the somatic cells obtain and maintain their individual function and morphology is still a mystery. The key regulator for this is chemical modification of the genomic DNA (i.e., DNA methylation). In vertebrates, the 5th position of cytosine base in the cytosine–guanine dinucleotides sequence is methylated. The function of DNA methylation is inhibition of gene expression. Thus, genes unnecessary for the cell are highly methylated. Somatic cells obtain cell-type specific DNA methylation patterns during cell differentiation. The cell-type specific DNA methylation pattern is inherited after every cycle of DNA replication as well as genomic information (i.e., DNA methylation maintenance). Therefore, epithelial and hepatocyte cells remain the same even after cell revision. Thus, DNA methylation functions as a "cellular memory" that regulates gene expression to maintain cell-type specific functions and morphology. Aberration of the DNA methylation pattern is intimately linked with tumorigenesis [1]. Therefore, revealing the underlying mechanism of DNA methylation maintenance is an important research field.

Two proteins play a pivotal role in DNA methylation maintenance. DNMT1 is a DNA methyltransferase that catalyzes the transfer of methyl group to the cytosine base in DNA. UHRF1 is a multi-functional protein; it recruits the DNMT1 to the hemi-methylation site (mentioned later) and has an enzymatic activity that covalently links a 76-amino acid protein (ubiquitin) to substrate proteins. This modification is called, "ubiquitination."

After DNA replication, hemi-methylated DNA, where only cytosine in a one (parental) strand is methylated

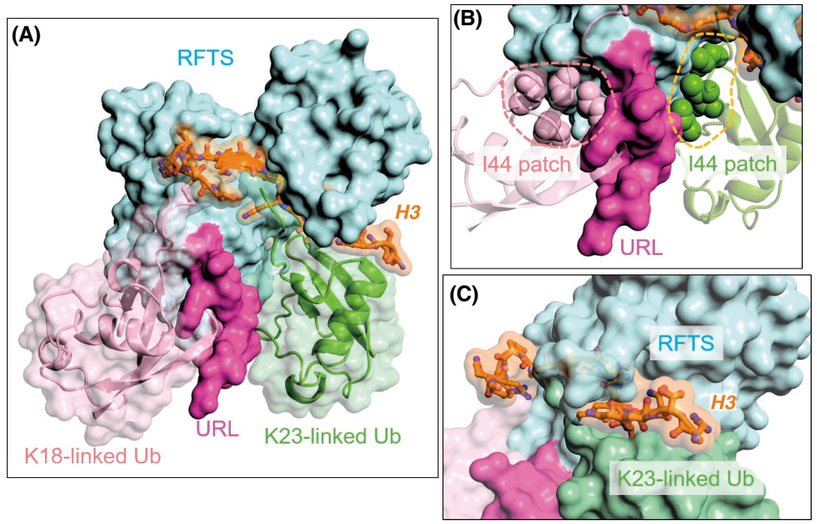

Figure 1: Crystal structure of RFTS in complex with K18 and K23 mono-ubiquitylated histone H3. **(A)** Overall structure of the complex. DNMT1 is shown as a cyan surface model. Histone H3, K18- and K23-linked ubiquitins are depicted as orange, light pink, and green illustrations superposed on transparent surfaces, respectively. Two ubiquitins are separated by the URL region of DNMT1, colored magenta. **(B)** Close-up view of interaction between DNMT1 and K18- and K23-linked ubiquitins. I44 patches of ubiquitins are tightly interacted with DNMT1. **(C)** Recognition of histone H3 tail by DNMT1 and K23-linked ubiquitin. H3 tail is sandwiched between DNMT1 and K23-linked ubiquitin.

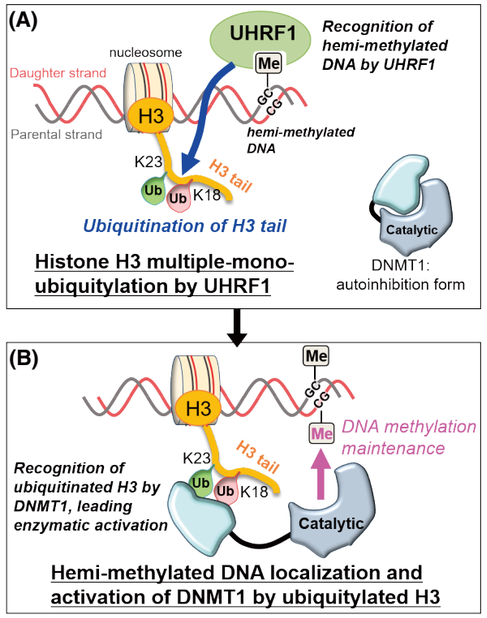

Figure 2: Schematic model of hemi-methylated site recruitment and activation of DNMT1. **(A)** UHRF1 recognizes hemi-methylated DNA and ubiquitylates the K18 and K23 on histone H3. In this situation, DNMT1 forms a closed conformation, which is low enzymatic activity. **(B)** Binding of DNMT1 to ubiquitylated histone H3 changes its structure from closed to open, facilitating DNA methylation activity.

[Fig. 2(A)], is transiently generated, because DNA polymerase can only inherit the genomic sequence, not DNA methylation. The first step of the DNA methylation maintenance is that UHRF1 specifically recognizes hemi-methylated DNA [Fig. 2(A)] [2, 3]. Subsequently, UHRF1 catalyzes the ubiquitination of the lysine residues in the amino terminal tail of histone H3 (H3 tail), which is a component of nucleosome, a basic unit of chromatin structure. The DNMT1 preferentially binds to the ubiquitylated histone H3, which enables DNMT1 to recruit on the hemi-methylation sites [4]. Finally, DNMT1 transfers the methyl group to cytosine in the hemi-methylated DNA.

Molecular mechanism of DNA methylation maintenance has been elucidated. However, it remains unclear how UHRF1 ubiquitylates the histone H3 tail and how DNMT1 recognizes the ubiquitylated histone H3 tail.

Mass spectrum analysis demonstrated that multiple lysine residues (K14, K18, and K23) in the histone H3 tail were simultaneously mono-ubiquitylated by UHRF1, which is a very unique ubiquitination, because it is known that a poly-ubiquitin chain generally functions as a biological signal in the cells. Biochemical analysis shows that DNMT1 can bind to K18 and K23 mono-ubiquitylated histone H3 with high affinity and specificity. Crystal structure of DNMT1 in complex with the K18 and K23 double mono-ubiquitylated histone H3, determined using BL-1A, showed that K18- and K23-linked ubiquitins in histone H3 were bound to DNMT1 with large binding interface [Fig. 1(A)]. A canonical binding surface, I44 patch (L8, I44, V70), of K18-linked ubiquitin was recognized by previously identified ubiquitin interacting motif of DNMT1. By contrast, the I44 patch of K23-linked ubiquitin was bound to novel hydrophobic binding surface of RFTS [Fig. 1(B)]. In addition to the hydrophobic interactions, the ubiquitin recognition loop (URL) of RFTS, which is disordered in free-form RFTS structures, physically separated two ubiquitins and contributed to hydrophilic interactions with them [Fig. 1(B)]. Histone H3 tail was inserted in the shallow cleft. Notably, residues 2–9 of histone H3 were sandwiched by DNMT1 and K23-linked ubiquitin [Fig. 1(C)].

In addition to the recognition of the ubiquitylated histone H3, the crystal structure also revealed a novel activation mechanism of DNMT1: the structural change of DNMT1 by binding of ubiquitylated histone H3 coupled with its enzymatic activation. It is known that DNMT1 alone forms autoinhibition structure [Fig. 2(A)] [5]. However, upon binding of K18 and K23, double mono-ubiquitylated histone H3 undergoes substantial conformational change of DNMT1, leading to open form of DNMT1. This structural rearrangement allows the DNMT1 to bind the hemi-methylated DNA, facilitating its methylation [Fig. 2(B)].

Whereas the importance of DNA methylation as a cellular memory is widely known, the mechanism underlying the localization and activation of DNMT1, an essential protein for DNA methylation maintenance, has remained a mystery. This report revealed that ubiquitination of histone H3 is a key event for DNA methylation maintenance. Ubiquitylated histone H3 not only recruits the DNMT1 to the hemi-methylated DNA region, but it also enhances DNA methylation activity for DNMT1. The novel mechanism provides new insight into the inheritance of cellular memory and DNA methylation.

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