Structural Basis for Recognition of Methylated DNA Ligase 1 by UHRF1

DNA methylation defines the gene expression patterns of somatic cells and is faithfully inherited after each cycle of replication to maintain cell identity. UHRF1 is an essential factor for the maintenance of DNA methylation, and recruits DNA methyltransferase to the methylation sites. Recently, replication factor DNA ligase1 (LIG1) has been shown to bind to UHRF1 and recruit UHRF1 to the replication sites. Here, we determined the crystal structure of UHRF1 in complex with methylated LIG1, which reveals the details of the interaction between UHRF1 and LIG1. SAXS analysis demonstrated the structural change of UHRF1 from closed to open form upon binding of methylated LIG1. These structural data provide novel insights into the molecular mechanism of maintenance of DNA methylation coupled with DNA replication.

A fundamental role of biology is robust inheritance of genomic information. In addition to the genomic sequence, DNA methylation, which occurs at the C5 position of a cytosine in the CG dinucleotide sequence, is also known to be inheritable information.

DNA methylation contributes to gene silencing through heterochromatin formation. DNA methylation defines tissue-specific gene expression patterns, therefore, somatic cells have a unique feature. Importantly, DNA methylation patterns in the cells are inherited after each cycle of DNA replication to upfold the cell identity. UHRF1 is an essential factor for the maintenance of DNA methylation. UHRF1 has five functional domains: UBL, TTD, PHD SRA and RING domains. After DNA replication, UHRF1 binds to hemimethylated DNA, which is methylated at only the parent strand, and subsequently ubiquitinates histone H3. The ubiquitylated histone H3 functions as a binding platform for DNA methyltransferase, DNMT1. Finally, DNMT1 is recruited to hemimethylation sites by the ubiquitylated histone H3 and remethylates the nascent strand. Maintenance of DNA methylation occurs immediately after DNA replication, however, the mechanism underlying the functional and structural relationship between replication and maintenance of DNA methylation is unclear.

DNA ligase 1 (LIG1) has emerged as a factor for recruiting UHRF1 to replication sites, thus LIG1 links replication and maintenance of DNA methylation [1]. LIG1 accumulates at replication sites during S-phase and catalyzes the ligation reaction of Okazaki fragments in lagging strands. Intriguingly, the N-terminal of LIG1 has a histone H3-like sequence and the side chain of Lys126 is methylated by lysine methyltransferase, G9a/GLP (H3: ^{4}KQTAR$\underline{K}^{9}$, LIG1: ^{121}RRTAR$\underline{K}^{126}$, underline means methylated lysine residue). UHRF1 tandem tudor domain (TTD) binds to the methylated LIG1. The UHRF1 TTD domain is known to function as a hub that is involved in inter- and intramolecular interactions, such as histone H3 containing trimethylated K9 (H3K9me3; known as an epigenetic mark for heterochromatin formation) and UHRF1 linkers [2]. Interestingly, despite the sequence similarity, UHRF1 TTD domain binds to trimethylated K126 in LIG1 (LIG1K126me3) with the dissociation constant K_d of 9 nM, whereas the K_d between TTD and H3K9me3 is 1 μM. How can LIG1K126me3 bind to UHRF1 TTD domain with high affinity?

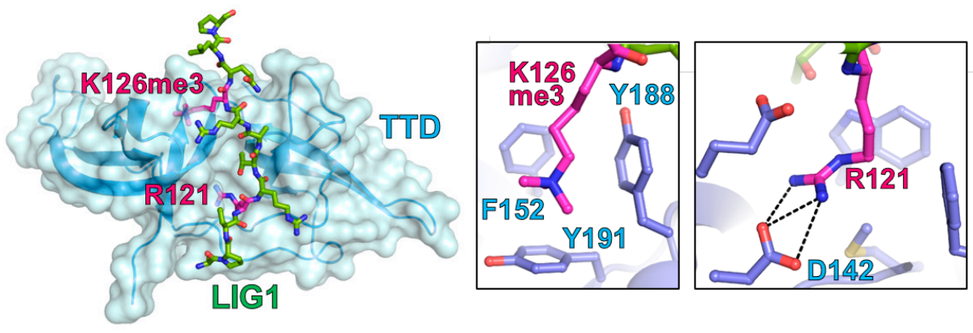

Figure 1: Crystal structure of UHRF1 TTD (blue cartoon model overlaid on surface model) bound to the LIG1K126me3 peptide (green stick model). Arg121 and K126me3 of LIG1 are colored magenta. Bottom panels are magnified views of recognition of Arg121 and K126me3 of LIG1 by UHRF1 TTD.

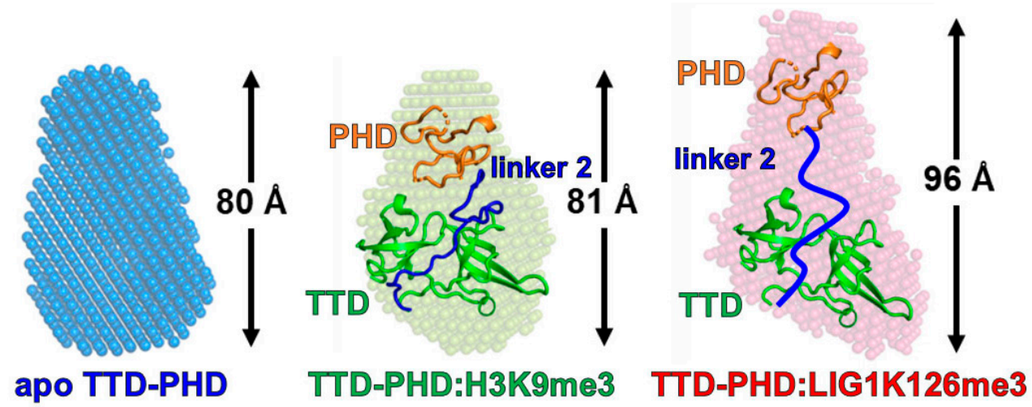

Figure 2: Low-resolution beads models of apo TTD-PHD (left) and when bound to H3K9me3 (middle) and LIG1K126me3 peptide (right). The crystal structure of TTD-PHD in complex with H3K9me3 and the open-form model of TTD-PHD upon binding of LIG1K126me3 are superimposed on the corresponding beads models, respectively.

To reveal the mechanism of the high affinity binding, we determined the crystal structure of UHRF1 TTD bound to the LIG1K126me3 peptide at BL-17A [3]. LIG1 binds to the groove of TTD (TTD groove) (**Fig. 1**). Two dense contacts were observed: K126me3 in LIG1 is recognized by an aromatic cage comprising F152, Y188 and Y191 of UHRF1 TTD, and Arg121 in LIG1 forms hydrogen bonds with Asp142 in the acidic pocket of UHRF1 TTD (**Fig. 1**). Structural and mutational analysis clearly showed that the interaction between Arg121 of LIG1 and the acidic pocket of UHRF1 TTD is critical for the high affinity binding, because only one amino-acid residue mutation (Lys4 to Arg, corresponding to Arg121 in LIG1) in histone H3 greatly enhanced the binding affinity to UHRF1 TTD with K_d of 22 nM, which is nearly identical to that of LIG1K126me3.

We also revealed the structural change of UHRF1 upon binding to LIG1K126me3 by SEC-SAXS at BL-10C (**Fig. 2**). The TTD-PHD module of UHRF1 has a compact structure in which the linker 2 between TTD and PHD interacts with the TTD groove [4]. The TTD-PHD module is known to bind to H3K9me3, where residues 1–4 and K9me3 are simultaneously recognized by PHD and TTD, respectively. SEC-SAXS analysis showed that binding of H3K9me3 leads to no structural change of TTD-PHD, which is still a compact structure. In contrast, binding of LIG1K126me3 to the TTD-PHD module is limited to the TTD region, as a result of which the structure of the TTD-PHD module is changed from closed to open form (**Fig. 2**).

Taken together, this study clarified the structural mechanism by which replication factor LIG1 could recruit UHRF1 to the replication site. In addition, we identified the important interaction point for the high affinity binding between UHRF1 and LIG1. It has been shown that overexpression of UHRF1 in various cancer cells is involved in aberrant DNA methylation which leads to silencing of tumor suppressor genes. The small molecule that binds to the acidic pocket in the TTD groove of UHRF1 may inhibit the interaction with LIG1, paving the way for the development of cancer drugs.

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BEAMLINES

BL-10C and BL-17A

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