Recognition of Hemi-Methylated DNA by the SRA Protein UHRF1 Using a Base-Flipping Mechanism

DNA methylation is a heritable epigenetic mark regulating tissue-specific gene expression during development, and its dysregulation has been implicated in many diseases, including cancer. The inheritance of an epigenetic DNA methylation pattern relies on maintenance DNA methylation during replication, in which the newly synthesized strand is methylated depending on the methylation status of the template strand. A hemi-methylated DNA binding protein, UHRF1, plays a key role in maintenance DNA methylation. The crystal structures of the SRA domain of UHRF1 were determined in both free and hemi-methylated DNA bound states, and provide a new insight into the molecular mechanism underlying maintenance DNA methylation.

DNA methylation is one of the major epigenetic marks that regulate the tissue-specific gene expression accompanied by modulation of chromatin structure. In mammals, DNA methylation occurs at the C5 position of cytosine in CpG dinucleotides, and is generally coupled with heterochromatin formation and transcriptional repression. A DNA methylation pattern unique to each cell type is established during embryogenesis and defines the differentiation state and function of cells. The differentiation states are secured by the inheritance of the DNA methylation pattern from mother to daughter cells through somatic cell division, which is termed maintenance DNA methylation. The protein UHRF1 plays an essential role in the first step of maintenance DNA methylation. UHRF1 recognizes hemi-methyl CpG sites through its SRA (SET and RING associated) domain and directs the maintenance DNA methyltransferase, Dnmt1, to these sites to methylate the newly synthesized strand [1, 2]. To gain structural insight into maintenance DNA methylation, we determined the crystal structures of the SRA domain (amino acids 405-613) from mouse UHRF1 in a free form and in a complex with hemi-methylated DNA [3].

Triclinic crystals of the unliganded SRA domain were grown using the hanging-drop vapor-diffusion method with PEG3350 as a precipitant. The SRA-DNA complex was crystallized in two crystal forms using different 12 base-pair oligonucleotide duplexes that contained a single hemi-methylated CpG site. The monoclinic form belongs to a C2 space group with cell dimensions of a=89.6 Å, b=104.0 Å, c=65.6 Å, and β=99.2°, while another form belongs to a tetragonal space group $P4_12_12$ with cell dimensions of a=b=58.9 Å and c=182.3 Å. X-ray diffraction data sets were collected on BL-5A and were processed using HKL2000 software. The unliganded structure was solved at 1.77 Å by using a molecular replacement method, with the MOLREP program from the CCP4 suite using the crystal structure of the human UHRF1 SRA domain determined by the Structural Genomics Consortium in Toronto (PDB entry, 3BI7) as a search model. The complex structures of the monoclinic and tetragonal forms were determined by molecular replacement using the unliganded structure, at 1.6 Å and 2.6 Å resolution, respectively. These complex structures are essentially identical.

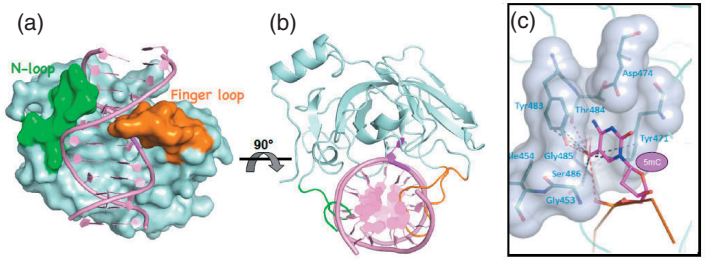

Figure 1
Crystal structure of SRA domain bound to hemi-methylated DNA (a) Molecular surface of the SRA domain bound to hemi-methylated DNA. The N-loop (amino acids 404-411) and finger loop (amino acids 489-499) are colored in green and orange, respectively. (b) Ribbon representation of the SRA domain bound to hemi-methylated DNA. The methylated cytosine that is flipped out of DNA duplex is shown in magenta. (c) Recognition of the methylated cytosine by protein residues. Red and black broken lines show possible hydrogen bonds and hydrophobic interactions between the methyl group of the methyl cytosine and SRA, respectively.

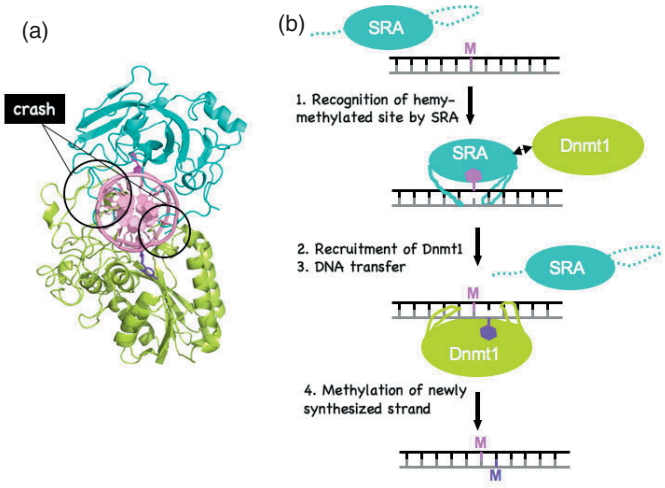

Figure 2
A successive DNA transfer model for maintenance DNA methylation by UHRF1 and Dnmt1. (a) A comparative model of the catalytic domain of Dnmt1(light green) bound to DNA. The model is overlaid on the structure of the SRA-DNA complex. The methylated cytosine is shown in magenta, and the target cytosine for Dnmt1 in purple. (b) Schematic model showing cooperative action by UHRF1 and Dnmt1 for maintenance methylation. Pre-existing and newly synthesized DNA strands are indicated in black and gray, respectively. 'M' represents the methyl cytosine.

The SRA domain adopts a novel saddle-shaped fold harboring a basic concave surface, which accommodates the hemi-methylated DNA in the complex [Fig. 1(a)]. Upon binding to DNA, conformational changes are induced in the N- and finger loops of the SRA so that DNA is tightly held at the hemi-methyl CpG site from both sides [Fig. 1(b)]. Interestingly, the methylated cytosine base is flipped out of the DNA duplex, and is recognized in the hydrophobic pocket located in the concave surface [Fig. 1(c)]. The base flipping out of double-stranded DNA has been observed in catalytic actions of bacterial DNA methyltransferases and DNA repair enzymes, by which the enzymes gain access to the substrate base. The SRA domain exhibits no structural similarity to theses enzymes, and is seemingly the first non-enzymatic domain that utilizes base flipping for rigorous base recognition.

Furthermore, the coordinated action between UHRF1 and Dnmt1 was investigated using a model-building approach. The comparative model of the catalytic domain of Dnmt1 bound to the hemi-methylated DNA was built based on the crystal structure of the bacterial methyltransferases-DNA complex (PDB entry, 1MHT). Superposition of the comparative model onto the structure of the SRA-DNA complex revealed a steric interference in the case of simultaneous binding of SRA and Dnmt1 to the hemi-methylated DNA [Fig. 2(a)], implying that the methyl-cytosine recognized by the SRA and the target cytosine for Dnmt1 swing out successively from the helix during transfer of the DNA from UHRF1 to Dnmt1 [Fig. 2(b)]. Together with such a concerted action of UHRF1 and Dnmt1, the base flipping out in the recognition of the hemi-methylated site by SRA may ensure that methylation marks are faithfully copied from the template to the newly synthesized strand.

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K. Arita[1], M. Ariyoshi[1], H. Tochio[1], Y. Nakamura[2], M. Shirakawa[1] ([1]Kyoto Univ., [2]The Univ. of Tokyo)