UHRF1, a modular multi-domain protein, regulates replication-coupled crosstalk between DNA methylation and histone modifications

Hideharu Hashimoto, **John R. Horton**, **Xing Zhang**, and **Xiaodong Cheng**[*]
Department of Biochemistry; Emory University School of Medicine; Atlanta, Georgia USA

Abstract

Cytosine methylation in DNA is a major epigenetic signal, and plays a central role in propagating chromatin status during cell division. However the mechanistic links between DNA methylation and histone methylation are poorly understood. A multi-domain protein UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) is required for DNA CpG maintenance methylation at replication forks, and mouse UHRF1-null cells show enhanced susceptibility to DNA replication arrest and DNA damaging agents. Recent data demonstrated that the SET and RING associated (SRA) domain of UHRF1 binds hemimethylated CpG and flips 5-methylcytosine out of the DNA helix, whereas its tandem tudor domain and PHD domain bind the tail of histone H3 in a highly methylation sensitive manner. We hypothesize that UHRF1 brings the two components (histones and DNA) carrying appropriate markers (on the tails of H3 and hemimethylated CpG sites) ready to be assembled into a nucleosome after replication.

Keywords

Introduction

In mammals and other vertebrates, DNA methylation occurs at the C5 position of cytosine (5mC), mostly within CpG dinucleotides. The mammalian DNA nucleotide methyltransferases (Dnmts) include four members, in two families that are structurally and functionally distinct.[1] The Dnmt3 family establishes the initial CpG methylation pattern de novo, whereas Dnmt1 maintains this pattern during chromosome replication[2] and repair.[3] These enzymes utilize a conserved base flipping mechanism[4] that has been studied best in the bacterial 5mC methyltransferase (MTase) M.HhaI.[5] Briefly, this mechanism involves MTase binding to the DNA, eversion of the target nucleotide so that it projects out of the double helix ("base flipping"), covalent attack of a conserved Cys nucleophile on cytosine C6, transfer of the methyl group from S-adenosyl-ʟ-methionine to the activated cytosine C5, and the various release steps. This methylation, together with histone modifications, plays an important role in modulating chromatin structure, thus controlling gene expression and many other chromatin-dependent processes.[6]

As befits a maintenance MTase, Dnmt1 has a 30-40-fold preference for hemimethylated sites (reviewed in refs. [7] and [8]). However, Dnmt1 is necessary but not sufficient for proper

[*]Correspondence to: Xiaodong Cheng; Emory University; Department of Biochemistry; 1510 Clifton Rd; Atlanta, Georgia 30322 USA; Tel.: 404.727.8491; Fax: 404.727.3746; Email: xcheng@emory.edu

maintenance methylation. An accessory protein UHRF1 targets Dnmt1 to hemimethylated replication forks (and presumably repair sites).[9-11] The murine ortholog of this protein is also known as NP95 (nuclear protein of 95 kDa);[12-15] the human ortholog is called ICBP90 (inverted CCAAT binding protein of 90 kDa).[16] UHRF1 harbors at least five recognizable functional domains: an ubiquitin-like domain (UBL) at the N terminus, followed by a tandem tudor domain, a plant homeodomain (PHD), a SET and RING associated (SRA) domain, and really interesting new gene (RING) domain at the C terminus (Fig. 1A). At the time of this writing (October, 2008), structures are available for all five individual domains, either from human or mouse UHRF1 or its homolog UHRF2 (Fig. 1B). However, we do not know whether these domains act independently or in a cooperative fashion.

SRA Domain Structure

The crystal structure of the SRA domain of UHRF1 in complex with DNA containing a hemimethylated CpG site was recently determined.[17-19] It reveals that the SRA domain contains two twisted β sheets packed together forming a crescent moon-like structure, whose basic inner surface binds DNA (Fig. 2A). The inner surface of the crescent contains two loops, responsible for CpG recognition (R496-associated loop) and promotion of base flipping (V451-associated loop). The tips of these loops approach the DNA from opposite directions contacting major (R496 loop) and minor grooves (V451 loop), and are stabilized at the center of the DNA helix by van der Waals contacts between the R496 and V451 side chains (Fig. 2B). The 5-methylcytosine (5mC) flips completely out of the DNA helix and positions in a binding pocket with planar stacking contacts, and both Watson-Crick polar hydrogen bonds and van der Waals interactions specific for 5mC (Fig. 2C). The methyl group at the C5 position in 5mC is in van der Waals contact with Cα and Cβ atoms of S486 (see insert in Fig. 2C), make tight-fit shape recognition that distinguishes 5mC from cytosine. The structure also suggests an explanation for the preference for hemimethylated sites. In the major groove side, the backbone carbonyl oxygen of N494 is so close to the C5 ring carbon of the unmethylated cytosine, that addition of a methyl group to the C5 of the cytosine would cause a steric clash.

Base Flipping Mechanism

Base flipping is a conserved mechanism that is widely used by nucleotide modifying enzymes, including DNA MTases,[4,5] DNA repair enzymes,[20-22] and RNA modification enzymes.[23] This mechanism, first discovered in the bacterial 5mC MTase M.HhaI,[5] involves enzyme binding to the DNA and eversion of the target nucleotide so that it projects out of the double helix and into the active-site pocket. The SRA domain is the first-discovered non-enzymatic sequence-specific DNA binding protein domain that uses the base flipping mechanism in its interaction with DNA.

One unexpected finding from our original structure[19] is that the adenine (Ade) two bases 3′ to the 5mC, also adopts an extrahelical conformation and stacks against a hydrophobic surface patch. Concurrently, the end of DNA near the flipped Ade is destabilized, due to the lack of interactions with SRA domain. To address (1) whether this second flipping outside of CpG dinucleotide is Ade specific and (2) how the crystallographic restraining forces influence the stability of DNA ends, we determined three additional crystal structures using an oligonucleotide containing a thymine (Thy) at the position corresponding to flipped Ade, i.e., two bases 3′ to the 5mC (Table 1). The complexes crystallized either in the space group $C222_1$ (with two different cell dimensions, crystal forms 1 and 2) or $P2_1$ (crystal form 3), with resolution ranging from 1.41 Å to 2.29 Å. Survey of the three structures revealed three modes of protein-DNA crystallographic packing forces (Fig. 3A-C). The high packing mode involves two DNA molecules stacking head-to-head surrounded by eight total SRA molecules, two interact specifically and six non-specifically (Fig. 3A) (crystal forms 1 and 2). The intermediate

packing mode involves two head-to-head DNA molecules bound specifically by two SRA molecules plus two SRA capping on two ends of the DNA molecules, respectively (Fig. 3B) (crystal form 3). The low packing mode (only observed in crystal form 1) does not involve DNA-DNA stacking interactions. Instead, each DNA has one SRA molecule specifically bound at CpG site and another one non-specifically bound only at one end of DNA molecule, leaving the other end unattended (Fig. 3C)—a situation very similar to the crystal structure in $P4_12_12$ space group where Ade flipping occurred.[19] In contrast to the previously described, the Thy is intrahelical with normal base paring under all three modes of protein-DNA crystallographic packing forces.

Superimposition of the intrahelical Thy and the extrahelical Ade indicates that the flipped Ade is associated with increased interstrand phosphate-phosphate distance (Fig. 3D). Trapping the flipped Ade is possible due to the existence of a hydrophobic surface patch nearby. Base flipping of an Ade nucleotide outside of recognition sequence (or non-target base) has been observed in HinP1I endonuclease,[24] EcoP15I DNA MTase,[25] EcoRV DNA MTase[26] and *E. coli* MutY adenine glycosylase.[27] Interestingly, the interaction between a hydrophobic surface patch and a flipped Ade is reminiscent of a structure of the *E. coli* β clamp on a primed DNA template,[28] where the nucleotide (an Ade) of the terminal base pair flips out (Fig. 3E).

Comparison with HhaI MTase and MBD Domains

There is no apparent sequence or structural similarity between SRA and enzymes that flip base such as DNA MTase domains or DNA repair enzymes. However, several features are shared among these base-flipping proteins: the phosphodiester backbone pinching[29] due to extensive protein-phosphate contacts surrounding the flipped nucleotide, the use of two loops to approach DNA from the major and minor grooves simultaneously, and the binding of the flipped base in a concave pocket (Fig. 4A and B).[30] Whereas enzymes use base flipping to gain access to a DNA base to perform chemistry on it, the SRA domain probably uses base flipping to increase its protein-DNA interface [19] and to prevent the SRA domain from linear diffusion away from the target site on DNA. This may be particularly important for the SRA domain, as its recognition sequence is only two base pairs. In comparison, the surface area buried at the SRA-DNA interface is approximately 70% more than the buried surface at the methyl-binding domain (MBD) of MeCP2-DNA (Fig. 4C) and MBD1-DNA interfaces (Fig. 4D) that do not involve base flipping[31,32] but also recognize only CpG sequence. We therefore suggest that the SRA-DNA interaction (through recognition and flipping of the 5mC) serves as an anchor to keep UHRF1 at a hemimethylated CpG site where it recruits Dnmt1 for maintenance methylation.

UHRF1-DNMT1 Interactions

There are two studies on the direct interaction between UHRF1 and Dnmt1, but they differ in the regions involved in the interaction.[10,11] One study involves the SRA domain,[11] whereas the other involves the PHD domain.[10] From the side of Dnmt1, three regions have been implicated, residues 1-446, or 401-615, or 1081-1408.[10,11] Additional data will be required to settle this point. It remains to be shown whether the binding of UHRF1 affects the affinity and/or specificity of DNMT1 to its substrate, hemi-methylated DNA.

UHRF1-histone Interactions

Besides the SRA domain, the Structural Genomics Consortium at Toronto has solved three additional domain structures by X-ray crystallography (Fig. 1B): the N-terminal ubiquitin-like domain (PDB 2FAZ[33]) and the tandem tudor domain with and without bound histone H3K9me3 (PDB 3DB3 and 3DB4,[34]) for human UHRF1, and the C-terminal RING domain (PDB 1Z6U[35]) of human UHRF2, whereas the RIKEN Structural Genomics/Proteomics

Initiative at Japan solved an NMR structure for the PHD domain of human UHRF2 (PDB 2E6S[36]). Individual domain functions have been suggested for the PHD that may be involved in histone H3 tail binding,[12,16] and for the RING domain that may confer E3 ubiquitin ligase activity on histones.[12]

The new structure of the tandem tudor domain bound with histone H3 tail contains three structured H3 residues, H3R8-H3K9me3-H3S10 (PDB 3DB3) (Fig. 5A). Similarly only two structured histone residues, H4R19 and H4K20me2, were observed in the structure of another tandem tudor domain of 53BP1,[37] (Fig. 5B). Structural comparison of two tandem tudor domains reveal that (1) the histone peptide runs along the shortest dimension of the domain, a possible reason that only 2-3 substrate peptide residues are structured; (2) the histone peptide is bound between the two individual tudor domains with the methyl-lysine binding aromatic cage formed by residues from a single tudor domain; and (3) a glutamate or aspartate residue in (or near) the binding cage seems to be a common mode of interaction involving methyl-lysine.[38]

Conclusion and Perspectives

The methylation of mammalian DNA, primarily at CpG dinucleotides, has long been recognized to play a major role in controlling gene expression among other functions. Given their importance, many basic questions remain to be answered about the proteins responsible for this methylation, particularly how DNA methylation coordinates with the parallel chromatin-marking system that modifies histones. The fact that UHRF1 contains modules, within the same polypeptide, recognizing both DNA silencing mark (via the SRA) and histone silencing marks (via the Tudor and/or PHD) suggests it maybe a key component to couple the preservation of histone-modification through the cell cycle with maintenance DNA methylation (Fig. 6). In this context the missing key experiment is whether these domains act independently or in a cooperative fashion. For example, does binding of UHRF1 SRA domain to hemi-methylated DNA improve the binding of UHRF1 Tudor and/or PHD domains to histone tail? Furthermore, the E3 ubiquitin ligase activity,[12] residing in the C-terminal RING domain, may add an additional level of modification, such as H2A ubiquitylation that is enriched with inactive genes.[39] Without understanding the interactions and spatial relationships between such modular domains, or whether inter-domain interactions contribute to target specificity, it is not possible to construct a temporal sequence of events or causal relationships in gene silencing.

The experimental characterizations of Dnmts and their associated protein factors are providing a rapidly and convergent picture of the kinetic mechanisms (activities of oligomers[40]), binding partners (UHRF1-Dnmt1,9,[10] and Dnmt3L-Dnmt3a[41]), chromatin recognition[42] (histone binding such as H3K4me0,[43] and H3K9me3,[44]), RNA-directed DNA methylation.[45,46] However, understanding the basis for establishing, maintaining and disturbing DNA methylation patterns will require a much better understanding of the union between structure and function in the Dnmts and their associated protein factors than we currently possess.

Acknowledgements

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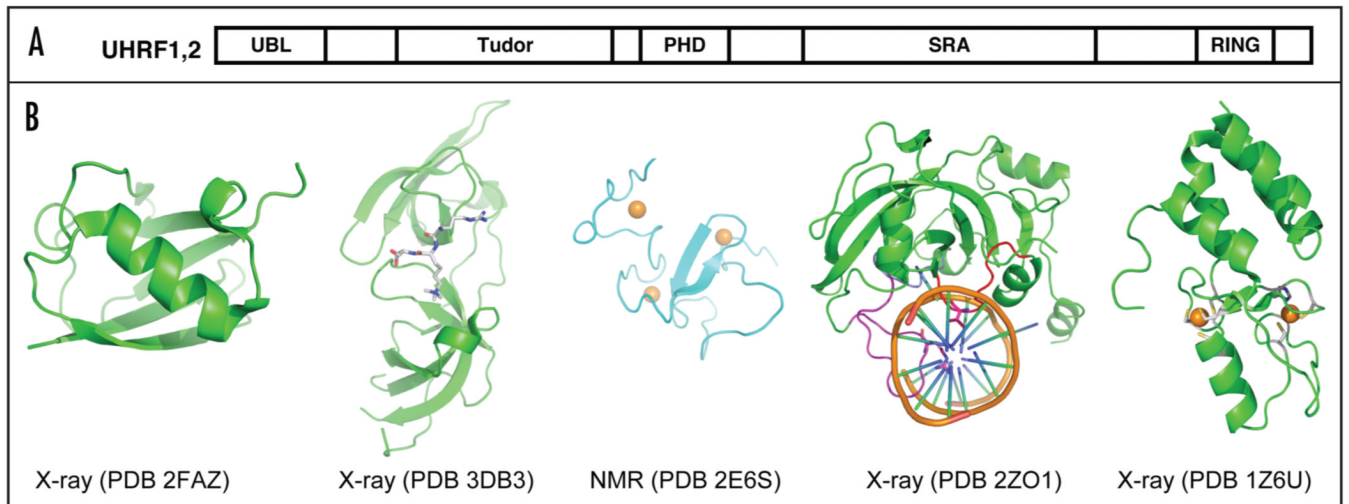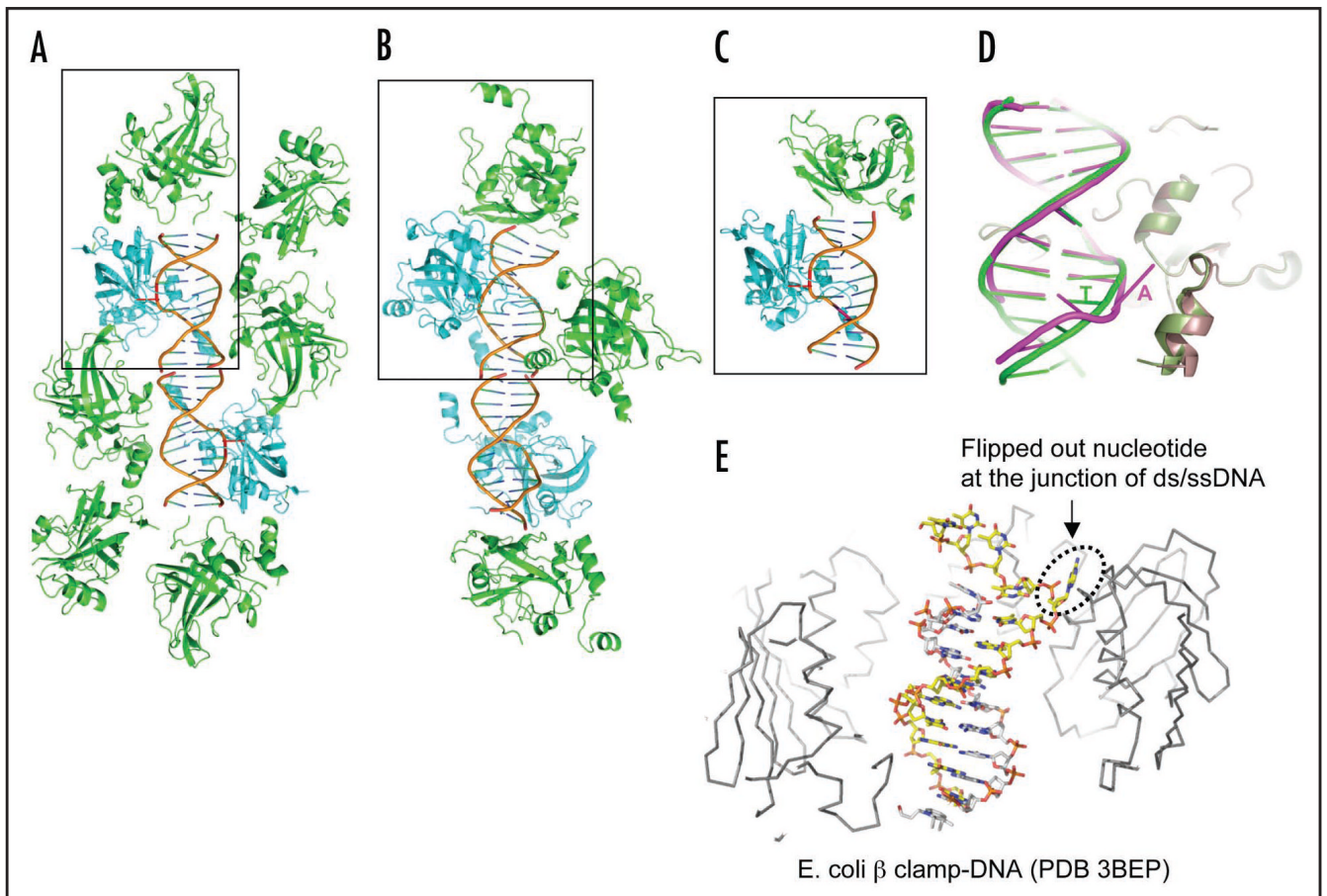
Figure 1.
UHRF1—a multi-domain protein. (A) Schematic representation of UHRF1 and its homolog
UHRF2. (B) Five domain structures are currently available.
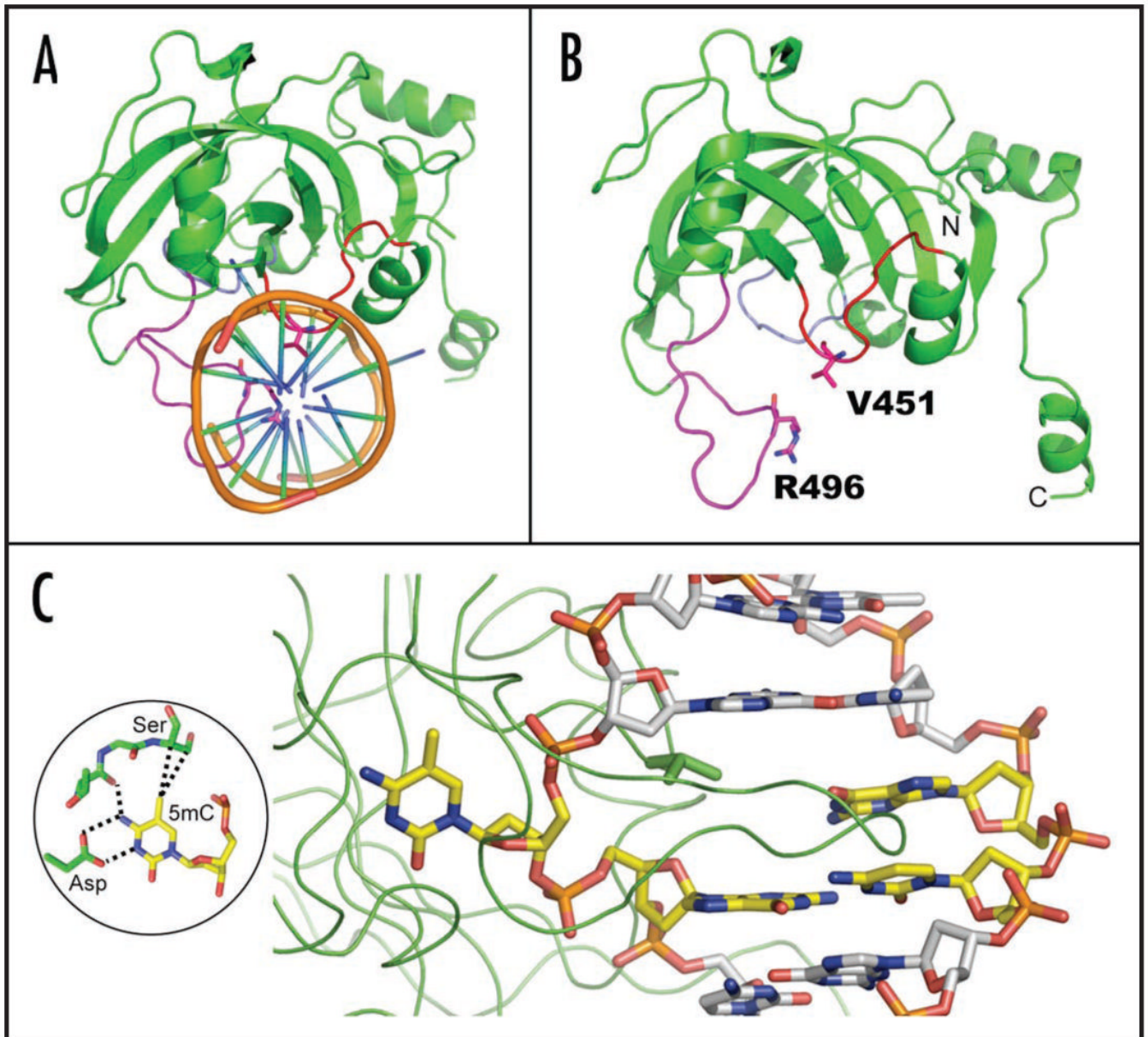
Figure 2.
Structure of SRA-DNA complex. (A) The two loops—CpG recognition and base flipping—penetrate into the DNA helix from opposite directions. Adapted from ref.[19] (B) The side chains of V451 of base flipping loop and R496 of CpG recognition loop are in direct van der Waals contact. Adapted from ref.[19] (C) The 5mC flips out and is bound in a cage-like pocket.
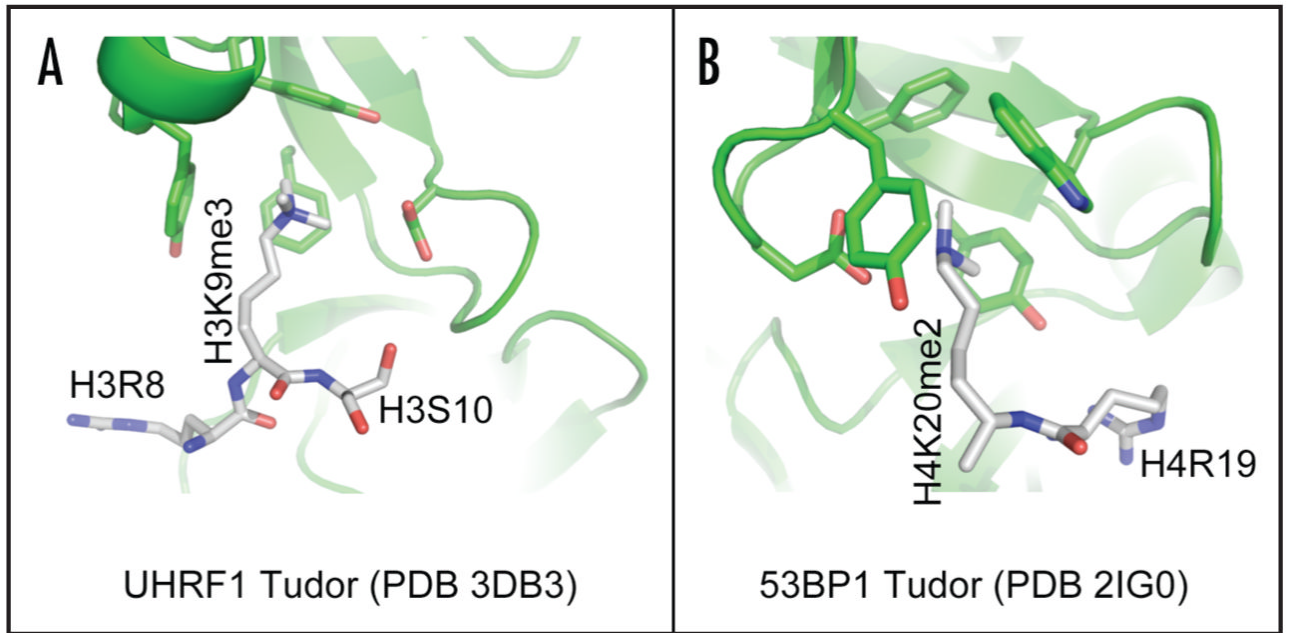
Figure 3.
Three new X-ray structures in which the thymine two bases 3′ to the 5mC are intrahelical (see Table 1). (A and B) Crystal packing force involving two DNA molecules stacking head-to-head. The SRA domain molecules involved in specific interactions are shown in blue, and the SRA domain in non-specific interactions in green. (C) Crystal packing force involving a single DNA molecule. (D) Structural comparison of DNA containing an extrahelical Ade (magenta) and intrahelical Thy (green). (E) Structure of *E. coli* β clamp-DNA shows a flipped-out nucleotide at the junction of double-strand (ds) and single-strand (ss) DNA.
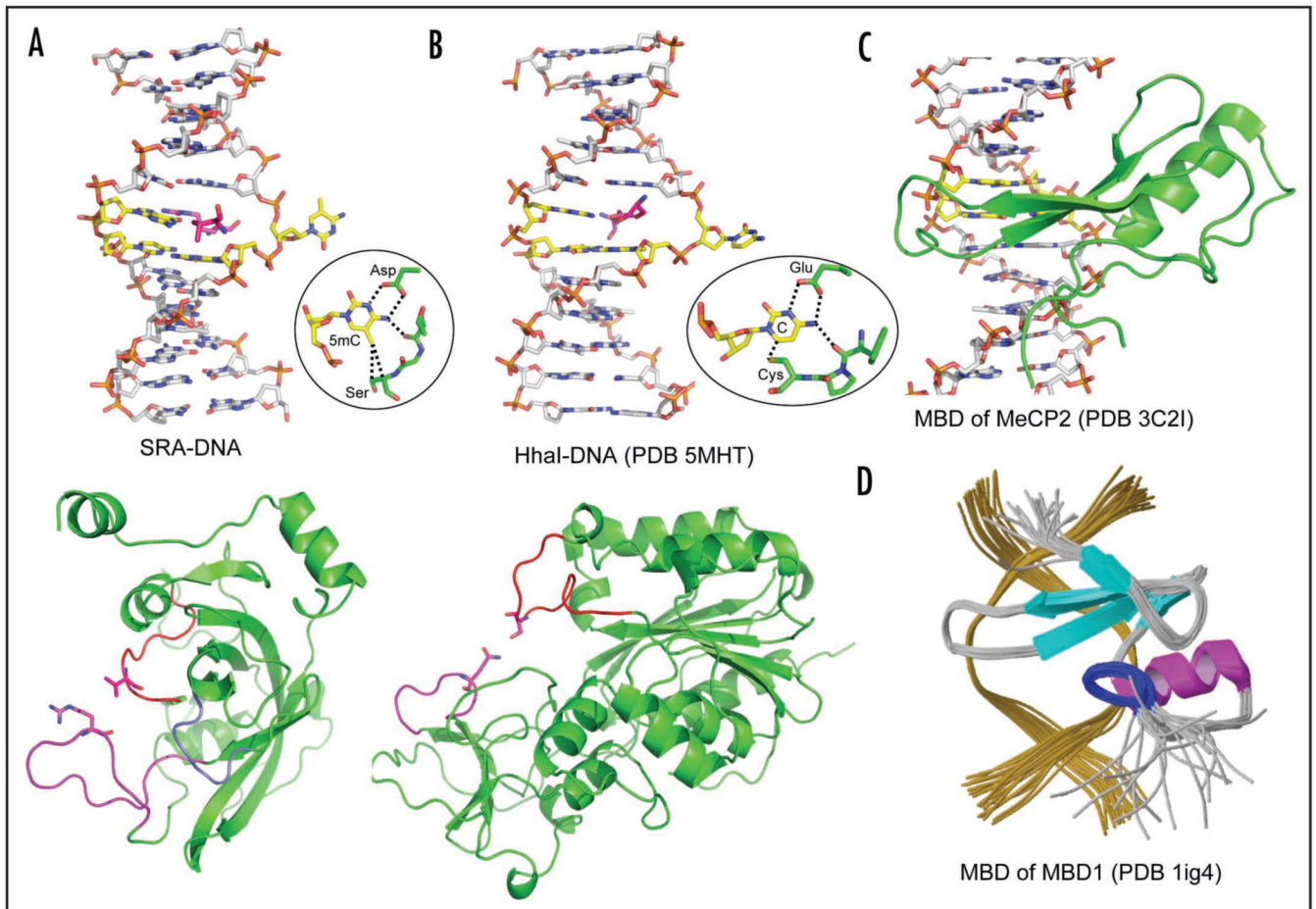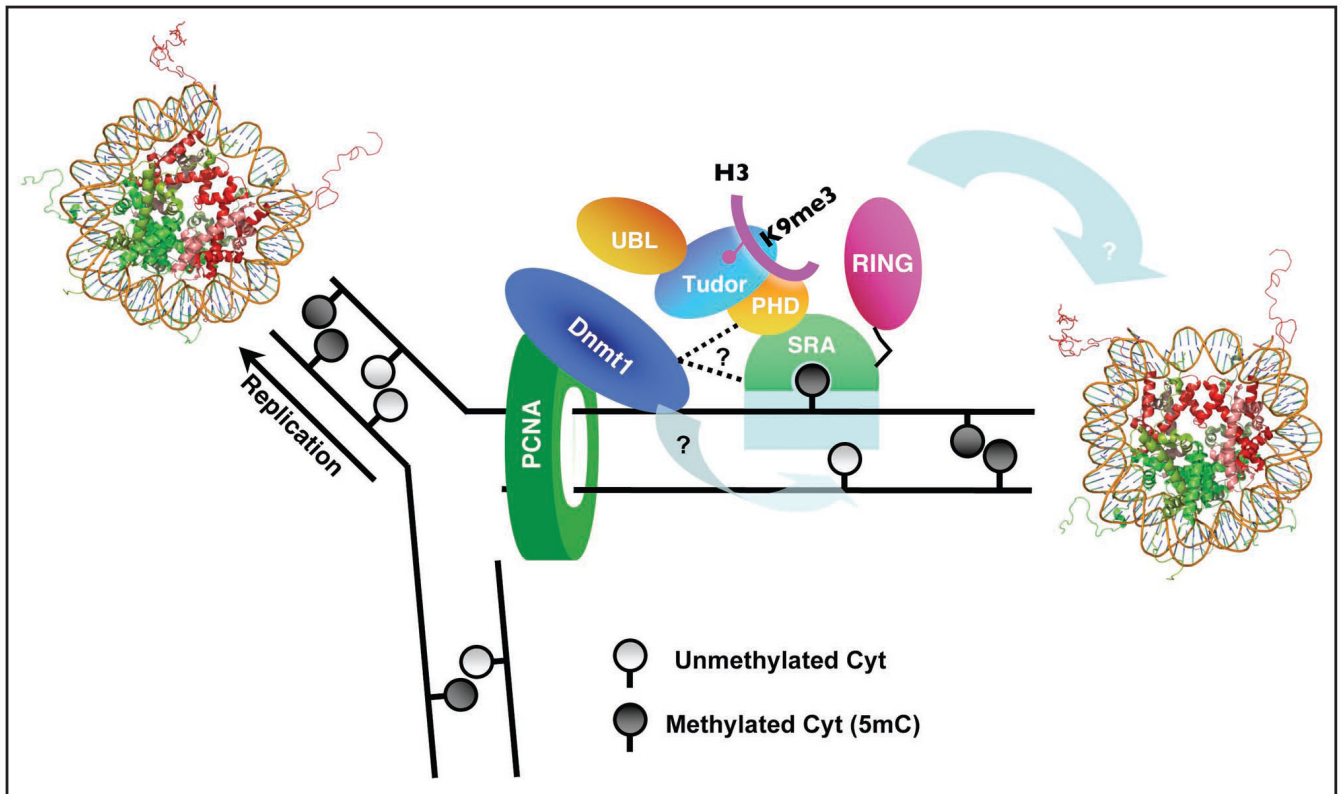
Figure 4.
Comparison of base flipping by the SRA domain and HhaI MTase. (Top) DNA structures bound by SRA (A) and HhaI (B) show a flipped nucleotide. The intercalating amino acids are shown in each case. (Bottom) Structures of SRA (A) and HhaI (B) show the two opposite-side DNA-approaching loops. Inserted are the major determinants of 5mC (A) and cytosine (B). (C) X-ray structure of MeCP2-DNA and (D) the NMR structure of MBD1-DNA shows MBD domain inserts a beta-hairpin through the DNA major groove. The methyl-binding domains of MBD1,[31] and MeCP2,[32] instead of using a base-flipping mechanism, recognize changes in hydration of the major groove of a fully methylated CpG rather than detecting methyl groups directly.

Figure 5.
Methyllysine binding cage in tandom todor domain of (A) UHRF1 and (B) 53BP1.

Figure 6.
Hypothetical model of UHRF1-mediated replication-coupled crosstalk between DNA methylation and histone modifications. Existence of both silencing mark readers recognizing DNA (via the SRA) and histone (via the Tudor and/or PHD) facilitates the idea of maintenance and conversion of epigenetic silencing marks on both DNA and histone modifications.

Table 1

New crystal structures of SRA-DNA complexes[#]

Data collection	Crystal 1	Crystal 2	Crystal 3
Space group		C222$_1$	P2$_1$
Cell dimensions		(α = β = γ = 90°)	α = γ = 90°, β = 98.9°
a (Å)	84.678	61.67	50.08
b (Å)	103.691	91.59	62.42
c (Å)	149.838	118.51	113.45
Asymmetric unit	2 complexes	1 complex	2 complexes
DNA		5′-CCATG**MGCT**GAC-3′ 3′-GGTAC**GCG**ACTG-5′	
Beamline (SERCAT)		APS 22-ID	
Wavelength (Å)		1.00000	
Resolution (Å)[*]	32.79-1.41 (1.45-1.41)	29.91-1.99 (2.06-1.99)	34.53-2.29 (2.37-2.29)
R$_{sym}$ or R$_{merge}$[*]	0.095/0.771	0.102/0.568	0.102/0.352
I/σI[*]	10.9/1.0	9.0/3.6	10.5/2.3
Completeness (%)[*]	97.0/69.9	99.8/99.1	97.5/90.5
Redundancy[*]	5.1/1.7	9.6/6.5	3.4/2.9
Observed reflections	662,990	224,631	102,763
Unique reflections[*]	121,098	23,488	30,041
Refinement			
Resolution (Å)	1.41	1.99	2.29
No. reflections	111,836	22,237	27,777
R$_{work}$/R$_{free}$	0.149/0.186	0.198/0.232	0.222/0.265
Number of atoms			
Protein	3220	1609	3208
DNA	974	487	974
Heterogen	64 (ethylene glycol)	54 (glycerol)	-
Water	596	162	150
B-factors (Å^2)	17.8	28.8	35.1
R.m.s. deviations			
Bond lengths (Å)	0.004	0.005	0.006
Bond angles (°)	1.1	1.1	1.1
Dihedral angles (°)	22.0	22.2	22.7
Improper angles (°)	0.99	0.96	1.08
Estimated coordinate error			
From Luzzati plot (Å)	0.20	0.22	0.31

[*] Highest resolution shell is shown in parenthesis.

[#] Protein production, crystallization, X-ray data collection, structure determination and refinement were made as described {in ref. [19]}.