RFTS-dependent negative regulation of Dnmt1 by nucleosome structure and histone tails

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Introduction

The 5th position of genomic Cytosine is frequently methylated. In gene promoters, methylation represses transcription, whereas there is a positive correlation with expression when methylation is located in gene bodies [1]. DNA methylation is crucial for normal development [2]. Once DNA methylation patterns are established, they are faithfully maintained through cell divisions. Maintenance DNA methylation is essential not only for differentiated cells but also for epigenetic reprogramming from primed to naïve pluripotent cells [3]. When maintenance methylation is impaired, aberrantly differentiated or apoptotic cells are observed [4,5]. Despite numerous analyses of methylation patterns, the regulatory mechanism of DNA methylation is still largely unknown.

Dnmt1 is the enzyme primarily responsible for the accurate perpetuation of DNA methylation patterns following cell division. It can target unmethylated DNA methylation in promoter regions represses gene expression and is copied over mitotic divisions by Dnmt1. Dnmt1 activity is regulated by its replication foci targeting sequence (RFTS) domain which masks the catalytic pocket. It has been shown that Dnmt1 activity on unmethylated DNA is inhibited in nucleosome cores. In the present study, we aimed to assess the effect of nucleosome formation on maintenance methylation at single CpG resolution. We show that Dnmt1 fully methylates naked linker DNA in dinucleosomes, whereas maintenance methylation was repressed at all CpG sites in nucleosome core particles. Deletion of RFTS partly released obstruction of Dnmt1 activity in core particles. Histone H3 tail peptides inhibited Dnmt1 in an RFTS-dependent manner and repression was modulated by acetylation or methylation. We propose a novel function of RFTS to regulate Dnmt1 activity in nucleosomes.

Abbreviation
Dnmt1, DNA methyltransferase 1; λ exo, λ exonuclease.
DNA but has a preference for hemimethylated substrate [6,7]. Dnmt1−/− knockout in mice is lethal at early embryonic stage [8]. Dnmt1 is a multidomain protein [9,10], containing an independent replication foci targeting sequence (RFTS) domain. RFTS masks the catalytic pocket, and this intramolecular interaction increases the activation energy of Dnmt1 [9]. Dnmt1 interacts directly and/or indirectly with histone tail regions. In egg or cell lysate, the RFTS domain binds to ubiquitin, ubiquitylated histone H3, and unmodified H3 [11–13]. However, the physiological meaning of the interaction between RFTS and histone H3 is unclear.

The direct effect of nucleosomal structure on Dnmt1 activity in vitro has been reported in several studies, however, the described effects are controversial. The maximal activity of Dnmt1 toward mononucleosomes reconstituted with unmethylated DNA and histones prepared from HeLa cells was unchanged compared to naked DNA but $K_{max}$ values on substrates were increased by nucleosome structure [14]. When mononucleosomes were reconstituted with recombinant histones and unmethylated DNA, Dnmt1 activity toward nucleosomes was slightly reduced compared to naked DNA [15]. In another study, mononucleosomes including some hemimethylated CpG sites were used. DNMT1 showed an intrinsic ability to methylate CpG sites packaged into nucleosomes but the activity was DNA sequence dependent [16]. Analysis of mononucleosomes with unmethylated DNA by bisulfite sequencing showed that Dnmt1 de novo methylation was inhibited in nucleosome cores but not on linker DNA. Dnmt1 activity toward hemimethylated nucleosomal arrays was also reported to be lower than that toward naked DNA [17].

Dnmt1 methylates hemimethylated DNA in a processive manner, whereas unmethylated substrate is modified distributively [7]. In the present study, we therefore specifically address the effect of nucleosomal structure on Dnmt1 maintenance methylation activity by using reconstituted dinucleosomes in which all CpG sites are hemimethylated. Using bisulfite sequencing, we mapped Dnmt1 activity at single CpG resolution. As previously shown for de novo methylation [17], core regions in the nucleosomes were refractory to maintenance methylation while linker DNA was not. On naked DNA, histone tail peptides repressed Dnmt1 activity at low micromolar concentration. This repression was lost upon removal of the RFTS domain. Acetylation of histone H3 peptide abolished interaction with RFTS and Dnmt1 inhibition. Our results suggest that histone presence regulates Dnmt1 maintenance methylation activity in two ways. Firstly, nucleosomes obstruct Dnmt1 access to DNA and this steric hindrance is partially released by RFTS removal. Secondly, the interaction with unmodified histone tails can inhibit Dnmt1 activity directly by stabilizing RFTS-dependent autoinhibition.

Results

Repression of the methylation activity of Dnmt1 in the nucleosome core region

At single base resolution, analysis of Dnmt1-catalyzed methylation has so far been performed on nucleosomes prepared with unmethylated DNA [17]. To elucidate the effect of nucleosomal structure on mouse Dnmt1 activity on its intrinsic target, we prepared high-purity hemimethylated DNA which can be analyzed by sodium bisulfite sequencing (Fig. 1A). More than 95% of CpG sites on the upper strand in purified hemimethylated DNA were methylated, while less than 5% of CpG sites on the lower strand were sporadically methylated (Fig. 1B). The N-terminal 290 amino acids of Dnmt1 contain a sequence which binds to PCNA...
during replication. Dnmt1 291 lacking this domain is functionally similar to full-length Dnmt1 \textit{in vivo} \cite{18} and in terms of enzymatic activity on naked DNA \cite{6}.

At first we analyzed the methylation activity of Dnmt1 291 on naked hemimethylated DNA. More than 90\% of CpG sites were methylated by Dnmt1 291 and we
did not observe specific sites exempt from methylation (Fig. 1C), indicating that all CpG sites are recognized by Dnmt1 291 in equal manner.

We then reconstituted dinucleosomes from hemimethylated DNA and recombinant histones. In the nucleosomes, contamination by free DNA was negligible (Fig. 2A), nucleosome core particles were positioned as expected (Fig. 2B), and the size of DNA wrapped on the core particles was similar to unmethylated template (Fig. 2C). The prepared nucleosomes were then used in a methylation reaction with Dnmt1 291 (Fig. 2D). To monitor the integrity of the nucleosomes during the reaction, bacterial methyltransferase M. AluI was added [19]. In three independent experiments, CpG sites in both core particle regions core 1 (CpG number 6–18) and core 2 (CpG 22–34) were scarcely methylated, while more than 90% of CpG sites in all linker DNA regions (linkers 1, 2, and 3) were methylated (Fig. 2E and Fig. S2).
Full-length Dnmt1 (Dnmt1 FL), as well as Dnmt1 291, methylated more than 90% of CpG sites in the naked hemimethylated DNA (compare Fig. 3A and 1C). The prepared nucleosomes were then methylated with Dnmt1 FL (Fig. 3B). CpG sites in both core 1 and core 2 were scarcely methylated, while more than 90% of CpG sites in all linker DNA regions were methylated (Figs 3C and S3). The methylation pattern introduced by Dnmt1 FL was comparable to that by Dnmt1 291 (compare Figs 3C and 2E). In addition, no significant difference between the percentage of DNA methylation in each CpG catalyzed by Dnmt1 FL and Dnmt1 291 was observed (Fig. 3D). These suggest that the N-terminal region (amino acids 1–290) does not contribute to Dnmt1 inhibition by nucleosome structure.

Increase in Dnmt1 access to nucleosome entry/exit sites by removing RFTS

In Xenopus egg lysate, the N-terminal region (amino acids 180–470) lacking the PCNA binding domain but including the RFTS region of Xenopus Dnmt1 has been reported to bind to unmodified histone H3 [13]. We also detected binding of GST-mouse Dnmt1 to the N-terminal region of histone H3 (amino acids 1–30) which is limited to the RFTS domain (amino acids 291–602) (Fig. 4A). Crystal structure suggests that RFTS interacts with the catalytic pocket to block access to substrate DNA (Fig. 4B), thereby increasing activation energy [9]. We examined whether the truncation of RFTS changes the inhibitory effect of nucleosomes, by preparing Dnmt1 with a deletion of amino acids 1–601 (Dnmt1 602) (Fig. 4C). As for Dnmt1 291, we observed that the methylation activity of Dnmt1 602 was repressed by nucleosome structure (Figs 4D, S4, and 5A). The percentage of methylated CpGs in core regions methylated with Dnmt1 602 was slightly higher compared to Dnmt1 291 (compare Fig. 5A and 2E). On closer inspection, methylation in the central region of the nucleosome cores was unchanged, while in the marginal regions close to the entry/exit sites of both nucleosome cores methylation

Fig. 3. Nucleosome structure-dependent DNA methylation activity of Dnmt1 FL. Hemimethylated naked DNA (A) or dinucleosomes (B) were methylated by Dnmt1 FL. Two additional independent experiments were performed, and shown in Fig. S3. (C) Percentage of CpG methylation in the regions (linker 1, 2, 3, and core 1, 2) was shown as average ± SD (n = 3) (data from panel B and Fig. S3), as Fig. 2E. (D) Single-base analysis of DNA methylation. The percentage of DNA methylation in each CpG was plotted to the DNA sequence. Dinucleosomes were methylated with Dnmt1 291 (green line) or Dnmt1 FL (blue line) The percentage ± SD (n = 3) is shown. The data are derived from Figs 2D and S2 (Dnmt1 291), panel B and Fig. S3 (Dnmt1 FL).
was increased (Fig. 5B). Both Dnmt1 291 and 601 methylated all CpG sites in the naked DNA (Fig. 1C and 4D), indicating that the effect was not due to their individual DNA sequence specificity. The methylation level within the marginal region of both nucleosomes was summarized in Fig. 5C, showing that removal of
RFTS increased the methylation in the M2 marginal region by around twofold. Within the M2 regions, increased methylation was restricted to the two outermost CpG sites (17, 18, 33, and 34), which are 12 and 18 bp apart from the nucleosome exit. No change was seen at CpGs 16 and 32 in the M2 region or CpG
Repression of Dnmt1 activity by unmodified histone tail post-translational modifications

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It has been reported that Dnmt1 binds to histone deacetylases [25–27] as well as histone H3 methyltransferases SUV39H1 and G9a [28,29]. Furthermore, Dnmt1 binds to HP1 and Uhrf1 (Np95) which recognize K9 methylated H3 and release RFTS-dependent inhibition [28,30]. We therefore examined whether post-translational modifications on H3 (1–30) can change the inhibitory effect of the H3 peptide. Acetylation of lysine residues in H3 (1–30), H3 (1–30)ac, abolished the repressive effect on Dnmt1 291 activity in the tested concentration range (Fig. 7A). In contrast, H3 (1–30) K9me3 repressed Dnmt1 291 activity, although to a lower extent than the unmodified peptide (Fig. 7B). Thus, histones and their modifications can modulate the activity of Dnmt1. In consistency with the effect on Dnmt1 activity, RFTS did not bind to H3 (1–30) ac, but to H3 (1–30)K9me3 (Fig. 7C).

Dnmt1 processively methylates hemimethylated DNA, whereas unmethylated substrate is modified distributively [7]. We next tested whether the observed repression by H3 tail was due to specific inhibition of the processive reaction. We therefore used 42-bp oligonucleotides as substrate in which only one CpG site was hemimethylated (42 bp/1hmCpG) (Table S2). Toward 42 bp/1hmCpG, the activity of Dnmt1 291 was also repressed (Fig. 7D), suggesting that processive and nonprocessive methylation were equally affected by H3 (1–30).

To further narrow down histone tail regions required for inhibition, we prepared truncated H3 peptides. H3 (10–30) did not inhibit Dnmt1 291 activity, while H3 (1–20) showed a biphasic effect (Fig. 8A). Dnmt1 291 activity was slightly stimulated at less than 10 μM H3 (1–20), but the stimulatory effect was lost at higher concentration. H3 (5–30) or (1–25) repressed Dnmt1 291 activity, but the effect was milder than H3 (1–30). Loss of inhibition with truncated peptides suggests that a large binding interface is needed for Dnmt1 repression by histone H3.

A peptide (H3 rev) in which the amino acid sequence of H3 was inverted (Table S1) inhibited Dnmt1 activity with similar potency (Fig. 8B), suggesting that not the amino acid sequence but the total charge of the peptide is essential for the repression. We further examined whether the repressive effect is dependent on amino acid sequence. H4 (1–30), with a pI similar to H3 (10–30) (Table S1), inhibited the activity of Dnmt1 291 (Fig. 8C), however, the repressive effect was weaker than H3 (1–30) (compare to
Histone H2B (1–27) which has a lower pKa than H3 (10–30) (Table S1), did not inhibit the activity of Dnmt1 291 (Fig. 8D). In summary, histone tails can modulate Dnmt1 activity in a histone molecule-dependent manner, and their inhibitory effect is influenced by post-translational modifications.
Discussion

Dnmt1 activity toward nucleosomes reconstituted with unmethylated or hemimethylated DNA has been reported [14–17]. Schrader et al. examined Dnmt1 activity toward nucleosomes including hemimethylated DNA, and reported an overall repressive effect, however, not resolved by nucleosome regions [17]. As Dnmt1 switches from distributive to processive methylation when presented with hemimethylated substrate, we analyzed Dnmt1 maintenance methylation activity at single CpG resolution on dinucleosomes containing hemimethylated DNA [7]. We found that Dnmt1

![Graphs and diagrams showing the effect of acetylation and methylation on Dnmt1 activity](image)

**Fig. 7.** Detailed analysis of repression properties of Dnmt1 291. (A) Acetylation released the repression by H3 (1–30). Acetylation sites (ac) are shown in upper panel. (B) Repression of Dnmt1 291 by H3 (1–30) K9me3. Trimethylation site (me3) is shown in upper panel. The effect of modified H3 (1–30) on Dnmt1 291 activity was determined as described in Fig. 6, by using 42 bp/12hmCpG as substrates. (C) The effect of post-translational modifications on the interaction between RFTS and H3 (1–30). The interaction was monitored as in Fig. 4A. Recombinant GST-tagged Dnmt1 (291–601) was mixed with 5.6x (lanes 2 and 6 and 10), 17x (lanes 3, 7 and 11), and 56x (lanes 4, 8 and 12) molar excess of unmodified histone H3 (lanes 1–4) histone H3 (1–30)K9me3 (lanes 5–8) or histone H3 (1–30)ac (lanes 9–12). (D) Effect of histone H3 (1–30) on DNA methylation activity of Dnmt1 291 toward oligonucleotide 42 bp/1hmCpG, was examined. The activity in the absence of the peptide was taken as 100%, and the average ± SD (n = 3) is shown.
maintenance methylation activity was blocked at CpGs in nucleosome cores and the repression was partially released by removal of the RFTS domain. The RFTS guides Dnmt1 to replication foci in S-phase [31] and increases activation energy by intramolecular binding to the catalytic domain via hydrogen bonds [9,23]. The interaction between RFTS and the catalytic domain of Dnmt1 is released by interaction with the SRA domain of Uhrf1 [23], and Uhrf1 is crucial for DNA methylation [30]. Although several roles of RFTS in Dnmt1 function have been reported, its function in a nucleosomal context remains to be elucidated. In the present study, we observed that RFTS obstructs Dnmt1 access to nucleosome entry/exit sites. We also found that RFTS is directly bound by the N terminus of histone H3 and that this interaction inhibits Dnmt1 catalytic activity, potentially by stabilizing RFTS-dependent autoinhibition. Thus, RFTS regulates Dnmt1 activity not only by intramolecular interaction [9,32] but it also mediates Dnmt1 regulation by histones. Furthermore, as free, modified histones not incorporated in chromatin are observed in the nucleus [33], the repression of Dnmt1 by histone tails could not be limited to nucleosome structures.

In contrast to Dnmt1, de novo DNA methyltransferase Dnmt3a, as well as the Dnmt3a/Dnmt3L complex methylate linker DNA preferentially in a nucleosomal context [34,35]. The histone H3 tail stimulates the enzymatic activity of Dnmt3a in vitro and stimulation was abrogated by H3K4 methylation [35–37]. We showed that maintenance methylation by Dnmt1 is inhibited by histone H3, H3 methylated at lysine 9 and H4, and that the inhibitory effect of histone H3 is released by acetylation. Thus, de novo methyltransferase Dnmt3a and maintenance DNA methyltransferase Dnmt1 are oppositely regulated by unmodified histone H3.

We observed that H3 peptide with inverted amino acid sequence inhibited Dnmt1 with similar potency as the native H3 tail. Therefore, the polykationic nature of the peptide might be more important for inhibition than the peptide structure, possibly affecting protein or DNA conformation.

**Fig. 8.** Effect of truncated H3 tail. H3 rev, H4 tail, and H2B tail on Dnmt1 291 activity. The effect of H3 (1–20) (open circles with solid line), (1–25) (open circles with broken line), (5–30) (closed circles with broken line), and (10–30) (closed circles with solid line) (A), H3 rev (B), H4 (1–30) (C), and H2B (1–27) (D), (amino acid sequences are shown in Table S1) on Dnmt1 291 activity toward 42 bp/12hmCpG was verified as in Fig. 5. The relative activity was shown as average ± SD (n = 3).
In conclusion, this study shows a role of RFTS in regulating Dnmt1 function in a nucleosomal context and in mediating inhibition of Dnmt1 by histone tails. Future investigation should address the interaction between histone modifications and Dnmt1 to elucidate the regulation of DNA maintenance methylation.

Materials and methods

Preparation of hemimethylated DNA

Hemimethylated DNA was prepared basically as described by Hermann et al. [38] but including a purification step to reduce contamination by fully methylated DNA in the product. The 601.2 sequence (Fig. S1) was amplified with primer A and B, and then 2 mg of DNA was methylated with 0.2 mg of M. SsSI (NEB). Methylated DNA (2 mg) was treated with 3 μg of λ exo at 37 °C for 2 h to remove the lower strand, and then purified. The purification was performed using 6% acrylamide disk gel in Mini Prep Cell (BioRad, Tokyo, Japan) with 1 watt. The size of eluted DNA was monitored with 5% native PAGE in 1× TBE buffer. The fractions containing only single-stranded DNA were collected, annealed with primer C by incubation at 95 °C for 2 min, and cooled down to 37 °C with a 15 min linear gradient. The annealed DNA (3 μg) was mixed with 1.3 μg of T4 polymerase in a buffer containing 7 mM MgCl₂, 15 mM (NH₄)₂SO₄, 0.5 mM DTT, 0.1 mM EDTA, 0.5 mM dNTP, and 50 mM Tris-HCl, pH 8.5, and incubated 37 °C for 5 min to fill in the lower strand. The filled in DNA was digested with HindIII and BamHI and the product was purified with 9% acrylamide disk gel in Mini Prep Cell. The preparation steps were summarized in Fig. 1A.

Preparation of recombinant proteins and peptides

For truncated Dnmt1, Sf9 insect cells were infected with baculoviruses harboring cDNA of Dnmt1 which encodes amino acids 291–1620 or 602–1620. The expressed Dnmt1 was purified as described by Takeshita et al. [9]. For GST-Dnmt1 (291–601), cDNAs corresponding to GST and the Dnmt1 fragment were sequentially subcloned into an expression plasmid, pFASTBAC1 (Invitrogen, Tokyo, Japan) in frame, and baculovirus was prepared as described [6]. Sf9 cells infected with the virus were suspended in buffer A (0.3 M NaCl, 0.3% (w/v) Nikkol (Nikko Chemicals, Tokyo, Japan), 3 mM MgCl₂, 1 mM sucrose, 5 mM β-mercaptoethanol, 1/2000 (v/v) protease inhibitor cocktails (PI) (Nacalai Tesque, Kyoto, Japan), 20 mM Tris-HCl, pH 7.4), and centrifuged at 10 000 g for 5 min. Supernatant was purified with glutathione Sepharose (GE Healthcare, Tokyo, Japan). For GST or GST-Dnmt1 (1–118), pGEX or pGEX with the respective cDNA inserts were transformed into BL21, and cultured at 18 °C. GST or GST-Dnmt1 (1–118) was purified as described for GST-Dnmt1 (1–291).

cDNA coding for T4 polymerase was amplified by PCR from T4 phage DNA and subcloned in frame into pET30a plasmids via BamHI and SalI sites. The expression plasmid was transformed into BL21 RIL, and its expression was induced by adding 0.5 mM IPTG at 18 °C. Bacteria were suspended in buffer B (0.5 M NaCl, 0.1% (w/v) Triton X-100, 5 mM β-mercaptoethanol, 1/2000 (v/v) PI, 10 mM Tris-HCl, pH 7.4), and sonicated. After centrifugation (10 000 g for 10 min), the supernatant was loaded onto HisAccept (Nacalai Tesque). The column was washed with buffer B containing 10 mM imidazole, and eluted with buffer B containing 250 mM imidazole. The eluate was diluted 10 times with 10 mM Tris-HCl, pH 7.4, loaded onto a HitrapQ FF column (GE Healthcare), and eluted with a gradient (50–800 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.1% (w/v) Triton X-100, 1/1000 (v/v) PI, 5 mM β-mercaptoethanol). For preparing expression vector of λ exonuclease (λ exo), cDNA was cloned into pBAD vector with a His-tag at the N terminus of λ exo. The expression vector was transformed into ER1821 and expression was induced by 0.2% (v/v) arabinose for 16 h at 18 °C. Bacteria were suspended in buffer C (0.1% (w/v) Triton X-100, 5 mM β-mercaptoethanol, 0.3 M NaCl, 1 mM MgCl₂, 10 mM Tris-HCl, pH 7.4), and sonicated. After centrifugation (10 000 g for 10 min), the supernatant was loaded onto HisAccept. The column was washed with buffer C containing 10 mM imidazole, and eluted with buffer C containing 250 mM imidazole. Recombinant histones were expressed and purified as described, and histone octamer was reconstituted as described by Luger et al. [39]. Modified and unmodified histone H3 peptides were synthesized and purified as described by Kawakami et al. [40]. Histone (1–27) was provided from Scrum Inc. (Tokyo, Japan).

Reconstitution of nucleosomes

Dinucleosomes were prepared and purified as described by Mishima et al. [41]. Briefly, histone octamers and DNA were mixed in buffer containing 2 M KCl, and salt concentration was gradually reduced by dialysis. After reconstitution, contaminating free DNA was removed by ultracentrifugation with a glycerol gradient. The nucleosome concentration was determined via absorbance at 260 nm.

Enzymatic digestion of reconstituted nucleosomes

Nucleosomes (35 ng of DNA) were digested with either Alul (5 units) or ScaI (6 units) as described elsewhere [41]. Nucleosomes (35 ng of DNA) were treated with indicated
concentration of micrococcal nuclease, as described elsewhere [41].

Gel shift assay

GST-Dnmt1 (291–601), GST-Dnmt1 (1–118), or GST (180 nm) were mixed with 0–10 μM of amino terminal histone H3 peptide in buffer containing 0.01 (w/v) Tween20, 50 mM NaCl, 15% (v/v) glycerol, 20 mM Tris-HCl, pH 7.5 and incubated on ice for 30 min. The mixture was separated in 6% native PAGE. Proteins were detected with Coomassie Brillant Blue staining.

DNA methylation assay

Radioisotope-dependent assay of steady state methylation was performed as described by Takeshita et al. [9]. Briefly, annealed DNA (100 nM) was methylated with Dnmt1 (2 nm), in the presence of 2.2 μM [3H]-SAM (PerkinElmer Life Sciences, Yokohama, Japan) in 25 μL of buffer F (5 mM EDTA, 2.7 mM glycerol, 0.2 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, and 20 mM Tris-HCl, 7.4), at 37 °C for 1 h. Radioactivity incorporated in DNA was measured by liquid scintillation counter, as described by Takeshita [9].

For sodium bisulfite (BS) sequence analysis, 1.4 nm dinucleosomes or naked DNA were methylated with 30 nm of the indicated Dnmt1 versions, under conditions as described above for the methylation assay. To monitor the nucleosome deposition, 0.1 Unit μL⁻¹ of bacterial DNA methyltransferase M. AluI (NEB) was added in the reaction mixture. An aliquot of the reaction mixture was treated with Epitect bisulfite kit (Qiagen, Tokyo, Japan). The bisulfite-treated DNA was amplified by PCR with primers (SBS upper sd Fwd and SBS upper sd Rev, or SBS lower sd Fwd and SBS lower sd Rev) (Table S2). The amplified DNA was subcloned into pBlueScriptII and its sequence was determined by ABI 3100 DNA sequencer. CpG methylation was analyzed using QUMA [42].

F-oligo binding to Dnmt1

The two complementary oligonucleotides (20 bp/1CpG-F sense M and 20 bp/1CpG-antisense in Table S1) were annealed, and the annealed oligonucleotides (200 nm) were methylated with full-length or truncated Dnmt1 (80 nm), under the same conditions as for measuring methylation activity. After incubation at 37 °C for 1 h, the reaction mixtures were separated by 7% SDS/PAGE. Proteins were stained with Lumitein (Biotium, Inc, CA, USA), and bands were detected with a fluoro-imager, FLA9500, as described by Mishima et al. [43]. The percentage of shifted Dnmt1 over input Dnmt1 was calculated.

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Author contributions

IS conceived and supervised the study; LB, ST, JO, MS, and IS designed experiments; YM, LB, TK, KA, SO, ST, and HH performed experiments; AS and MW contributed to the final version of the manuscript; IS and LB wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest associated with this manuscript.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Amino acids sequence of H3 and H4 peptides.

Table S2. Oligonucleotides used for analyzing Dnmt1 activity.

Table S3. Oligonucleotide for PCR and fill-in reaction.

Fig. S1. Sequence of hemimethylated DNA.

Fig. S2. Repeatability of Dnmt1 291 methylation activity.

Fig. S3. Nucleosome structure-dependent DNA methylation activity of Dnmt1 FL.

Fig. S4. Repeatability of Dnmt1 602 methylation activity toward naked DNA or dinucleosomes.