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Preferential 5-Methylcytosine Oxidation in the Linker Region of Reconstituted Positioned Nucleosomes by Tet1 Protein

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Preferential 5-methylcytosine oxidation in the linker region of reconstituted positioned nucleosomes by Tet1 protein

Seiichiro Kizaki,^[a] Tingting Zou,^[a] Yue Li,^[a] Yong-Woon Han,^[b] Yuki Suzuki,^[b] Yoshie Harada,^[b] and Hiroshi Sugiyama^{*[a,b]}

Abstract: Tet (ten-eleven translocation) family proteins oxidize 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), and 5-carboxycytosine (caC), and are suggested to be involved in the active DNA demethylation pathway. In this study, we reconstituted positioned mononucleosomes using CpG-methylated 382 bp DNA containing the Widom 601 sequence and recombinant histone octamer, and subjected the nucleosome to treatment with Tet1 protein. The sites of oxidized methylcytosine were identified by bisulfite sequencing. We found that, for the oxidation reaction, Tet1 protein prefers mCs located in the linker region of the nucleosome compared with those located in the core region.

Introduction

In eukaryotic cells, the nucleosome is the fundamental unit of chromatin. The nucleosome is formed by wrapping ~146 bp of DNA around a histone octamer comprising two pairs each of H2A, H2B, H3, and H4. The nucleosome is the central site for the epigenetic regulation of gene expression, and histone modifications on nucleosomes affect the level of gene expression. In the nucleosome, DNA is wrapped in ~1.75 turns around the histone octamer.^[1]

Many studies have reported on the chemical and biological reactions with DNA in reconstituted nucleosomes. For example, Dervan *et al.* investigated the DNA-binding ability of pyrrole-imidazole polyamide, a DNA sequence-selective minor groove-binding molecule, with nucleosomal DNA, and discovered that polyamide can bind to nucleosomal DNA facing away from or even partially facing the histone octamer.^[2] Trzupek *et al.* compared the alkylation properties of yatakemycin and duocarmycin SA, which bind to the minor groove of DNA and alkylate adenine in free and nucleosomal DNA. Both compounds showed a relatively unaltered ability to alkylate nucleosomal DNA in terms of both the alkylating efficiency and sequence

selectivity.^[3] By contrast, Zou *et al.* recently reported that duocarmycin B₂ preferentially alkylates linker DNA over core DNA.^[4]

The presence of a histone octamer prevents access of proteins to DNA and hinders the access of transcriptional regulatory factors and elongating polymerase to DNA, hence controlling the process of gene expression.^[5–8] Using reconstituted nucleosomes, Takeshima *et al.* compared the activities of two mammalian *de novo* DNA methyltransferases, Dnmt3a and Dnmt3b, and found that Dnmt3a had higher DNA methylation activity than Dnmt3b toward the linker DNA region. Dnmt3a scarcely methylated the core DNA region, whereas Dnmt3b significantly methylated this region, although the activity was low.^[9]

Tet (ten-eleven translocation) family proteins can oxidize 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC) and further to 5-formylcytosine (fC) and 5-carboxycytosine (caC).^[10–12] 5-hydroxymethylcytosine is prevalent in mouse stem cells^[10,13] and the central nervous system (CNS).^[14] 5-formylcytosine and caC are also found in stem cells and in some organs.^[11,15] A number of methods to detect and sequence mC^[16,17], hmC^[18–23], fC^[24–29], and caC^[30] are reported. Previously, we evaluated the effects of mC and hmC on nucleosome structure and found that replacing mC with hmC slightly relieves the packing of the nucleosome.^[31] Tet proteins are suggested to play important roles in the reprogramming of somatic cells to generate induced pluripotent stem cells (iPSCs).^[32,33] We also investigated the DNA sequence selectivity of Tet protein and showed that mC in the CpG sequence is most easily oxidized by Tet protein.^[34] However, it remains unknown how the nucleosome affects the activity of Tet proteins. Here we evaluated the activity of Tet1 protein in reconstituted mononucleosomes.

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Figure 1. Preparation of CpG methylated nucleosome, followed by Tet treatment and bisulfite sequencing.

Results and discussion

Generally, nucleosome positioning is heterogeneous, and histone octamer sliding and repositioning after nucleosome reconstitution can complicate experiments.^[35,36] In the present study, we used PCR to prepare 382 bp DNA containing the Widom 601 sequence,^[37] which provided a well-positioned mononucleosome with core and linker DNA regions.

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5' ATTTAGGTGACACTATAGAATACTCAAGCTTGCATGCCTGCAGGTCCGGGATCCTAAT
3' TAAATCCACTGTGATATCTTATGAGTTCGAACGTACGGACGTCCAGGCCTAGGATTA

GACCAAGGAAAGCATGATTCTTCCACCGAGTTTCATCCCTTATGTGATGGACCTATACGC
CTGGTTCCCTTCGTACTAAGAAGGTGGCTCAAGTAGGGAATACACTACCTGGGATATCGC

GGCGCGCCCTGGAGAATCCCGGTGCGAGGCGCTCAATTGGTCGTAGACAGCTCTAGCAC
CGCGCGGGACCTCTTAGGGCCACGCTCCGCCGAGTTAACCGATCTGTCGAGATCGTG

CGCTTAAACCGCACGTACCGCTGTCCCGCGCTTTTAACGCCAAGGGGATTACTCCCTA
CGGAATTTCGCTCGATCGCGACAGGGGCGCGAAAATTGCGGTTCCTTAATGAGGGAT

GTTCAGGCACGTGTGAGATATATACATCTGTGCATGTATTGAACAGCGACCTTGCGCG
CAAGGTCCGTGCACAGTCTATATATGATAGGACACGTACATAACTTGTCGTGGAACGCGC

TGCCAGTCGGATAGTGTCGCGCTCCCGACTCTAGAGGATCCCGGGTACCGAGCTCGAA
ACGGTCACGCTATCACAAGGCCGAGGGCTGAGATCTCCTAGGGCCCATGCTGAGGCTT

TTCGCCCTATAGTGAGTCGTATTA3'
AAGCGGGATATCACTCAGCATAAT5'

```

Figure 2. All CpG sites of 382 bp DNA were methylated with M.SssI. Bold regions are core DNA and the other parts are linker DNA. CpG sites are underlined and shown in red

A total of 28 CpG sites were methylated using prokaryotic DNA methyltransferase (M.SssI) (Figs 1, 2). We conducted bisulfite sequencing to confirm that all cytosines on CpG sites were methylated in the 382 bp DNA fragment (Supplementary Fig. 1).^[38,39] The nucleosome was then reconstituted using the “salt-jump” dialysis method.^[40–42] After dialysis, the formation of the nucleosome was confirmed using a gel mobility shift assay (Supplementary Fig. 2-4) and atomic force microscopy (AFM) observation (Supplementary Fig. 5). Densitometric analysis of the gel mobility shift assay demonstrated that ~96% of the reconstituted nucleosome was obtained.

Figure 3. Bisulfite treatment of DNA. (A) fC and caC are converted to uracil by sodium bisulfite treatment, although mC and hmC do not react with sodium bisulfite. **(B)** Examples of the quantification of the formed fC and caC. The percentages of formed fC and caC were calculated based on the area of the two peaks (blue: cytosine, red: thymine).

The reconstituted nucleosome was treated with mTet1 protein (catalytic domain) for 1 hour at 37 °C, and the Tet-treated nucleosome was subjected directly to bisulfite treatment. A previous report suggested that mC is converted to fC and caC under this condition.^[43] Because fC and caC are converted to uracil after bisulfite treatment and read as thymine (Fig. 3A),^[22,29,44] it is possible to estimate the degree of oxidation of mC in a concentration-dependent manner (Fig. 3B). Using chromatograms of capillary sequencing, we estimated the percentages of formed fC and caC (Fig. 4 and Supplementary Fig. 6). At the core DNA region the percentages were lower than 20% (except for site6), and at the linker DNA region the percentages were higher than 40% (Fig. 4B). Free DNA was treated with mTet1 under the same conditions and using the same concentration of mTet1 protein. However, no striking reactivity difference between core DNA region and linker DNA region was observed. This suggests that, when on the nucleosome, Tet protein prefers to oxidize mCs located in the linker DNA region compared with those located in the core DNA region. The bulky nucleosome core may hinder the access of Tet protein to mCs in the core DNA region.

Figure 4. Quantification of mC oxidation by Tet protein. (A) Sequence of DNA **(B)** Conversion percentage for each site in top strand DNA. Free DNA and nucleosomes were treated with 3.31 μM Tet protein.

Conclusions

We found a significant difference in the activity of Tet proteins toward mCs located in the core and linker DNA regions. In cells, the activity of Tet protein should be stringently regulated. The inefficient oxidation by Tet protein of the core DNA region may be important for preventing aberrant DNA demethylation. During the reprogramming process, mCs in CpG islands are demethylated in a wide region of the genome.^[45] The assistance of chromatin remodeling factors to enable the access of Tet protein to DNA seems essential to this process.

Experimental Section

1) mTet1 and its oxidative substrate DNA preparation:

mTet1 active domain (1367-2039) was purchased from Wisegene (USA), stocked in 20 mM HEPES (pH 7.4), NaCl 50 mM, glycerol 50%.

The 382 bp DNA fragment containing Widom 601 sequence was amplified by PCR using forward 5'-dATTAGGTGACACTATAGAATAC-3' and reverse 5'-dTAAATCGACTCACTATAGG-3' primers from pGEM-3z/601. After the reaction, the amplified DNA was purified using GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, MO, USA). Then the DNA was treated with M.SssI (New England Biolabs, Ipswich, MA, USA).

2) Nucleosome reconstitution:

CpG-methylated 382 bp DNA (200 nM) and recombinant human histone octamer (EpiCypher, Davis Dr, Durham, NC, USA) (300 nM) were mixed together in 2 M NaCl and 20 mM HEPES KOH (pH 7.5) (total volume 50 μ L), and placed in Oscillatory Cup (MWCO: 8,000) (COSMO BIO, Tokyo, Japan). The dialysis tube was immersed into 500 mL of 2 M NaCl and 20 mM HEPES KOH (pH 7.5) for 2 hours at 4 °C, followed by 1.5 M NaCl (overnight), 1.0 M NaCl (8 hours), 0.75 M NaCl (overnight), and 0 M NaCl (8 hours) (each contains 20 mM HEPES KOH (pH 7.5)). After dialysis, the sample was collected from the tube and stored at 4 °C until use.

3) mTet1 oxidation and bisulfite sequencing:

The reconstituted nucleosome (14 nM) and CpG-methylated 382 bp DNA (14 nM) were incubated with 3.31 μ M of mTet1 protein in 50 mM HEPES (pH 8.0), 100 mM NaCl, 2 mM L-ascorbic acid, 1 mM 2-oxoglutarate disodium salt hydrate, 105 μ M Fe(NH₄)₂(SO₄)₂ 6H₂O, 1.2 mM ATP and 2.5 mM DTT at 37 °C for 1 hour in 20 μ L of reaction. Then the samples were treated with sodium bisulfite using EpiTect Bisulfite Kit (QIAGEN, Hilden, Germany). After bisulfite treatment, PCR amplification was carried out using four different PCR primer-sets (primer-set1: forward 5'-dATAGAATATTTAAGTTTGTATGTTTGTAGG-3' and reverse 5'-dTAAACTAAAAATAATCCCCTTAAC-3', primer-set2: forward 5'-dGTTTAATTGGTYGTAGATAGTTTGTAGTAT-3' and

reverse 5'-dATACCCRAAAATCCTCTAAAATC-3', primer-set3: forward 5'-dGGTATTYGGGGATTTTTAGAGT-3' and reverse 5'-dCTCAATTAATCCTAAACAACCTCTAACACC-3' primer-set4: forward 5'-dTGTTTGGGAATTAGGGAGTAATTTTT-3' and reverse 5'-dCAAAAAAACATAATTCTTCCACC-3' primers). After the reaction, DNA was purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Cycle sequencing was carried out with BigDye® Terminator Kit (Applied Biosystems, Foster City, CA, USA). 3130 Genetic Analyzer (Applied Biosystems) was used for sequencing. The chromatographic images were imported into ImageJ (<http://rsb.info.nih.gov/ij/>) and analyzed.

4) AFM imaging:

The reconstituted nucleosome was diluted to a concentration of 0.5 ng/ μ L in a buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, and 1 mM EDTA, and 3 μ L of the sample was immediately deposited onto freshly cleaved mica discs (ϕ 1.5 mm) pretreated with 0.1% (3-Aminopropyl)triethoxysilane (APTES). After 1 min incubation, the sample was rinsed with 2 $\times$ 10 μ L washes of the buffer and then imaged in the same buffer without the drying step. The AFM experiments were performed using Nano Live Vision (RIBM, Tsukuba, Japan). The sample was imaged in buffer solution at ambient temperature with a small cantilever of dimensions L $\times$ W $\times$ H = 10 $\times$ 2 $\times$ 0.1 μ m³ (BL-AC10EGS, Olympus, Tokyo, Japan). These cantilevers had a spring constant of 0.1-0.2 N/m with a resonant frequency in water of 400-1000 kHz and 320 $\times$ 240 pixel images were obtained at the scan rate of 0.2 frames per second.

Keywords: Tet •5-hydroxymethylcytosine•5-methylcytosine•nucleosome

References

- [1] K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **1997**, *389*, 251–60.
- [2] J. M. Gottesfeld, C. Melander, R. K. Suto, H. Raviol, K. Luger, P. B. Dervan, *J Mol Biol* **2001**, *309*, 615–629.
- [3] J. D. Trzupsek, J. M. Gottesfeld, D. L. Boger, *Nat. Chem. Biol.* **2006**, *2*, 79–82.
- [4] T. Zou, S. Kizaki, G. N. Pandian, H. Sugiyama, *Chem. - A Eur. J.* **2016**, DOI 10.1002/chem.201600950.
- [5] Y. Lorch, J. W. LaPointe, R. D. Kornberg, *Cell* **1987**, *49*, 203–210.
- [6] T. Matsui, *Mol Cell Biol* **1987**, *7*, 1401–1408.
- [7] J. L. Workman, R. G. Roeder, *Cell* **1987**, *51*, 613–622.
- [8] J. A. Knezetic, D. S. Luse, *Cell* **1986**, *45*, 95–104.
- [9] H. Takeshima, I. Suetake, H. Shimahara, K. Ura, S. I. Tate, S. Tajima, *J. Biochem.* **2006**, *139*, 503–515.
- [10] M. Tahiliani, K. P. Koh, Y. Shen, W. a Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L. M. Iyer, D. R. Liu, L. Aravind, et al., *Science* **2009**, *324*, 930–5.
- [11] S. Ito, L. Shen, Q. Dai, S. C. Wu, L. B. Collins, J. A. Swenberg, C. He, Y. Zhang, *Science* **2011**, *333*, 1300–3.
- [12] Y. He, B.-Z. Li, Z. Li, P. Liu, Y. Wang, Q. Tang, J. Ding, Y. Jia, Z. Chen, L. Li, et al., *Science* **2011**, *333*, 1303–7.

- [13] S. Kriaucionis, N. Heintz, *Science* **2009**, 324, 929–30.
- [14] M. Münzel, D. Globisch, T. Brückl, M. Wagner, V. Welzmler, S. Michalakakis, M. Müller, M. Biel, T. Carell, *Angew. Chem. Int. Ed. Engl.* **2010**, 49, 5375–7.
- [15] T. Pfaffeneder, B. Hackner, M. Truß, M. Münzel, M. Müller, C. a. Deiml, C. Hagemeyer, T. Carell, *Angew. Chemie* **2011**, 123, 7146–7150.
- [16] J. von Watzdorf, K. Leitner, A. Marx, *Angew. Chem. Int. Ed. Engl.* **2016**, 55, 3229–32.
- [17] S. Beck, V. K. Rakyen, *Trends Genet.* **2008**, 24, 231–237.
- [18] A. Okamoto, K. Sugizaki, A. Nakamura, H. Yanagisawa, S. Ikeda, *Chem. Commun. (Camb.)* **2011**, 47, 11231–3.
- [19] W. a. Pastor, U. J. Pape, Y. Huang, H. R. Henderson, R. Lister, M. Ko, E. M. McLoughlin, Y. Brudno, S. Mahapatra, P. Kapranov, et al., *Nature* **2011**, 473, 394–7.
- [20] J. Terragni, J. Bitinaite, Y. Zheng, S. Pradhan, *Biochemistry* **2012**, 51, 1009–1019.
- [21] C.-X. Song, T. a. Clark, X.-Y. Lu, A. Kislyuk, Q. Dai, S. W. Turner, C. He, J. Korlach, *Nat. Methods* **2012**, 9, 75–7.
- [22] M. J. Booth, M. R. Branco, G. Ficiz, D. Oxley, F. Krueger, W. Reik, S. Balasubramanian, *Science* **2012**, 336, 934–7.
- [23] M. Yu, G. C. Hon, K. E. Szulwach, C.-X. Song, L. Zhang, A. Kim, X. Li, Q. Dai, Y. Shen, B. Park, et al., *Cell* **2012**, 149, 1368–80.
- [24] W. Mao, J. Hu, T. Hong, X. Xing, S. Wang, X. Chen, X. Zhou, *Org. Biomol. Chem.* **2013**, 11, 3568–72.
- [25] T. Tian, X. Zhang, B. Fu, Y. Long, S. Peng, S. Wang, X. Zhou, X. Zhou, *Chem. Commun. (Camb.)* **2013**, 49, 9968–70.
- [26] E.-A. Raiber, D. Beraldi, G. Ficiz, H. E. Burgess, M. R. Branco, P. Murat, D. Oxley, M. J. Booth, W. Reik, S. Balasubramanian, *Genome Biol.* **2012**, 13, R69.
- [27] C.-X. Song, K. E. Szulwach, Q. Dai, Y. Fu, S.-Q. Mao, L. Lin, C. Street, Y. Li, M. Poidevin, H. Wu, et al., *Cell* **2013**, 153, 1–14.
- [28] J. Hu, X. Xing, X. Xu, F. Wu, P. Guo, S. Yan, Z. Xu, J. Xu, X. Weng, X. Zhou, *Chemistry* **2013**, 19, 5836–40.
- [29] M. J. Booth, G. Marsico, M. Bachman, D. Beraldi, S. Balasubramanian, *Nat. Chem.* **2014**, 6, 435–40.
- [30] X. Lu, C. Song, K. Szulwach, Z. Wang, P. Weidenbacher, P. Jin, C. He, *J. Am. Chem. Soc.* **2013**, 135, 9315–9317.
- [31] S. Kizaki, Y. Suzuki, T. Takenaka, M. Endo, H. Sugiyama, *Biomater. Sci.* **2014**, DOI 10.1039/c4bm00113c.
- [32] C. a. Doege, K. Inoue, T. Yamashita, D. B. Rhee, S. Travis, R. Fujita, P. Guarnieri, G. Bhagat, W. B. Vanti, A. Shih, et al., *Nature* **2012**, 3–6.
- [33] Y. Costa, J. Ding, T. W. Theunissen, F. Faiola, T. a. Hore, P. V. Shliaha, M. Fidalgo, A. Saunders, M. Lawrence, S. Dietmann, et al., *Nature* **2013**, DOI 10.1038/nature11925.
- [34] S. Kizaki, A. Chandranan, H. Sugiyama, *Chembiochem* **2015**, DOI 10.1002/cbic.201500646.
- [35] a. Flaus, K. Luger, S. Tan, T. J. Richmond, *Proc. Natl. Acad. Sci. U. S. A.* **1996**, 93, 1370–1375.
- [36] a. Flaus, T. J. Richmond, *J. Mol. Biol.* **1998**, 275, 427–441.
- [37] P. T. Lowary, J. Widom, *J. Mol. Biol.* **1998**, 276, 19–42.
- [38] M. Frommer, L. E. McDonald, D. S. Millar, C. M. Collis, F. Watt, G. W. Grigg, P. L. Molloy, C. L. Paul, *Proc. Natl. Acad. Sci. U. S. A.* **1992**, 89, 1827–31.
- [39] H. Hayatsu, Y. Wataya, K. Kai, S. Iida, *Biochemistry* **1970**, 9, 2858–65.
- [40] M. Tomschik, M. A. Karymov, J. Zlatanova, S. H. Leuba, *Structure* **2001**, 9, 1201–1211.
- [41] Y. Zivanovic, I. Duband-Goulet, P. Schultz, E. Stofer, P. Oudet, A. Prunell, *J. Mol. Biol.* **1990**, 214, 479–495.
- [42] Y.-W. Han, Y. Tsunaka, H. Yokota, T. Matsumoto, G. Kashiwazaki, H. Morinaga, K. Hashiya, T. Bando, H. Sugiyama, Y. Harada, *Biomater. Sci.* **2014**, 2, 297.
- [43] S. Kizaki, H. Sugiyama, *Org. Biomol. Chem.* **2014**, 12, 104–7.
- [44] M. J. Booth, T. W. B. Ost, D. Beraldi, N. M. Bell, M. R. Branco, W. Reik, S. Balasubramanian, *Nat. Protoc.* **2013**, 8, 1841–51.
- [45] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, *Cell* **2007**, 131, 861–72.

Entry for the Table of Contents

COMMUNICATION

CpG-methylated mononucleosome with linker DNA was treated with Tet1 protein. The sites of oxidized methylcytosine were identified by bisulfite sequencing. The results revealed that Tet1 protein prefers to oxidize mCs located in the linker region of nucleosomes compared with mCs located in the core region.

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Supplementary Information

Figure S1. Sequencing chromatograms of bisulfite-treated CpG-methylated DNA.

Sequencing chromatograms obtained by capillary sequencing using (A) primer-set1 forward primer, (B) primer-set2 forward primer, (C) primer-set3 forward primer, and (D) primer-set4 forward primer.

(A)

AGAA AAC C AATT CCC AAAC TTAA AAAC CGG AAA AAAAT C GCT ATT TCAAT TACA TACA CAA A

ATAT AT ATAT TCTAACAC GTACCTC AAAAC TAAAAAATAAT CCCC TTAAC GATT A

AACGC GAA AACAA CGCG TACGTACGTTTAAAC GATACTAAAAC TATTCTACAA C

CAATTAAACA

(C)

(D)

Figure S2. Gel mobility shift assay of reconstituted nucleosome. Lane 1: free DNA, Lane 2: CpG-unmethylated nucleosome, Lane 3: CpG-methylated nucleosome, 6% polyacrylamide gel, Electrophoresis was conducted at 4 °C for 1 hour (100 V) and the gel was stained with ethidium bromide.

Figure S3. Gel mobility shift assay of reconstituted nucleosome. Lane 1: free DNA, Lane 2: CpG-methylated nucleosome, Lane 3: CpG-methylated nucleosome (the gel was stained with Coomassie Brilliant Blue staining solution), 6% polyacrylamide gel, Electrophoresis was conducted at 4 °C for 1 hour (100 V).

Figure S4. Nuclease digestion of reconstituted nucleosome. Lane 1: free DNA, Lane 2: CpG-methylated nucleosome, Lane 3: CpG-methylated nucleosome treated with micrococcal nuclease (NEB) at 37 °C for 10 min, 6% polyacrylamide gel, Electrophoresis was conducted at 4 °C for 1 hour (100 V) and the gel was stained with ethidium bromide.

Figure S5. AFM image of reconstituted CpG-methylated nucleosome.
Reconstituted nucleosome was observed in 20 mM Tris-HCl (pH 7.5).

100 nm

Figure S6. Quantification of mC oxidation by Tet protein. Conversion percentage for each site in bottom strand DNA. Free DNA and nucleosome were treated with 3.31 μ M Tet protein.