J|A|C|S COMMUNICATIONS

Published on Web 01/22/2010

DNA Methylation Increases Nucleosome Compaction and Rigidity

John S. Choy,[†,II] Sijie Wei,[‡] Ju Yeon Lee,[‡] Song Tan,[§] Steven Chu,[†] and Tae-Hee Lee*[,‡]

Department of Physics, Bio-X Program, Stanford University, Stanford, California 94305, and Department of Chemistry and Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

Received December 4, 2009; E-mail: txl18@psu.edu

DNA in eukaryotic cells is packaged into arrays of nucleosomes, each assembled from ~147 base pairs of DNA wrapped around a histone octamer and folded into higher-order chromatin structures. Enzyme-mediated chemical modifications of DNA and histones regulate various genome transactions by modulating access to the DNA. One example of these epigenetic modifications is methylation of CpG dinucleotides, which is often associated with gene repression.[1] While CpG methylation is essential in development, inappropriate CpG methylation in tumor suppressor genes is associated with cancer.[2] A better understanding of how CpG methylation affects nucleosome structure will provide insights into the mechanism of gene repression and the development of novel drugs to treat methyl-CpG-related diseases.

Two mechanisms have been proposed to explain how methylation might repress gene transcription. First, methylated CpGs may prevent transcriptional activators from binding the DNA target.[3] Second, transcriptional repressors with methyl-CpG binding domains may associate with methylated CpGs and block transcription by modifying the surrounding chromatin or prevent interaction by activators.[4,5] Since access to the underlying DNA is largely governed by DNA−histone interactions,[6,7] a third possibility is that the nucleosome structure is changed by methylation, leading to a more closed state. To test this, we used a single-molecule approach to monitor methylation-induced conformational changes in mononucleosomes.

The single-molecule system shown in Figure 1A, which measures linker DNA end-to-end distance and flexibility by fluorescence resonance energy transfer (FRET) and fluorescence polarization, was constructed (see Methods in the Supporting Information). Two unevenly populated nucleosome bands from reactions that contained 5S rDNA, histone octamers, and the NAP1 histone chaperone were observed in a native gel analysis, but in the absence of NAP1, both products were absent (Figure 1B). Consistent with the native gel analysis, we identified two unevenly distributed populations of nucleosomes, one with a low 0.29 (30%) FRET state and the other with an intermediate 0.4 (60%) FRET state (Figure 1C,D). No FRET signals were observed when the DNA was free of histones. A small population (8%) of nucleosomes made excursions between the 0.29 and 0.4 FRET states (Figure 1G). These excursions were observed mostly once in ~5 min long FRET traces. These data are consistent with reports that the histone core can assemble nucleosomes at two translational positions on DNA, either near the center of the DNA or biased toward one end.[8] Crossing the energetic barrier between these two states requires high-heat treatment.[9] This is consistent with only a small population of complexes making excursions between the 0.29 and 0.4 FRET states at a very low rate at room temperature (Figure 1G).

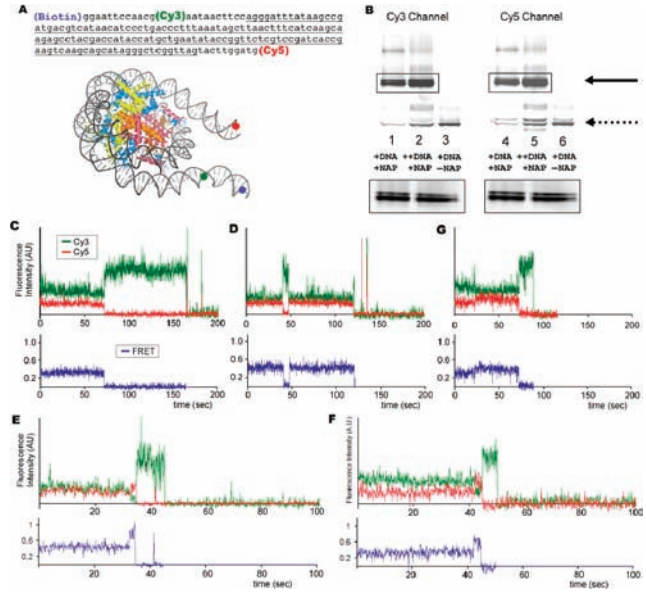

Figure 1. (A) Experimental system for studying the effects of DNA methylation on nucleosome structure. Solid circles in blue, green, and red indicate the positions of biotin, Cy3, and Cy5, respectively, on the DNA. (B) Native polyacrylamide gel analysis of nucleosomes. The solid arrow indicates assembled nucleosomes, and the dotted arrow indicates DNA. (C−G) Sample FRET traces.

Next, we addressed the question of how DNA methylation might modulate nucleosomal structure. Changes in DNA−histone interactions as a result of CpG methylation would likely alter the linker DNA end-to-end distance. We predicted that a decrease in DNA−histone contacts would lead to a more open structure and a longer linker DNA end-to-end distance. Conversely, an increase in DNA−histone contacts would lead to a more closed structure and a shorter DNA linker end-to-end distance. Nucleosomes were immobilized as in the previous experiment, after which *M.SssI* CpG methyltransferase was added. Strikingly, after a 90 min incubation, the population of nucleosome complexes that made excursions to a high 0.74 FRET state increased from 0.4 to 16% (Figures 1E,F and 2A). Importantly, the appearance of nucleosomes with excursions to 0.74 FRET states depended on the methyl donor S-adenosylmethionine (SAM), consistent with methylation of DNA (Figure 2A). The 40-fold increase in complexes with a high 0.74 FRET state suggests that upon CpG methylation, the DNA end-to-end distance shortens, possibly through more DNA−histone contacts, forming a more compact nucleosome structure (Figure 3 top). However, we cannot completely rule out the possibility that the DNA may take an alternative path to shorten the end-to-end distance. This would require unwrapping more internal regions of the DNA to shorten the end-to-end distance without forming a more compact nucleosome structure. Previously, FRET studies showed

[†] Stanford University.
[‡] Department of Chemistry, The Pennsylvania State University.
[§] Department of Biochemistry & Molecular Biology, The Pennsylvania State University.
[II] Present address: National Cancer Institute, Bethesda, MD 20889.

10.1021/ja910264z

that wrapping and unwrapping of DNA ends around the histone core were responsible for end-to-end distance changes.[10,11] Thus, we favor the former interpretation that a more compact nucleosome structure is induced by CpG methylation. A higher polarization of the high-FRET state also supports our interpretation (see the discussion below).

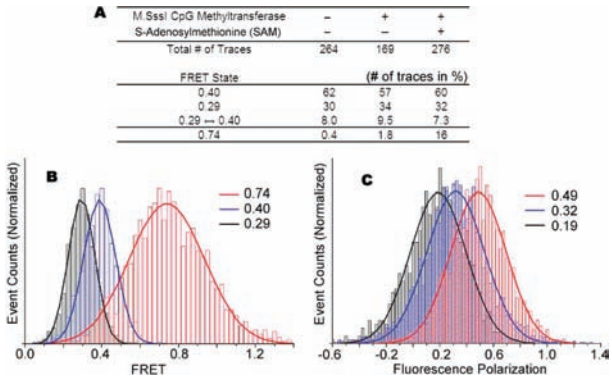

Figure 2. Effects of DNA methylation on nucleosome structure. (A) Statistics on the FRET states of nucleosomes. (B) FRET efficiency distributions of the three FRET states. (C) Cy5 fluorescence polarization of the three FRET states.

Finally, we examined whether a more compact structure may be correlated with having a more rigid DNA wrapped around the histone core. Fluorescence anisotropy has long been utilized to determine the rotational-diffusion time scale of a biological molecule.[12] A heavier, restricted, or rigid molecule yields a higher anisotropy of fluorescence emission than a lighter, unrestricted, or flexible molecule. The fluorescence polarization, which is a measure of the anisotropy in the fluorescence emission, from the FRET acceptor Cy5 represents the structural flexibility of the Cy5-labeled end with respect to the Cy3-labeled region of the nucleosomal DNA. Consequently, a higher Cy5 emission polarization suggests a greater rigidity of the DNA wrapped around the histone core (further details are given in the Supporting Information).

As shown in Figure 2, the high-FRET state (0.74 FRET) has higher polarization (0.49) than the two lower-FRET states (0.32 and 0.19 for the 0.40 and 0.29 FRET states, respectively). The polarization difference for the 0.40 and 0.29 FRET states may reflect the difference in the structural flexibility caused by different translational positioning, i.e., one end of DNA in one translational positioning may be longer and more flexible than in the other, where the ends are equal and less flexible. The two bands in the native gel also suggest different flexibilities of the two states (Figure 1B). The highly polarized emission from the 0.74 FRET state suggests that nucleosomal DNA motion around the histone core is more restricted when the DNA is methylated. On the basis of the highly polarized FRET acceptor emission, we conclude that DNA methylation enhances the interaction between DNA and the histone core, resulting in a more rigid nucleosomal structure.

On the basis of our findings, we propose that CpG methylation induces excursions to a closed and rigid nucleosomal conformation, resulting on average in a more tightly wrapped nucleosome structure (Figure 3). Therefore, in addition to action as "molecular handles"

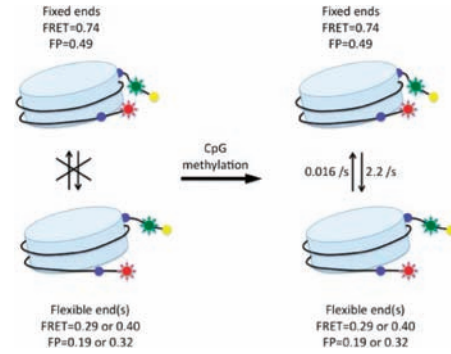

Figure 3. Summary of the CpG methylation effect on nucleosomal structure. Data analysis is included in the Supporting Information.

recognized by methyl-CpG binding proteins or as a means to prevent association of transcription factors by masking the underlying sequence,[1,13,14] a third mechanism of function of CpG methylation involves direct alteration of the structural dynamics of nucleosomes.

To our knowledge, this is the first report to show how DNA methylation changes the structure and dynamics of mononucleosomes, leading to a more compact and rigid structure. Our single-molecule system has allowed us to observe changes in structure and dynamics that otherwise would be challenging to detect with traditional biochemical methods. In conclusion, this report strongly suggests that CpG methylation may contribute to the repression of gene transcription and other genome processes by inducing a more compact and rigid nucleosome conformation.

Acknowledgment. The authors thank J. Huh, R. Dutnall, M. Mattson, and A. Islas for NAP1 expression and advice and J. Cotes for 5S rDNA. J.S.C. was an American Cancer Society Postdoctoral Fellow. T.-H.L. was supported by a Searle Scholar Award and the Camille and Henry Dreyfus New Faculty Award.

Supporting Information Available: Methods and two supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bird, A. *Genes Dev.* **2002**, *16*, 6.
(2) Esteller, M. *Nat. Rev. Genet.* **2007**, *8*, 286.
(3) Tate, P. H.; Bird, A. P. *Curr. Opin. Genet. Dev.* **1993**, *3*, 226.
(4) Jones, P. L.; Veenstra, G. J.; Wade, P. A.; Vermaak, D.; Kass, S. U.; Landsberger, N.; Strouboulis, J.; Wolffe, A. P. *Nat. Genet.* **1998**, *19*, 187.
(5) Nan, X.; Ng, H. H.; Johnson, C. A.; Laherty, C. D.; Turner, B. M.; Eisenman, R. N.; Bird, A. *Nature* **1998**, *393*, 386.
(6) Razin, A.; Cedar, H. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2725.
(7) Solage, A.; Cedar, H. *Biochemistry* **1978**, *17*, 2934.
(8) Luger, K. *Curr. Opin. Genet. Dev.* **2003**, *13*, 127.
(9) Luger, K.; Rechsteiner, T. J.; Richmond, T. J. *Methods Enzymol.* **1999**, *304*, 3.
(10) Gansen, A.; Toth, K.; Schwarz, N.; Langowski, J. *J. Phys. Chem. B* **2009**, *113*, 2604.
(11) Toth, K.; Brun, N.; Langowski, J. *Biochemistry* **2006**, *45*, 1591.
(12) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006.
(13) Pennings, S.; Allan, J.; Davey, C. S. *Briefings Funct. Genomics Proteomics* **2005**, *3*, 351.
(14) Weber, M.; Schubeler, D. *Curr. Opin. Cell Biol.* **2007**, *19*, 273.

JA910264Z