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Effects of DNA Methylation on the Structure of Nucleosomes

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Supporting Information

ABSTRACT: Nucleosomes are the fundamental packing units of the eukaryotic genome. Understanding the dynamic structure of a nucleosome is a key to the elucidation of genome packaging in eukaryotes, which is tied to the mechanisms of gene regulation. CpG methylation of DNA is an epigenetic modification associated with the inactivation of transcription and the formation of a repressive chromatin structure. Unraveling the changes in the structure of nucleosomes upon CpG methylation is an essential step toward the understanding of the mechanisms of gene repression and silencing by CpG methylation. Here we report single-molecule and ensemble fluorescence studies showing how the structure of a nucleosome is affected by CpG methylation. The results indicate that CpG methylation induces tighter wrapping of DNA around the histone core accompanied by a topology change. These findings suggest that changes in the physical properties of nucleosomes induced upon CpG methylation may contribute directly to the formation of a repressive chromatin structure.

Nucleosomes are the fundamental packing units of the eukaryotic genome. Genes wrapped in nucleosomes are further compacted into chromatin and eventually into chromosome. The structural organization of the eukaryotic genome is associated with regulated access to the genetic information and protection of the genes against physical damage.[1] A nucleosome core particle comprises ~147 base pair (bp) DNA in a left-handed superhelical format with 1.65 turns around an octameric histone core containing two copies of H2A, H2B, H3, and H4.[2] Covalent modifications of DNA and histones play important roles in regulating various genome activities.

Modifications of DNA that alter its physical properties will most likely affect the structure of nucleosomes.[3,4] It has been suggested that the sequence-dependent mechanics of wrapped nucleosomal DNA plays a role in the positioning and dynamics of nucleosomes.[5] We recently reported that DNA methylation induces compaction and rigidity of the linker DNA region that may imply suppressed dynamics of nucleosome structure and consequently reduced gene accessibility.[6] DNA methylation typically takes place in a CpG dinucleotide context where the cytosine is methylated to 5-methylcytosine, and it is associated with the formation of heterochromatin and silencing of genes critical for the regulation of growth and proliferation.[7,8] Abnormal CpG methylation leads to fatal diseases and defective development. Most CpG islands are hypomethylated in normal somatic tissues, while most tumor types display hypermethylated CpG islands in tumor suppressor regions.[9−11] Methylation of CpG islands found in promoter regions is important in gene repression during X-chromosome inactivation and in genomic imprinting.[12,13]

Studies suggest that the extent of CpG methylation is closely tied to the modulated accessibility of genes through establishing and maintaining repressive or transcriptionally inactive structures of chromatin. CpG methylation has been implicated in repressive chromatin mainly in two ways. First, transcriptional repressors or related factors block transcription by binding to the methyl-CpG moiety.[14,15] Second, the binding of transcription activation factors to DNA becomes inefficient because of the compaction and rigidity of the chromatin structure induced by CpG methylation.[16] Both hypotheses are supported by experimental evidence. However, a clear link between CpG methylation and the chromatin structure is yet to be unraveled. As nucleosomes are the fundamental building blocks of chromatin, the effects of CpG methylation on the structure of a nucleosome are at the core of this problem.

Here we present the effects of CpG methylation on the structure of the internal regions of nucleosomal DNA as determined by single-molecule and ensemble-averaged fluorescence measurements. Single-molecule methods provide an efficient means to probe subpopulation dynamics in a heterogeneous mixture of diverse species and states, which is particularly useful when the change of interest is small and easily lost during ensemble averaging. Utilizing this unique benefit of single-molecule methods, we studied the changes in the structure of nucleosomes induced upon CpG methylation.

We designed two nucleosomal DNA constructs based on the Selex 601 sequence containing 15 CpG dinucleotides (Figure 1A). The 601 + 39 DNA construct is labeled with Cy5 at the +39th nucleotide from the dyad and with Cy3 at the −38th nucleotide. The 601 + 29 DNA construct is labeled with Cy5 at the +29th nucleotide and with Cy3 at the −38th nucleotide (Figure 1B,C). DNA was constructed by ligating oligonucleotides (IDT DNA, Coralville, IA), some of which had already been labeled with fluorophores or modified by methylation. Nucleosomes were reconstituted from the purified DNA constructs and *Xenopus laevis* histones with yeast Nap1[17] and analyzed with 5% native polyacrylamide gel electrophoresis (Figure 1D). The nucleosomes showed one major band, indicating homogeneous positioning of histones along the DNA. The methylated nucleosomal DNA displayed slow migration relative to the unmethylated DNA because of the increased mass due to the additional 30 methyl groups. The nucleosomes assembled

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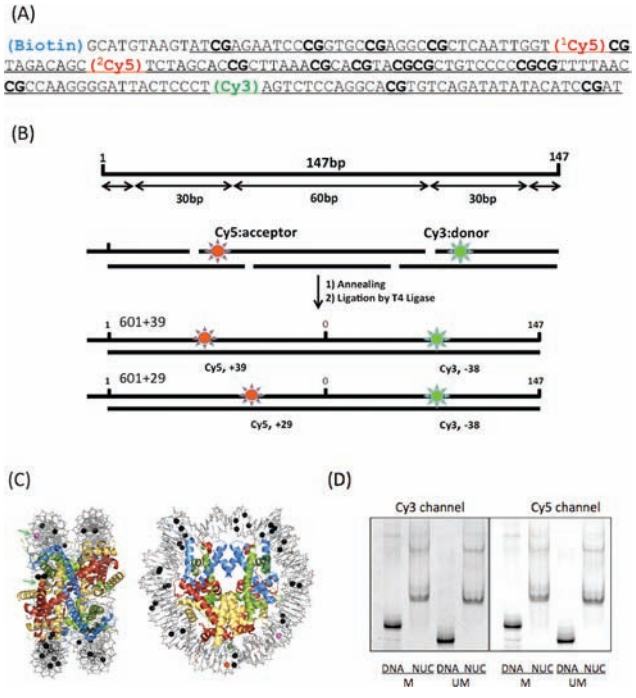

Figure 1. Nucleosomal DNA constructs based on the Selex 601 sequence and nucleosome reconstitution. (A) Selex 601 DNA sequence. The locations of Cy3 (FRET donor) and Cy5 (FRET acceptor) are marked. ^{1}Cy5 is at the +39th base from the dyad (cytosine, 74th) (601 + 39 DNA) and ^{2}Cy5 is at the +29th base from the dyad (601 + 29 DNA). Cy3 is located at the −38th base from the dyad. The underlined sequence represents the double-stranded DNA region. (B) The 147 bp DNA fragment comprises ~60 bp of $(H3-H4)_2$ tetramer binding site at the center and ~30 bp of H2A−H2B dimer binding sites near the termini. (C) The locations of fluorophores [Cy3 (green), ^{1}Cy5 (red), ^{2}Cy5 (purple)] and methylation (black) in a crystal structure (PDB entry 3MVD) are shown in a sphere representation. (D) Unmethylated (UM) or methylated (M) nucleosomes were reconstituted with yeast nucleosome assembly protein 1 (Nap1) and analyzed on a 5% native polyacrylamide gel.

with the methylated DNA also showed a lower mobility than the unmethylated nucleosomes.

We immobilized the nucleosomes on a functionalized microscope slide via biotin−streptavidin conjugation and recorded the fluorescence resonance energy transfer (FRET) signal from the individual nucleosomes. The FRET efficiency can be approximated as $I_{acceptor}/(I_{donor} + I_{acceptor})$, where I_{donor} and $I_{acceptor}$ denote the fluorescence intensities of the donor and acceptor, respectively. Figure S1 in the Supporting Information (SI) illustrates typical FRET signals, and Figure 2 shows the FRET efficiency histograms of the two nucleosomes before and after CpG methylation.

Figure 2 reveals that each nucleosome showed one major FRET efficiency and that the FRET efficiency of the M601 + 39 nucleosome (0.71) was lower than that of the unmethylated counterpart UM601 + 39 (0.84), suggesting that methylation affects the structure of a nucleosome. The decreased FRET efficiency of the methylated nucleosomes indicates the extent to which DNA wrapping or the conformation of DNA is altered upon the methylation. As the increased distance between the two fluorophores in the 601 + 39 nucleosomes could result from either over- or underwrapping of the DNA around the histone core, we further examined the FRET change with the 601 + 29 nucleosomes. The FRET efficiency of the UM601 + 29

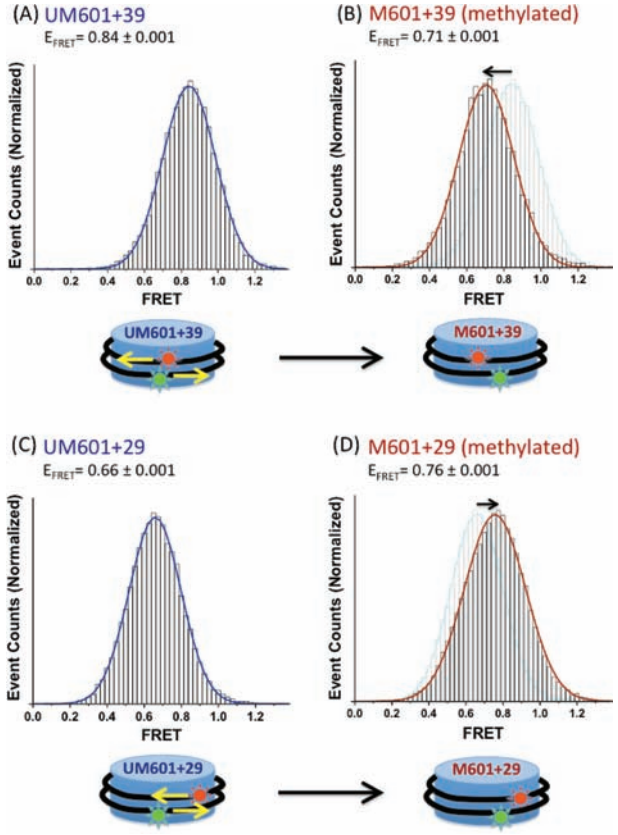

Figure 2. Effects of CpG methylation on the structure of assembled nucleosomes, as shown by FRET efficiency histograms of (A) unmethylated 601 + 39 nucleosomes, (B) methylated 601 + 39 nucleosomes, (C) unmethylated 601 + 29 nucleosomes, and (D) methylated 601 + 29 nucleosomes. The pale-colored histograms in (B) and (D) are replicas of the histograms in (A) and (C), respectively.

nucleosome was 0.66, which is lower than the value of 0.84 observed for the UM601 + 39 nucleosome, as expected according to the difference in the labeling positions of the fluorophores in the two constructs. The M601 + 29 nucleosomes displayed an increased FRET efficiency of 0.76. These results indicate that CpG methylation induces overwrapping of DNA around the histone octamer in a nucleosome. Under- or overwrapping of DNA would accompany a topology change, as we previously reported for histone-acetylated nucleosomes.[17] We examined the topology change of nucleosomal DNA upon CpG methylation by measuring the fluorescence anisotropies of the FRET pairs (Table 1). The results indicate a significant difference (7.0 ± 1.5°) in the dipole alignment angles (β) for UM601 + 39 and M601 + 39 nucleosomes. Detailed information on the calculation is included in the SI. The decrease in β from 46.7 to 39.7° upon methylation would have resulted in increased FRET efficiency if the distance between the FRET pair did not change. The decreased FRET efficiency upon the methylation therefore confirms that the distance between the FRET pair must have been decreased. These results indicate that the topology of nucleosomal DNA changes upon CpG methylation, which accompanies overwrapping.

We previously reported the transient formation of a compact and rigid nucleosome structure induced by CpG methylation of 5S rDNA with *M.SssI* CpG methyltransferase,[6] which was supported by an earlier study that postulated stabilization of nucleosomal occupancy upon methylation.[18] This transient

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Table 1. Fluorescence Anisotropies of Unmethylated and Methylated Nucleosomes and Dipole Alignment Angles (β) of the FRET Pairs

	fluorescence anisotropy at λ_{ex} (nm)/λ_{em} (nm)			
	532/570 (Cy3)	600/670 (Cy5)	532/670 (Cy5 via FRET)	β (deg)
UM601 + 39	0.33 ± 0.01	0.33 ± 0.01	0.07 ± 0.01	46.7 ± 0.97
M601 + 39	0.34 ± 0.01	0.33 ± 0.01	0.13 ± 0.01	39.7 ± 1.12

structure is represented by the shortening of the end-to-end distance in the linker DNA region, which suggests tight wrapping of DNA around the histone core. The current system enabled us to investigate the structural changes in the internal regions of DNA upon CpG methylation. Multiple studies have reported that CpG methylation reduces the flexibility of free DNA.[19−21] For instance, C_9pG_{10} methylation in a DNA dodecamer containing an *Eco*RI restriction site reduces the flexibility of the phosphate−sugar backbone at the C9 position.[20] A 32-mer nucleotide with the cAMP respective element (CRE) having methylated nucleotides at the eighth and 16th positions showed increased stiffness, possibly due to restriction of the conformational space by the bulky methyl groups.[21]

As the rigidity of DNA is increased upon the methylation, we may expect the nucleosomal DNA to be less twisted in the methylated form. The change in the FRET efficiency upon CpG methylation indicates that methylation induces overwrapping of the internal regions of DNA around a histone octamer. Our results from the anisotropy measurements confirm the topology change upon CpG methylation, suggesting that the reduced extent of twist upon methylation causes the overwrapping of DNA around the histone core. A Monte Carlo simulation study also suggested an underwinding of DNA with methylated CpG sequences, resulting in an increased helical repeat from 10.5 to 11.0 bp/turn.[19] The increased helical repeat would imply that the histone content in a methylation rich region in the genome would be increased, which is beneficial for the formation of a repressive chromatin structure.

It has been reported that subtle rotational or translational changes of nucleosomal DNA in the promoter regions can play a role in gene regulation. For instance, the ability of a TATA-binding protein to bind to a nucleosomal target site depends on the rotational setting of the site.[22] A nucleosome on the TATA box of a PHO5 gene whose translational positioning was shifted by a few base pairs displayed altered accessibility to the TATA box.[23] Therefore, the accessibility to a promoter region close to CpG islands can be sensitive to changes in the DNA topology upon methylation, which may suggest another role for CpG hypermethylation in blocking promoter regions of tumor suppressors in cancerous cells.[10] The sequence used in our measurements had a GC content of 55% and an observed CpG/expected CpG ratio of 1.29, which meet the classic definition of a CpG island[24] except that the length is shorter than 200 bp.

Our study has unraveled the following effects of CpG methylation on the structure of nucleosomes: (i) tighter wrapping of DNA around histones and (ii) a topology change of nucleosomal DNA. Both of these can contribute to the formation of a repressive chromatin structure.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure S1 and supplementary methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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