Single-Molecule Micromanipulation Studies of Methylated DNA

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Introduction

5-methyldeoxycytosine (5-mC) is a major DNA modification in higher eukaryotes[1]. In metazoan genomes, DNA methylation has been traditionally considered to be restricted to symmetric modification of 5-mC at CpG loci. However, the extensive methylation of human mitochondrial DNA in a non-CpG context has been reported recently[2]. The systematic non-CpG cytosine methylation with relatively low frequency is evidenced in stem and embryonic cells[3], neurons[4], and cancer cells[5]. There are multiple lines of evidence suggesting that the local DNA topology determines the selective usage of certain genomic regions as regulatory elements[6]. Yet, physical properties of asymmetrically methylated DNA that exist transiently during replication or maintained at regions flanking CTCF/cohesin binding sites as a hemimethylation signature of progenitor cells[7] are understudied. In order to fill in these knowledge gaps, we designed a study to determine the effect of non-clustered cytosine methylation in the CpG and non-CpG context on the micromechanics behavior of long dsDNA.

Results

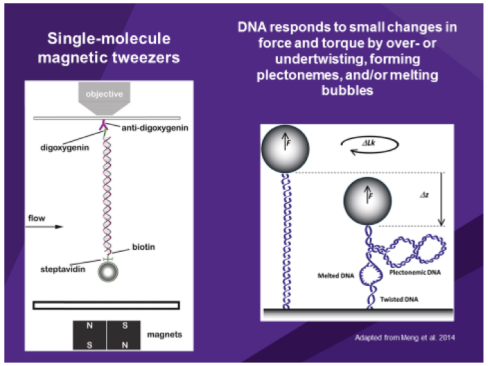
Three types of molecules were studied: the control PCR construct with 23% deoxycytidines lacked methylation altogether. This control sequence was enzymatically methylated by *M.SssI* at the average frequency of one CpG element per 18 base pairs. A second methylated case was prepared using m-5C-PCR, with methylation randomly distributed at an average rate of 1 per 15 bps within any context.

Using force-extension measurements at physiologically relevant stretching forces and supercoiling densities and the classical worm-like chain model of entropic elasticity, we have demonstrated that enzymatic methylation at CpG dinucleotides decreases the persistence length of dsDNA from ~44 to 36 nm. The persistence length of PCR-created DNA with a diverse methylation layout is ~23 nm.

Negative torsional strain occurring during transcription and replication causes interconversions between twisted, plectonemic, and melted DNA states that are related to DNA resilience and flexibility. In our study, sparse random methylation of mostly non-CpG deoxycytidine increased the force (~0.75 pN vs ~0.6 pN for unmethylated counterpart) needed to separate methylated dsDNA strands and create denaturation bubbles during negative supercoiling at physiologically relevant superhelical density -0.06.

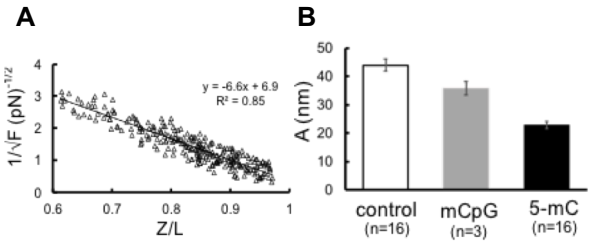
Discussion

Our data provide a foundation for further study of how intrinsic DNA shapes and their nanomechanics might influence the organization of functionally distinct epigenetic landscapes[8]. A key step will be to examine how the different DNA methylation profiles change binding properties of architectural and gene-regulatory proteins.



Schematic of magnetic tweezers setup and illustration of the conformational changes of DNA molecule

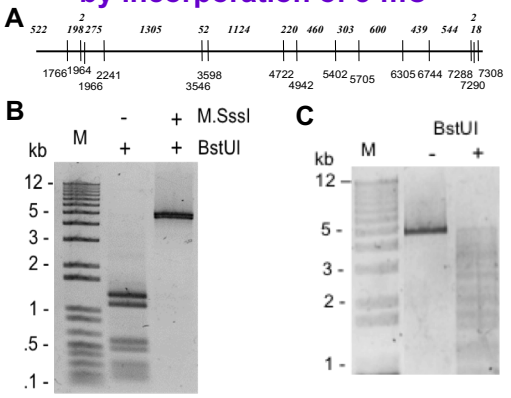
5-mC decreases DNA persistence length



(A). The persistence length (A) value obtained from the force (F)-extension curves of control DNA. 1/√F is plotted as a function of z/L (extension normalized to contour length) and A is determined from the slope of least-squares fit of data. The solid line represents the best fit of linear regression.

(B). Comparisons of the persistence length values of control (c, n=16), enzymatically methylated mCpG (n=3), and PCR-methylated (5-mC, n=16) DNA; the number of experiments n for each construct is indicated.

Protection against *BstUI* digestion by incorporation of 5-mC

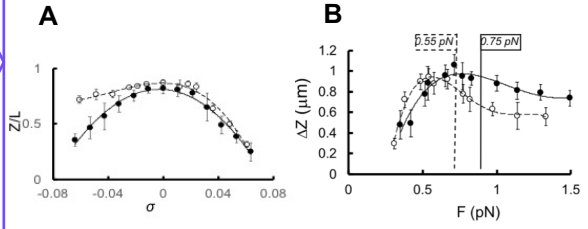


(A). Methylation-sensitive *BstUI* restriction sites and pFOS1 fragment sizes.

(B). The extent of enzymatic (*M.SssI*) CpG methylation (mCpG) of PCR-created construct (control) verified by digestion with *BstUI*.

(C). *BstUI* digestion of randomly methylated by PCR construct (5-mC) produces a range of DNA fragments sizes.

5-mC increases DNA duplex stability against unwinding



(A). Rotation curves. Control (empty circles; dashed line) and methylated (filled circles; solid line) DNA constructs held at constant force 0.6 pN at various superhelical densities σ.

(B). Melting curves for control and methylated DNA. Difference in extension (ΔZ= Z(-0.06)-Z(+0.06)) at the superhelical density σ +/-0.06 as a function of applied stretching force.

References

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