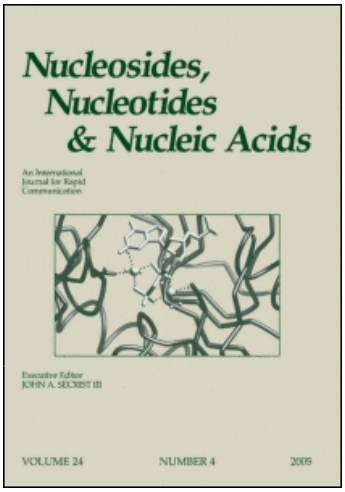

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

5-Methylcytosine and 5-Azacytosine Containing 25Mer Duplexes: Synthesis and Investigation of their Interaction with HeLa Nuclear Protein Extracts

Yacoob Padia[a]; Mario Ariatti[a]; Peter A. Jones[b]
[a] Department of Biochemistry, University of Durban-Westville, Durban, South Africa [b] Department of Biochemistry and Molecular Biology, University of Southern California School of Medicine, Los Angeles, California

To cite this Article Padia, Yacoob , Ariatti, Mario and Jones, Peter A.(1995) '5-Methylcytosine and 5-Azacytosine Containing 25Mer Duplexes: Synthesis and Investigation of their Interaction with HeLa Nuclear Protein Extracts', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1091 — 1092
To link to this Article: DOI: 10.1080/15257779508012541
URL: http://dx.doi.org/10.1080/15257779508012541

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1091-1092 (1995)

5-METHYLCYTOSINE AND 5-AZACYTOSINE CONTAINING 25MER DUPLEXES : SYNTHESIS AND INVESTIGATION OF THEIR INTERACTION WITH HeLa NUCLEAR PROTEIN EXTRACTS.

Yacoob Padia, Mario Ariatti* and Peter A. Jones+

Department of Biochemistry, University of Durban-Westville, Private Bag X54001, Durban, 4000, South Africa. +Department of Biochemistry and Molecular Biology, University of Southern California School of Medicine, Los Angeles, California 90033

Abstract: DNA duplexes each containing a single hemimethylated, fully methylated or 2'-deoxy-5-azacytidine (dz^5C) hemi-methylated CpG sequence and their interactions with partially purified methylated DNA binding proteins and methyltransferase from HeLa cell nuclei are described.

The methylation status of cytosine residues in key CpG sequences of a number of eukaryotic genes is believed to play a role in regulating gene expression. This may be mediated, in part, through the binding of a sequence-specific methylated DNA binding protein (MDBP). It is also known that the antileukemic agent 5-azacytidine (z^5C) may induce suppressed genetic information, an effect believed to arise through the inhibition of post-replicative DNA methylation following its incorporation into DNA. Tight complexes between nuclear DNA binding proteins and $2'dz^5C$ containing DNA from a C3H 10T½ derived cell line have also been demonstrated[1].

An attempt has been made here to study these events at a single CpG palindrome in oligodeoxynucleotide 25mer duplexes containing a hemimethylated, a dz^5C hemimethylated and a fully methylated CpG at equal distance from the two termini (Figure 1 A, B and C, respectively).

Duplexes were obtained by Klenow enzyme catalysed extension of an hexameric primer annealed to a 25mer template with appropriate 2'dNTP building blocks. For this purpose $2'dz^5CTP$ was synthesized by

5'AAATTCAACCAZGTTCCCTCTCTCC3'
TTTAAGTTGGTGXAAGGGAGAGAGG

FIG. 1 Duplexes : A(X = C); B(X = z^5C); C(X = m^5C); Z = m^5C

TABLE 1 : KLENOW ENZYME CATALYSED SYNTHESIS OF OLIGO-DEOXYRIBONUCLEOTIDE DUPLEXES

SYSTEM[1]		TEMPLATE STRANDS COPIED (%)[2]
2'dCTP	(A)	63 (77)
2'dz^{5}CTP	(B)	12 (14)
2'dm^{5}CTP	(C)	50 (20)
-2'dCTP		2.5
-primer		<0.1

[1]Identity of duplex synthesized given in brackets. [2]Values in brackets obtained in 10x scale-up (120pmoles template, 6μmoles primer).

5'phosphorylation of 2'dz^{5}C followed by pyrophosphorylation of 2'dz^{5}CMP. Hence, in reaction mixtures (100μl) containing 12 pmoles template, Hepes (200mM), $MgCl_2$ (5mM), Tris-HCl (50mM pH 8.0), dATP, dGTP, dCTP (20μM each), [^{3}H]dTTP (2μM, 1μCi) and Klenow enzyme (2U), the optimal primer : template mole ratio was found to be 50:1, while maximum incorporation was achieved after 4 hours at room temperature. At best, 77% of template strands were copied in an overnight incubation to afford A. Lower yields of duplexes B and C may be ascribed to poorer suitability of 2'dm^{5}CTP and 2'dz^{5}CTP as Klenow enzyme substrates (Table 1).

Unmethylated and hemimethylated λ DNA was used in binding and methylation assays on DEAE-Sephacel fractions of a 0.3M NaCl HeLa nuclear protein extract to locate MDBP and DNA methyltransferase activity. Poor binding of duplexes to MDBP extracts under conditions of high and low stringency was observed. This may be ascribed to the partial homology of duplexes to the 14 bp MDBP consensus sequence which displays dyad symmetry[3]. In a methyltransferase assay with S-adenosyl-L-[methyl-3H] methionine, duplex associated radioactivity was found to be greatest with the dz^{5}C hemimethylated duplex (B) suggesting the presence of a 'transition state' adduct between the enzyme and the duplex although this was not confirmed.

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