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Effect of cytosine methylation on the cleavage of oligonucleotide duplexes with restriction endonucleases *Hpa*II and *Msp*I

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The pattern of eucaryotic DNA methylation is commonly determined by restriction analysis with methylation-sensitive enzymes (e.g.1). Due to the likely biological significance of hemimethylated CpG dinucleotides (2,3), we investigated the MspI and HpaII hydrolysis of synthetic 29-mer oligos (fig.1a), unmethylated, hemimethylated or fully-methylated at the internal C residue of their recognition site CCGG. As shown in fig.1b,

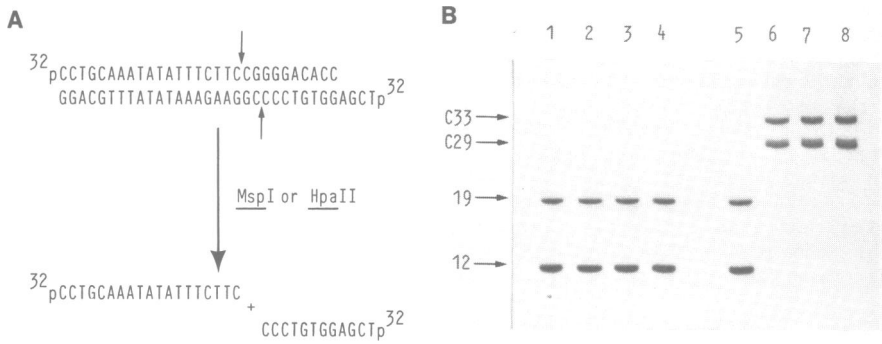

Fig.1: A: Oligos C29/C33 and fragments (C19 and C12) obtained after digestion. The arrows indicate the sites of cleavage. B: Digestions (4) with MspI (lanes 1-4) or HpaII (lanes 5-8) Lanes: 1,5 C29/C33; 2,6: mC29/C33; 3,7: mC29/mC33; 4,8: C29/mC33.

MspI cleaved all the four substrates, irrespective of their state of methylation. HpaII failed to digest the symmetrically-methylated duplex (mC29/mC33), while the unmethylated duplex (C29/C33) was completely and efficiently digested. Surprisingly, neither cleavage nor nicking of the unmethylated strand were observed with the hemimethylated substrates. These results demonstrate that the use of HpaII in the search for regions of active, unmethylated chromatin would fail to identify hemimethylated CpG sites, recently shown to be involved in the process of transcriptional gene activation (3).

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